Uncomplicated Urinary Tract Infection in Primary Care; Evaluation of Point of Care Tests and Patient Management

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Thesis submitted for the degree of PhD

2015

Cardiff University

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Acknowledgements

I would like to thank my supervisors, Professor Christopher Butler and Dr Micaela Gal for their encouragement, guidance and wisdom.

I am grateful to the South East Wales Trials Unit (SEWTU), particularly the POETIC trial team, I thoroughly enjoyed working on this trial and with this team over the past three years. A special thank you to Professor Kerry Hood and Tim Pickles, for their help with the data analysis.

Thank you to Professor Jones, Dr Deakin and the team at Liverpool University for their collaboration on the chromatic sensing part of my thesis.

I am grateful to all the GPs, microbiologists and laboratory staff who participated in the research, particularly Dr Mandy Wootton and the team at the Specialist Antimicrobial Chemotherapy Unit for their expert advice and continuous support.

Thank-you to Mala Mann, Angela Watkins and colleagues at the Cochrane Institute of Primary Care and Public Health I have fully enjoyed the past three years working in such a supportive environment.

A big thank you to all the friends I have made since moving to Cardiff to start my PhD, you have made the move to Wales a joy and I am sure I have made friends for life. In particular Anwen, Fiona and Jon - thank you for proof reading my thesis!

Finally, thank you to my family and friends back home for supporting and encouraging me throughout my PhD.

Thesis Summary

Antibiotic resistance is an increasing global public health problem. Resistance is increasing sharply in gram-negative organisms, including *Escherichia coli (E. coli),* the main causative organism for community-acquired urinary tract infection (UTI). Antimicrobial stewardship strategies in primary care to help contain antibiotic resistance include supporting general practitioners (GPs) in deciding whether to prescribe an antibiotic for UTI and selecting the most appropriate antibiotic.

In this thesis, I aim to describe the management of uncomplicated UTI in primary care and evaluate potential point of care tests (POCT) to assist the diagnosis and/or appropriate prescribing of antibiotics for uncomplicated UTI. The program of work includes:

1. Laboratory evaluation of a culture-based test that allows the quantification, identification and susceptibility profile of infecting bacteria from urine (Flexicult^{TM}).
2. Evaluation of a novel chromatic sensing technique to identify bacterially infected urine compared to visual assessment of urine turbidity and urinalysis dipsticks.
3. Systematic review and analysis of data (descriptive and multi-level modelling) from an international primary care based observational study to describe UTI management.

I identified unwarranted variation in clinical management of UTI between countries and between general practices within countries. Empirical antibiotic prescribing for UTI in Europe is high and treatment is generally prescribed for longer than guidelines recommend. Flexicult^{TM} was comparable to UK 'reference standard' NHS laboratory microscopy and culture for identifying bacterial UTI. The use of Flexicult^{TM} in practice may support GPs in screening out negative samples reducing the proportion of patients that are prescribed antibiotics empirically. Chromatic sensing and visually assessing turbidity were equally useful at identifying negative urine samples and both improved the analytic performance of urinalysis dipsticks. The chromatic sensing system requires development prior to further evaluation.

Glossary Terms and Abbreviations

95% CI	95% Confidence Interval
ASP	Any small particles (microscopy)
ATCC	American Type Culture Collection (biological resource company)
AUROC	Area under receiver operating characteristic
BMS	Biomedical scientist
BNF	British National Formulary
BSAC	British Society for Antimicrobial Chemotherapy
CRD	Centre for reviews and dissemination
CRF	Case Report Form
CSU	Catheter specimen of urine
DIC	Deviance information criterion
DID	Defined Daily Dose
EQA	External Quality Assurance
ESAC	European Surveillance of Antimicrobial Consumption
ESBL	Extended Spectrum Beta Lactamase; enzymes produced by bacteria that are resistant to certain antibiotics
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
EUCAST MIC	European Committee on Antimicrobial Susceptibility Testing Minimum Inhibitory Concentration
EUG	European Urinalysis Guidelines
GI	Gastrointestinal tract
GP	General Practitioner
HPA	Health Protection Agency; now Public Health England and Public Health Wales
ICC	Intraclass correlation
IDSA	Infectious Diseases Society of America
IQR	Inter-quartile range
ITU	Intensive treatment unit
LE	Leucocyte esterase
LHR or LR	Likelihood ratio
LRTI	Lower Respiratory Tract Infection
Maldi-TOF	Matrix Assisted Laser Desorption Ionization Time-of-Flight
MCMC	Markov chain Monte Carlo estimate
MDR-GNB	Multidrug resistant Gram negative bacteria
MIC	Minimum Inhibitory Concentration
MQL	Marginal quasi-likelihood estimate
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSU	Mid-stream urine

NHS	National Health Service
NLR	Negative likelihood ratio
NPV	Negative Predictive Value
OD; BD; TDS; QDS	Pharmaceutical dosing terminology for: once daily; twice daily; three times a day; and four times a day
OR	Odds Ratio
PHW	Public Health Wales
PICO	Population/Intervention/Comparison/Outcome
PIL	Patient Information Leaflet
PLR	Positive likelihood ratio
POCT	Point Of Care Test
POETIC study	Point of care testing for urinary tract infection in primary care
PPV	Positive Predicitve Value
PQL	Penalized (predictive) quali-likelihood estimate
RBC	Red blood cell
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
R-GNOSIS	Resistance in Gram-Negative Organisms: Studying Intervention Strategies
ROC	Receiver operating characteristic
SD	Standard Deviation
SE	Standard Error
SIGN	Scottish Intercollegiate Guidelines Network
SMAC	Standing Medical Advisory Committee (UK Department fo Health)
SMI	Standard for Microbiology Investigations
SOP	Standard Operating Procedure
SPA	Suprapubic aspirate
SSI	Statens Serum Institut, Denmark
UHW	University Hospital Wales
UKECA	United Kingdom Ethics Committee Authority
UTI	Urinary Tract Infection
VPC	Variance partition coefficient
WBC	White blood cell
WHO	World Health Organisation

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Section 1: Introduction

Chapter 1: Introduction, aims and objectives

Overall research aim

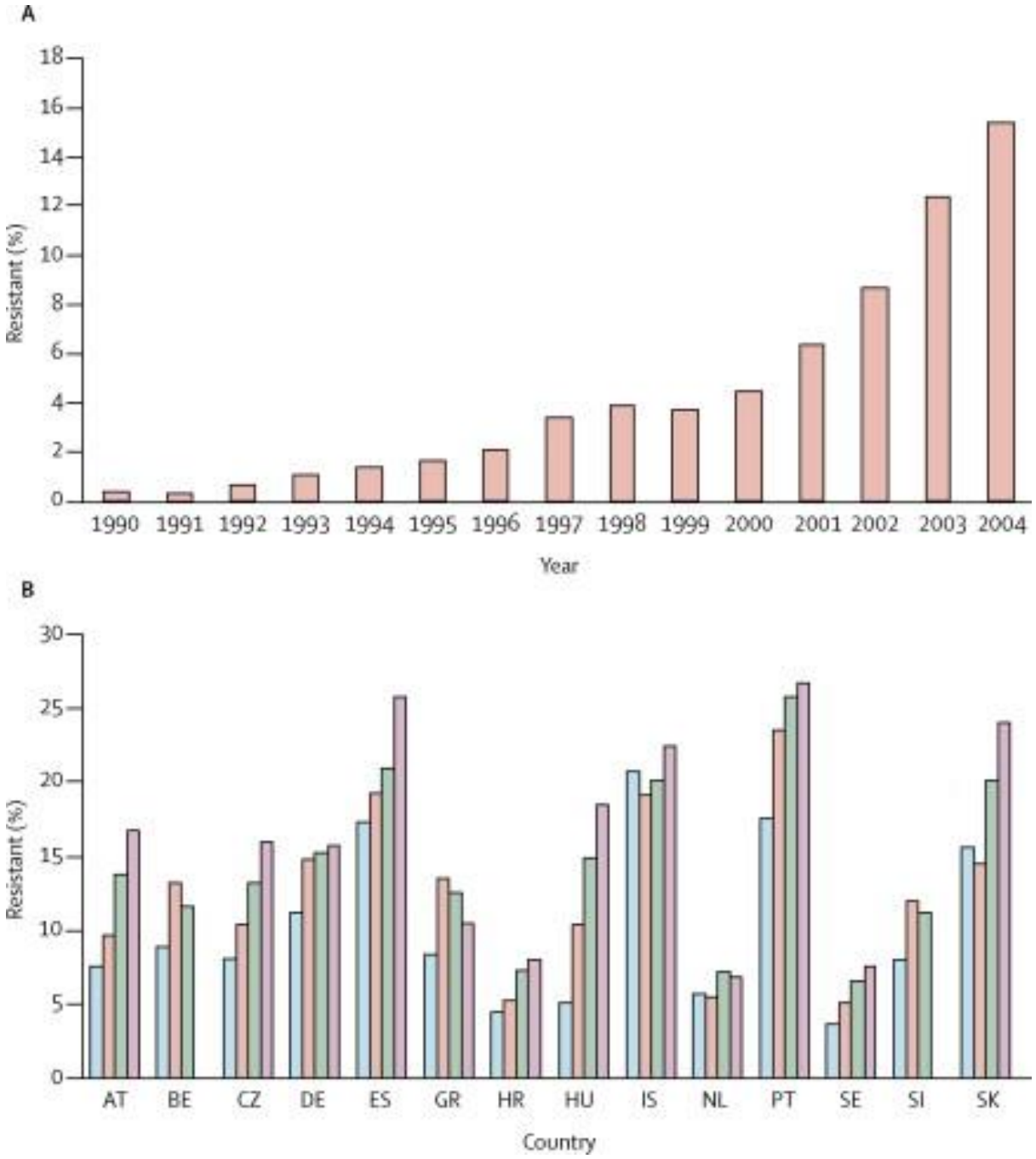
The overall aim of this thesis is to describe the management of patients presenting with symptoms of uncomplicated urinary tract infection (UTI) in primary care, and to evaluate potential point of care tests to assist clinicians in the diagnosis of uncomplicated UTI and/or the appropriate prescribing of antibiotics for uncomplicated UTI in this setting.

The importance of this research: antibiotic resistance

Antibiotics have been used for decades to save millions of people from life threatening infections. Indeed, the practice of technologically advanced medicine has been possible only with the aid of antibiotics, and food animals can now be reared more intensively with the use of antibiotics as growth promoters and herd/flock therapy (1). However 70 years after the introduction of antibiotics (the mass production of penicillin was in the early 1940's) we find ourselves on the edge of a post-antibiotic era, where there would be limited options for the treatment of infections and prophylaxis for surgical procedures, increased length of hospital stays and increased patient morbidity and mortality due to the global emergence of antibiotic resistance in bacteria. A 2009 European Centre for Disease Control and European Medicines Agency joint report stated that 25,000 deaths in Europe were a direct consequence of multi-drug antibiotic resistance (2). Likewise in the Unites States of America a report by the Centre for Disease Control estimated that 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 die each year as a result of these infections (3). The World Health Organisation (WHO) Global Tuberculosis Report (2013) estimated that world-wide 170,000 people died from multi-drug resistant tuberculosis in 2012 (4).

A key finding from the WHO antimicrobial resistance global report on surveillance 2014 included information that resistance to one of the most widely used antibacterial - the fluoroquinolones - is very widespread. In the 1980s, when these drugs were first introduced, resistance was virtually zero. Today, there are countries in many parts of the world where this antibiotic is now ineffective in more than half of patients (5). Until recently, it was considered that the hospital, and especially the intensive care units, were the major source of antibiotic resistant bacteria, with many reporting problems worldwide with *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, the Enterobacteriaceae and Staphylococci (1). However resistance is increasing sharply in *Escherichia coli,* the main causative organism of urinary tract infection and an organism where infections are acquired in the community, or, even if they arise in hospital, usually involve strains from the patients endogenous gut flora. In accord with the WHO report, resistance to fluoroquinolones in *E. coli*, are increasing steadily as shown in Figures 1.1.1 (A) for England and Wales (data from the Health Protection Agency), and (B) Europe respectively (data from European Antimicrobial Resistance Surveillance System) (6).

Figure 1.1.1. (A) Resistance trends to ciprofloxacin in *E. coli* from bacteraemias in England and Wales, 1990–2004. (B) Resistance trends to fluoroquinolones in *E coli* from European countries, 2001–04. Sources: (A) Health Protection Agency, data on file; (B) European Antimicrobial Resistance Surveillance System (http://earss.rivm.nl).(6)



AT=Austria, BE=Belgium, CZ=Czech Republic, DE=Germany, ES=Spain, GR=Greece, HR=Croatia, HU=Hungary, NL=Netherlands, PT=Portugal, SE=Sweden, SI=Slovenia, SK=Slovakia. Countries with incomplete reporting over the 3 years (e.g., France, Italy, and Ireland) are excluded. For each county the bars, in sequence left to right, represent the 4 years.

The first 30 or so years of antibiotic use saw the introduction of a multitude of new antibiotic agents, yet in the last 25-30 years, only one new family, the oxazolidinones, has been introduced (1). The discovery and development of new antibiotics remains vital. However many pharmaceutical companies have left this area of research possibly due to antibiotics being for short-term consumers (even if there is wide usage) and the

knowledge that new antibiotics may rapidly be compromised by emerging resistance (6, 7). Instead companies will be developing specifically targeted antibiotics i.e. strain specific Gram-negative antibiotics but in order to use these treatments appropriately better POCTs need to be developed so that these infections can be clearly identified prior to treatment. The WHO is also calling attention to the need to develop new diagnostic tests, antibiotics and other tools to allow healthcare professionals to stay ahead of emerging resistance. Included in the report are the following strategies that can be undertaken by various facilitators;

Health workers and pharmacists can help tackle resistance by:

- enhancing infection prevention and control;
- only prescribing and dispensing antibiotics when they are truly needed;
- prescribing and dispensing the right antibiotic(s) to treat the illness;

Policymakers can help tackle resistance by:

- strengthening resistance tracking and laboratory capacity;
- regulating and promoting appropriate use of medicines;

Policymakers and industry can help tackle resistance by:

- fostering innovation and research and development of new tools;
- promoting cooperation and information sharing among all stakeholders.

A key feature of these strategies (to limit antibiotic resistance) is the promotion of better antibiotic prescribing. A report from the UK Department of Health's Standing Medical Advisory Committee (SMAC) Sub-Group on Antimicrobial Resistance in 1998 identified that 80% of antimicrobial prescribing for patients was in the community for common infections and recommended to clinicians 'Four things you can do to make a difference', namely;

(i) no prescribing of antibiotics for simple coughs and colds;

(ii) no prescribing of antibiotics for viral sore throats;

(iii) limiting prescribing for uncomplicated cystitis to 3 days in otherwise fit women;

(iv) limiting the antibiotic agents prescribed over the telephone to exceptional cases (8).

As part of my research I am interested in the promotion of more appropriate antibiotic prescribing for community acquired uncomplicated urinary tract infection. Necessary steps to manage this situation include better surveillance to assess accurately the extent of problems (i.e. inappropriate prescribing, unwarranted variation in prescribing practice and use of diagnostics), more prudent use of the available antibiotics to conserve valuable therapeutic resources and improved infection control to limit the spread of resistant organisms (4). A complete picture of infectious disease management allows for inter-country comparison and analyses of drivers of change (9). The surveillance of the management of uncomplicated UTI in primary care (including antibiotic prescribing) and evaluation of point of care tests to guide prudent use of available antibiotics for uncomplicated UTI are the foundation of my research.

Policies to reduce inappropriate antibiotic prescribing in primary care, thereby reducing the pressure for the emergence and spread of antibiotic-resistant pathogens, have had some success, but substantial variations in prescribing rates between- and within-countries persist. The European surveillance of Antimicrobial Consumption (ESAC) project, monitoring outpatient antibiotic use from 33 countries revealed the total outpatient antibiotic use in 2009 varied by a factor of 3.8 between the countries with the highest (38.6 defined daily dose (DID) in Greece) and lowest (10.2 DID in Romania). A significant increase was found in total outpatient antibiotic use, as well as a significant seasonal variation, which decreased over time from 1997 to 2009 (10). Current policies are based largely on ecological relationships between prescribing and resistance, and there are only limited data at the level of the individual patient that are not subject to the serious selection bias of using routinely collected patient data and that are adjusted for potential confounders (11). I will be presenting the results of a narrative systematic review of observational studies of the management of uncomplicated urinary tract infections in primary care in Europe. Additionally, I will present and discuss some of the results from a multi-centre, multi-national observational study (phase 2 of a larger ‘POETIC study’ discussed further in Section 3, Chapter 2) of the presentation and

management of patients presenting with suspected uncomplicated urinary tract infection in primary care centres in England, Wales, Spain and the Netherlands.

Another important strategy to reduce antibiotic use is to support general practitioners (GPs) in deciding both whether or not to prescribe an antibiotic for patients presenting with symptoms of uncomplicated UTI and in selecting the most appropriate antibiotic. This need could be met by a diagnostic point-of-care test (POCT) that could rapidly identify the presence of either infecting bacteria or surrogate markers of bacterial infection (host or pathogen biomarker), and ideally also indicate the antibiotic susceptibility profile of any infecting bacteria. A POCT is defined as any test performed by or on behalf of the treating doctors, on-site at the time of consultation, which allows the test result to be used to guide immediate decisions about patient treatment (12). A recent study of clinicians' and patients' views of POCTs (for lower respiratory tract infection) in primary care showed that the GPs most common reported advantages include managing patient expectations for antibiotics and enhancing patient satisfaction where clinicians felt that patients were generally reassured by technology that augmented clinical assessment. POCTs can also be helpful in clinicians' own decision making process and contribute to more robust diagnosis and prognosis (13, 14). Concerns raised by GPs include test performance sensitivity, specificity and reliability and misdiagnosis of serious infections. Problems interpreting values, deciding cut-off points, or false positives and negatives are also a concern (14). Patient views are generally supportive of POCT use stating that POCTs would be useful and give GPs more information to make better decision about their treatment and to establish if antibiotics are necessary. Concerns from patients include anxiety waiting for a result, aversion to needles or a blood sample being taken and problems of time and cost (14).

The development stages of any new diagnostic test for infectious disease include identifying a clear need for a new test, clearly defining where and how a test is to be used, discovery and validation of candidate biomarkers (to incorporate into a new diagnostic test) and extensive evaluation of the test. Clinical specimens such as urine

are an essential prerequisite for both biomarker discovery and the development and evaluation of new diagnostic tests. A systematic approach is needed to test:

(i) analytical validity, whether the test measures what it is claimed to measure;

(ii) clinical validity, whether the test answers the clinical question being asked (e.g., screening, diagnosis, prognosis, monitoring);

(iii) clinical usefulness, whether the test leads to better outcomes; and

(iv) social context, including ethical, economic, and legal issues (15).

I will be presenting the analytic performance and discussing the clinical usefulness of UK Flexicult™ SSI-Urinary kit; a culture based diagnostic and antibiotic susceptibility point of care test for urinary tract infections. Additionally I will be presenting preliminary analytic performance data on chromatic sensing; a novel approach (and potential POCT) to aid GPs in the diagnosis of bacterial UTI.

Thesis layout

This thesis is divided into four sections: Section 1 – Introduction; Section 2 – Analytic Performance of UK Flexicult™ SSI-Urinary Kit, chromatic sensing, visual turbidity assessment of urine and dipstick urinalysis in the diagnosis of suspected urinary tract infection; Section 4 - Management of uncomplicated UTI in Primary Care; and Section 5 – Overall Discussion.

Summary of research aims and objectives

The primary aim of this research is to describe the management of uncomplicated urinary tract infection (UTI) in primary care and to evaluate potential point of care tests to assist in the diagnosis of uncomplicated UTI and/or the appropriate prescribing of antibiotics for uncomplicated UTI in this setting.

Secondary aims are;

1. To evaluate the analytic performance of UK Flexicult™ SSI-Urinary kit by comparing performance with routine NHS microbiology laboratory testing, and enhanced urine culture techniques.
2. To explore chromatic sensing technology as a potential point-of-care test to help GPs in their decision to prescribe antibiotics to patients with suspected urinary tract infection.
3. To systematically identify and review published studies that have evaluated the routine management of adult women with suspected uncomplicated UTI attending primary care in different European countries; and to summarise these data to determine variation in management between countries.
4. To evaluate whether a patients' resident country, age, history of UTI, symptom severity, days off work, days with symptoms, dipstick urinalysis testing, urine appearance and oral temperature are associated with different management decisions (including the use of POCT, requesting a urine culture, antibiotic prescribing and follow-up recommendations).

Research objectives:

- Compare the diagnostic accuracy in determining a bacterial UTI using UK Flexicult POCT to routine NHS microbiology laboratory methods, and evaluate discrepancies using enhanced urine culture and identification techniques;
- Evaluate Biomedical Scientists (BMS) and General Practitioners (GP) inter-observer variation on the interpretation of the UK Flexicult POCT;
- Determine if diluting turbid urine (potential for over-inoculation) could improve identification of predominant uropathogens; and improve Flexicult susceptibility concordance with NHS susceptibility results;
- Assess the analytic performance of chromatic sensing in the laboratory diagnosis of UTI with routine NHS microscopy and culture as the reference standard; and compare the analytic performance of chromatic sensing to currently available POCTs for UTI including urine dipsticks and visual turbidity assessment;

- Conduct a narrative systematic review of the literature including critical appraisal and data extraction of included studies evaluating the routine management of women with suspected uncomplicated UTI in primary care;
- To describe patient demographics and signs and symptoms at presentation in primary care (with symptoms attributable to UTI) and clinical management decisions for those patients included into the POETIC observational study of uncomplicated UTI in four participating countries;
- To examine the individual (patient) level factors associated with differences in patient management using multilevel statistical modelling.

Chapter 2: Background

In this chapter I will summarise: the evaluation of diagnostics tests; the classification and prevalence of uncomplicated UTI; current management options including prescription of antibiotics and available point of care tests; and the laboratory diagnostic process for UTI in the UK.

Evaluation of diagnostic tests

Clinical samples are a vital requirement for enabling and facilitating the development and evaluation phases of any diagnostic test. These phases include identifying and validating candidate biomarkers, establishing proof-of-principle for the new test, establishing methodology for sample preparation and evaluating the new test prototype. Once a new test has been developed and before it can be recommended for use in routine care, the evaluation data should demonstrate that the test is at least as good as the current 'gold standard' test. Therefore, the test must be compared directly with the existing best practice method using the same clinical samples.

The absolute 'gold standard' for diagnosis of UTI is the culture of bacteria from urine obtained by suprapubic needle aspiration. This is not generally feasible and can cause unnecessary anxiety or pain to patients, so the next best clinical specimen is a mid-stream urine. There is no bacterial count that can be taken as an absolute 'gold standard' for the diagnosis of UTI and guidelines vary on this. Due to the limitations of microbiological microscopy and culture it may be more appropriate to consider the 'gold standard' diagnostic test for UTI as the 'reference standard'.

When assessing and comparing diagnostic accuracy of a test there are several analyses that can be used and are described in later chapters but predominantly sensitivity, specificity, positive and negative predictive values are used to assess the tests diagnostic accuracy. Sensitivity shows the likelihood of a positive test result if an individual were to truly have the disease. The specificity shows the likelihood of having

a negative test result if an individual does not have the disease. The sensitivity and specificity are characteristics of the test itself and are independent of the disease prevalence. Disease prevalence is combined with sensitivity and specificity to create the positive and negative predictive values. The positive predictive value (PPV) is the probability that the patient has the disease when the test result is positive. The negative predictive value (NPV) is the probability that the patient does not have the disease if the test result is negative.

Classification and prevalence of urinary tract infections

Urinary tract infection (UTI) refers to the presence of microbial pathogens within the urinary tract. Infections can be classified according to their location within the urogenital tract. The different parts of the urinary tract, however, communicate with each other to some degree. As a result, bacteria in one area can move to infect other areas. For practical clinical reasons UTIs (and infections of the male genital tract) are classified according to the predominant clinical symptoms:

• uncomplicated lower UTI (cystitis; bladder)

• uncomplicated pyelonephritis (kidney)

• complicated UTI with or without pyelonephritis (bladder ± kidney)

• urosepsis (blood stream)

• urethritis (urethra)

• special forms: prostatitis (prostate), epididymitis (epididymis) and orchitis (testes).

Asymptomatic bacteriuria is another term defined as the isolation of bacteria from the urine in significant quantities consistent with infection, but without the local or systemic genitourinary signs or symptoms. Asymptomatic bacteriuria only requires treatment in specific populations such as pregnant women (16). Asymptomatic bacteria is common in nursing homes.

Factors that suggest a potential complicated UTI include: male sex; elderly; hospital-acquired infection; pregnancy; indwelling urinary catheter; recent urinary tract intervention; functional or anatomical abnormality of the urinary tract; recent antibacterial

use; symptoms for > 7 days at presentation; diabetes mellitus; and immunosuppression (17). Table 1.1.1 describes five different categories of urinary tract infection, their clinical presentation and laboratory diagnostic criteria (although this is not universal as discussed later in the microbiological diagnosis section).

The clinical presentation and management of different UTI categories may vary during life and may depend on the patient's condition. Therefore, the requirements of special patient groups (the elderly, those with underlying diseases and the immunocompromised) also need to be considered at the time of consultation (17).

Uncomplicated UTI is classified as UTI without structural and functional abnormalities within the urinary tract (uropathies), without relevant kidney diseases (nephropathies) and without relevant comorbidities favouring more serious adverse outcome (18). There seem to be no long-term adverse effects on renal function nor increased mortality associated with acute uncomplicated UTI/cystitis (in the non-pregnant population), not even in women with frequent recurrences. Untreated cystitis rarely progresses to symptomatic upper urinary tract infection and is limited to symptoms, which can, however, affect quality of life considerably (18).

Uncomplicated UTIs affect up to 15% of women each year. More than 25% of women who have had an infection will experience a recurrence in their lifetime (19). The probability of cystitis in a woman with symptoms of dysuria, urinary frequency, or gross haematuria is about 50% in primary care settings (19, 20). Specific combinations of symptoms (for example, dysuria and frequency without vaginal discharge or irritation) raise the probability of cystitis to more than 90%. When a woman who has previously had cystitis has symptoms suggesting a recurrence, there is an 84% - 93% chance that an infection is present (19). In an American study of a random digit dialling survey of 2000 women representative of the United States population on the incidence (and associated costs) of UTI; 11% (95% CI: 9.4, 12.1%) of women aged 18 and older reported at least one presumed UTI during the past 12 months, with the majority of the cases occurring among women with a history of two or more UTI episodes in their life. They estimate that by age 26, one-third of women will have at least one physician-

diagnosed presumed UTI with a lifetime risk of 60% (21). Within three to four months of an initial UTI, 20% to 30% of women will experience a recurrence of the infection with additional concomitant short-term morbidity (16).

Table 1.1.1. Criteria for the diagnosis of a UTI, as modified according to IDSA/ESCMID guidelines (17, 22).

Category	Description	Clinical Features	Laboratory Investigations[1]
1	Acute uncomplicated UTI in women; acute uncomplicated cystitis	Dysuria, urgency, frequency, suprapubic pain, no urinary symptoms in four weeks before this episode	≥ 10 WBC/mm^3 ≥10^3 cfu/mL*
2	Acute uncomplicated pyelonephritis	Fever, chills, flank pain; other diagnoses excluded: no history or clinical evidence of urological abnormalities (ultrasonography, radiography)	≥ 10 WBC/mm^3 ≥10^4 cfu/mL*
3	Complicated UTI	Any of symptoms from categories 1 or 2 above; one or more factors associated with a complicated UTI[2]	≥ 10 WBC/mm^3 ≥10^5 cfu/mL* in women ≥10^4 cfu/mL in men, or in straight catheter urine in women
4	Asymptomatic bacteriuria	No urinary symptoms	≥ 10 WBC/mm^3 ≥10^5 cfu/mL* in two consecutive MSU cultures ≥ 24 hours apart
5	Recurrent UTI (antimicrobial prophylaxis)	At least three episodes of uncomplicated UTI documented by culture in the last 12 months; women only; no structural or functional abnormalities	≥ 10 WBC/mm^3

ISDA – Infectious Diseases Society of America; ESCMID – European Society for Microbiology and Infectious Diseases ; UTI - urinary tract infection; MSU – mid-stream urine; WBC – white blood cells; 1 – laboratory diagnostic criteria may vary between countries and laboratories; 2 – as described in text; *Uropathogen in MSU culture. All pyuria counts refer to unspun urine.

Management of uncomplicated UTI in primary care

Guidelines recommend treating uncomplicated UTI empirically if there are severe or multiple relevant symptoms or the patient has a history of UTI (brief summary in Table1.1.2). If there is any uncertainty or if the symptoms are mild, further diagnostic tests should be undertaken. These vary across countries and include evaluating urine turbidity, dipstick urinalysis (generally nitrites and leucocytes), dip-slides, urine sediment/microscopy and in some countries C-reactive protein tests. Laboratory urine culture is generally not recommended for uncomplicated urinary tract infection (diagnostic tests are discussed in more detail later).

Table 1.1.2. Guidelines on management of uncomplicated urinary tract infections in women

Guidelines	Treat in presence of	First Choice Drugs	Duration
Health Protection Agency (England and Wales)	Severe or ≥3 symptoms alone or if mild symptoms cloudy urine and dipstick	Trimethoprim	200mg bid 3 days
		Nitrofurantoin	100mg bid 3 days
Infectious Diseases Society of America and the European Society of Microbiology and Infectious Diseases	Empirical treatment for women with symptoms of uncomplicated cystitis and able to take oral medication	Nitrofurantoin	100mg bid 5 days
		Trimethoprim-sulfamethoxazole	160/800mg 3 days
		Fosfomycin Trometamol	3gm single dose
		Pivmecillinam	400mg bid 5 days

Short-term, higher dose antibiotic therapy (3 days or less) can be considered as the treatment of choice because of many advantages: better compliance; less collateral effects; lower cost; but as efficient as conventional therapy (11, 17, 18). Another aspect relates to adverse effects in the patient. In general, the antibiotics commonly prescribed cause only mild and moderate adverse effects such as vaginal candidiasis and

gastrointestinal disorders. Several fluoroquinolones, however, had to be withdrawn from the market because severe adverse effects, for example, liver damage. Some antibiotics still in use have the potential risk of severe, but very rare adverse effects, for example, Lyell syndrome (trimethoprim and sulfamethoxazole) or severe pulmonal and neuronal effects (nitrofurantoin) (18). Table 1.1.3 outlines the main antibiotics used for uncomplicated urinary tract infections, their potential for causing ecological collateral damage and their recommended use. “Collateral damage” is a term used to refer to ecological adverse effects of antibiotic therapy; namely, the selection of drug-resistant organisms and the unwanted development of colonization or infection with multidrug-resistant organisms; it is the cornerstone of antibiotic stewardship. There are different levels of collateral damage which can be evaluated such as antibiotic use in persons infected with an antibiotic-resistant organism (individual level), antibiotic use in an institution correlated to rates of antibiotic resistance in that institution (group level), and interventions aimed at limiting use of antibiotics of various classes to decrease selection pressure leading to antibiotic resistance (23). Costelloe et al. performed a systematic review investigating subsequent antibiotic resistance in individuals prescribed antibiotics in primary care and found that prescribing for a respiratory or urinary infection individuals develop bacterial resistance to that antibiotic. The effect is greatest in the month immediately after treatment but may persist for up to 12 months. This effect not only increases the population carriage of organisms resistant to first line antibiotics, but also creates the conditions for increased use of second line antibiotics in the community (24). Nitrofurantoin is fast becoming the first-choice antibiotic for uncomplicated UTI in many countries, such as the UK, Germany, Spain, The Netherlands, Italy, Croatia, Sweden, and the USA (22, 25-31). Trimethoprim is still first-choice in some countries as long as the local resistance rates are less than 20% (22, 25). Other first-choice antibiotics include fosfomycin and pivmecillinam but these are not licensed in all countries (including the UK) (18, 22, 25).

Empirical antibiotic prescribing for uncomplicated UTI, however, may often be unnecessary, since 34% - 60% of patients with suspected UTI have been shown not to

have a microbiologically proven UTI (32-34). This does not necessarily rule out a bacterial infection as the diagnostic criteria for UTI is variable, laboratory techniques are often basic and subjective, and urine samples can be poor quality/contaminated. Also, many women, even those with a bacteriologically proven UTI, will recover at a similar rate regardless of whether they received antibiotic treatment or not (35) and some women presenting with symptoms of uncomplicated UTI accept or actually prefer to be managed without immediate antibiotics for example having the option of delayed back-up antibiotic prescription (36). In a prospective cohort study of 176 women across 20 general practices in the Netherlands, Knottnerus BJ *et al*. concluded that more than a third of women with UTI symptoms are willing to delay antibiotic treatment when asked by their GP and the majority of delaying women report spontaneous symptom improvement after one week (37).

The management of UTI in primary care is evaluated and discussed further in Section 3. Current POCTs used for UTI and routine diagnostic processes are described further below.

Table 1.1.3. Description of antibiotics used to treat UTI.

Antibiotic	Collateral* Considerations (18)	Further Comments and Recommended Use (22)
Aminopenicillins	Restricted impact on Enterobacteriaceae in the gastrointestinal (GI) tract, but provides anti-enterococcal activity	Amoxicillin and ampicillin should not be used for empirical treatment given the relatively poor efficacy and very high prevalence of antibiotic resistance to these agents worldwide.
Cephalosporins	Reduce Gram-negative Enterobacteriaceae but select Enterococci in the GI tract because of their inherent enterococcal resistance. This mechanism may also cause an overgrowth of *Clostridium difficile* and Extended Spectrum Beta Lactamase (ESBL) producing bacteria.	β-Lactam agents, including co-amoxiclav, cefdinir, cefaclor and cefpodoxime-proxetil, in 3 – 7 days regimens are appropriate choices for therapy when other agents cannot be used. Other β-lactams, such as cephalexin, are less well studied but may be appropriate in certain settings. The β-lactams generally have inferior efficacy and more adverse effects, compared with other antibiotics. For these reasons, β-lactams other than pivmecillinam should be used with caution for uncomplicated cystitis.
Fluoroquinolones	Very high impact on the gastrointestinal and skin flora by eradicating susceptible and selectively resistant bacterial strains, which are not only fluoroquinolone –resistant strains, but also ESBL-producing bacteria, methicillin resistant *S. aureus* (MRSA) and *C. difficile*.	Ofloxacin, ciprofloxacin, and levofloxacin highly efficacious in 3-day regimens but have propensity for collateral damage* and should be reserved for important uses other than acute cystitis and thus should be considered alternative antibiotics for acute cystitis.
Fosfomycin	Low impact on the GI flora.	3g in single dose; minimal resistance but appears to have inferior efficacy compared with other short-course regimens according to data submitted to the US Food and Drug Administration (FDA). Not licenced globally (e.g. U.K).
Nitrofurantoin	Does not concentrate in the gastrointestinal tract. The selection of resistant mutants in the GI tract is, therefore, generally not a problem.	100 mg twice daily for 5 days has efficacy comparable to 3 days trimethoprim-sulfamethoxazole.
Pivmecillinam	Only limited impact on the GI flora.	Appropriate choice for therapy in regions where available (only available in Scandinavian countries, the Netherlands, Austria and Canada), minimal resistance but may have inferior efficacy compared with other therapies (400mg 2dd 3 – 7days).
Trimethoprim and trimethoprim/sulfamethoxazole	High impact on the GI flora, resulting in pronounced decrease of Gram-negative Enterobacteriaceae. Selection of resistant *E. coli* has been shown to occur easily. The resistant genes are usually plasmid encoded and easily spread.	Use if local resistance is less than 20% or if infecting strain known to be susceptible. Trimethoprim (200 mg 2dd 3 days) considered equivalent to trimethoprim-sulfamethoxazole (160/800mg 2dd 3 days).

* Collateral damage, a term describing ecological adverse effects of antibiotic therapy, such as the selection of drug-resistant organisms and colonization or infection with multidrug-resistant organisms and colonization or infection with multidrug resistant organisms, has been associated with use of broad spectrum cephalosporins and fluoroquinolones (22).

Current point of care tests for UTI

In the UK at present only visual assessment of the turbidity of urine and urinalysis dipsticks are used as POCTs for UTI in primary care (as recommended by the HPA guidelines (25)).

Dip-slides and rapid microscopy

POCTs including urine dip-slides and rapid microscopy are used for diagnosis of UTI in primary care in other European countries. A dip-slide is a type of culture technique which can allow the user to determine bacterial counts and identification in clinical specimens such as urine after a 24 hour test incubation period. Dip-slides are sticks coated with culture media, which get dipped into urine and come complete with an all-in-one transport container and growth chamber. Dip-slides decrease the time between urine collection and test inoculation which increases bacterial recovery and accuracy of results. Dip-slides are suitable for identifying negative urine specimens (no bacterial growth) and those with significant growth of *E. coli* and in these instances are useful in guiding a GP in whether to prescribe antibiotics or not. At high bacterial concentrations, confluent growth of bacteria on the test necessitates a subculture for identification, mixed flora may also be missed and *S. saprophyticus* cannot be isolated (38). Additionally the sensitivity and specificity of dip-slides read in daily general practice decreases when compared to the dip-slides inoculated with the same urine read by experienced microbiologists; for reliable results, reading by laboratory professionals, or special training for clinical personnel is recommended (38, 39). Dip-slides need to be cultured for 24h prior to reading the result and do not provide additional information on antibiotic susceptibility; although the identification of certain bacterial isolates and the known antibiotic resistance levels in that geographical region may aid the general practitioners (GPs) prescribing choice.

Traditional urine sediment microscopy may still be used in primary care. Without laboratory procedures including staining the sample, some of the elements (crystals, casts, squamous cells, epithelial cells, blood cells and bacteria) that may be present in urine may not be seen and with little knowledge of the differentiation of these

elements/particles misclassification is common (38). Even if using Gram-stained microscopy the sensitivity in detecting UTI with $<10^5$ cfu/mL is still low (40). Using the above methods also requires the provision and maintenance of additional equipment such as incubators and microscopes.

Currently culture based and microscopy POCT are not universally used and although they can be useful in guiding a GP as whether to prescribe antibiotics or not, these tests do not help the user choose the most appropriate antibiotic and can be difficult to interpret for less experienced users.

Turbidity of urine

For women with mild symptoms of UTI, the HPA guidelines advise considering another diagnosis to UTI if a urine sample does not appear visually cloudy (i.e. urine is clear or non-turbid); this is based on studies reporting Negative Predictive Values (NPV) of 97%, 91% and 97% (25, 41-43). All of the studies conclude that by disregarding clear urines as UTI positive, the number of samples requiring further evaluation is significantly reduced. However, the patients included in all these studies are not necessarily reflective of the patient demographic for uncomplicated UTI in primary care and none of these studies were pragmatic trials so were not performed under the routine processes that the HPA guidelines are focused on i.e. routine management of uncomplicated UTI in primary care. The study by Bulloch et al., was in an emergency department of a children's hospital in Ohio, USA. The age range was four months to 19 years with both males and females recruited. The sampling was not randomised or consecutive and was considered a 'convenience' sample as only when investigators were in the emergency department were patients recruited. The sample size was 159, and the sample used for visual inspection was from both mid-stream urine and catheters. Conversely Flanagan et al. recruited elderly patients (63 – 100 years, male and female) admitted to a geriatric ward in a hospital in Belfast, UK. The sample size was larger with 418 patients but there was limited information published on the recruitment process. The final study by Phillips et al. recruited adult patients, both male and female, attending nephrology out-patient clinics

with a sample size of 363. All three studies varied on their classification of culture positive UTI and in the methods they used to determine a visually turbid urine. The studies were published between 1989 and 2000 therefore the HPA guidelines are not based on recent scientific research. A 2010 study by Little et al., recruiting adult female patients in primary care with suspected uncomplicated UTI reported that clinicians can be reasonably confident that a patient with cloudy urine has microbiologically proven UTI (based on European Urinalysis Guideline criteria) but must be cautious of excluding patients without cloudy urine (44). This opposes the guidelines by ruling out clear urine as negative although it encourages further research specifically evaluating urine turbidity as a valid preliminary screening tool for UTI when combined with patients' signs and symptoms.

The European urinalysis guidelines do not discuss visual appearance of urine in great detail but give a summary of the most common causes for abnormal appearance of urine that are not always caused by bacteria (Table 1.1.4) (38).

Table 1.1.4. Characteristic appearances of urine (38).

Appearance	Cause	Remarks
Colourless	Dilute urine	Polyuria, non-fasting specimen
Cloudy, turbid	Phosphates, bicarbonates, urates, leukocytes, RBC, bacteria, yeasts, spermatozoa, mucin, crystals, pus, tissue, faecal contamination, radiographic dye	Rectovesical fistula possible
Milky	Pyuria Chyluria Paraffin	Infection Lymphatic obstruction Vaginal cream
Blue-green	Biliverdin *Pseudomonas* infection Drugs: arbutin, chlorophyll, creosote, Indicans, guaiacol, flavins, methylene blue, Triamterene, enteral nutrition (if blue dye added)	Small intestine infection Mouth deodorants
Yellow	Flavines (acriflavine, riboflavine)	Vitamin B ingestion
Yellow-orange	Concentrated urine Urobilin, bilirubin Rhubarb, senna Drugs: Salazosulfapyridine, Phenacetin, Pyridine derivatives, rifampicin	Yellow foam Alkaline pH
Yellow-green	Bilirubin-biliverdin Roboflavin Thymol	Yellow foam
Yellow-brown	Bilirubin-biliverdin Drugs: nitrofurantoin	Beer brown
Red or brown	Haemoglobin, RBC Myoglobin Methaemoglobin Bilifuscin Urobilin Porphyrin Beets, rhubarb, carotene Fuchsin, aniline derivatives Certain drugs such as chloroquine, napthole, metronidazole, nitrite, Nitrofurantoin, phenacetin, thymol.	Positive strip result, menstruation Positive strip also; muscle injury Acid pH Result of unstable haemoglobin May be colourless Alkaline pH Foods, candy
Red-pink	Urate	May be associated with (massive) crystalluria
Red-orange	Drug: Rifampicin	
Red-purple	Porphyrins	May be colourless
Brown	See above	
Brown-black	Methaemoglobin Homogentisic acid Melanin/melanogen	Blood, acid pH Alkaptonuria (alkaline pH) Rare
Darkening upon standing	Porphyrin, homogentisic acid, melanogen, Serotonin Drugs: cascara, chlorpromazine, methyldopa, metronidazole, phenacetin, imipenem	

There is no agreed standard method for assessing urine turbidity. Some of the methods used in published studies include;

- Assessment against a bright background (42);
- Assessment against a white background with black printed 11 font text, which is then viewed under normal fluorescent lighting conditions. To be considered a clear urine, the printed text had to be as legible through the urine as through water (41);
- Urine collected in an aluminium bowl; sharpness of metal folds in the wall of the bowl below as compared to above urine surface level; clear – perfectly transparent; cloudy – loss of transparency irrespective of degree (43);
- Use of a double beam turbidimeter (45).

All of the methods dichotomised the results into cloudy or clear and all methods gave a sensitivity or NPV >90%. Some studies also added acetic acid to the urine to dissolve any phosphates, however this was shown not to have any effect on the outcomes (41, 45).

The Scottish Intercollegiate Guidelines Network (SIGN) guidelines (46) do not recommend using urine clarity as an indicator for no infection due to observer error and suggest it may not be a useful discriminator. This has also been highlighted in other studies. Lindsay and Johnston (47), although agreeing that there is value in using visual assessment of urine to screen out non-infected urine, commented on the difficulty in interpreting turbid versus clear urine. They found a disagreement of 21 % (57 out of a total 267 samples; only one of which was significant for bacteriuria) in interpretation between GPs and laboratory staff (samples assessed within three hours of GP). This could be due to change in the appearance of the urine over time between assessing turbidity (as some bacteria will continue replicating in urine at room temperature etc.) or difficulty in judging clarity. Bulloch et al., had a second observer performing the same

visual inspection, and when there was a discrepancy, the specimen was considered cloudy. There were disagreements in 8/159 urines with a Cohen's kappa value 0.876 indicating an almost perfect agreement between the two observers (only 3 of the clear urines were found to be significant for bacteria but they did not state whether these were the urines causing disagreement between the observers) (41).

Although visual inspection of urine is recommended in UK based guidelines there is little evidence to show this is being done in practice. Further evaluation ideally in a pragmatic study may clarify its usefulness in practice and reassure GPs.

Urinalysis dipsticks

Urinalysis dipsticks are often used in primary care to diagnose UTI and are the most widely used POCT in primary care. Urinalysis dipsticks (multistix or multiple test strips) are used simply by dipping into urine, waiting for a few minutes (specified by the manufacturer and varies for each tab/test on the dipstick) and comparing the colour changes for each test/tab to the colour chart on the dipstick container. Urinalysis dipsticks are designed to detect multiple components of urine including glucose, ketones, specific gravity, blood (haemolysed and non-haemolysed), pH, protein, nitrites and leucocytes. Of most interest for urinary tract infections are the presence of nitrites, leucocytes, protein and blood in the urine. Nitrite is based on activity of nitrate reductase enzyme that is present in most Gram-negative uropathogenic rod-shaped bacteria, such as *E. coli*. However the enzyme is not present in other uropathogenic bacteria such as *Enterococcus spp* and *Staphylococcus spp*. The positive detection of bacteria also requires nitrate in the patients' diet (vegetables), its excretion into urine and sufficient bladder incubation time to allow the bacterial enzyme to act. The presence of leucocytes is detected by Leucocyte esterase (LE) presence in the urine which is detected on the basis of indoxyl esterase activity released from a patients lysed neutrophil granulocytes and macrophages cells on the dipstick test pad and can be associated with non-infectious renal disease as well as a UTI (38). Total urine protein is a mixture of high molecular weight proteins (e.g. albumin, transferrin, intact immunoglobulins, α_2-

macroglobulin) and low molecular weight proteins (e.g. α_1-microglobulin, retinol-binding protein, immunoglobulin light chains) sieved from plasma, from the kidney and from the urinary tract (38). Red blood cells reflect pre-renal, renal or post renal disease, but can also occur due to menstruation or strenuous exercise (38). The European Urinalysis group suggest that the combined positivity 'either nitrite or leukocyte result positive' is generally useful for diagnosing a bacterial UTI. Specificity of the combination is reduced compared to nitrite alone, because not all patients with leukocyturia have bacteriuria.

The HPA recommends performing a dipstick test in patients with mild symptoms and cloudy urine (25). If the test result is positive for nitrite, leucocyte and blood or if positive for nitrite alone a UTI is probable (based on Little et al. (48)). If it is negative for nitrite but positive for leucocyte it may or may not be a UTI, and further testing (urine culture), treatment with antibiotics or delayed antibiotic treatment is recommended depending on the severity of the symptoms. If the dipstick reads negative for nitrite, leucocyte and blood or negative for nitrite and leucocytes (positive blood and protein) a UTI is unlikely (25).

The HPA guidelines for dipstick use have been based on a limited number of studies. The HPA primarily focuses on the study by Little et al. whereby women (17 – 70 years old) with suspected UTI attending primary care were randomised into one of five management approaches. The approach of interest here was to target antibiotics based on dipstick results. Only nitrite, leucocyte esterase and blood independently predicted a bacterial UTI. A dipstick rule based on having nitrite or both leucocytes and blood was moderately sensitive (77%) and specific (70%) with a positive predictive value (PPV) 81% and negative predictive value (NPV) 65%. Predictive values were improved by varying the cut-point: the NPV was 73% for all three dipstick results being negative, and the PPV was 92% for having nitrite and either blood or leucocyte esterase (LE) (48).

SIGN recommend using dipsticks for otherwise healthy women less than 65 years old with mild or two or less symptoms of UTI. If there is only one sign/symptom present and the dipstick is positive for nitrite or leucocyte this is associated with a higher probability (80%) of UTI. Negative dipstick results do not completely exclude bacteriuria. However,

the probability of having a UTI reduces to ~20% (46). The SIGN guidelines are primarily based on a literature review from 1991 which used Receiver Operating Characteristic (ROC) function of 2 x 2 outputs from selected papers assessing the use of nitrite and leucocyte esterase for predicting UTI. The overall conclusions were that nitrite or LE function (disjunctive pairing) had the best diagnostic accuracy (49). A more recent systematic review also shows if nitrite is detected, this increases the probability of a UTI, with a likelihood ratio of 2.6 – 10.6. In contrast, the detection of leukocyte esterase increases the probability to a lesser degree (LR 1.0 – 2.6) (40).

A study in the Netherlands recruited female patients (n=1993) with symptoms of an acute uncomplicated UTI in primary care reported that the nitrite test had a high positive predictive value (PPV) (96%) and specificity (94%); and a negative nitrite with a positive LE test showed a fairly high PPV (79%) and sensitivity (82%). Almost all patients (94%) with a nitrite-positive urine sample and the majority of patients (71%) with urine samples negative for the nitrite test but positive for the Leucocyte Esterase (LE) test received antibiotic therapy. Only one-fifth of the patients with urine samples showing both negative nitrite and negative LE tests were prescribed antibiotics. However when both nitrite and LE tests were negative approximately 50% of the samples were found to be culture positive ($\geq 10^3$ cfu/mL). Overall 70% of patients were prescribed antibiotics (50).

In summary, it has been shown that although a dipstick rule does improve diagnostic precision, clinicians still need to take into account the limited negative predictive value (NPV) (44). The poor NPV suggests that the offer of some kind of antibiotic safety net – such as delayed antibiotic prescribing – to women with negative dipsticks results is reasonable. Antibiotic use targeted with dipstick testing with a delayed prescription as a backup or empirical delayed prescription has also been shown to reduce antibiotic use (33). However, the low NPV of dipstick testing could equally support empirical treatment with antibiotics for all women with symptoms, reserving investigation for those failing to respond. Particularly if presenting symptoms respond to empirical antibiotic treatment regardless of dipstick result (51).

Microbiological diagnosis of UTI in the UK

The routine reference standard for diagnosis of UTI is to submit a sample of the patients' urine to a microbiology laboratory. At the laboratory the urine is usually microscopically examined and if microscopy positive the urine is cultured to detect the presence of significant pathogenic bacteria (uropathogens). Following positive culture for potential pathogens, the antibiotic susceptibility profile of the pathogen is established. This process usually takes an average of 24-48 hours.

Urine samples

The quality of a urine sample will affect the ability to detect relevant bacteria and confirm a diagnosis of UTI. Specimens can be divided into those with high risk of contamination (clean catch, CSU or midstream urine samples; MSU), or low risk (suprapubic aspirate (SPA) or operatively obtained urine from ureter or kidney) (46). Due to the invasive nature of collecting low risk samples, MSU are the routine sample type requested from women suspected of having a UTI in primary care.

MSU or clean catch urine is the middle portion of voided urine. Ideally this should be collected from the first morning sample as the urine will be more concentrated and the overnight incubation time allows for bacterial growth in the bladder. However, this may not be possible as most women (in the UK) will only provide a urine sample after a GP requests it at the time of consultation. The urine may then be affected by the ingestion of food and fluid and other daily activities.

There is a need to avoid contaminating fluids and commensal organisms present on the skin and surrounding areas. Women may be asked to clean the genital area prior to collecting the sample, to separate the labia when urinating, void the first section of urine and to avoid other contaminating factors women should be provided with a sterile urine container to collect the urine. Collection devices such as the Whizz® mid-stream device (JBOL ltd, Oxford, UK) may aid women in collecting urine and have been shown to reduce contamination upon culture (52). To prevent damage or death of diagnostically relevant bacteria, the urine should ideally be examined in the laboratory within 2 hours of collection (38). This is usually not feasible in primary care and therefore urines should

either be refrigerated or collected into tubes containing boric acid (stabilises the white cell number and bacterial concentration) and processed in the laboratory within 24 or 48 hours respectively (38).

Laboratory process

Once the urine samples are received in the laboratory the diagnostic process in the UK involves automated microscopy which will detect bacteria, red blood cells (RBC), white blood cells (WBC), epithelial cells and other small particles in the urine (53). If the urine meets the laboratory defined criteria for microscopy the urines will be cultured.

Urine culture is a basic manual process that involves inoculating a set amount of urine onto a section of an agar plate (using for example a 1µL calibrated loop, filter paper strip or multipoint technology). Often chromogenic UTI agar plates are used as this allows bacterial species to grow generating different colour colonies to aid identification. After at least 18 hours (usually overnight incubation) at 37°C the microbiologist will then count the number of bacterial colonies grown (from viable bacterial cells) on the agar section and from this calculate the number of colony forming units per millilitre (cfu/mL). Colony forming units are used instead of cells/mL as the number of cells that make up a colony cannot be determined using this method. This culture method is considered a semi-quantitative method as the accuracy in quantification is limited and the detection limit restricted (for example using 1 uL urine the detection limit is 1×10^3 cfu/mL; 1 colony). For most uncomplicated UTI's one single causative bacterial species will be cultured. If the culture has mixed bacteria this may be due to contamination but true infections with two or three bacterial species on culture do occur (38), however, predominance needs to be determined. The criteria for predominance has not been validated and may vary between laboratories and methods.

If there is positive growth of a pure or predominant uropathogen (Table 1.1.5.) antibiotic susceptibility testing is generally performed to guide the clinician in prescribing the most appropriate antibiotic for that patient.

Table 1.1.5. The pathogenicity and frequency of micro-organism in mid-stream urine (38).

	Frequency (percent of isolates)			
	A. Common (>10%)	B. Fairly common (1-10%)	C. Uncommon (0.1 – 1%)	D. Rare (<0.1%)
Primary pathogens	*E.coli*	*S. saprophyticus*		*E.coli* CO^2-dependent, *Salmonella* spp.[a] (*Leptospira*, mycobacteria)
Secondary pathogens		*Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *P. mirabilis*, *P. aeruginosa*	*Citrobacter* spp., *M. morganii*, *P. vulgaris*, *Serratia* spp., *S. aureus*	*Corynebacterium urealyticum*, *Haemophilus* spp.[b], Pneumococci[b]
Doubtful pathogens		GBS[c], Yeast, CNS (others)[d]	*Acinetobacter* spp., *Pseudomonas* spp., *Stenotrophomonas maltophilia*	A great number of reported cases have been published with exceptional cases of infections caused by other species
Usually urethral or genital flora[e]		α streptococci, *Gardnerella vaginalis*, Lactobacilli, etc.	*Bifidobacterium* spp., « Diphtheroid » rods, etc.	

[a] Low concentrations are reported even if they are most likely caused by contamination during specimen collection.
[b] Most often isolated from children.
[c] GBS= group B streptococci.
[d] CNS= coagulase-negative staphylococci.
[e] No identification and susceptibility testing (only exceptionally, if especially indicated).

Susceptibility testing can be performed directly using urine at the same time as culture (for example if there is a high white blood cell count i.e. WBC>100 after microscopy) or by doing a second culture of the cultured predominant bacteria. If performed directly from urine the growth of bacteria must be semi-confluent and of a single species otherwise the results should be disregarded. One of the most commonly used methods is the disc diffusion test; this involves placing commercially prepared, paper discs containing a fixed concentration of antibiotic onto an inoculated agar plate. After overnight incubation the zones of growth inhibition around each of the antibiotic disks are measured to the closest millimetre; the diameter of the zone is related to the susceptibility of the isolate (54).

Depending if the sample is from an in-patient (hospital) or from an out-patient/GP (community) referral and the species of bacteria being tested; different antibiotic disc combinations (sets) are used. The guidelines for these processes are based on the UK Standards for Microbiology Investigations (SMIs) and British Society for Antimicrobial Susceptibility (BSAC) working party and are a comprehensive referenced collection of recommended algorithms and procedures for clinical microbiology (55).

More enhanced methods for culture of bacteria in urine include (but are not limited to) serial dilution of urine and spiral plating onto agar plates which allows for total counts to be performed (quantitative culture). Chemical tests for identification of bacteria such oxidase test (positive for *Pseudomonas*), Indole test (positive for coliforms), catalase test (positive *S. saprophyticus*), serology (positive for *Salmonella*); and Maldi-TOF (Matrix-assisted laser desorption ionization time-of-flight). The MALDI Biotyper identifies microorganisms using MALDI-TOF mass spectrometry to measure a unique molecular fingerprint of an organism. Specifically, the MALDI Biotyper measures highly abundant proteins that are found in all microorganisms. The characteristic patterns of these proteins are used to reliably and accurately identify a particular microorganism by matching the respective pattern with an extensive database to determine the identity of the microorganism down to the species level (www.bruker.com). For antibiotic susceptibility testing the E-test gradient diffusion method or broth/agar dilution methods to determine minimum inhibitory concentration (MIC), the lowest concentration of an antibiotic that will inhibit the visible growth of a microorganism after overnight incubation, may offer a superior testing method to the disc diffusion method (it is often used to calibrate disc diffusion method).

Definition of a bacterially positive urinary tract infection

Standard laboratory processing of urine samples is confined to a single initial specimen per patient, with detection of conventional aerobic bacteria normally at a value of $\geq 10^5$ cfu/mL (46) of pure or predominant growth of a uropathogen classified to be a UTI.

The criterion for the presence of significant bacteria was established from early work by Kass *et al* in the 1950's comparing SPA against MSU specimens in women suffering

either from acute UTI or who had asymptomatic UTI during pregnancy. A single positive MSU reliably determined the presence of a UTI at 10^5 cfu/mL in 80% of cases studied with two samples improving this to 95% (46, 56-58). Despite the fact that the criteria were developed for acute pyelonephritis and asymptomatic bacteriuria in women, this cut-off is being used (in the UK) generally for symptomatic lower urinary tract infection to this day.

The HPA guidelines only recommend urine culture for women with failed antibiotic treatment or persistent symptoms; their criteria for a positive culture is a single organism ≥ 10^4 cfu/mL; ≥ 10^5 mixed growth with one predominant organism; or ≥ 10^3 CFU/mL *Escherichia coli* or *Staphylococcus saprophyticus* (25).

The European Urinalysis Guidelines also recommend different significant concentrations of bacterial colonies depending on the symptomatic status of the patient and the organism identified. For women with symptoms of UTI a significant colony concentration of 10^5 cfu/mL should be used for mixed growth of two secondary pathogens such as *Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *P. mirabilis, P. aeruginosa*, Citrobacter spp., or one doubtful pathogen such as group B streptococci, coagulase-negative staphylococci, *Acinetobacter* spp. and *Pseudomonas* spp. For growth of one secondary pathogen a significant concentration of 10^4 cfu/mL is required and finally for primary pathogens such as *E. coli* and *S. saprophyticus* a growth of 10^3 cfu/mL should be classed as significant. For asymptomatic women growth of at least 10^5 cfu/mL should be classed as significant for all uropathogens (38).

Problems with the microbiological diagnostic process

Over half of the samples submitted routinely to UK microbiology laboratories are urine samples; the most common of which are mid-stream urine from community patients. The most common symptoms for sending urine samples are frequency and dysuria (59, 60). Based on published data from 2000, resource costs approximately £3.50 - £12.50 per sample for basic culture to susceptibility testing (expert opinions) compared to a cost of general empiric treatment (trimethoprim 200mg bd; 3 day course) at £0.05 - £0.50 (61). The NHS covers the costs of laboratory procedures and prescriptions but does not cover

the cost of all POCTs used in general practice. The use of the microbiology laboratory service is difficult to balance; underuse of the laboratory may lead to under-ascertainment of UTI and over prescribing of antibiotics, while over-use may unnecessarily increase laboratory and primary care workload costs (59).

As already described the quality of the urine sample submitted to the laboratory is very important; poor quality urines result in false negative or false positive results and contamination is a major issue; all of which lead to unnecessary costs and strain the laboratory service, and result in incorrect or delayed treatment of patients.

Routine culture is a basic method which could result in additional false negatives and to a lesser extent false positives; the method is highly subjective and may vary between microbiologists and/or laboratories.

The use of the diagnostic laboratory by General Practices takes 2-3 days for the results to become available (62). Therefore, patients are often treated with empirical antibiotics for a presumed UTI based only on their presenting symptoms, either without sending urine for microbiological testing or before these results are available (63).

Section 2: Analytic performance of UK Flexicult™ SSI-Urinary Kit, chromatic sensing, visual turbidity assessment of urine and dipstick urinalysis in the diagnosis of suspected urinary tract infection

Chapter 1: UK Flexicult™ SSI-Urinary Kit Introduction and Methods

Introduction

The UK Flexicult research in this section has been published in the European Journal of Clinical Microbiology and Infectious Diseases (64).

Flexicult™ SSI Urinary kit

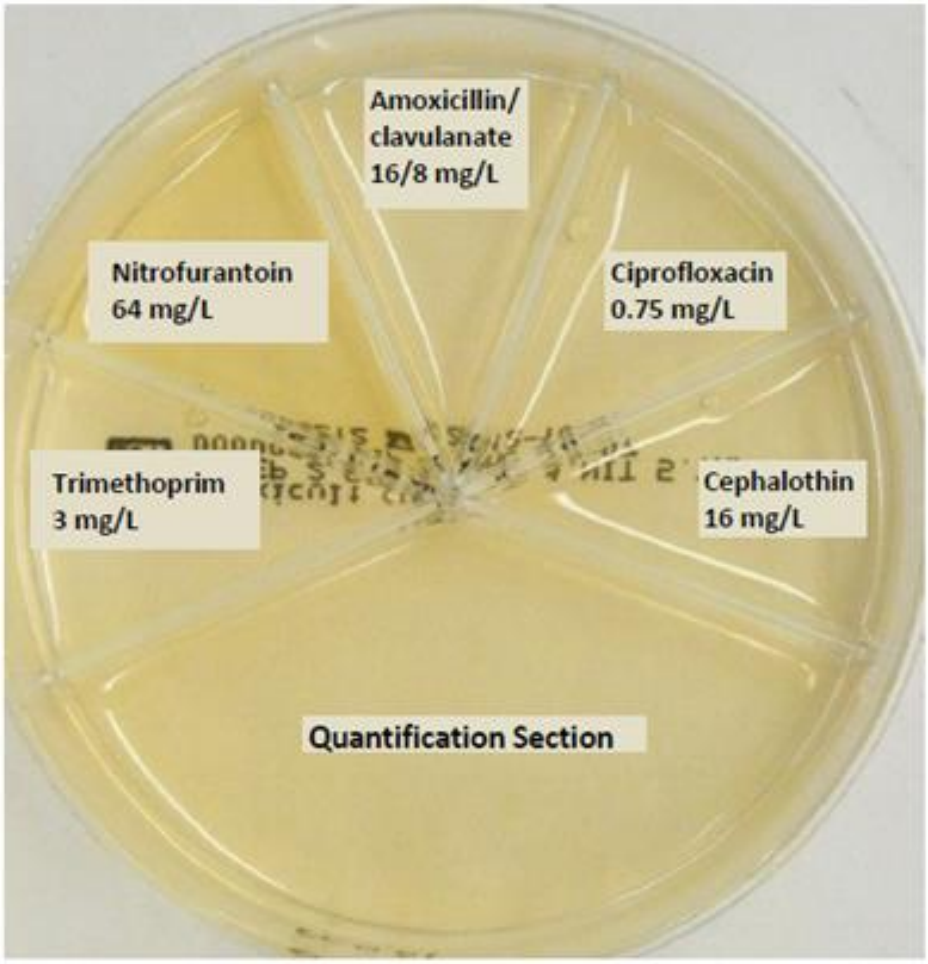
The Flexicult kit was developed by the Statens Serum Institut in Denmark in light of the increasing antibiotic resistance of urinary pathogens and as the overall societal costs of UTI is related to the appropriateness of empiric UTI treatment.

The kit is a point-of-care test for the diagnosis and susceptibility testing of urinary tract pathogens and is already in clinical use in primary care practices in Denmark. It is a chromogenic agar plate divided into 6 sections; 1 large section for quantification and identification of bacteria (this section contains no antibiotics) and 5 smaller sections for susceptibility testing which contain specific concentrations of different antibiotics (Figure 2.1.1). The kit has an incubation period of at least 18h (generally overnight) after inoculation.

There has only been one publicised evaluation of the Flexicult test; both an in-house and 'field study' of the Danish Flexicult kit were performed. The in-house validation involved quantification and susceptibility testing using the Flexicult test for 116 bacterial strains collected from a previous study which had identification, quantification and susceptibility results performed using validated routine laboratory culture methods. In a 'field trial', 19 Danish GP's were asked to use the test along with routine diagnostic procedures for patients with symptoms of UTI (urine samples were also collected and sent for routine culture), and a total of 121 diagnostic specimens were processed. The in-house study showed quantification equivalent to 10^3 cfu/mL if there were ~15 – 20 bacterial colonies in the control section; 10^5 cfu/mL if semi-confluent bacterial growth and 10^7 cfu/mL if confluent growth was observed in the control section. Results from the antibiotic susceptibility sections were compared with the minimum inhibitory concentration (MIC)

value of the strain for each antibiotic; 90% and 96% resistant and susceptible strains respectively were correctly identified.

Figure 2.1.1. UK Flexicult™ SSI-Urinary Kit



The field trial showed discrepancies in 16% of the quantification results between GP's reading of the plates and the laboratory culture results. The susceptibility profiles showed an overall accordance of 93% with the isolated bacterial MIC's. It was also reported that the GP's found the kit to be easy to use and read (65). This study states that identification of bacterial isolates is not a feature of this kit; however, the kit brochure does provide detailed guidance on this, and the chromogenic nature of the agar is designed for this purpose.

Although providing useful information on the use and applicability of this test particularly with the in-house validation; this study had limited urine sample numbers and participating GPs in the field trial. The 19 GPs (and presumably the 121 patient samples)

were recruited from one county in Denmark, there is no information on the number of samples each GP tested or if the GPs were part of one or numerous practices. Additionally, the GPs volunteered to participate; this may not be representative of the general GP population. The study report does not provide information on the urine samples apart form that they were collected from patients with suspected UTI. Clinical data are not given. When using the kit the GPs were asked only to quantify the bacteria and interpret the antibiotic susceptibility profiles; they were not required to identify the bacteria and therefore mixed cultures or contaminants would not have been identified. Routine practice in Denmark also involves using microscopy, dipsticks and/or dip-slides and the GPs participating in this study were advised to follow routine procedure in addition to using the Flexicult kit; this may have guided the GPs interpretation of the Flexicult quantification results. Other than error rate (which was calculated to include 12 discrepant samples as acceptable due to contaminants or other reasons rather than remaining discrepant or being removed from the calculation) neither measures of diagnostic accuracy or statistical uncertainty were provided, nor was reproducibility which would be important for evaluation of a subjective test such as this. There was no information on any training or guidance that GPs were given to help them interpret the plates. There are also no published studies on the Flexicult kit enabling more appropriate prescribing to patients, and no economic evaluation or report on clinical outcomes when using this kit compared to routine practice.

A new version of Flexicult has now been developed specifically for evaluation in UK primary care. This kit differs from that being used in Denmark by the inclusion of some different antibiotics more commonly used in the UK for UTI; cephalothin, ciprofloxacin and co-amoxiclav instead of sulfamethoxazole, ampicillin and mecillinam; nitrofurantoin and trimethoprim are included in both kits.

Prior to clinical implementation, performance of a new test should be evaluated against the appropriate reference standard using appropriate clinical samples. Routine NHS methods including automated microscopy, culture and sensitivity testing of urine is the current ‘reference standard’ testing being done in the UK.

Rationale

The availability of rapid diagnostic tests that can be done at the point of care (e.g. GP surgery) has the potential to enable faster initiation of appropriate therapy, guide narrow-spectrum antibiotic use for UTI, and reduce antibiotic prescribing where the cause is not bacterial. The current diagnostic tests available for UTI leave considerable room for improvement and laboratory referral and diagnosis takes too long.

The UK Flexicult SSI-urinary kit will be evaluated as part of a randomised controlled trial (RCT) in the POETIC study (study website: http://www.poetic-study.co.uk/). However, due to the limited published data on the Danish Flexicult kit and the fact that the UK Flexicult kit has not been validated with clinical samples previously; this study will provide useful preliminary data on the use and analytic performance of the kit prior to being evaluated for patient management in the RCT. The UK Flexicult test results should be comparable to standard UK laboratory tests; microscopy, semi-quantitative culture and susceptibility testing and as the test itself is subjective some measure of reproducibility should be investigated.

Hypothesis

The UK Flexicult SSI-urinary kit uses a similar method to standard NHS urine culture and susceptibility testing. The analytic laboratory performance of this test in determining a microbiologically positive urine sample is expected to be similar to the standard method.

Aim

The aim of this study was to;

- Evaluate the analytic performance of UK Flexicult™ SSI-Urinary kit by comparing performance with routine NHS laboratory testing and enhanced urine culture techniques following the STARD standard [12];
- Explore the effect of urine over inoculation of Flexicult on the interpretation of positive UTI and antibiotic susceptibility;

- Evaluate the subjective nature of the interpretation of the kit result by experienced microbiologists and naive general practitioners for whom the test is intended.

Study Objectives

The objectives of this study are to:

- Use the UK Flexicult urinary kit determine the quantification, identification and susceptibility profiles of sequential urine samples selected from those routinely submitted to a single NHS microbiology laboratory;
- Compare the diagnostic accuracy in determining a bacterial UTI using UK Flexicult to routine NHS processing methods (as the reference standard) and use an enhanced urine culture and identification technique to compare discordant results.
- To determine if diluting turbid urine (potential for over-inoculation) could improve identification of predominant uropathogens using Flexicult;
- To determine if diluting turbid urine could improve Flexicult susceptibility result concordance with NHS susceptibility results.
- To evaluate bacteriology Biomedical Scientists (BMS) and General Practitioners (GPs) inter-observer variation on the interpretation of UK Flexicult;
- To compare variation when reading 'real Flexicult tests' vs. corresponding 'Flexicult test images' (accuracy of evaluating images rather than actual tests that may be used for GP training if actual tests unavailable);

Methods

Setting

All testing was performed in collaboration between Cardiff University and the Public Health Wales Microbiology Department, University Hospital Wales (UHW), Cardiff. The routine microbiology testing was performed by NHS staff based in the Clinical

Microbiology Laboratory, University Hospital Wales. The spiral plate culture on chromogenic medium and Flexicult culture were performed by myself in the Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Microbiology, Cardiff.

Ethical approval (12/NE/0306) and Public Health Wales Research Risk Review Committee approval (2012PHW0018) were obtained prior to commencement of the study (appendix 2.1.1).

Urine samples

Urine samples submitted from primary and secondary care in the course of routine patient care were used for the evaluation and validation procedures. Urine selection was not based on any clinical or microbiological information. Rather this was a convenience sample with urine samples selected for analysis by Public Health Wales (PHW) laboratory staff. The urines came from the general pool of urines submitted from inpatients (hospital wards) and outpatients (primary care, outpatient clinics and A&E) to this hospital clinical microbiology laboratory. Only excess urine samples (surplus to routine NHS laboratory testing requirements) were used for the flexicult evaluation and validation procedures. The flexicult and spiral plate culture, were both performed on the urines on the same day as NHS routine processing. The only exclusion criteria were urine samples collected in boric acid (as this may interfere with the antibiotic sections of FSUK) and urines with less than 5mL volume after routine processing.

Routine tests such as microscopy and culture were carried out on the urine samples according to local NHS standard operating procedures (following UK standards for Microbiology Investigations B41 Investigations of Urine (66)) in the NHS laboratory. The Flexicult culture and spiral plate culture on chromogenic medium, were both performed on the urines within 24 hours of receipt in the laboratory; I performed both tests. For each urine sample the order of process was inoculation onto Flexicult followed by serial dilution of the urine and then spiral plating onto the chromogenic medium. The Flexicult

plates were interpreted and recorded prior to the quantification and identification of the corresponding spiral plate cultures.

Sample size

A sample of 200 was chosen based on a prevalence of laboratory diagnosed UTI between 20% and 30% [approximate usual proportion, correspondence with Dr Robin Howe, Director and National Lead for Microbiology Services, Public Health Wales, University Hospital of Wales] and a Flexicult^{TM} SSI urinary kit error rate of 4.72% (based on quantification only)(65). This would give sufficient power to detect sensitivity of 0.95 (95% CI 0.82 – 0.99), and specificity between 0.98 - 0.99 (95% CI 0.93 – 0.99). A total of 211 urine samples were processed and 200 were included in the analysis; 10 were excluded as they were processed 48h after receipt in the laboratory (heavy growth of pathogenic and contaminating bacteria could lead to false positives or mixed growth not comparable with the routine culture methods performed at the time of receipt in the laboratory) and one sample had missing NHS microscopy and culture results.

NHS microbiology data collection

NHS microscopy and culture results (if applicable) of the routinely submitted samples were provided on a weekly basis by a member of the Public Health Wales Microbiology team after the evaluation tests had been performed, read and recorded by myself.

Routine urine processing through the NHS Microbiology Laboratory first involves automated microscopy (iQ200 Sprint), the criteria for positive selection for culture, is as follows:

1. ≥ 5 Bacteria
2. ≥100 White Blood Cells (WBC)
3. ≥20000 ASP (any small particles)
4. ≥50 WBC + ≥ 2000 ASP
5. ≥50 WBC + ≥1000 ASP + ≥ 3 Bacteria
6. ≥3 WBC + ≥6000 ASP

Bacteria equates to 3 or 5 x 10^3 cfu/mL from culture. In addition, any samples from antenatal clinics, Intensive Treatment Units and children < 2 years old are all automatically cultured.

Any microscopy positive specimens are plated onto ¼ plate of Chromogenic UTI agar (Oxoid Chromogenic Brilliance UTI clarity agar) using a 1μL microbiology loop. After overnight incubation at 37°C the plates are read as follows;

1 colony = 1 x 10^3 cfu/mL.

10 colonies = 1 x 10^4 cfu/mL

100 colonies = 1 x 10^5 cfu/mL

There are no defined criteria for diagnosing a UTI microbiologically and microscopy and clinical evaluation is usually also required. However, the urine samples were associated with little useful clinical information on the request form apart from suspicion of UTI. Microbiological findings considered positive for a UTI in this study were >10^5 cfu/mL pure or predominant growth (x1000) of a clinically significant UTI pathogen.

Antibiotic susceptibility testing is performed on significant bacterial isolates (from culture positive urine samples) using the standard disc diffusion method (67) and with the appropriate urine antibiotic disc sets as described in Table 2.1.1 (only antibiotics relevant to Flexicult have been listed).

Table 2.1.1. Antibiotic disc sets for NHS disc diffusion method.

Gram-Negative Bacteria[1] (In-patients)	**Gram-Negative Bacteria[1] (General Practice)**	***S. saprophyticus***	***Enterococci* spp.**	***Pseudomonas* spp.**
Trimethoprim 4 – 2mg/L Co-amoxiclav 32 mg/L Nitrofurantoin 64 mg/L Ciprofloxacin 1 – 0.5 mg/L	Trimethoprim 4 – 2 mg/L Co-amoxiclav 32 mg/L Nitrofurantoin 64 mg/L Ciprofloxacin 1 – 0.5 mg/L	Nitrofurantoin 64 mg/L Trimethoprim 4 mg/L	Nitrofurantoin 64 mg/L	Ciprofloxacin 1 – 0.5 mg/L

Cefpodoxime	Cefpodoxime
1 mg/L	1 mg/L
	Cephalexin
	16 mg/L

[1] *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp..

Flexicult test procedure

Sufficient urine from each sample was poured onto the Flexicult test to submerge all the compartments (approximately 5ml). If the urine sample did not cover all the agar compartments, the plate was tilted in a circular motion so that all the compartments came into contact with the urine. After approximately 5 seconds the excess urine was poured off the Flexicult test. The Flexicult tests were incubated aerobically overnight at 36°C ± 1°C with the bottom of the plate facing upwards and lid down. After incubation overnight, each test was first inspected for bacterial growth. This procedure follows the methodology described in the manufacturer's brochure and is the process that would be used if the test were performed in routine practice.

The UK Flexicult SSI Urinary Kit brochure was used to interpret the quantification, identification, predominance and susceptibility results. If growth was visible the number of colonies in the quantification section of the test was quantified and the size and colour recorded. This was performed for all different colonies grown (i.e. if mixed growth) if possible. For each group of colonies seen the bacteria were identified according to the colour and size using the references shown in the (UK Flexicult SSI Urinary Kit) brochure. If an amount of $\geq 10^3$ cfu/mL (approximately 20 colonies) of a urinary tract pathogenic bacterium alone or in predominant quantity (greater than 10x growth of any other bacteria; as indicated in the brochure) was found in the control section of Flexicult, the antibiotic resistance profile was also read. Growth in the antibiotic compartments was compared to growth in the large quantification compartment. If growth in one antibiotic compartment was much lower than in the quantification compartment – or if there was no growth at all – the bacterium was considered susceptible to the antibiotic. If the bacterium in the antibiotic compartment had grown to a similar quantity as the

quantification compartment, it was considered resistant (procedure taken directly from the manufacturers' brochure instructions). The amount of growth in each compartment was recorded as well as the corresponding susceptibility.

Once the plates had been read, an image of each plate was captured using an Olympus 620UZ camera; the images were taken using the same camera, settings and approximate position for each plate. The plates were disposed of following local laboratory guidelines.

Quality control

All new batches/Lot numbers of Flexicult plates underwent quality control by inoculating with 0.5 McFarland suspension of the following American Type Culture Collection (ATCC) organisms; *Escherichia coli* (#25922), *E. coli* (extended spectrum beta-lactamase (ESBL) #35218), *E. coli* (ESBL #13353), *Psuedomonas aeruginosa* (#27853) and *Enterococcus faecalis* (#29212) (one organism suspension per Flexicult plate). These strains have known antibiotic susceptibility profiles. These strains are used in the PHW laboratory for quality control purposes of the routine antibiotic susceptibility testing procedures and were therefore used for the quality control of the Flexicult plates. Each isolate was maintained on columbia agar with 5% horse blood routinely by PHW staff following local laboratory procedures. For each isolate I used a 10µL loop to pick colonies from the agar and inoculate 10 mL sterile water to get a suspension resembling 0.5 McFarland standard turbidity which equates to 1.5 X 10^8 CFU/mL. Two batches or separate Lot numbers of Flexicult tests were used during the study; 2852 (expiry 10/12/2012) and 2212 (expiry 07/10/2012) both of which had an eight week shelf life. Both Lots of Flexicult plates were inoculated at weekly intervals to determine any growth prior to the expiry date. The inoculation and interpretation of Flexicult follows the same procedure as inoculating Flexicult with urine described above.

Enhanced culture technique: spiral-plating onto colorex orientation UTI medium

Urine samples were serially diluted to 10^3 and 10^6 in sterile water. Using the Whitley Automated Spiral Plater (WASP) 50 µL of each dilution 10^6, 10^3 and neat urine were

spiral plated onto Colorex Orientation UTI medium plates (E&O Laboratories Ltd) within 15 minutes of preparation. The plates were left until dry, inverted and incubated overnight at 35°C.

After incubation the plates were inspected for growth. For each urine sample only the most appropriate plate dilution was counted; this was the dilution that had distinct countable colonies visible. A clear perspex counting grid was used to count colonies in sectors and the corresponding counting tables used to calculate the CFU/mL. The laboratory provided a local SOP to follow for this procedure.

Identification of bacteria was primarily performed by comparing the colour of the colonies to the Colorex Orientation UTI medium guidelines. For bacteria that were unable to be identified by colour further identification was performed using Matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF Bruker) mass spectrometry following the manufacturer's instructions (www.bruker.com).

Analysis

Data was captured using worksheets developed by myself (appendix 2.1.2) and entered into Excel and SPSS spread sheets.

Both the UK Flexicult and the routine NHS culture process use semi-quantitative subjective methods for determining a microbiologically positive urine sample. Due to the different methodologies the comparisons are not exact i.e. UK Flexicult had four quantification categories: no growth; $<10^3$ cfu/mL; $10^3 – 10^4$ cfu/mL; $\geq 10^5$ cfu/mL (which included $\geq 10^7$ cfu/mL) and the results from the NHS culture were: no growth; no significant growth; $10^4 – 10^5$ cfu/mL; $>10^5$ cfu/mL. Samples that were 'not cultured' through the NHS (due to negative microscopy) were included as 'no growth' (negative for microbiological UTI). When determining predominant growth, the NHS method used growth of one organism at 1000x greater than any other as predominant, and the UK Flexicult method used 10x greater growth as predominant. Spiral plating was used to compare discordant results between the reference standard (routine NHS testing) and the UK Flexicult as total quantification and more advanced identification could be performed.

A variety of diagnostic performance evaluations were calculated including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and error rates (as described in appendix 2.1.3). Confidence intervals (95%) were calculated using a spread-sheet (CIPROPORTION) developed by a Cardiff University statistician Robert Newcombe (http://medicine.cf.ac.uk/media/filer_public/2012/11/01/ciproportion.xls)(68).

Urine dilution study method

Urine dilution was subsequently used to investigate the potential effects of over inoculation on UK Flexicult plates. An additional 79 turbid urines (urine samples with any degree of visual uniform turbidity) from primary care received in the Clinical Microbiology Laboratory, University Hospital Wales were diluted 1:1000, and both undiluted and diluted urine inoculated onto UK Flexicult as described above. The results between undiluted and diluted urines were compared to determine any differences in identification of UTI and susceptibility. The results were also compared to the corresponding NHS results to identify any changes in analytical performance.

If there were any discordant susceptibility results with the NHS after dilution the minimum inhibitory concentration (MIC) for those organisms were established using standardised methods namely gradient E-test (ciprofloxacin, co-amoxiclav, nitrofurantoin and trimethoprim) and agar dilution method (cephalexin – used instead of cephalothin which was unavailable for this study) as described below.

MIC: E-Test (co-amoxiclav, nitrofurantoin, trimethoprim)

- Mueller Hinton agar plates were inoculated with a 0.5 McFarland suspension of the test organisms and sensitive *E. coli* strain #ATCC 25922 (and #35218 for co-amoxiclav) using a cotton swab;
- Individual E-test strips containing the relevant antibiotics were placed onto the inoculated plates;

- Plates were inverted and incubated at 37°C over-night;
- After 18h the plates were read; the point along the antibiotic E-test strip at which the bacterial growth is inhibited is the MIC.

MIC: Agar Dilution (cephalexin used for cephalothin MIC)

- Cephalexin was prepared at 2560 mg/L (adjusted for 94% potency); 15.6mg dissolved into 5mL sterile water (suspension A). A further dilution was made to 80 mg/L by adding 0.5mL antibiotic suspension to 15.5 mL sterile water (suspension B).
- Plates were prepared by melting Mueller Hinton agar in a water bath, once cooled to the touch the antibiotic suspension was added to each agar bottle at the following concentrations (immediately after mixing, the agar was poured into petri dishes and left to set);

Dilution	mg/L
1 mL of A into 20mL agar	128
500 µL of A into 20 mL agar	64
250 µL of A into 20 mL agar	32
125 µL of A into 20 mL agar	16
62.5 µL of A into 20 mL agar	8
1 mL of B into 20 mL agar	4
500 µL of B into 20 mL agar	2
250 µL of B into 20 mL agar	1
125 µL of B into 20 mL agar	0.5
62.5 µL of B into 20 mL agar	0.25
No antibiotic added	0 (control)

- 0.5 McFarland suspension of the test organisms and sensitive *E. coli* strain (#ATCC 25922) were made up in sterile water (this equals approx. 10^8 cfu/mL).
- Using multipoint inoculum replicating apparatus, 180 µL sterile water was added to the wells of the inoculum plate and 20 µL of the bacterial suspension added to each well. The automated apparatus then inoculated each plate with 1 µL bacterial suspension (i.e. each inoculum at 10^4 cfu/spot). The plates were left to dry, inverted and incubated for at least 18h at 37°C.
- The plates were read the following day; the MIC for each inoculum is the cephalexin plate/agar concentration with <10 colonies per inoculum spot.

Inter-observer reproducibility study method

Urine samples and Flexicult inoculation

Anonymised excess urine samples were used for this study. A total of 60 urine samples submitted routinely to the Public Health Wales (PHW) Microbiology Laboratory, University Hospital Wales were chosen by a member of PHW staff. As this study required a focus on positive samples ideally of varying quantification and organisms grown, 54 microscopy positive samples and six microscopy negative samples were selected. Flexicult tests were inoculated on the same day as routine culture; following the method described above.

After overnight incubation the Flexicult plates were firstly read by three qualified bacteriology Biomedical Scientists individually and blinded to each-others results (all employed in the PHW Microbiology Laboratory, UHW); this was during the morning. In the afternoon the same Flexicult plates were then be read by myself and three GPs; all Cardiff University Clinical Academic Fellows from the Institute of Primary Care and Public health who volunteered to participate in the study. Results were recorded for the quantification, identification and susceptibility onto a specifically designed results form (appendix 2.1.4). All assessors were asked to refrain from discussing the Flexicult kits and results; as I was present during the GP session I know that they adhered to this

request. The SSI UK Flexicult brochure was the only guide available when reading the plates at this initial session although a link to the POETIC website (http://www.poetic-study.co.uk/) which provides further information on interpretation of Flexicult was sent to all the assessors prior to this session; it was their decision whether to access it. The assessors were asked to record if they read the brochure and/or website and if so for how long.

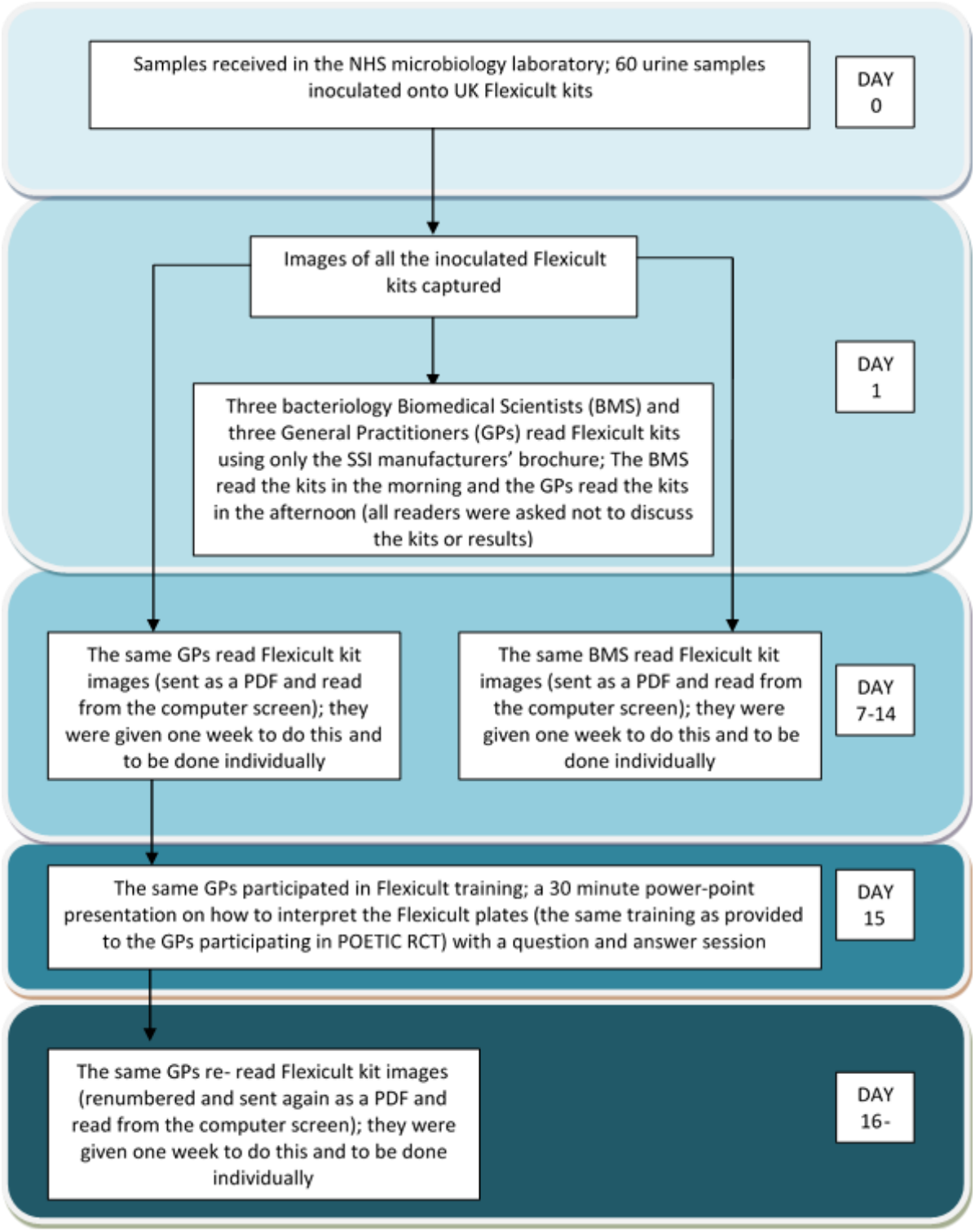
Reading Flexicult™ Plates

Images of the Flexicult plates were captured once the GPs had finished their session which was not time restricted (using the camera function of the Apple iPhone 4 v7.1.1; flash off; all images captured in the same position and lighting).

Reading Flexicult™ images

The images of the Flexicult plates were renumbered and put into a PDF document. This document was then sent to all of the above assessors a week after the initial session to interpret once again with only the SSI UK Flexicult brochure and access to the POETIC website as a guide; it was again up to the assessors if or for how long they used these guides. The assessors were asked to interpret the images of the plates from the computer screen (not to print out the PDF as the colours of the plates may change depending on the printer), the same worksheet template as used in the initial session were provided to record the results. All assessors were given one week to read and record the results from plate images. They were asked to do this individually and blinded from the other assessor's results.

Figure 2.1.2. Reproducibility Design Flow Diagram



Flexicult™ training

Once the results of the Flexicult™ images had been returned from all participants, the GPs participated in a training session which I led. This involved approx. 30 minute power-point presentation on the quantification, identification and susceptibility interpretation of various Flexicult plates, followed by a question and answer session (it was the same power-point training provided to the participating GPs in the POETIC randomised controlled trial (RCT)).

Repeat reading of Flexicult™ images

Once the Flexicult™ training was complete the GPs were asked to again read and interpret the images of the Flexicult™ plates. All GPs were given one week to do this and were asked to do this individually and blinded from the each-others results. The images were once again re-numbered to avoid recall (memory effects) and sent in a PDF document to be interpreted from the computer screen, the worksheet template was provided to record results.

The SSI UK Flexicult™ brochure and access to the Flexicult training website was available when re-reading the images of the tests.

Analysis

All the data was entered into Microsoft Excel spreadsheets and STATA 12.1 and analysed for accuracy as compared to my interpretation (agreed with my Supervisors as I have experience with the interpretation of the UK Flexicult plates). The strength of the agreement was calculated using the Kappa statistic (with 95% confidence intervals) using STATA 12.1 data analysis program.

The kappa statistic corrects for chance agreement and informs us of the possible agreement over and above chance which the assessors have achieved (69). The interpretation of the Kappa statistic is outlined in Table 2.1.2;

Table 2.1.2. Qualitative classification of kappa values (69).

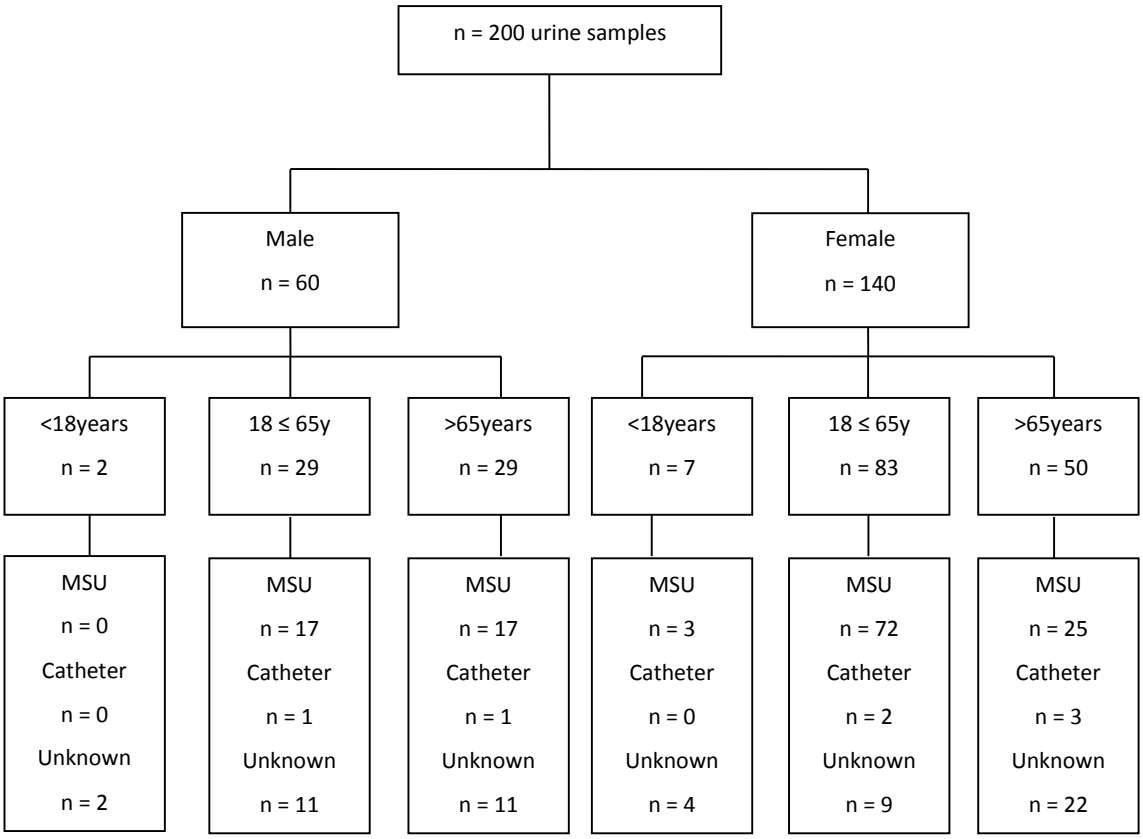
Kappa value	Degree of agreement beyond chance
0.0	None
0.00 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.00	Almost perfect

Chapter 2: UK Flexicult™ SSI-Urinary Kit Results

Urine samples

Approximately ten urine samples were selected by laboratory staff and passed onto me each day of the evaluation (Monday – Thursday), over a period of seven weeks from September to November 2012. 200 samples were included in the comparison of the Flexicult kit with NHS routine culture: 124 (62.0%) were from outpatients; 72 (36.0%) from inpatients; and 4 (2.0%) unknown. Fig 2.2.1 details further available information on the urines included in the study. Out of the total 200 urine samples; 70% were from female patients; 95.5% were from patients aged 18 years or older; and 67% were mid-stream urine samples (29.5% unknown collection method and 3.5% from catheters). For this study all samples were included in the analysis.

Figure 2.2.1. Summary of urine samples evaluated in study



MSU = mid-stream urine; n= number of samples; unknown = unknown sample collection method.

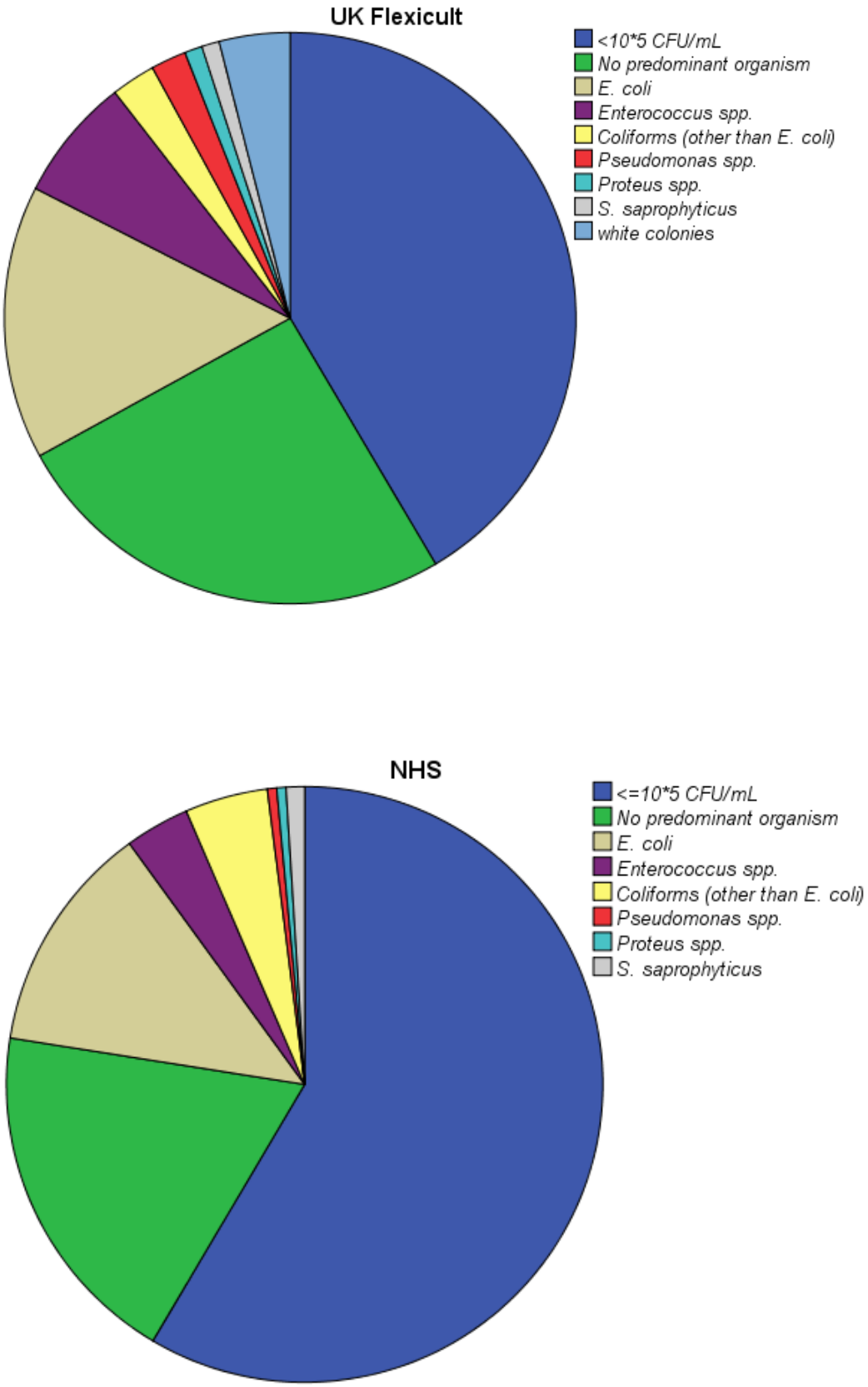
Analytic performance of UK Flexicult compared to the reference standard (NHS)

In total the NHS routine culture identified that 45 (22.5%) urine samples were microbiologically positive compared to 66 (33.0%) using the UK Flexicult method; with a sensitivity of 86.7% (95% CI 73.8 – 93.7) for all samples and a sensitivity of 87.0% (95% CI 67.9 – 95.5) on a sub-group analysis of outpatients samples only. 19.0% of the samples cultured using the NHS method showed contamination (no predominant organism) compared to 25.5% using UK Flexicult. The most prevalent organism identified through both methods was E. coli; 25/45 (55.6%) NHS and 31/66 (47.0%) UK Flexicult (Figure 2.2.2 shows a breakdown of the culture results using each method).

Table 2.2.1 details the number of concordant and discordant positive and negative samples for UK Flexicult compared to the NHS processing, as well as the analytic performance measurements. This also includes a sub-group analysis for outpatients only. When using the UK Flexicult method 13.5% urine samples would be classed as microbiologically positive when in current routine NHS processing they would be negative. Using UK Flexicult, 3.0% of samples would be classed as microbiologically negative, while when processed in current routine practice they would be considered positive.

By comparing the 27 discordant positive results to the spiral plating method; 16 samples were found to be negative (agreeing with the NHS results) and 11 positive (agreeing with the Flexicult results). Of these 16 discordant results (Flexicult positive) the spiral plating method found 10 samples to have growth $<10^5$ cfu/mL and six samples to have growth $\geq 10^5$ cfu/mL but no predominant organism (mixed growth indicating contamination rather than true positive).

Figure 2.2.2. Culture results using both UK Flexicult and NHS culture methods



When comparing the six discordant negative results; the spiral plating method found four to be positive (agreeing with the NHS results) and two to be negative (agreeing with the Flexicult results). Of the four spiral plate/NHS positive results the Flexicult method found three to have no predominant growth (contamination) and one to have no growth. A figure showing images of concordant and discordant spiral plate cultures and corresponding Flexicult culture plates are in included in appendix 2.1.5.

Table 2.2.1. Cross tabulation of UK Flexicult™ SSI urinary kit results compared to routine NHS culture in determining microbiologically positive urine samples

	NHS Results	UK FSUK Results (All patients; n=200)	UK FSUK Results (Outpatients only; n=124)
Concordant positives (%)	45 (22.5)	39 (19.5)	20 (16.1)
Concordant negatives (%)	155 (77.5)	128 (64.0)	84 (67.8)
Discordant positives (%)		27 (13.5)	17 (13.7)
Discordant negatives (%)		6 (3.0)	3 (2.4)
	Statistical measures		
Sensitivity (95% CI)		86.7 (73.8 – 93.7)	87.0 (67.9 – 95.5)
Specificity (95% CI)		82.6 (75.8 – 87.7)	83.2 (74.7 – 89.2)
Positive Predictive Value (95% CI)		59.1 (47.1 – 70.1)	54.1 (38.4 – 69.0)
Negative Predictive Value (95% CI)		95.5 (90.6 – 97.9)	96.6 (90.4 – 98.8)
Error Rate (%)		16.5	16.1

By using a lower cut-point for the UK Flexicult (≥10^3 cfu/mL) test the sensitivity did not improve (86%; 95% CI 73.8 – 93.1). However, by using only quantification irrespective of identification or predominance (remaining at the higher cut-off of UK Flexicult ≥10^5 cfu/mL) the sensitivity improved to 95.6% (95% CI 85.2 – 98.8) with two false negative samples and over half (109/200) found to be true negatives. The sensitivity increases to 100% (95% CI 92.1 – 100.0) if the lower UK Flexicult cut-off value of ≥10^3 cfu/mL is used for positive UTI. However, this would reduce the number of true negatives identified from

109 to 51/200.

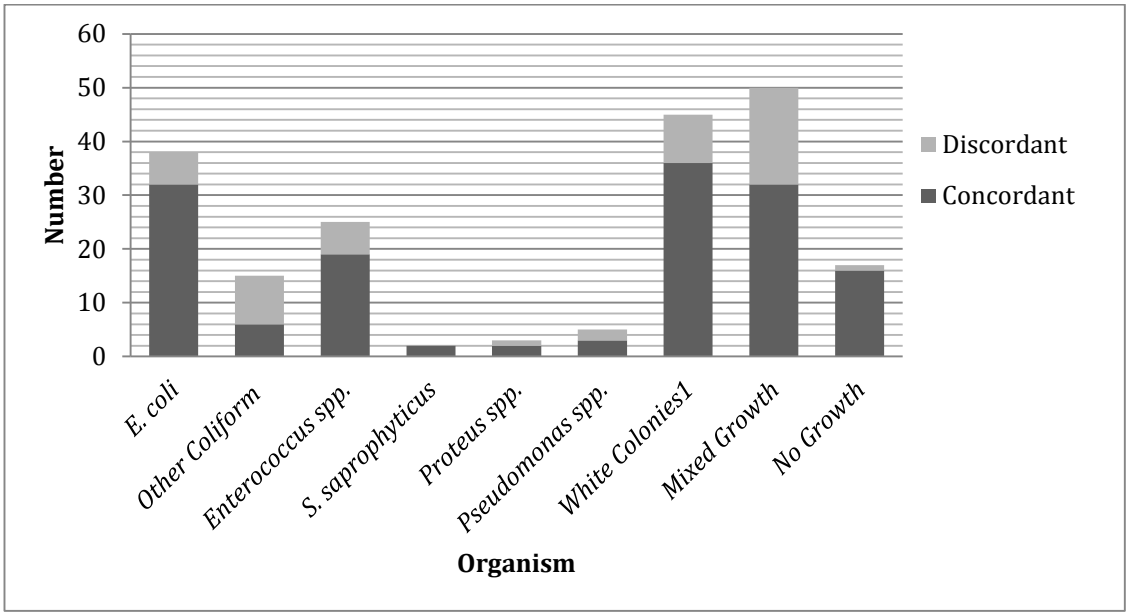
Presumptive identification of bacteria (colour and size of colonies)

Of the *E. coli* (n=25) samples identified by the NHS, 25 were also identified as *E. coli* using UK Flexicult kits. Of the coliforms (*Klebsiella* sp. and *Enterobacter sp.)* (n=9) identified by the NHS, four were identified as coliforms using Flexicult however of the remaining five; three were identified as no predominant organism (confluent mixed growth) and two were inconclusive for identification using the UK Flexicult guidelines. The remaining seven *Enterococcus* spp., two *Staphylococcus saprophyticus*, one *Proteus* sp. and one *Pseudomonas* sp. were correctly identified using UK Flexicult (as compared to NHS identification results). Overall the same pure/predominant organisms were identified using UK Flexicult as by the NHS method in 40/45 (88.9%) samples.

The identification of bacteria using UK Flexicult kit compared to the more advanced SP and MALDI-ToF methods gave an accuracy of 84.2% for *E. coli*; 76% for *Enterococcus* spp.; 40% for coliforms (*Klebsiella* sp. and *Enterobacter*); 66.7% for *Proteus* sp.; 60% for *Pseudomonas* sp and 100% for *S. saprophyticus*. The concordance for each organism is shown in Fig 2.2.3.

Figure 2.2.3. Concordance in identification of organisms using UK Flexicult™ SSI urinary

kit against Spiral-plating onto Colorex Orientation UTI Medium[1].



Urine Dilution Study

Diluting the urine samples resulted in a reduction of false positive samples; eight of 13 samples which resulted in mixed growth (no predominance) using undiluted urine resulted in a positive growth of a predominant organism when urine was diluted as shown in Table 2.2.2.

Table 2.2.2. Cross tabulation of undiluted versus diluted urines inoculated onto UK Flexicult™ SSI urinary kit

		1:1000 Diluted Urine			Total
		Negative	No Predominance	High positive (>10^5 cfu/mL)	
Undiluted Urine	Negative	27	0	0	27
	No Predominance	4	1	8	13
	Low positive(≥10^3 to <10^5 cfu/mL)	10	0	0	10
	High positive (≥10^5 cfu/mL)	5	2	22	29
Total		46	3	30	79

[1] Unable to accurately identify 'white colonies' using UK Flexicult; therefore grouped together.

Diluting the urine would not reduce the number of discordant positive results when compared to routine culture and if using the UK Flexicult kit in clinical practice the higher levels of positivity would remain. Diluting the urine marginally reduced the proportion of discordant negative samples (Table 2.2.3).

Table 2.2.3. Cross tabulation of NHS routine processing versus UK Flexicult^{TM} SSI urinary kit inoculated with undiluted and diluted urine (1:1000) in determining microbiologically positive urine (at ≥ 10^5 cfu/mL pure or predominant organism)

	NHS Routine Processing (Reference Standard)	**Undiluted Urine Flexicult^{TM} SSI urinary kit**	**Diluted Urine Flexicult^{TM} SSI urinary kit**
Raw Results			
Concordant Positives (%)	23 (29.5)	20 (25.6)	21 (26.9)
Concordant Negatives (%)	55 (70.5)	47 (60.3)	47 (60.2)
Discordant Positives (%)	NA	8 (10.3)	8 (10.3)
Discordant Negatives (%)	NA	3 (3.8)	2 (2.6)
Statistical Measures			
Sensitivity (95% CI)		87.0 (67.9 – 95.5)	91.3 (73.2 – 97.6)
Specificity (95% CI)		85.5 (73.8 – 92.4)	85.5 (73.8 – 92.4)
Positive Predictive Value (95% CI)		71.4 (52.9 – 84.8)	72.4 (54.3 – 85.3)
Negative Predictive Value (95% CI)		94.0 (83.8 – 97.4)	95.9 (86.3 – 98.9)
Error Rate (%)		14.1	12.8

Antibiotic Susceptibility Testing

The comparison of antibiotic susceptibility disc testing (NHS) and UK Flexicult was performed on 40 UTI positive (NHS) samples. Overall susceptibility discrepancies between the study NHS results and UK Flexicult kit results were; 22.2% 1st generation cephalosporins; 0% ciprofloxacin; 47.6% co-amoxiclav; 15.4% nitrofurantoin; and 6.3% trimethoprim.

Table 2.2.4. Summary Table of Susceptibility Results for both NHS and UK Flexicult™ SSI urinary kit

Antibiotic	1st Generation Cephalosporin	Ciprofloxacin	Co-amoxiclav	Nitrofurantoin	Trimethoprim
Study Prevalence (the number of resistant samples identified by each test within this study)					
NHS Resistance (N)	55.6%[2] (9)	16.1% (31)	16.7% (30)	7.7% (39)	53.1% (32)
Flexicult Resistance (N=40)	52.5%	20%	47.5%	20%	52.5%
Accuracy (of the susceptibility profiles of the organisms identified using UK Flexicult compared to the NHS method considered the gold standard for this study)					
Flexicult Sensitive/ NHS Sensitive	2/4 (50%)	26/26 (100%)	11/25 (44%)	31/36 (86.1%)	14/15 (93.3%)
Flexicult Resistant/ NHS Resistant	5/5 (100%)	5/5 (100%)	5/5 (100%)	2/3 (66.7%)	16/17 (94.1%)

Diluting the urine made no difference in interpreting Flexicult™ SSI urinary kit susceptibility with ciprofloxacin, co-amoxiclav or nitrofurantoin. On the trimethoprim section one sample was interpreted as susceptible after dilution and cephalothin had seven urines that were interpreted as susceptible after dilution (resistant with undiluted urine). There were still a number of remaining discordant co-amoxiclav (n=7) and cephalothin results (n=4) compared to the NHS routine testing. The MICs of the organisms showing discordancy (all *E. coli*) were all 8 mg/L for both co-amoxiclav and

[2] 5/5 Cephalexin resistant; 4/4 Cephradine sensitive.

cephalothin and according to the Flexicult™ SSI urinary kit antibiotic break-point concentrations should be susceptible (16/8 mg/L for co-amoxiclav and 16mg/L for cephalothin).

Flexicult quality control

There were quality control failures of the UK Flexicult plates during this study; both batches (Lots) of plates showed growth of susceptible *E. coli* ATCC strain 25922 (>10^5 cfu/mL) on the cephalothin and co-amoxiclav sections prior to the expiry date; the batch of plates with Lot number 2852 showing growth >10^3 cfu/mL on the cephalothin section at week seven (29/11/212) and growth ≥10^5 cfu/mL on the co-amoxiclav section at week six (22/11/2012).

Reproducibility study results

Urine samples and corresponding NHS results

In total 73.3% urine samples were from female patients; 76.6% mid-stream urine with 41.7% from patients aged 16-65 years and 53.3% from patients >65 years.

Overall the NHS results for these samples showed 10% (n=6) no significant growth; 25% (n=15) no predominant organism; 36.7% (n=22) *E.coli*; 11.7% (n=7) Coliform (*Klebsiella* spp., *Enterobacter* spp.); 5% (n=3) *Pseudomonas* spp.; 3.3% (n=2) *S. saprophyticus*; 1.7% (n=1) *Enterococcus* spp.; 1.7% (n=1) *Proteus* spp.; and 5.1% (n=3) other organisms (unable to be identified using Flexicult). The resistance levels for the positive samples (n= 31 to 35 depending on the antibiotic) were 6.7% resistance to 3rd Generation cephalosporin; 6.7% resistance to ciprofloxacin; 5% resistance to co-amoxiclav; 8.3% resistance to nitrofurantoin; and 20% resistance trimethoprim.

Use of Flexicult brochure and POETIC website

The average time spent reading the Flexicult brochure prior to interpreting the plates for the Biomedical Scientists was 23 minutes; compared to 11 minutes for the GPs. Only one BMS and one GP accessed the POETIC website prior to the evaluation. All of the GPs continued to read the Flexicult brochure before interpreting the images both prior to

and after training. On average it took the GPs 67 seconds to read each Flexicult plate before training and 44 seconds after training (although after training these were images rather than actual plates). The BMS took on average 57 seconds to read each plate.

Accuracy and agreement of Flexicult interpretation

Identification of a UTI

The agreement between the Biomedical Scientists and myself on the identification of a positive UTI (pure or predominant growth of a uropathogen at ≥ 10^5 cfu/mL) using Flexicult plates ranged from 76.7% - 78.3% with a moderate strength of agreement. The within group Kappa agreement for the BMS was substantial and the BMS within group agreement including myself was also substantial.

The agreement (prior to training) between the GPs and myself on the identification of a positive UTI using Flexicult plates ranged from 55.0% - 60.0% with a slight strength of agreement between myself and each GP. The within group Kappa agreement for the GPs was fair and the GP within group agreement and myself was also fair.

After training the agreement between the GPs and myself on the identification of a positive UTI using the images ranged from 61.0% to 72.9% with slight to moderate strength agreement. The within group agreement for the GPs was moderate as was the within group agreement with the GPs and myself. These results are shown in Table 2.2.5.

Susceptibility results

For all the antibiotic susceptibility profiles interpreted by the Biomedical Scientists and myself the strength of agreement was almost perfect (Kappa). For the GPs and myself prior to training the strength of agreement varied from moderate to fair and after training from substantial to almost perfect. Only the GPs interpretation of nitrofurantoin susceptibility compared to mine remained as a substantial agreement rather than almost perfect agreement after training, although the 95% confidence interval for co-amoxiclav also remains within substantial agreement after training (Table 2.2.6).

Table 2.2.5. Agreement in Identification of Positive UTI (≥10^5 cfu/mL pure or predominant uropathogen) using Flexicult

	Agreement (%)	Kappa statistic	95% CI	Kappa statistic	95% CI	Kappa statistic	95% CI
	Individual compared to experienced reader			**Within group agreement**		**Within group and experienced reader agreement**	
BMS 1	76.7	0.533	0.337-0.730				
BMS 2	78.3	0.567	0.364-0.769	0.707	0.656-0.806	0.620	0.550-0.679
BMS 3	76.7	0.533	0.327-0.740				
GP1	55.9	0.114	-0.130-0.358				
GP2	60.0	0.200	-0.010-0.410	0.362	0.338-0.432	0.223	0.125-0.343
GP3	55.0	0.100	-0.095-0.295				
GP1 (after training)	72.9	0.456	0.231-0.681				
GP2 (after training)	66.1	0.318	0.090-0.546	0.535	0.480-0.638	0.428	0.332-0.498
GP3 (after training)	61.0	0.216	-0.022-0.454				

Table 2.2.6. Agreement in interpreting susceptibility profiles of uropathogens using Flexicult

	Kappa statistic (95% CI) Within group and experienced reader agreement				
	Cephalothin	**Ciprofloxacin**	**Co-amoxiclav**	**Nitro-furantoin**	**Trimethoprim**
Biomedical Scientists	0.954 (0.914-1.000)	0.942 (0.918-1.000)	0.959 (0.954-0.962)	1.000	0.937 (0.859-1.000)
GPs prior to training	0.653 (0.588-0.702)	0.590 (0.363-0.648)	0.654 (0.649-0.731)	0.631 (0.598-0.673)	0.579 (0.533-0.614)
GPs after training	0.864 (0.814-0.957)	0.886 (0.840-0.972)	0.833 (0.707-0.890)	0.671 (0.656-0.787)	0.861 (0.807-0.930)

Interpretation of plates versus interpretation of images

The interpretation of growth (no significant growth, mixed growth or pure/predominant growth); Quantification (≥10^5 cfu/mL yes or no); Identification (*E.coli*, coliforms, *Enterococcus* spp., *S. saprophyticus*, *Pseudomonas* spp., *Proteus* spp., unsure); and susceptibility profiles (resistant, susceptible or unsure) on the Flexicult plates compared to the corresponding images, read individually by the each Biomedical Scientist and myself showed moderate to substantial agreement for the growth, quantification and identification and substantial to almost perfect agreement for the susceptibility profiles.

Biomedical scientists and General Practitioners comments on interpreting Flexicult

Direct comments from BMS;

- 'Difficult to tell if culture is pure or mixed due to heavy growth'
- 'Descriptions of colony colour and identification vague'
- 'Very difficult to be accurate (normally on the bench confirmatory testing would be performed)'
- 'More definitive colony counting guidelines would be useful (very subjective)'
- 'Difficult to read plates from images – particularly for low/no/faint/white/mixed growth'

Direct comments from GPs;

- 'Definitely need training on interpretation'
- 'Is there any need to differentiate between the two Enterococci?'
- 'Difficult test – hard to know where to look at colour – on the agar or on the colonies'
- 'Hard to interpret when different colours in the different antibiotic sections'
- 'Colonies did not match the guide – may be due to mixed growth?'
- 'Sometimes there were no colonies and the agar had changed colour'
- 'Much harder to interpret pictures than the real specimens, especially for mixed growth'
- 'Following training I identified more mixed growth; I also looked more at the antibiotic sections to determine if growth was mixed rather than just using larger section (as instructed in brochure)'
- 'In pure growth determining sensitivities is easy, when growth is mixed this is much harder'

Chapter 3: Chromatic sensing, visual turbidity and dipstick urinalysis introduction and methods

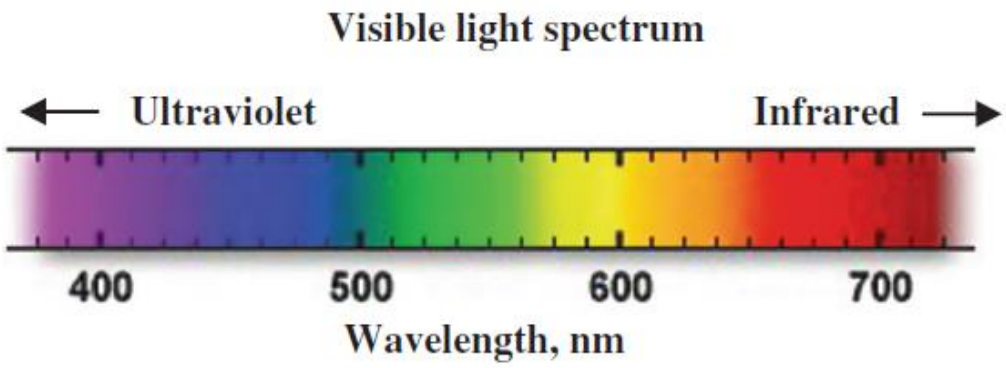
Introduction

The chromatic sensing research in this section has been published in the Journal of Physiological Measurement (70).The development and set-up of the chromatic sensing system is described in appendix 2.3.1. The current use of visual assessment of urine turbidity and urinalysis dipsticks as point of care tests for UTI in primary care have been discussed in section 1 chapter 2.

Colour science and chromaticity

Chromaticity refers to the aspect of colour that includes consideration of its dominant wavelength and purity. Chromatic intelligent monitoring relates to vision and optics; physically, human vision is based on rods and cones in the eye (sensors and filters) responding to visible light of different wavelengths (colour) to provide signals conveying information in terms of the primary colours red, green and blue (Fig 2.3.1). Being based on colour science, chromatic processing applies physically to these signals and metaphorically in the information domain to aid in the interpretation of the significance of a signal (71).

Figure 2.3.1. Colour spectra of light in visible range (72).



Light has three characteristics by which a colour may be specified: hue, saturation and brightness. Hue is associated with the dominant wavelength in a mixture of light waves, i.e., it represents the dominant colour as perceived by an observer. Saturation refers to relative purity or the amount of white light mixed with a hue. Brightness (or lightness) is a subjective term, which embodies the chromatic notion of intensity. Hue and saturation taken together are called chromaticity. Therefore, a colour may be characterized by brightness and chromaticity (72).

Chromatic sensing uses information technology such as laptop computers and webcams or mobile phone systems to capture images of liquid samples, and specific software is used to analyse these images. The red, green and blue (RGB) pixels of images are translated into a quantitative signature for characterising a spectrum in terms of the three chromatic parameters: hue; saturation; and brightness. The RGB outputs can be transformed via algorithms from colour science into other representations that emphasize different relationships emerging from the relative proportions of RGB.

Additionally, when light falls on an object or in this study, liquid, it may be reflected, transmitted, or absorbed. Reflected light is the part of the incident energy that is bounced off the liquid surface, transmitted light passes through the liquid, and absorbed light constitutes the part of the incident radiant energy absorbed within the liquid. The degree to which these phenomena take place depends on the materialistic nature of the liquid and on the particular wavelength of the electromagnetic spectrum being used (72) . The refractive index, particle size, homogeneity, and concentration of the liquid contribute to its disposition and are all considered when developing a chromatic analytical system for miscible liquids.

Chromatic sensing applications

Chromatic sensing applications include neonatal bilirubin monitoring (skin colour); direct monitoring of a neonates skin colour using a digital camera image and extracting the hue, light/brightness and saturation information directly from the RGB pixels in the image. Hue represents skin colour with increased yellowness indicating raised bilirubin levels associated with jaundice, blueness indicates blood oxygenation, and redness indicating

blood circulation); the brightness and saturation of these hues are also analysed (71). Chromatic sensing has also been used to differentiate between illicit and authentic brands of liquor by simply analysing a photographic image of the liquor. This system uses a laptop computer and webcam; chromatic outputs of the webcam directly provide a chromatic signature while the luminosity of the computer screen is used to set and diffuse the light source aimed at a liquor sample contained within a bottle. Different liquors preferentially absorb the light at various wavelengths to different degrees, the output of which (RGB or HLS) can be captured and mathematically analysed and compared (73). The above are two examples of chromatic sensing applications most comparable to using the system for medical diagnostics and using urine as a miscible liquid. Although details of the chromatic process and application on both these examples have been published there were no published data on the diagnostic/statistical accuracy of these methods. Additionally, no work has been done on the feasibility of applying chromatic sensing to determine the presence of bacteria in human samples such as urine.

Rationale for exploring chromatic sensing as a POCT for UTI

In 70 – 95% cases the infectious agent responsible for acute uncomplicated UTI is *E coli* (74). *E. coli* absorbs light in the UV region of the spectrum (75); we should, therefore, be able to capture the tail of this UV region through the blue spectrum of the urine images and chromatically map differences between bacterial positive and negative urine samples. Alternatively there may be some other elements such as metabolites in bacterially infected urine that are different to non-infected urine, and may draw a chromatically different picture.

It is reported that clinicians can be reasonably confident that symptomatic patients with cloudy urine do have a UTI, but they should be cautious about excluding patients based on the absence of cloudiness (44). Chromatic sensing can separate spectral analysis of the turbidity as well as the liquid components of the urine (without prior manipulation) and thus possibly make a more accurate interpretation than by human vision alone.

If we are able to show that chromatic sensing can distinguish between a bacterial positive and negative sample, it's function as a POCT will have a number of added benefits that are known to be essential for successful implementation into clinical practice. These benefits include: non-invasive sampling; a turnaround time of a few minutes; simple use without the need of expensive reagents or technically trained staff; low cost by using standard laptop computers or mobile phone technology; and automatically generated conclusive results.

Objectives

- To assess the analytic performance of chromatic sensing in the laboratory diagnosis of UTI with NHS microscopy and culture as the reference standard;
- To evaluate the analytic performance of chromatic sensing compared to currently available POCTs for UTI including urine dipsticks and visual turbidity assessment.

Methods

Setting, samples, NHS microscopy and culture

As this study was performed at the same time and with the same samples as the Flexicult evaluation study (Section 2 Chapter 1) information on the samples, approvals and routine laboratory methods (NHS microscopy and culture) are described there. I have only used one cut-off for positive growth of a pure or predominant uropathogen (bacteria infecting the urinary tract) $>10^5$ cfu/mL.

Chromatic sensing procedure

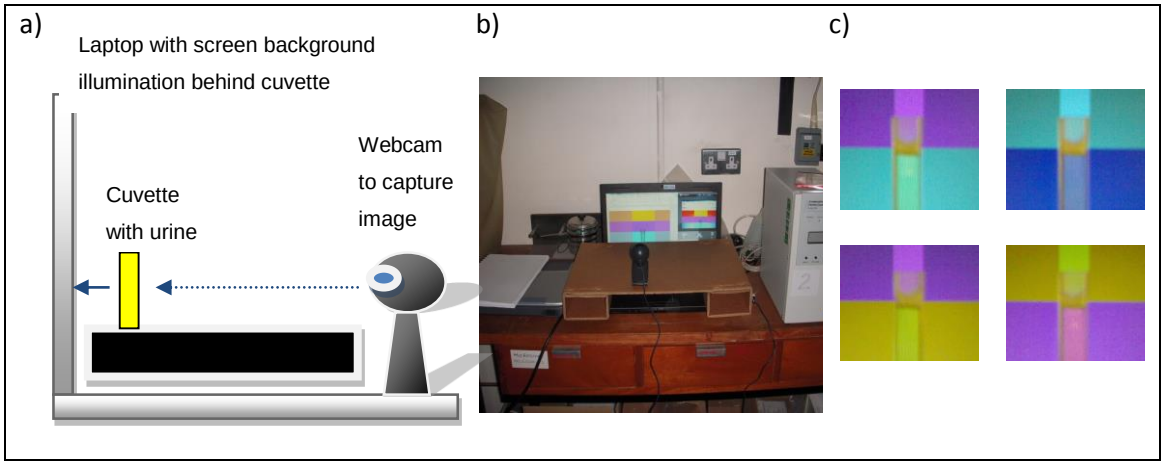
The chromatic sensing analysis was delayed up to 48 hours after receipt in the laboratory due to time constraints in being able to undertake all the diagnostic tests during routine processing hours.

For each urine sample two clear, polystyrene, spectrophotometer cuvettes (C5677-100EA Sigma-Aldrich) were filled with 2.5 mL urine. The two cuvettes containing the urine were then placed onto a stand containing a UV light (developed and provided by

Liverpool University). It was decided by the team at Liverpool University to use two cuvettes instead of one to increase the optical path length and in so doing be better able to discriminate between the changes in lower *E. coli* concentrations.

The set-up consisted of a portable computer (Toshiba), whose VDU provided the main illumination and a web-cam connected to the computer which captured images of the cuvettes containing the urine. The chromatic information was extracted via software installed in the computer. The stand holding the cuvettes was placed approximately 2 cm in front of the laptop screen, with the required background (Microsoft Office PowerPoint template on screen, developed and provided by Liverpool University, appendix 2.3.2) and the webcam positioned so that the screen was visualised through the urine (in the double cuvettes). The equipment set-up is illustrated in figure 2.3.2 and details of all equipment and corresponding settings used are outlined in Table 2.3.1.

Figure 2.3.2. Set-up and image capture of liquid samples using a laptop and webcam: a) diagram of set-up; (b) photograph of an example of the laptop set-up; (c) example of images captured using this set-up.



The screen light source (transmitted light – chromatic indication) and ambient light source (scattered light – chromatic turbidity) were analysed for all samples. The red, green and blue (RGB) outputs from images captured under these different light sources were collected. The images and red, green and blue outputs for each urine sample were sent to Liverpool University, where the outputs were analysed according to their developed mathematical algorithms. The chromatic data was returned in dichotomous form (positive or negative) for chromatic indication (a determination of the liquid

component of the urine by measuring transmitted light) and continuous numeric form for chromatic turbidity (determining the amount of scattered light by measuring the reflection of ambient light).

Table 2.3.1. Details of the equipment and settings used for chromatic image capture of urines.

Laptop/Computer	Toshiba Portege R500 Laptop
Webcam	Creative PD1110
Camera Settings	Auto; Exposure 67, Brightness 20, Contrast 20, Saturation 50, Sharpness 3, Gamma 3
Screen View (Microsoft Power Point) Template)	UrineTestBackgroundXWhite for EB CIMCOM 240912(2) (appendix 2.3.2)
Number of Cuvettes	2
Volume of urine	2.5mL per cuvette

For the chromatic indication analysis, the first 67 samples were analysed un-blinded by the Liverpool team; (they were aware of the NHS culture results for each samples). This enabled them to check the mathematical algorithms in distinguishing between bacterially positive and negative urine. The remaining 128 samples were analysed blinded to the culture results.

Visual turbidity procedure

Visual turbidity was performed within 24 hours of receipt of the urine in the laboratory. All urines were visually assessed for turbidity and colour. The urine samples were assessed in 20mL clear, plastic, universal containers, inverted twice and held against white paper with writing on; the colour was recorded as seen and the turbidity was categorised into one of the following groups;

1) Clear
2) Clear but with material present (no uniform turbidity)
3) Slightly turbid (can easily see through urine)
4) Turbid (difficult to see through urine)

5) Very turbid (cannot see through urine)

Urinalysis dipstick procedure

Dipstick urinalysis was performed within 24 hours of receipt of the urine in the laboratory and immediately after visual assessment of turbidity.

All urine samples had dipstick testing performed according to the manufacturer's instructions (Bayer Multistix® 8 SG). The urine samples were inverted to mix and then the urinalysis dipsticks were dipped into the urine sample, ensuring all the test pads were immersed. The dipstick was immediately removed from the urine, tapped onto the side of the urine container to remove excess urine and the test pads read by comparing to the corresponding row of colour blocks on the urinalysis bottle at the indicated time for colour development (from 30 seconds for glucose to 2 minutes for leucocytes). The following test pads were included on each urinalysis dipstick; glucose, ketone, specific gravity, blood, pH, protein, nitrite and leucocytes

Analysis

Data was entered into Microsoft Excel and SPSS spread sheets. Diagnostic performance evaluations were calculated for sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), likelihood ratios, diagnostic odds ratio and accuracy. Outcomes were analysed and compared to the reference standard laboratory testing (routine NHS microscopy and culture). ROC curves were analysed to evaluate the additive value of chromatic sensing and/or dipstick testing to turbidity of urine. Confidence intervals (95%) were calculated using a spread-sheet (CIPROPORTION) developed by a Cardiff University statistician Robert Newcombe (68).

Chapter 4: Chromatic sensing, visual turbidity and dipstick urinalysis analytic performance results

In total 195 samples were included in the analysis; 125 (64.1%) bacterially negative and 70 (35.9%) bacterially positive urine samples (>10^5 cfu/mL pure or predominant uropathogen).

Visual turbidity

Of the 195 urine samples visually assessed 22.5% were categorised as clear, 7.5% clear with material in, 18.3% slightly turbid, 43% turbid and 8.7% very turbid (shown in Table 2.4.1 below).

Table 2.4.1. Frequency of urine samples categorised into 5 levels of turbidity through visual assessment.

Turbidity classification	Frequency	Per cent	Cumulative Per cent
1) clear	44	22.5	22.5
2) clear but with material in	15	7.5	30.0
3) slightly turbid	36	18.3	48.3
4) turbid	84	43.0	91.3
5) very turbid	17	8.7	100.0
Total	195	100.0	

To dichotomise the data I further sub-categorised the results into not turbid (urines that were clear and clear with material in it) and turbid (urines classed as slightly turbid, turbid and very turbid); 30.3% were classed as not turbid and 69.7% as turbid.

Cross-tabulation of the urines categorised into not turbid and turbid with NHS culture results (positive if >10^5 cfu/mL) are shown in Table 2.4.2.

Table 2.4.2. SPSS cross-tabulation of non-turbid and turbid urine samples with NHS quantification results.

		NHS Quantification		Total
		<=10^5 cfu/mL	>10^5 cfu/mL	
Visual Turbidity	not turbid	59	0	59
	turbid	66	70	136
Total		125	70	195

When using this categorisation of visual turbidity the sensitivity was 100% (95% CI 94.8; 100); specificity is 47.2% (95% CI 38.7; 55.9); positive predictive value 51.5% (95% CI 43.5; 59.7) and negative predictive value 100% (95% CI 93.9; 100). All urines classed as non-turbid (59/195) could be safely classed as negative for a UTI. Due to the low specificity, all the turbid urines (136/195) could be either classed as positive or negative for a UTI. The absence of turbidity may therefore be useful in ruling out a UTI.

Urinalysis dipstick

From this evaluation leucocytes were found to be the most predictive of UTI followed by nitrite and then blood and protein (Table 2.4.3). Both nitrite and leucocyte urinalysis are better predictors of not having a UTI with NPV of 70.8 (95% CI 63.2 – 77.4) and 82.1% (95% CI 74.0 – 88.1) respectively and both having low PPVs.

When combining tests my results give a PPV for ‘positive nitrite, leucocytes and/or blood’ of 82% and 76% respectively. Nitrite alone had a PPV of 62%.

A urinalysis result negative for nitrite, leucocytes and blood gave a NPV of 84% and the highest sensitivity of 83.1%. However, there were 12 false negatives samples and therefore does not completely rule out a UTI.

The sensitivity, specificity, PPV and NPV for various urinalysis tests and combinations of tests are shown in appendix 2.3.3.

Table 2.4.3. Performance of urinalysis tests in predicting a laboratory UTI diagnosis.

	True positives n (%)	True negatives n (%)	False positives n (%)	False negatives n (%)	% Correctly classified	Likelihood ratios (LR+, LR-)	Odds ratio
Nitrite	26 (13.3)	109 (55.9)	15 (7.7)	45 (23.1)	69.2	3.03, 0.72	3.93
Leucocyte ≥+	44 (22.6)	100 (51.3)	24 (12.3)	27 (13.8)	73.8	3.20, 0.47	6.79
Blood, haemolysed ≥trace	39 (20.0)	84 (43.1)	40 (20.5)	32 (16.4)	63.1	1.70, 0.67	2.56
Protein ≥trace	36 (18.5)	73 (37.4)	51 (26.2)	35 (17.9)	55.9	1.23, 0.84	1.47

Visual turbidity and dipstick urinalysis prediction rules

In this study the sensitivity of visual turbidity was 100%, therefore I could safely eliminate any clear or non-turbid samples from further testing. However, with the remaining samples (approx. 70% in this sample set) it was not possible to safely determine whether these were positive or negative for a UTI by turbidity assessment alone. By combining the urinalysis dipstick testing with turbidity testing, I hoped to improve the diagnostic performance of these manoeuvres for these samples. The remaining 136 samples (59 removed as non-turbid) were first evaluated using a prediction rule; if nitrite, leucocyte AND blood (haemolysed) are negative the urine is unlikely to be positive for UTI. The results are as follows; combined sensitivity 82.9% (95% CI 72.4 – 89.9); specificity 70.4 % (95% CI 61.9 – 77.7); PPV 61.1% (95% CI 51.0 – 70.3) and NPV 88.0% (95% CI 80.2 – 93.0). Out of 195 samples; 100 would have been deemed negative based on non-turbidity combined with nitrite, leucocyte and blood urinalysis negative, the risk would be 12/100 (12.0%), 95 samples would have been considered probable for a UTI with a risk of 37/95 (38.9%).

Another prediction rule I tested was that if the urine was visually turbid AND nitrite OR leucocyte positive, it was likely to be positive for a UTI. The results were as follows; sensitivity 77.1% (95% CI 66.1 – 85.4); specificity 80.0% (95% CI 72.1 – 86.1); PPV 68.4% (95% CI 57.5 – 77.6) and NPV 86.2% (95% CI 78.8 – 91.3). Out of 195 samples; 116 would be considered negative with a risk of 16/116 (13.8%), 79 samples would be considered probable for a UTI with a risk of 25/79 (31.6%).

Table 2.4.4. Performance of various visual turbidity and urinalysis prediction rules in determining UTI.

	True positive n (%)	True negative n (%)	False positive n (%)	False negative n (%)	Correctly classified (%)	Likelihood ratios (LR+, LR-)
Visual Turbidity	70 (36.0)	59 (30.2)	66 (33.8)	0 (0)	66.2	1.89, 0
VT negative and/or NLB negative	58 (29.7)	88 (45.1)	37 (19.0)	12 (6.2)	74.8	2.8, 0.243
VT and N or L positive	54 (27.7)	100 (51.3)	25 (12.8)	16 (8.2)	79.0	3.86, 0.286

VT = visual turbidity, N = Nitrite, L = Leucocyte, B = Blood

The superior prediction rule based on likelihood ratios was the visually turbid urine with either positive nitrite or leucocyte (Table 2.4.4). A positive result in this combination of tests is approximately 4 times more likely to occur in a patient that does have an NHS laboratory diagnosed infection compared to one who does not. The sensitivity and specificity for this prediction rule is 77.1% (95% CI 65.6 – 86.3%) and 80.0% (95% CI 71.9 – 86.6%) respectively.

Chromatic sensing

Chromatic indication (transmission)

The chromatic indication (evaluation of transmission of light) overall gave a sensitivity of 97.1% (95% CI 90.2 – 99.2); specificity 54.4% (95% CI 45.7 – 62.9); PPV 54.4% (95% CI 45.7 – 62.9) and NPV 97.1% (95% CI 90.2 – 99.2) as detailed in Table 2.4.5.

Although the accuracy of the results (number of correctly classified samples) decreased after blinding from 76.1% to 66.4%.

Table 2.4.5. Performance of chromatic indication in determining UTI on samples with unblended and blinded culture results.

Chromatic Indication	True positives, n (%)	True negatives, n (%)	False positives, n (%)	False negatives, n (%)	Sensitivity (95% CI)	Specificity 95% CI)	PPV (95% CI)	NPV (95% CI)
Unblinded (n=67)	23	28	16	0	100 (85.7 – 100)	63.6 (48.9 – 76.2)	59.0 (43.4 – 72.9)	100.0 (87.9 – 100)
Blinded (n=128)	45	40	41	2	95.7 (85.8 – 98.8)	49.4 (38.8 – 60.1)	52.3 (41.9 – 62.6)	95.2 (84.2 – 98.7)
All samples (n=195)	68	68	57	2	97.1 (90.2 – 99.2)	54.4 (45.7 – 62.9)	54.4 (45.7 – 62.9)	97.1 (90.2 – 99.2)

Chromatic turbidity (dichotomising continuous data)

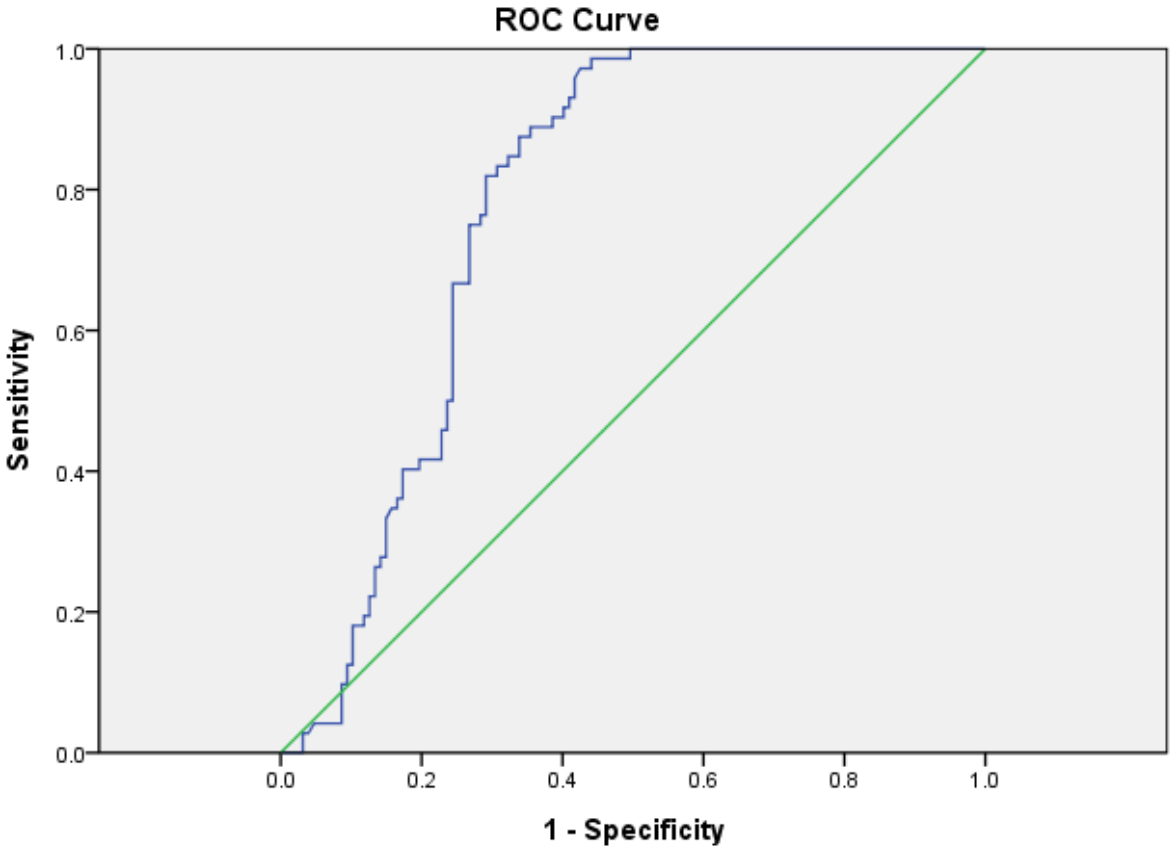
ROC and area under the curve

Looking at the co-ordinates of the ROC curve and to give optimum sensitivity a cut-off of 0.152 was selected to dichotomise the data (Figure 2.4.1));

CT < 0.152 negative (low turbidity)

CT≥ 0.152 positive (high turbidity)

Figure 2.4.1. ROC curve of chromatic turbidity results



Using this cut-off chromatic turbidity had a sensitivity of 100%; specificity of 50.4%; PPV of 53.0% and NPV of 100%. When comparing the chromatic turbidity results with the visual turbidity results (analysing the same sample set) the outcomes were very similar as shown in Table 2.4.6.

Table 2.4.6. Performance of chromatic and visual turbidity in determining UTI (as compared to NHS culture >10^5 cfu/mL)

	True positives, n (%)	True negatives, n (%)	False positives, n (%)	False negatives, n (%)	Sensitivity (95% CI)	Specificity 95% CI)	PPV (95% CI)	NPV (95% CI)
Chromatic Turbidity	70 (35.9)	63 (32.3)	62 (31.8)	0 (0)	100 (94.8 – 100)	50.4 (41.8 – 59.0)	53.0 (44.6 – 61.3)	100 (94.3 - 100)
Visual Turbidity	70 (35.9)	59 (30.3)	66 (33.8)	0 (0)	100 (94.8 – 100)	47.2 (38.7 – 55.9)	51.5 (43.2 – 59.7)	100 (93.9 – 100)

Chromatic indication and chromatic turbidity

Chromatic indication and chromatic turbidity were combined using the following rule; any sample with chromatic indication AND chromatic turbidity positive, was considered positive; if the sample is either chromatic indication OR chromatic turbidity negative it is deemed negative. This gave the following results: sensitivity of 97.1%; specificity of 64.8%; PPV of 60.7%; NPV of 64.8%; with a 'positive' group compromising of 112 (57%) of the samples with a risk of 44/112 (39.3%), and a 'negative' group compromising of 83 (43%) of the cases with a risk of 2/83 (2.4%).

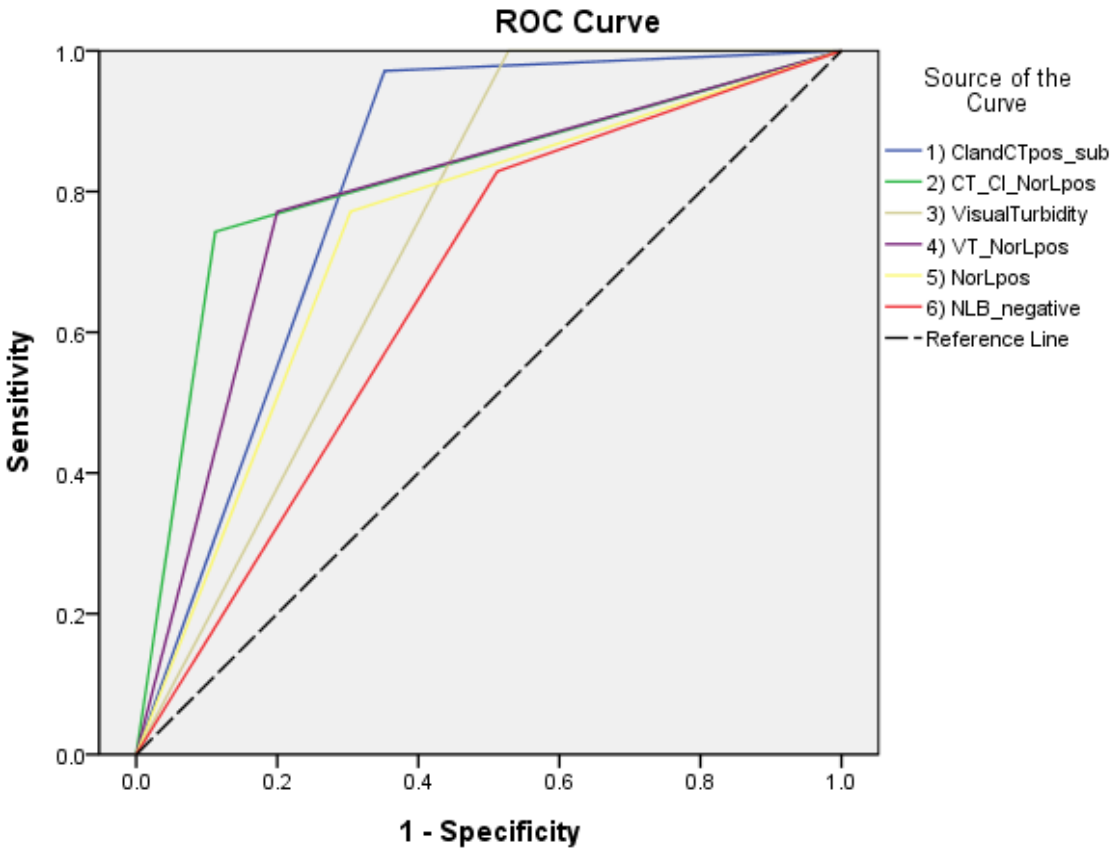
Chromaticity and urinalysis

As visually turbid urine combined with the urinalysis result of either positive nitrite or leucocyte (≥+) gave the best differentiation between bacterial positive and negative urine, I repeated the same rule but using Chromatic Indication and Chromatic Turbidity positive with positive nitrite or leucocyte (≥+). The results gave a sensitivity of 74.3% (95% CI 63.0 – 83.1); specificity of 88.8% (95% CI 82.1 – 93.2); PPV of 78.8% (95% CI 67.5 – 86.9) and NPV of 86.0% (95% CI 79.0 – 91.0). Out of 195 urine samples 66 (33.8%) would be categorised as positive (or likely positive for UTI) with a risk of 14/66 (21.2%) and 129 (66.2%) would be categorised as negative (or likely negative for a UTI) with a risk of 18/129 (14.0%).

Using an ROC curve (Figure 2.4.2) the following 6 predictions rules were compared;

1) Chromatic Turbidity and Indication positive likely UTI
2) Chromatic Turbidity and Indication positive and nitrite or leucocyte (≥+) positive likely UTI
3) Visually turbid likely UTI
4) Visually turbid and nitrite or leucocyte (≥+) positive likely UTI
5) Nitrite or leucocyte (≥+) positive likely UTI
6) Nitrite, leucocyte and blood (haemolysed) negative unlikely UTI

Figure 2.4.2. ROC curves of 6 various chromatic, turbidity and urinalysis prediction rules for bacterially positive UTI (NHS culture >10^5 cfu/mL positive)



The AUROC of 0.815 (for Chromatic Turbidity and Chromatic Indication positive with nitrite or leucocyte (≥+) positive) indicated the highest discrimination out of all the rules, and the null hypothesis was rejected ($p<0.001$) for all (Table 2.4.7).

Table 2.4.7. Area under the curve (based on ROC curves in figure 2.4.2) for 6 prediction rules in determining a UTI

Prediction Rule	Area	Std. Error[a]	P-value	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
1)CI and CTpos_sub	.810	.031	.000	.750	.870
2)CT_CI_NorLpos	.815	.035	.000	.747	.884
3)VisualTurbidity	.736	.034	.000	.668	.804
4)VT_NorLpos	.786	.036	.000	.716	.856
5)NorLpos	.734	.038	.000	.660	.808
6)NLB_negative	.658	.040	.000	.581	.736

(CI – chromatic indication; CT – chromatic turbidity; N – nitrite; L – leucocyte; VT- visual turbidity; NLB – nitrite, leucocyte, blood)

Chapter 5: Section 2 Discussion

UK Flexicult™ SSI urinary Kit

The completed STARD checklist for reporting the Flexicult diagnostic accuracy study is shown in appendix 2.3.4.

The UK Flexicult results were comparable to routine NHS urine processing in identifying microbiologically positive urine samples which may aid the diagnosis of UTI. UK Flexicult produced few false negatives (3% when compared to routine NHS method) which is important for primary care clinicians: not missing patients that may benefit from antibiotics is generally more important in clinical care than prescribing antibiotics to patients with culture negative urine. The higher false positivity could lead to over-prescribing in primary care, although this study did not compare UK Flexicult to empirical prescribing in general practice and may still reduce unnecessary antibiotic prescribing compared to diagnosis based on clinical symptoms and/or urinalysis dipstick results alone. The semi-quantitative nature of Flexicult, over-inoculation or delay in processing (allowing organisms within the urine to multiply) may have caused the higher false positivity as it is more difficult to differentiate between a cut-off of less than or greater than 10^5 cfu/mL or determine a predominant organism when there is a high level of growth. The only other published study of the Flexicult™ SSI urinary kit by Blom et al. which analysed 121 urine samples from patients who consulted participating primary care clinicians with suspected UTI in Denmark. The samples were tested for bacteriuria using the (Danish) Flexicult kit and the results were compared with urine samples obtained simultaneously for control purposes and found an overall error rate of 4.7% [11]. The error rate from this study was 16.5%, however, this was based on detection of a pure/predominant clinically significant organism as well as quantification which was not done in the previous study.

Identification of the most common uropathogens was comparable to NHS and spiral plating methods when using UK Flexicult. However, the inability to differentiate white colonies may cause some patients to be treated for a UTI when commensal organisms

or *Candida* spp. are cultured or conversely patients that may have less common uropathogens such as *S. aureus* or *Acinetobacter* spp. may not be treated appropriately. By diluting turbid urine samples (1:1000) prior to inoculation onto the UK Flexicult there was an improvement in the determination of predominance (by diluting out the contaminating organisms). However diluting the urine had no effect on reducing discordant positive results with the NHS and minimal effects on reducing the discordant negative results; this is likely due to the improved sensitivity of UK Flexicult when using diluted urine. Flexicult has a detection limit of ~5 x 10^2 cfu/mL[11] and by diluting the urine 1:1000 the kit would have a detection limit of >10^5 cfu/mL which, depending on the definition of a microbiologically positive UTI may not be appropriate.

The higher NHS resistance levels in this study compared to the most recent (2013) reported prevalence for community acquired coliforms in Wales [9] may be due to the small sample size and the sample population used for this study which included hospital inpatient samples and samples that may have been selected due to concerns over antibiotic resistance. Trimethoprim is a recommended first-line antibiotic for uncomplicated UTI in the UK and the accuracy using UK Flexicult is encouraging. The resistance levels using both methods is also similar for 1st generation cephalosporins, however the NHS cephalosporin results had a very small sample size and may not be comparable to UK Flexicult. Ciprofloxacin had 100% accuracy compared to the NHS testing and similar overall resistance levels. However, compared to the resistance levels of the other antibiotics included in UK Flexicult the lower overall proportion of ciprofloxacin resistance may encourage prescribing of this antibiotic which is not recommended for general use due to its ecologically adverse effects [17]. The resistance levels of both co-amoxiclav and nitrofuantoin were much higher than the NHS results and this was reflected in the higher number of false resistant samples. Co-amoxiclav is not a recommended first-line antibiotic for uncomplicated UTI and clavulanate is known to be unstable in vitro making it unreliable for a POC based test which ideally requires an extended shelf-life. However, nitrofurantoin is a recommended first-line antibiotic for uncomplicated UTI and the higher resistance determined by UK Flexicult kit could lead

to its underuse as an alternative to trimethoprim and escalation of other 2nd or 3rd line antibiotics. The high false positive resistance levels to co-amoxiclav and 1st generation cephalosporin could be due to degradation of the antibiotics in the kit; and further testing of the antibiotic concentrations at various time-points (following inoculation) under different shipment and storage conditions should be evaluated further. The only way to be certain of the resistance of each isolate would be to determine the MIC for the corresponding antibiotic showing resistance on UK Flexicult which was not performed as part of this study.

By using quantification only, irrespective of predominance or identification, the sensitivity of UK Flexicult increased from 86.7% to 95.6% at the $\geq 10^5$ cfu/mL and from 86% to 100% at the $\geq 10^3$ cfu/mL cut-points for positivity. Whiting et al reviewed studies that discussed factors that may affect clinicians' decisions to order a test, and found in primary care tests are more commonly used to rule out a condition or to help the doctor to make a decision about referral. Conversely, in secondary care, tests are more often used with the aim of reaching a definite diagnosis (76). The usefulness of the POCT culture approach may lie mainly in screening out negative urine samples prior to antibiotic prescribing and/or sending samples for routine culture. The higher cut-off used in this study would screen out over 50% of the samples with only 1% of true positives being missed if this POCT was used in determining whether the sample would meet the laboratory definition of a UTI.

The inter-observer reproducibility study aimed to assess the subjective nature of Flexicult. Some of the considerations I had for the design of this study included; the feasible number of available suitably qualified 'assessors', I required more than two from each group (experienced microbiologists and naïve GPs) but was limited to the number of assessors who offered to participate. The assessment of the Flexicult plates in 'real-time' on the same day but independently; over time the growth on the plates may change so it was important that the assessors were reading the same plates at the same time. The feasible number of plates to be read by assessors with varying types of growth; there are numerous types of uropathogens that may be identified in practice both of pure or

mixed growth and of varying quantification levels. Therefore, I wanted to include as many different plates as possible (from real infections rather than spiked urine) without over whelming the assessors with too many to read in one session. I also wanted to look at the potential benefit of training the GPs so I needed to repeat the assessment of the same plates but after a period of time to avoid recall (memory) effects; therefore the images of the same plates were captured, re-numbered and sent for interpretation after a period of time.

Biomedical scientists experienced in quantifying bacteria and using chromogenic agar for identification had a much higher percent agreement (77%) and strength of agreement (substantial) in identifying positive UTIs using Flexicult compared to the GPs (57% agreement; fair strength). Even after training the strength of inter-observer agreement between the GPs and myself was only moderate with an increased agreement of 67%. The Flexicult kit is subjective and appropriate training, guidelines and user experience is critical for the diagnostic accuracy and inter-observer reliability of the test.

A concern I had from this study is that the GPs participating in it did volunteer to do so but may not have put in as much time or effort in using the brochure/website and interpreting the plates as they would have done in practice when a patient is expecting appropriate care. This study also raised some concerns regarding the brochure and training for use with Flexicult; I think both could be substantially improved and would be fundamental to the success of this test in practice. Depending on the outcomes of the clinical trial it may be worth investigating potential user guides or a web-based diagnostic tool to be used in the interpretation of Flexicult until the user is confident in their own ability to read this test. In Denmark clinicians are invited to send plates back to SSI or contact them for help in interpretation of the tests (personal communication with SSI) whether this would be practical and effective in other European countries would need to be investigated.

Chromatic sensing

Chromatic sensing provides a novel approach to predicting bacterial infections in urine and in so doing potentially predicting UTI in patients has been described. The system set-up uses everyday technical equipment such as laptops/computers and webcams (or even mobile phones although not assessed in this study) and a non-invasive, non-specialised approach to analysing urine samples. The system set-up, although easy for one person using the same equipment and location (same bench in the laboratory) would need to be evaluated based on various users, equipment and locations, particularly as the fundamental process of this system uses ambient light and screen illumination. Changes in room lighting (both natural and artificial) and laptop screen brightness, hue and other parameters may completely change the chromatic results. Although this has been taken into consideration by providing reference areas on the laptop screen (either side of the sample) to be used as a quality control for the analysis when using a standardised substance such as water, it has not been tested with multiple equipment and users setting up the system. The economic aspect of this technology would also need to be evaluated.

For this study the images and RGB outputs were captured by the user (myself) but the actual analyses were performed by the chromatic experts at Liverpool University; for this to be an effective POCT in practice the system would have to be automated with immediate results which would require further development at this stage.

Chromatic analysis is intended as a POCT to avoid unnecessary antibiotic prescribing and/or reduce the number of samples referred to the NHS microbiology laboratories. Currently in the UK only visual turbidity and/or urinalysis dipstick testing are recommended (along with patient demographics and clinical signs and symptoms) at the point of care for predicting uncomplicated UTI and therefore prescribing antibiotics appropriately. I found chromatic indication and chromatic turbidity to perform as well as visual assessment of urine turbidity in predicting negative urine samples. The SIGN guidelines do not recommend using visual assessment of turbidity because of observer error, suggesting it may not be a useful discriminator (46). Chromatic analysis has the benefit of providing a quantifiable and potentially reproducible (although this would need

to be investigated) measurement of turbidity. It could also give a finer scale of turbidity than the broad categories of human visual assessment as well as quantifying additional chromatic parameters which could indicate other conditions of the urine sample. Neither chromatic analysis (both indication and turbidity) nor visual turbidity predict positive urine samples sufficiently accurately.

For this study I used dipstick results that were interpreted by the human eye. There is potential to use chromatic analysis to interpret dipstick colour change and in one computerised program analyse the urine for chromatic indication and turbidity and dipped urinalysis stick for nitrite and leucocyte positivity, producing results within a few minutes.

Visual turbidity

Assessment of the visual turbidity of urine gave a sensitivity of 100% from this study. If this is reflected in practice this could allow GPs to exclude any clear urines from further diagnostic tests or from empirical prescribing of antibiotics.

This study involved one assessor using the same method of interpreting the visual turbidity of the urines. In current practice it may be that the GPs ask the patients for their own interpretation of the turbidity of their urine or if a sample is requested (for instance for dipstick urinalysis) the GP or nurse may evaluate the turbidity of urine themselves; either way the method for interpretation most likely varies within and between practices. In the determination of sputum colour for lower respiratory tract infections there is some concern over the ability of patients to determine their own sputum colour or purulence. A study has demonstrated that sputum colour assessed with a sputum colour chart is a better marker for bacterial involvement than sputum colour reported by patients; assessed sputum colour (using chart) was also shown to be clearly related to bacterial load in sputum and, in particular, systemic inflammation consistent with infection, whereas patient reported sputum colour was not (77). This may have some parallels with the visual assessment of urine; perhaps a chart or comparison with pre-prepared urines of varying turbidity read by the clinical staff rather than the patient may be more pertinent in avoiding observer error. A larger study or pragmatic evaluation involving the correct

sub-set of patients and assessors (GPs or nurses) in routine clinical practice would need to be undertaken before any final conclusions can be drawn.

Dipstick testing

This study showed that a urinalysis result negative for nitrite, leucocytes and blood gave a NPV of 84% and the highest sensitivity of 83%. However, there were 12 false negatives samples and therefore does not completely rule out a UTI.

The HPA recommend (for women with mild symptoms of UTI) if a dipstick test is positive for nitrite, leucocyte and blood or if positive for nitrite alone a UTI is probable. If the dipstick reads negative for nitrite, leucocyte and blood or negative for nitrite and leucocytes (positive blood and protein) a UTI is unlikely (25). SIGN recommend if the dipstick is positive for nitrite or leucocyte this is associated with a higher probability (80%) of UTI. Negative dipstick results do not exclude bacteriuria however the probability of having a UTI drops to about 20% when dipstick tests are negative (46).

The results from my study showed a poorer performance of dipstick urinalysis when using nitrite compared to previous published studies. I included all uropathogens as positive for UTI (if $>10^5$ cfu/mL, pure or predominant).The recommendation by the HPA is based on the study by Little et al. (48), which only includes *E. coli* infection as positive for UTI, *E. coli* is a gram-negative bacteria and only Gram-negative bacteria produce nitrites. Gram-positive bacteria (such as *Enterococcus* spp. and *Staphylococcus* spp.) do not produce nitrites and could be a reason for the lower performance of nitrite urinalysis in my study. The urinalysis assessing leucocytes was marginally better than nitrites in my study and again may be for a similar reason (this is an indication of infection and may be caused by all bacterial species).

Reasons for false positive nitrite results include coloured urine and *in vitro* growth and false negative results could be due to no vegetables in the diet (beet ingestion, bilirubinuria), short bladder incubation time, presence of vitamin C and Gram positive bacterial infection. False positive leucocyte results could be due to oxidising detergents, formaldehyde (0.4 g/L), sodium azide and coloured urine and false negative results due

to vitamin C, protein > 5 g/L, glucose > 20 g/L, mucoid specimen, presence of cephalosporins or nitrofuantoin (38).

The guidelines only indicate using dipsticks for women with mild symptoms of uncomplicated UTI (≤ 2 symptoms). I did not have access to the symptoms of the patients included in this study and the samples were from men and women from primary and secondary care so comparing these results to the guidelines may not be applicable. However, on a fundamental basis of using a dipstick to identify (or exclude) a bacterial urine infection the results from this study are not indicative of this test being useful when used alone.

By combining visual turbidity with dipstick testing and using the rule that a person with turbid urine and either a positive nitrite or leucocyte dipstick result; the likelihood of them having a UTI increases four-fold. This may still not be considered an adequate diagnostic process for UTI as (from this study) 14% patients may not have been treated when they had a bacterial infection and 32% who did not have a bacterial infection would have been treated.

Limitations

The results of this study were included in the protocol submission for a larger randomised controlled trial of the UK Flexicult^{TM} SSI-urinary kit and as such the approvals process for this study needed to be as timely as possible. It was therefore decided to use anonymised, excess urine samples routinely submitted to the University Hospital Wales, Microbiology Laboratory. This did not require full ethical review (proportionate only) making the process quicker, and optimising the availability of urine samples (formal opinion from Ethics committee). However, this had limitations for this study; the samples analysed were not necessarily representative of patients presenting with uncomplicated UTI in general practice which is the population this test is aimed at. As clinicians generally submit urine samples to the laboratory from patients who have failed initial therapy, the study sample may have been bias towards higher positivity and proportion of resistant isolates. Additionally, as the samples were anonymised, no patient details (other than

gender or age) or symptoms were recorded and so could not be used in the evaluation. As boric acid can affect the antibiotic sections of the Flexicult plates urines collected this way were excluded; this may have biased the samples towards in-patient rather than out-patient samples. However, the sub-group analysis results of outpatient samples did not differ from the results using the total sample population.

From a practical perspective, there was a delay (because of the requirement for excess urine) between collection of the urines and NHS processing/culture and evaluation on Flexicult, by spiral plating culture (although Flexicult and spiral plating were performed at the same time), visual turbidity, dipstick urinalysis and chromatic sensing. The time from routine processing to diagnostic evaluation was within ~9 hours (i.e. the same day) for flexicult, spiral plating, visual assessment of urine turbidity and dipstick urinalysis and within 24 hours for the chromatic sensing procedure with the urine being stored at room temperature in the meanwhile. Ideally all tests should have been evaluated at the same time and close to the time of urine collection as this is the intended purpose of the POC tests; if this was not possible refrigeration of urines in the meantime would have been preferable. Time delays can result in multiplication of bacteria (both infecting and contaminating organisms) or damage/death of diagnostically relevant bacteria (38) and potential loss of resistant elements.

Some of the discrepancies in determining a microbiologically positive urine culture for UTI may be due to the different methods used for inoculation and quantification. Flexicult used a third of the test plate (which is flooded with urine), while the NHS method used a quarter of an agar plate and a 1 µL loop for inoculation which can only quantify down to 1×10^4 cfu/mL (10 colonies); identification of predominance of an infecting organism also varies. Both these methods are subjective, may have poor reproducibility and be prone to contamination and interpretation problems which would need to be evaluated further. This subjectivity may be of concern for UK Flexicult used in clinical practice when clinical staff inexperienced in microbiological test interpretation are reading the plates and/or there is inadequate training. In NHS and research microbiology laboratories diagnostic tests, process and equipment are assessed routinely through subscription to an external

quality assurance (EQA) scheme. This should be something to consider if UK Flexicult is implemented into routine primary care management. However, a study in Sweden evaluating an EQA scheme for the use of dip-slides in primary care found no improvement in GP user interpretation over a five year period and the need for training and continuous education of the clinical personnel using the dip-slides was highlighted (78).

Due to limited resources the spiral plating process was performed by myself as well as the UK Flexicult evaluation; ideally it would have been performed by someone blind to the study question. However I was blind to the results of the reference standard and only evaluated the spiral plate tests after recording the results of the UK Flexicult. No other information was available to myself as the reader of the diagnostic tests under evaluation.

Statistical analysis

The sample size calculation was based on potential sensitivity and specificity calculated using previous Flexicult errors rates from analysis of quantification only (from Blom et al.(65)). The sensitivity and specificity calculated in this study used both quantification and identification (as would be done in routine practice) therefore the actual results were not as good and had larger confidence intervals than when based on quantification alone. There was no previous published data on the number of false positives or false negatives to improve the accuracy of this original calculation, however, the prevalence of UTI found in this study was between 20 – 30% as indicated in the initial sample size calculation. The prevalence of bacteria at $>10^5$ cfu/mL from the field study in the Blom et al. paper was much higher at just over 50%; although there was no mention of purity or predominance and if not accounted for could result in a higher prevalence.

There may be concern over the number of comparisons used for these diagnostic evaluations and the potential for type I errors. The Bonferroni correction method can be used to counteract this problem by reducing the critical significance level according to the number of independent tests carried out in the study. However, this may also reduce the statistical power of the study and fail to detect true effects as well as false ones and

as such I have decided not to use this in my statistical analyses but rather highlight the potential issue as a limitation.

The implications for use of all these diagnostic tests in practice are discussed in the overall discussion in section 4.

Section 3: Management of uncomplicated UTI in Primary Care

Chapter 1: A systematic review of the management of uncomplicated urinary tract infection presenting in primary care in Europe

Introduction

Patients consulting in even the best general practices experience unwarranted variations in health care and health outcomes and much of clinical medicine remains empirical. Everyday practice is characterised by wide variations that have no basis in clinical science. Unfortunately even the best scientific research does not always get translated into routine practice and patient management. National guidelines (that may even vary between countries and be based on different scientific research) are often not translated into practice. Unscientific personal opinion (GP and patient), local medical opinion, and local supply of resources are often more important than science in determining how medical care is delivered (79).

Variation in antibiotic prescribing that does not improve outcomes for patients wastes resources, undermines self-care for similar conditions in the future, puts patients at unnecessary risk of side effects, and increases selection of resistant organisms, and so represents an opportunity for improved care through greater standardisation (80). Differences in prescribing in certain settings may be explained by other factors such as differences in severity of illness, the patients history of illness (and previous treatment) and use of more/different (conclusive) diagnostic tests (or even different use/interpretation of the same diagnostic test), or variation in resistance of circulating organisms. If factors such as the use of different diagnostic tests are the basis of different

prescribing practices then this may be worth standardising between practices, regions and countries for a universally better outcome.

Comparative studies evaluating different patterns of practice are important in identifying areas of unwarranted variation prior to development and implementation of scientific research. Section three describes the methods and results of a systematic review comparing observational studies evaluating the management of uncomplicated urinary tract infection in primary care in different countries in Europe. Secondly this section covers the comparative data analysis of patient management in general practices recruited within four networks in Southampton (England), Cardiff (Wales), Madrid/Catalonia (Spain) and Utrecht (the Netherlands) as part of an observational study of patients with suspected uncomplicated UTI (part of the POETIC study; Section 3, Chapter 2).

Hypothesis

Variations in the management of uncomplicated urinary tract infections in primary care exist between European countries and general practices within European countries.

Aim

To identify studies that evaluated the routine management of adult women with suspected uncomplicated UTI attending primary care in different European countries. Appraise and summarise these data to determine variation in management between countries, discuss if variation is warranted, and to contrast with the findings of the new, POETIC observational study data.

Objectives

1. To perform a systematic search of the literature, critical appraisal and data extraction of included studies evaluating the routine management of women with suspected uncomplicated UTI in primary care;

2. To describe the methods and interventions employed to manage uncomplicated urinary tract infection (UTI) in women attending primary care in studies conducted in Europe;
3. To summarise and compare the methods/interventions and discuss any variations in management between countries included in the review;
4. To discuss the findings in relation to the observational data from the POETIC study.

Systematic Review Method

Protocol and Registration

The systematic review protocol has been registered on the PROSPERO website, registration number CRD42014007433 http://www.crd.york.ac.uk/PROSPERO/. PROSPERO is an international database of prospectively registered systematic reviews in health and social care within the University of York, Centre for Reviews and Dissemination.

Eligibility Criteria

Table 3.1.1 outlines the eligibility criteria that defined the population, interventions, comparison and outcomes that made up the basis of this systematic review. The population consists of adult, females consulting to primary care in Europe with suspected uncomplicated urinary tract infection. This is based on the HPA (25) guidelines for uncomplicated UTI. The interventions include any diagnostic processes or medical treatment provided for the patient during the clinical consultation. As I am interested in observation of routine management there are no comparison groups as there would be in a randomised controlled trial, however, comparisons of management choices between studies and countries are included in the review.

Table 3.1.1. Inclusion and exclusion criteria listed (where applicable) for the population, interventions, comparisons and outcomes of interest in the systematic review.

PICO	Inclusion	Exclusion
Population	Women (and multi-gender studies); suspected uncomplicated/lower/acute UTI (cystitis); women presenting to primary care/general practice in the Europe.	Men only studies; children only studies (<16); pregnant women; associated with catheter; countries outside of Europe; secondary care; women with obvious co-morbidities; complicated UTI; studies not observing routine management i.e. randomised controlled trials.
Interventions	Any medical treatment; any diagnostic or medical tests for UTI; advice and follow up procedures.	Interventions that are not part of routine practice.
Comparison	Between studies: management choices.	NA
Outcomes	As well as describing the management decisions and processes, congruence to relevant guidelines.	Microbiological results/epidemiology of UTI.

Literature search

A search strategy was developed in OVID^{SP} Medline using the keywords and Medical Subject Headings (MeSH) outlined in Box 3.1.1. The search strategy was modified to search the remaining bibliographic databases.

Electronic sources (databases and websites)

Languages

- English language studies only (due to high translation costs).

Dates

- No publication date limit up to January 2014.

Databases

- Medline via Ovid; Medline in Process; British Nursing Index; CINHAL (Cumulative Index to Nursing and Allied Health Literature); The Cochrane Library; Embase; HMIC (Health Management Information Consortium); Scopus; Web of Science.

Web sites

- Google Scholar; I performed the general search 'UTI management' and checked the first 10 pages for any new references.

Journals

- Once the database searches had been performed I selected the most frequently used journals from the studies included in the review and searched for new references over the past two years (to January 2012). The journals included: Family Practice; British Medical Journal; Journal of Antimicrobial Chemotherapy; Scandinavian Journal of Infection; International Journal of Antimicrobial Agents; and Journal of Infection.

Additional searches

Snowballing methods

- I searched the reference lists of all included studies for potentially relevant papers (not already reviewed).

Study selection

Quantitative studies describing actual routine care (with real patients) that met the inclusion/exclusion criteria listed above were selected. No randomised controlled trials (RCTs), qualitative, vignette or case history studies were included as they were not representative of actual/observed routine practice.

After the search strategy was performed (for each database and website listed), I reviewed the titles and abstracts of each study after exporting to Endnote (primary researcher); any studies that could be excluded at this point (based on the inclusion/exclusion criteria) were. I then read the full article of all remaining studies; only

studies that met the inclusion/exclusion criteria were included for critical appraisal and data extraction.

Note: Studies were also included if the population included some participants that were not relevant to my review as long as the proportion was minimal (<25% of total population being analysed) or the outcomes were reported for a sub-group that met the inclusion/exclusion criteria listed above.

Box 3.1.1. OVID[SP] Medline search strategy performed on 30th January 2014.

OVID[SP] Medline Search Strategy (1946 to January week 3 2014);

1. exp Primary Health Care/
2. exp General Practice/
3. exp Family Practice/
4. exp Group Practice/
5. primary care.mp.
6. general practice.mp.
7. group practice.mp.
8. family practice.mp.
9. exp Physicians, Family/
10. exp Physician-Patient Relations/
11. primary healthcare.mp.
12. family physician*.mp.
13. primary health care.mp.
14. general practi*.mp.
15. family practi*.mp.
16. family doctor*.mp.
17. or/1-16
18. exp Urinary Tract Infections/
19. exp Bacteriuria/
20. exp Cystitis/
21. exp Cystitis, Interstitial/
22. exp Escherichia coli Infections/
23. exp Pyelonephritis/
24. bacteriuria.mp.
25. (urinary adj2 infection*).tw.
26. (Urinary Tract Infection* or UTI).mp.
27. cystitis.tw.
28. bladder infection*.mp.
29. or/18-28
30. (Albania or Andorra or Armenia or Austria or Azerbaijan or Belarus or Belgium or Bosnia & Herzegovina or Bulgaria or Croatia or Cyprus or Czech Republic or Denmark or Estonia or Finland or France or Georgia or Germany or Greece or Hungary or Iceland or Ireland or Italy or Kosovo or Latvia or Liechtenstein or Lithuania or Luxembourg or Macedonia or Malta or Moldova or Monaco or Montenegro or The Netherlands or Norway or Poland or Portugal or Romania or Russia or San Marino or Serbia or Slovakia or Slovenia or Spain or Sweden or Switzerland or Turkey or Ukraine or United Kingdom or Vatican City or Holland or Great Britain or Britain or England or Scotland or Wales or UK or welsh or scottish or irish).tw.
31. 17 and 29 and 30
32. "Pregnancy"/
33. pregnan*.mp.
34. exp Catheters/
35. or/32-34
36. 31 not 35
37. limit 36 to english language

Quality assessment

The quality assessment was conducted using a checklist I developed to describe the individual study's relevance to the review and a critical appraisal. The critical appraisal was based on recommendations and example checklists for observational studies from the following systematic review bodies: SysNet (The Cardiff University Systematic Review Network); TREND (Transparent Reporting of Evaluations with Nonrandomized Designs); CASP (Critical Appraisal Skills Program for cohort studies); and the Cochrane collaboration. As the studies included were not RCT's the main focus of the quality assessment was to ensure each study met the inclusion/exclusion criteria of the review protocol and that any bias was explicit in the findings (see appendix 3.1.1 for the critical appraisal and data extraction form).

Prior to undertaking the assessment, I discussed and worked through some of the studies with a second researcher (a research fellow based in the Institute of Primary Care and Public Health, Cardiff University) to finalise the assessment process and to ensure a high degree of agreement. A systematic review researcher and member of the Support Unit for Research Evidence, Cardiff University (SURE) reviewed my critical appraisal and data extraction forms (described in next section) prior to data synthesis.

I quality assessed all included studies but due to time constraints the second researcher assessed 55% (6/11). We discussed all studies reviewed by both assessors and any differences in opinion were resolved at that point. There was no need to gain consensus from a third party as all disagreements were resolved between the second researcher and myself.

Data extraction

Data extraction was performed on all included studies once the quality assessment was complete. I extracted the data on all the studies and 36% (4/11) were repeated by the second researcher blinded to my results. Comparisons between the duplicated studies were then discussed and any disagreements in the data extraction were resolved at this point.

The following information, where possible, was extracted from the review of each study (related to suspected uncomplicated UTI);

- Design, setting, population, aims and conclusions;
- Bias within and across studies (when synthesising and comparing data);
- Key signs/symptoms of patients with suspected uncomplicated UTI;
- If/what POCTs were used for diagnosing a UTI;
- If urine samples were sent for microbiological diagnosis (microscopy/culture);
- The proportion of patients prescribed antibiotics and choice of antibiotics prescribed (type, dose and duration if possible);
- The antibiotic management; delayed prescribing or change in prescription;
- Adherence to guidelines;
- Any other information deemed relevant from the review in the management of uncomplicated UTI.

Data synthesis

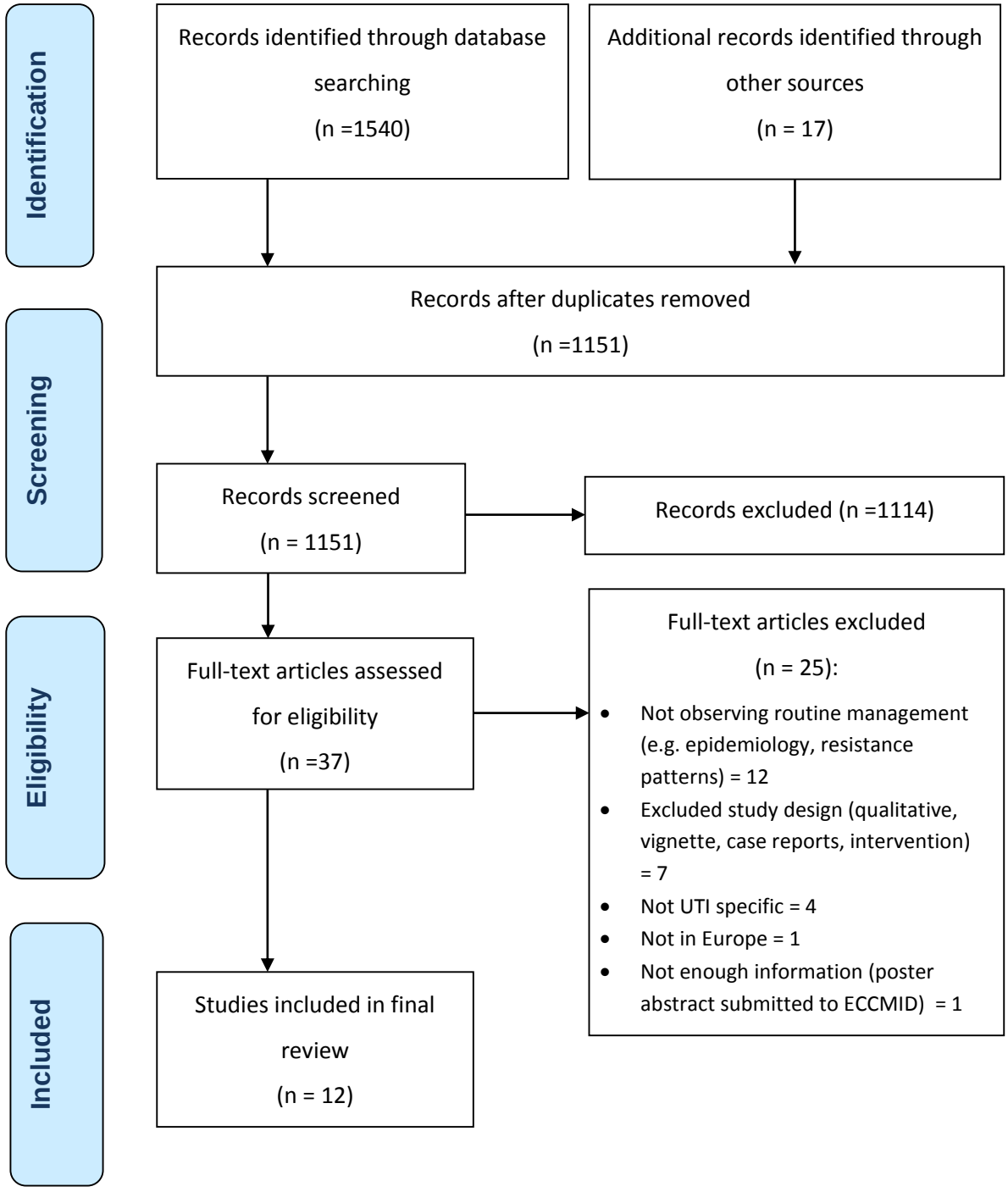
As the review describes actual observed practice and does not include any RCT/comparison studies the data synthesis was based on textual narrative synthesis. Where possible the scope, differences and similarities were used to draw conclusions across the studies included in the review. However, due to potential heterogeneity between studies in terms of setting, recruitment process, patient characteristics and study design this was not always possible and the results should be interpreted with caution. I have discussed bias in the data synthesis across studies. Comparisons between the results from this review and the results of the observational data analysis from the POETIC study are included in the overall discussion (section 4).

Results

Study Selection

Figure 3.1.1 shows the flow diagram of the literature search. Of the 1540 articles retrieved with the systematic search in OVID MEDLINE and the additional databases and of 17 articles identified otherwise (all by either reviewing the reference lists of included studies or checking the past two years of specific relevant journals), 389 were removed as they were duplicates and 1151 were screened by title and abstract only. From the preliminary screen 1114 studies were excluded and 37 were included in a full article assessment. 25 of these 37 articles were excluded for the following reasons: not observing routine management (for example, epidemiology studies describing resistance patterns); inappropriate study design (qualitative, vignette, case reports or interventions studies); not specific to UTI and unable to extract UTI data; not in Europe; and one study was a poster abstract submitted to the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) and did not have enough information to include in the review. In total 12 articles were included in the final review and their study characteristics are detailed in Table 3.1.2.

Figure 3.1.1. PRISMA Flow Diagram illustrating literature search and article selection.



Study Characteristics

Included in the final review were four studies from the UK (three from England and one from Wales); two from Spain; and one from each of the following; Ireland, Germany, Sweden, Italy, and Turkey. All were observational studies of routine management in primary care. The majority of studies involved GP reports at or just after the time of consultation (26, 28-30, 81-83), however, one study involved research students observing the consultations (84); one study collected data from a health database containing medical records (27); two studies had medical records reviewed by independent doctors (85) or members of the research team (86); one study had participants complete questionnaires (32) and one study in addition to GPs completing case report forms (CRFs) had patients complete two week diaries (82). The number of primary health care centers/practices and GPs participating varied greatly from study to study; Nazareth I et al. (83) only had two practices with six participating GPs whereas André et al. (28) had up to 155 practices with approximately 600 physicians participating. Nazareth I et al. (83) also had the smallest sample size (n=54) compared to the Italian study by Galatti L et al (27) which included 13,223 patients. The term 'uncomplicated UTI' was often not used, instead studies used 'lower' or 'acute' UTI/cystitis. A description of the study populations are given in Table 3.1.2. Ten of the studies allowed the participating GPs to diagnose UTI without input from the research teams, although André M et al. (28) and Skerk V et al. (30) used pre-set diagnosis lists to categorize patients with various types of UTI. All studies captured data on whether an antibiotic was prescribed, additional information on antibiotic type, dose and duration data varied between studies as did the availability of data on consultation, signs and symptoms, use of diagnostics and adherence to guidelines (described further under data synthesis). Table 3.1.2 describes the study design, setting, population, aims and conclusions for each study included in the review.

Table 3.1.2. Overview of the 12 studies included in the systematic review evaluating routine management of UTI in European primary care settings.

Author	Study Design	Study Setting	Study Population	Study Aim	Study Conclusion
André M et al.(28) *	Prospective study; pre-set diagnosis form for lower UTI/acute cystitis completed by GPs.	155 and 140 primary care centres in 5 counties in Sweden (to represent whole country) from 1 week period in November 2000 and 2005 respectively (~ 600 GPs).	Patients with suspected lower UTI; women (88.6%) and men (11.4%). Results separated out for lower UTIs in women (≥15 years), n=1012.	"To present some characteristics of patients diagnosed as having a UTI in primary care and to analyse the use of diagnostic tests and the treatment pattern."	"A change in antibiotic prescribing with improved adherence to national recommendations was observed. The current guidelines (from 1990) need to be updated and actively implemented in order to optimise treatment for UTIs."
Canbaz S et al. (84)*	Cross-sectional study; standardised form completed by research students observing the consultation.	8 rural health centres in Samsun, Turkey; 2 month period June – July 1999.	Patient with acute cystitis n=216; defined as otherwise healthy non-pregnant women, ≥12y, with a normal urinary tract (87).	"To evaluate the diagnostic and therapeutic approach to UTIs by GPs working in rural health centres in Samsun, Turkey."	"Polypharmacy widespread in this region; GPs need to review their knowledge about diagnosis and treatment of UTIs. Up-to-date information on rational use of antibiotics needed to guide GPs."
Fahey T et al.(81)**	Prospective cohort study; data collection sheets completed for patients with symptoms suggestive of UTI by GPs.	29 GPs from 8 primary care practices in Bristol, UK from July – September 2000.	135 non-pregnant women with suspected UTI; men and pregnant women excluded in some analyses, no mention of age range.	"To examine actual clinical practice of GPs when managing UTI."	"Individual symptoms of UTI are inadequate to guide antibiotic treatment decisions. Current clinical practice results in a large proportion of patients receiving unnecessary antibiotic treatment."
Galatti L et al.(27)**	Descriptive study using 'Health Search Database'; an Italian general practice research database containing electronic medical records of all patients registered in the lists of participating physicians whose diagnosis could be classified as acute or recurrent cystitis.	320 GPs from Northern, Central and Southern Italy from 1999-2002.	Non-pregnant women >16 ≤50 years whose diagnosis could be classified as acute uncomplicated cystitis, n=13223.	"To explore antibiotic prescribing pattern and patient-related variables associated with prescription for acute (and recurrent) cystitis."	"Data indicate considerable changes in treating acute and recurrent cystitis, with an evident rise in antibiotic use mostly related to fosfomycin trometamol. Prescriptive trend finds confirmation from the available evidence."

Author	Study Design	Study Setting	Study Population	Study Aim	Study Conclusion
Hummers -Pradier E et al.(29)[†]	Cross-sectional survey; short, structured form completed by GP at time of consultation.	36 teaching general practices of the Dept. of General Practice, University of Gottingen, Germany from November 2000 – February 2001.	Adult female patients with suspected UTI, age range; median 53, interquartile range 33 – 71. No other definitions given but some results separated for 'uncomplicated UTI'.	"To describe German GPs' management of female patients with symptoms of UTI."	"Most patients with urinary symptoms were not treated according to current guidelines, and GPs diagnostic and therapeutic accuracy was low. Patients with uncomplicated UTI were often treated for longer than recommended and second choice antibiotics were prescribed to a large proportion of all patients."
Little P et al. (82)**	Observational Study; CRFs completed by GPs at time of consultation and patient diaries completed by patients for up to 14 days after consultation.	117 GPs/nurses from 67 practices in Southern England, UK from January 2002 to February 2005.	Non-pregnant women (aged 18 – 75) presenting with a suspected 'uncomplicated UTI'. 843 women recruited; 541 completed diaries.	"To assess the natural course and the important predictors of severe symptoms in UTI and the effect of antibiotics and antibiotic resistance."	"Most women with UTI have multiple symptoms that they rate as moderately bad or worse, half feel unwell and have considerable restriction in daily activities. Antibiotic resistance and not prescribing antibiotics associated with >50% increase in the duration of more severe symptoms in women with uncomplicated UTI."
Llor C et al.(26)*	Cross-sectional study; GPs completed audit registry form.	110 primary care physicians across Spain, recruiting first six consecutive patients over an 8 week period, from March to July 2009.	Non-pregnant women >14 years. Uncomplicated UTI; no previous history of interest, isolated episode and foreseen to be cured with the usual short-course empiric treatment (n=545).	"To determine the diagnostic and therapeutic approaches undertaken by primary care practitioners for lower UTIs in women and to assess the adherence of GPs to evidence-based guidelines."	"Poor adherence to guidelines with high number of inappropriate urine cultures and low utilization of first line antibiotics."
Martinez MA et al.(85)*	Cross-sectional study; clinical records reviewed by independent doctors 48hours after selected study days.	10 emergency departments in public hospitals across 7 autonomous regions in Spain (not referred from primary care centres) from March 2003 – March 2004.	Women ≥14 years, diagnosed with acute community acquired UTI with a structurally and functionally normal urinary tract (n=1411). Sub-analyses for lower uncomplicated UTI in non-pregnant women.	"To assess the appropriateness of antibiotic prescription for UTI in several hospital emergency services and to evaluate the variability of antibiotic prescription among these."	"Physicians at Spanish emergency rooms prescribed an excessive number of second-choice antibiotics for UTI. There exists a high variability in antibiotic prescription among hospitals from different regions."

Author	Study Design	Study Setting	Study Population	Study Aim	Study Conclusion
Nazareth I et al.(83)**	Observational study; GPs completed a study specific questionnaire after each consultation.	2 primary care practices with 6 GPs from suburban London, UK over a 3 month period (no dates given).	Non-pregnant women, 16 - 45 years with symptoms of frequency or dysuria (n=54).	"To identify those factors in the management of lower urinary symptoms in women that assisted GPs in making a diagnosis and influenced the prescription of antibiotics."	"GPs take no particular regard of physical, psychological or menstrual factors in making their assessments. They were most accurate in their prediction of the result of urine analysis and least likely to prescribe antibiotics when they had good general knowledge of the patient. Doctors tended to be more conservative in their management of older women and those they knew less well."
O'Brien K et al. (32) *	Exploratory study (nested within larger study); participant questionnaires completed (self-completed, via telephone or face to face with study researcher).	9 general practices in SE Wales, patients recruited during last month of larger study, March 2004.	Non-pregnant women >17years with suspected uncomplicated UTI (n=113).	"To explore association between antibiotic prescribing and urine culture results when urine culture was performed in all symptomatic patients."	"Current strategies to target empirical antibiotic prescribing in clinically suspected, uncomplicated UTI require review."
Skerk V et al. (30)*	Prospective study; Family physicians completed specially designed questionnaires at time of consultation with later updates.	108 family medicine offices in 20 cities through-out Croatia from 1st October 2006 – 30th November 2006.	Patients with acute uncomplicated cystitis (non-pregnant women), age range from 10.2 – 99.4 years. (2.8% <18 years), n=1479.	"To investigate the epidemiology, aetiology, clinical presentation, complicating factors of UTIs treated by family physicians and present treatment choice for empirical antimicrobial therapy of UTI."	The authors conclude that the clinicians prescribe empirical antimicrobial therapy in accordance with national guidelines. However, only 15% patients with uncomplicated UTI prescribed first-line treatments and no information provided on the length or dose of treatment.
Vellinga A et al. (86)*	Prospective observational study; practices visited by study staff to collect demographic variables/ prescribing practices from patient charts from whom a urine sample was received in study laboratory.	22 GP surgeries in Galway region, Ireland from 14th September to 9th November 2009.	Eligible patients presenting with suspected UTI (n=866). Pregnant women excluded but men included (77.9% women). Some analyses for 'uncomplicated UTI'.	"To describe the current management of UTI in general practice including evaluation of appropriateness of antimicrobial treatment in relation to the laboratory results."	"The treatment of uncomplicated UTI was considered appropriate for 55% of the patients. There appears to be considerable scope to reduce the frequency and increase the quality of antimicrobial prescribing for patients with suspected UTI."

*1 reviewer;** 2 reviewers; ǂ 2 reviewers for critical appraisal, 1 reviewer for data extraction.

Individual Study Bias

Only five of the studies included practices in multiple geographic locations through-out their respective countries (26-28, 30, 85). The remaining seven studies had study sites within a single part of the country or specific town/city and therefore may not be representative of the whole country (29, 32, 81-84, 86). The majority of studies did not explain how the practices/physicians were selected. Galatti L et al. (27) selected patient records from GPs that had met a certain standard to participate and Vellinga A et al. (86) selected 22 GP practices with high level use of routine laboratory culture (25 highest ranking practices of 72 in the area). O'Brien K et al. (32) recruited nine practices; five were specifically chosen to balance prescribing rates, size and Townsend deprivation scores. For two studies the number of GPs/practices that agreed to participate was low; the study by Hummers Pradier E et al. (29) had 31% of eligible GPs participating; and Llor C et al. (26) had 62.5% of GPs who agreed to participate with no description of GPs who did not agree.

As all studies included in this review were observational; there was no randomisation involved and selection bias when recruiting patients may be unavoidable to a certain extent. Some studies recruited a consecutive sample of patients over a specified time period (29, 81) or until a certain sample size was reached (26). Limited information was provided on patients that agreed to participate versus those that did not or if all patients seen by the GPs were asked to participate. Little P et al. (82) analysed their results for only the sub-group of patients that returned their diaries and as O'Brien K et al. (32) used patient based questionnaires only patients that responded could be included in the analyses. Vellinga et al. enrolled all patients presenting with suspected UTI who submitted a urine sample with an opt-out methodology (86). The selection criteria for patients with UTI in some studies was very broad (often to allow maximum recruitment) with sub-analyses performed for different types of UTI (27, 29, 30, 81, 84-86); other studies asked GPs to recruit patients they suspected of having uncomplicated UTI (with minimal guidance) (26, 32, 82, 83). André M et al. (28) and Skerk V et al. (30) had pre-set study diagnosis lists that the recruiting GPs used to categorise patients with acute

cystitis/uncomplicated UTI which may not represent how the GP would routinely diagnose the patient. Five studies did not rely on the physicians themselves to record their management decisions; Galatti L et al (27), Martinez M et al. (85) and Vellinga A et al. (86) had independent reviewers go through medical records/charts to obtain the required information. O'Brien et al. (32) requested patients complete a questionnaire including some management decisions by the GP; this in itself could lead to recall bias. Canbaz et al. (48) had research students observe and document the GP/patient consultations. Galatti L et al. (27) and Martinez M et al. (85) designed studies in which neither the physicians nor patients were aware of being part of a study. The remaining studies may not be as representative of routine practice due to study requirements or changes in behaviour. As part of study procedures four of the studies (29, 32, 82, 86) requested urine samples to be collected from all patients for laboratory culture; this may have changed GP behaviour in waiting for culture results prior to antibiotic prescribing or changing prescription once culture results were available.

There are three studies which have been included but should be highlighted due to some particularly different aspects of their study design. Martinez et al. (85) designed a study based in hospital emergency rooms not general practice; patients were diagnosed with community acquired acute UTI, had not been seen prior to the visit to the emergency room and the GPs/residents that work in these units are not specialist and have a very similar profile to physicians who work in primary care clinics. The study by Nazareth et al. (83) was a particularly small study with only two practices; six GPs and 54 patients and was the earliest study included in the review (published in 1993). The study by Vellinga et al. (86) was more focused on appropriate prescribing with regard to microbiologically confirmed infection and as such the definition of 'uncomplicated UTI' was not explicit although this description was used in the conclusion. Men were included in the analyses but as they made up less than a quarter of the study population I decided to include this study in the review.

Key Findings from Individual Studies

The study by André M et al. (28) found 74% of all consultations for UTI were diagnosed as lower UTI with one or more diagnostic tests carried out for 98% women and 95% were prescribed antibiotics.

The study by Canbaz S et al. (84) found 58% patients diagnosed with acute cystitis and of these 98.6% were prescribed antibiotics; 74% prescriptions were consistent with current Turkish recommendations.

The Bristol, England based study (Fahey T et al.) found 87% women diagnosed with UTI were treated empirically with antibiotics and that GPs were far more likely to treat empirically patients with symptoms of dysuria and frequency or dysuria alone and were far less likely to perform diagnostic tests in patients with dysuria and frequency (81).

The study by Galatti L et al. (27) found 77% of patients with uncomplicated UTIs were prescribed at least one antibiotic with no regional variation. Antibiotic prescribing decreased from 81% to 66% with the use of diagnostic tests. However, antibiotic prescribing increased over four year period from 59% in 1999 to 86% in 2002. In 1999, of the antibiotics prescribed, norfloxacin was the most common (40%) which reduced to 11% in 2002. In 2002 fosfomycin trometamol was the most common antibiotic prescribed at 34% (from 5% 1999).

The study based in Gottingen, Germany (Hummers-Pradier E et al.) reported that GPs diagnosed UTI in 64% adult females and prescribed antibiotics to 56%. Dipsticks were performed in 92% of all cases; a positive nitrite test, dysuria and older age were the only predictive factors of culture-confirmed UTI, although the negative predictive value of dipsticks was low (29).

The second English study by Little P et al. (82) found 93% of women diagnosed with uncomplicated UTI were prescribed an antibiotic, and trimethoprim was the most common antibiotic prescribed (>75%). Among women in whom no microbiological UTI was confirmed there was a similar pattern of severity of symptoms to those with confirmed infections.

The first of two studies set in Spain (Llor C et al.) found the most frequent symptom in women with uncomplicated UTI to be dysuria (82%) followed by frequency (71%) and suprapubic pain (33%). Dipstick tests were performed for 84% women and laboratory cultures for 33%. In total 96% women with uncomplicated UTI were prescribed antibiotics and 18% of these women were prescribed first choice empiric antibiotic treatment (26).

The second Spanish study by Martinez MA et al. (based in hospital emergency departments) found almost all prescriptions were empirical (97% for uncomplicated UTI). Ciprofloxacin was the most commonly prescribed antibiotic (33%) followed by co-amoxiclav (28%). In total 51% were prescribed first choice antibiotics for lower uncomplicated UTI (fosfomycin, ciprofloxacin, ofloxacin, norfloxacin); 37% were prescribed an alternative choice (co-amoxiclav, cefuroxime); and 11% prescriptions were inappropriate (the category of antibiotic choice was based on expert opinion developed within the study) (85).

The final English study by Nazareth I et al. (83) found 69% women were prescribed antibiotics; equal proportions of women with positive and negative culture results received antibiotics, indicating over diagnosis by doctors for the group with negative results. When GPs did not know the patient they were four and a half times more likely to assume a clinically important infection. When they knew the patient well they were four times more likely to make a correct prediction of the test result and 12 times less likely to prescribe antibiotics. GPs were also six times more likely to prescribe antibiotics for older women (although the age range for recruitment onto this study was limited to16-45 years).

The fourth UK study based in South East Wales (O'Brien K et al.) found antibiotics were prescribed empirically in 61% cases of women with uncomplicated UTI. Of those prescribed empirical antibiotics 40% were subsequently found to have a positive urine culture. Overall 32% women had a positive culture; 75% had been prescribed an antibiotic where as 25% had not. Of the 68% women who had a negative culture result, 55% had been prescribed an antibiotic (32).

The study by Skerk V et al. (30) based in Croatia, reported 62% UTI patients were diagnosed with uncomplicated UTI, this occurred in all age groups but more frequently in women aged over 40 years. 92% patients (all types UTI) were prescribed antibiotics; 66% empirically and 34% targeted (based on culture). In total 21.3% of uncomplicated UTIs were microbiologically confirmed by culture.

The final study included in the review, based in Galway Ireland (Vellinga A et al.) reported that 56% of UTI patients were prescribed antibiotics; co-amoxiclav 33.1%; trimethoprim 26%; fluoroquinolones 17%; and nitrofurantoin nearly 12%. 37% of all prescriptions were in accordance with recommended first line treatment of nitrofurantoin or trimethoprim. The treatment for uncomplicated UTI was considered (microbiologically) appropriate for 55% of the patients. General practices showed preferences for certain antibiotics and prescribing differed considerably between practices; the proportion of patients receiving an antibiotic varied from 39% to 78% between practices and this was most apparent for the fluoroquinolones, which was the prescription of first choice in some practices (86).

Synthesis of results

Table 3.1.3 contains data from the 12 studies included in the review and their reported patient UTI signs/symptoms, routine use of diagnostics and antibiotic prescribing. Because of the variation in participant recruitment methods which may lead to bias between source populations in I have only included studies that recruited patients by GPs at the time of consultation; studies by Galatti et al., Martinez et al., O'Brien et al., and Vellinga et al. have been excluded from the synthesis of results.

Signs and Symptoms

Four of the studies report days with symptoms/time to consultation; the majority of patients consult within 10 days and on average experience around three days of symptoms prior to consultation. Two of the studies were in England (81, 82), one in Sweden (28) and one in Spain (26); there are no obvious differences between countries. Frequency and dysuria were reported by six studies. In the three English and one German study frequency was the most common symptom; although the German study reported a much lower level of prevalence (55% compared to ≥79%). In the Spanish and Turkish studies dysuria was the most common symptom reported (82% and 88% respectively).

Use of Diagnostic Tests

Data on the use of diagnostic test was limited however, four different diagnostic tests were mentioned; laboratory culture was reported in four studies (I have not included studies where culture was a mandatory study procedure); dipstick urinalysis in four studies; sediment microscopy in two studies and a C-reactive protein (CRP) in one study. Laboratory culture ranged from 0% in Turkey to 64% in Bristol, England (81). Where reported, dipstick urinalysis was performed in 44% of consultations in Bristol, England (81); up to 92% in Germany (29).

Antibiotic Prescribing

Prescribing rates could not be compared between studies as this would require the studies to evaluate antibiotic prescribing over a given period of time in a set population

and ideally at the same time. However, prescription of antibiotics was reported in every study included in the review to some extent which may provide some comparisons between countries. The German study (29, 86) reported the least antibiotic prescribing at 56% and highest was reported in the Turkish study at 99% (84). The German study was also the study with the lowest reported signs and symptoms and the higher prescribing studies the higher prevalence of reported signs and symptoms.

Seven of the studies described the most common antibiotics prescribed. Two of the English studies (81, 82) and the Swedish study (28) most commonly prescribed trimethoprim (first-line antibiotic). The studies in Germany (29) and Turkey (84) found cotrimoxazole (first-line antibiotic in Turkey although Germany recommends trimethoprim or nitrofurantoin) to be the most common antibiotic prescribed. The Spanish study (26) reported fosfomycin (first-line antibiotic) as the most common antibiotic prescribed and in the Croatian study it was cephalexin (first-line antibiotic) (30).

Five of the studies report dose/duration of antibiotic treatment. The study in Bristol, England (81) reported that overall 44% of prescriptions were for three days; 21% were for five days and 21% were for seven days. The German study (29) reported a median duration of antibiotic treatment of five days for all ages of women and that 70% of young patients with uncomplicated UTI were treated for more than three days. The Swedish study (28) reported treatment duration of seven to ten days irrespective of antibiotic prescribed. The Spanish study (26) reported (for uncomplicated UTI) short-course treatments were prescribed in 67% cases and long-course treatments in 33% cases. However fosfomycin trometamol (3g OD single-dose) was one of the recommended first-line treatments for uncomplicated UTI in Spain and only 19% were reportedly single dose where as 81% prescribed for two days. The Turkish study (84) reported faults in dosage interval in 19% cases; faults in duration in 24% cases; faults in dosage 10% cases; and overall irrational prescribing in 53% cases.

None of the studies reported delayed prescribing.

Adherence to Guidelines

Adherence to guidelines was not described by every study and there were mixed outcomes on those that did report this outcome. The German, Spanish and Turkish (26, 29, 84) based studies acknowledged poor adherence to their respective guidelines in terms of prescribing and additionally for the Spanish study inappropriate urine cultures being performed (26).

The Swedish (28) study reported improved adherence to guidelines and/or available evidence. The Croatian study (30) concluded that GPs were following prescribing guidelines, however, I would disagree as they also report only 15% patients with uncomplicated UTI were prescribed first-line treatment.

Both the Swedish study and the Turkish study recommend updated guidelines with active implementation to improve management of UTIs (28, 84).

Table 3.1.3. Data synthesis across all studies included in the systematic review including details on signs and symptoms, diagnostics and prescribing (the studies highlighted in red are excluded from the synthesis of results due to substantially different study design and recruitment processes).

Country (Reference)	England (81)	England (82)	England (83)	Wales (32)	Ireland (86)	Germany (29)	Sweden (28)	Italy (27)	Spain (26)	Spain (85)	Croatia (30)	Turkey (84)
Signs and Symptoms												
Days with symptoms	34%≤1d (IQR 1-5d)	Median 3d (IQR 3-7)					58% ≤3d 84%≤7d		Mean 3.4 ±6.8d			
Uncomplicated UTI/all UTIs							74%	39.2%	82.8%	37.1%	62%	58.2%
Dysuria	73%	64%[3]	79.6%			52%			81.9%			88.4%
Nocturia	28%	57%[3]										54.6%
Frequency/ polyuria	79%	78%[3]	85.2%			55%			70.5%			40.3%
Urgency	53%	63%[3]	13%			55%						
Suprabubic pain						14%			33.1%			48.6%
Blood in urine	15%	16%[3]	13%			17%						9.7%
Loin pain	30%		24.1%									31.5%
Fever			1.9%									9.3%
Diagnostics												
Dipstick Overall	44%					92%			83.7%			
Dipstick - Nitrite							76%					
Dipstick – Leucocyte							88%					
Laboratory Culture	64%						16%	26%	32.8%	18.6%[4]		0%
Other						44% SM	10% CRP					

							9% SM					
Country (Reference)	England (81)	England (82)	England (83)	Wales (32)	Ireland (86)	Germany (29)	Sweden (28)	Italy (27)	Spain (26)	Spain (85)	Croatia (30)	Turkey (84)
Prescribing												
Antibiotics prescribed (%)	87%	93%	68.5%	61%	56%	56%	96%	79.1%	95.6%	96.8%	69.5%[4]	98.6%
Trimethoprim	60%[1]	>75%			26%	13%	40%	6.6%[2]				
Cotrimoxazole						46%				2.3%		47.5%
Nitrofurantoin		✓			12%	2%	4%		0.3%		15%	
Fosfomycin								33.8%	52.7%	15.9%		
Co-amoxiclav		✓			33.1%				15.3%	27.8%	1.6%	
Amoxicillin		✓										
Cephalosporins		✓				2%	3%		1.1%	4.5%	79.6%	
(Fluoro-) /Quinolones		✓			17%	33%	18%	11.1%[2]	24.4%	46.2%		26.3%
Pivmecillinam							33%					
Other						4%	2%		6.2%	3.3%	3.8%	10.3%
Guideline recommendations (as reported in the study specific publications)												
First-line antibiotics					Trimethoprim nitrofurantoin	Trimethoprim, nitrofurantoin	Pivmecillinam trimethoprim nitrofurantoin, cephadoxil/cephalexin		Fosfomycin, nitrofurantoin		Nitrofurantoin 7d (alternative: co-amoxiclav 7d cephalexin 7d norfloxacin 3d)	Trimethoprim /sulfamethoxazole, quinolones

1 - includes two pregnant women and 13 men; 2 - data from 2002; 3 - symptoms grade moderately bad or worse by patient; 4 - all UTI; ✓ - indicated but not quantified; CRP – C Reactive Protein; SM - Sediment Microscopy.

Risk of bias across studies

I have tried to limit bias caused by study design and variation in recruitment/enrolment strategies by excluding studies that did not use data from patients recruited at the time of consultation from the synthesis of results. However, the remaining studies will have differences in study design and therefore bias with regard to source populations but this is unavoidable and why descriptive analysis has been used for this review.

The earliest study by Nazareth I et al. (83) was published in April 1993. However, the authors did not specify when the study actually took place but presumably it was 1992 or earlier. The remaining studies took place from 1999 (81) to 2009 (26, 86) covering a 10 year period; this may cause some bias when comparing studies as antibiotic resistance patterns may have changed and guidelines may be updated and training of GPs change as new research is published.

Although all studies are observational studies the aims and objectives of the various studies are different and I have tried to extrapolate data relevant to the aims and objectives of this review. This has resulted in limited data from some studies and to particular aspects of the management of uncomplicated UTI.

The definition of 'uncomplicated UTI' is not universally agreed and varies from country to country and study to study. All but one of the studies included only females; as already mentioned Vellinga et al. (86) also included men but this study was not included in the synthesis of results. Age ranges also vary between studies; one study describes only 'adult women' (81) while the others specify varying age ranges, for example, Nazareth et al. 16 to 45 years (83) and Skerk et al. 10 to 99 years (30). All studies excluded pregnant women and some studies mentioned exclusion criteria such as indwelling catheters and co-morbidities such as diabetes. When I originally ran the systematic search with one definition of uncomplicated UTI and strict inclusion/exclusion criteria I was limited to only three studies (27, 82, 83). Therefore I included studies with broader definitions of (uncomplicated/acute/lower) UTI/cystitis on the basis that the patients consult in primary care and the prevalence of patients <16 years, male, with co-

morbidities or indwelling catheters (if not explicitly excluded) are minimal and the data still reflects daily practice within their respective studies/settings.

Different studies used different reporting measures, for example for signs and symptoms Little P et al. (82) recorded prevalence of symptoms graded moderately bad or worse whereas the other studies reported prevalence of presence of symptoms without grading severity (26, 29, 81, 83, 84). I have not looked at management decisions based on fixed effects such as symptom severity, prior prescribing and age group.

Finally, different countries have different drug licences, different guidelines and different proportion of antibiotic resistance and therefore comparisons need to be considered with these factors in mind.

Main Findings

A total of 1540 articles were found using the search strategy and 37 full text articles were reviewed. Twelve studies met the inclusion criteria but only 8 studies were included in the synthesis of results.

Using the defined search criteria there are limited published studies evaluating the routine management of uncomplicated UTI in primary care in Europe. Within the published studies definitions of uncomplicated UTI vary and heterogeneity between study populations is apparent.

The only outcome reported by all studies was the proportion of antibiotic prescribing at the time of consultation; this was lowest in the German study and highest in the Turkish study (29, 84, 86). The German study (as part of the study design) had laboratory culture of urine samples for all the patients recruited; this may have resulted in the GPs delaying prescribing until the culture results were available and therefore having an overall lower level of empirical prescribing (although delayed prescribing was not reported). Conversely, the Turkish study reported that no urine laboratory cultures were requested by the GPs in their study (84).

For most of the studies that reported the type of antibiotic prescribed the most common was the first-line recommendation for their respective countries. The UK (based on HPA guidelines (25)) Sweden, Germany and Ireland all recommend trimethoprim or nitrofurantoin (and Croatia – nitrofurantoin only) as first-line antibiotic treatments, however, there was still a wide range of antibiotics prescribed (second choice and irrational) both within studies and across studies. Nitrofurantoin was particularly less commonly prescribed even though it was a recommended first or second choice antibiotic for the majority of countries included in the review (unable to confirm for Italy or Turkey). Prescription of broad –spectrum fluoroquinolones was also frequently reported in England, Ireland, Germany, Sweden, Italy, Spain and Turkey. Five of the studies reported antibiotic prescribing of increased duration and/or dose from the relevant recommendations. The data collected on the use of diagnostic tests was limited; in the studies that did report their use reduction in antibiotic prescribing was not apparent.

Limitations

Two major limitations in interpreting these results is the heterogeneity of the study populations across studies by the way in which patients were recruited and the limited availability of reported data that are relevant to my aims and objectives.

The aim of this review was to determine variation in management between countries but caution in interpreting the results needs to be made with regard to bias of source populations and it's representativeness of the countries' population as a whole.

Due to time and funding constraints my search was limited to English language only and I did not search the grey literature including unpublished studies. Limiting the search to English only may have restricted the number of studies included in the review particularly as Europe is such a multi-lingual continent. I do not feel publication bias would be a significant factor in my review as I only included observational studies of routine practice and as such 'negative findings' are implausible.

Robust systematic reviews should involve the participation of two or more reviewers for identifying relevant studies, critically appraising studies and extracting data. This review

was limited to two reviewers for a proportion of the critical appraisal and data extraction which may have limited findings or caused bias in the outcomes.

Chapter 2: Observational study – Presentation and management of patients with uncomplicated UTI in four European countries (POETIC Phase 2)

Introduction: R-GNOSIS and the POETIC study

R-GNOSIS (Resistance in Gram-Negative Organisms: Studying Intervention Strategies) funded by the EU Seventh Framework Programme (FP7), combines five international clinical intervention studies to determine - in the most relevant patient populations - the efficacy and effectiveness of interventions to reduce acquisition, carriage, infection and spread of Multi-Drug Resistant Gram-negative Bacteria (MDR-GNB). All information will be integrated by into innovative models to better understand and predict future trends and effects of interventions.

The POETIC (Point of Care Testing for Urinary Tract Infection in Primary Care) study is the only primary care work package within the R-GNOSIS study. POETIC is an evaluation of a novel Point of Care Test (Flexicult^{TM} SSI-Urinary Kit) guided urinary tract infection (UTI) management strategy for use in adult women presenting in primary care with suspected uncomplicated UTI. The Point of Care Test (POCT) will provide clinicians, at the point of care, within 24 hours, with a diagnosis of bacterial UTI and antibiotic resistance profiles of any identified pathogen to the antibiotics most commonly used for UTI in primary care. The study will determine whether or not this information aids clinicians to more appropriately prescribe antibiotics for uncomplicated UTI's (i.e. avoid the use of antibiotics for women where no bacterial infection is identified, and where a bacterial infection is identified, to ensure the narrowest spectrum antibiotic is prescribed appropriate to the sensitivity of the infecting organisms). The study is being carried out in 4 European countries: Wales, England, Netherlands and Spain, and is divided into four phases: 1) pilot study; 2) observational study; 3) randomised controlled trial; and 4) implementation study. My research focuses on the second phase, the observational

study, the purpose of which is to assess the variation in current management that could be changed (and potentially improved) by utilising a new POCT.

Hypothesis

There is variation in the management of patients with uncomplicated UTI in primary care in different countries including Wales, England, Spain and the Netherlands. The variation in management is not due to patient demographic or presenting sign or symptoms.

Aim

To evaluate whether the patients' resident country, age, history of UTI, symptom severity, days off work, days with symptoms, dipstick urinalysis testing, urine appearance and oral temperature are associated with different management decisions (including the use of a diagnostic POCT, requesting a urine culture, antibiotic prescribing and follow-up recommendations).

Objectives

- To describe the patient demographics and signs and symptoms (attributable to UTI) at presentation in primary care, and the clinical management decisions for patients included in the POETIC Observational study of uncomplicated UTI in the four participating countries;
- To examine the individual (patient) level factors associated with differences in patient management using multilevel statistical modelling.

Methods

Declaration

The study methods were developed and executed by the POETIC study team (of which I am part) and networks following the POETIC Observational study protocol. I have been involved in the POETIC study from its set-up; attended the meetings, provided comments on study design and helped to develop the data collection tools including case report forms (CRF) and patient diaries, contributed to clinical site training and the development

of the study website. The brief outline of the study methods below are for the readers' information and are taken from the study protocol.

The data analysis, statistics and interpretation included in this section are solely my own and undertaken with the approval of the POETIC study team.

Study Design and Population

The POETIC study was conducted in four study networks based in Southampton (England), Cardiff (Wales), Madrid/Catalonia (Spain) and Utrecht (the Netherlands). The study networks were selected on the basis of having well-established primary care research networks and may represent different European cultures and healthcare systems. Each network aimed to recruit in the region of 10 general practices. Each practice aimed to recruit 20 patients (total 800 participants).

POETIC phase 2 is an observational cohort study where clinicians registered sequential patients presenting with uncomplicated UTI symptoms. Participating clinicians and nurses identified eligible patients during routine general practice consultations. The participating clinician assessed eligibility, provided potential participants with a verbal description of the study, and if interested, provided a comprehensive participant information leaflet (PIL). Participants provided written informed consent prior to participation. Once a patient was consented, clinicians recorded information about their usual care diagnostic procedures, treatment and urine sampling on a study case report form (CRF). Information on the patients' clinical course was gathered through use of a symptom diary completed by the patients daily over a two week period.

Inclusion criteria

Female adult patients age 16 years and above presenting to primary care with at least one of three key urinary tract symptoms (dysuria, urgency including nocturia, and frequency) and where the clinician suspected uncomplicated UTI. Patients participating in the study must have been able to provide written informed consent.

Exclusion criteria

Women with one or more of the following were not eligible for inclusion:

- Terminally ill
- Currently receiving treatment for life-threatening cancer (e.g. basal cell carcinoma)
- Other severe systemic symptoms
- On long-term antibiotic treatment or have received antibiotics for urinary tract infection within the past four weeks
- History of bladder surgery (including cystoscopy) within the past four weeks
- Known or likely to have significant immune compromise (i.e. known immunodeficiency state, on long-term corticosteroid or chemotherapy treatment, insulin dependent diabetes)
- Known functional or anatomical abnormalities of the genitourinary tract
- History of pyelonephritis
- Known pregnancy

Ethical Approval

This study protocol was approved by the Research Ethics Committee (REC) recognised by the United Kingdom Ethics Committee Authority (UKECA) and relevant European Committees in the Netherlands and Spain.

Study Sample Size

The original sample size, calculated by the POETIC study statistician, was based around the precision of describing the proportion of population women presenting in primary care with suspected uncomplicated UTI that are prescribed antibiotics. A desired level of precision was such that a 95% confidence interval around an estimate of 50% is 45.0% to 55.0%; 50.0% was chosen as this gave the most conservative estimate (higher or lower percentages will have produced narrower confidence intervals). This required 385 participants before taking clustering (at the practice level) into account. Data on 800

participants was the aim, with approximately 200 in each network, with 10 practices per network and an average cluster (practice) size of 20 participants. From this, the relevant design effect is 2.082 so the maximum potential level of clustering accounted for is described by an intra-cluster correlation coefficient of ρ= 0.0570, or 5.70% (88). No additions were made to this sample size for potential dropout as data on prescribing antibiotics are collected at the initial baseline visit immediately after recruitment.

Data Collection

Case Report Forms (CRF)

From November 2012 through to October 2013 (for the Utrecht network this was extended until January 2014) participating clinicians were asked to register sequential patients with UTI symptoms, recording details of patient demographics and presenting symptoms, diagnostic procedures employed (i.e. dipstick testing) and treatment onto POETIC designed CRFs (appendix 3.2.2).

Follow-up

Patients were followed up using a two-week diary covering symptoms and medication use (appendix 3.2.3). If the patient diaries were not received by the study centre within an acceptable timeframe (within a month of the due date), participants were contacted by members of the study team to request the return of the diaries.

Missing data

All missing data were marked as -9 and considered as missing completely at random. No imputations were used for my analysis.

Data cleaning and outliers

Data cleaning was performed by the study's data management team prior to my data analysis. This process was completed through the database. Common items that were looked for in the data set were:

- Missing values

- Range checks
- Inconsistencies (e.g. do values cross check appropriately)
- Protocol violations (e.g. study visit outside acceptable range)

Once data cleaning was complete the data set was locked prior to the data and statistical analysis performed by myself.

If during my data analysis I found any additional outliers or unusual results these were discussed with the Chief Investigator and clinical Co-Investigators and any changes or exclusions indicated.

Guidelines

For each network, guidelines were developed by the POETIC study team clinicians based on country relevant guidelines for the management of women with suspected uncomplicated UTI. These were provided to all network participating practices during the study. The network specific guidelines are included the appendices (3.2.4 – 3.2.6).

Laboratory data

For sites in Wales and England, urine samples were sent to the Specialist Antimicrobial Chemotherapy Unit (SACU), Public Health Wales Microbiology Cardiff, at University Hospital of Wales. In The Netherlands, samples were sent to the Department of Medical Microbiology of the Universitair Medisch Centrum Utrecht. In Spain, the samples were sent to the Microbiological Departments of the Hospitals Ramón y Cajal (Madrid), Joan XXIII (Tarragona), and Bon Pastor (Barcelona). All laboratories were provided with a POETIC microbiology manual and standard operating procedures.

A urine (required for participation) sample was collected from participants on day 1 (day of recruitment). Participants were provided with written instructions on how to collect the urine samples. In the UK urine samples were collected using a mid-stream urine collection kit (Peezy MSU collection kit) to ensure a clean catch. Urine samples were transferred to a container containing boric acid for transport to the research laboratory, to arrive at the laboratory within 24 hours for identification and testing of antibiotic resistance.

Urine microscopy was performed using standard local procedures. Culture was performed on neat and diluted urine (10^{-3}, 10^{-6}) spiral-plated onto Columbia blood agar (CBA) (for total colony counts) and UTI Chromogenic agar (for species specific counts). Enumeration of bacteria was performed 18-24 hours following incubation at 35-37^{o}C. Bacterial counts were summarised into purity of growth (pure, predominant, mixed 2 organisms & >2 mixed organisms).

Pure or predominant organisms from positive urines were identified initially with chromogenic agar and confirmed using MALDI-ToF or other suitable laboratory methods. The definition of a UTI is defined for the POETIC trial as ≥10^{5} colony forming units per millilitre (cfu/ml) of a pure/predominant recognised uropathogen,

Susceptibility testing of positive isolates was performed following regional research laboratory procedures.

Data was recorded onto a standardised Microsoft excel spreadsheet provided to each laboratory.

Data Analysis

Software

The data was managed, and descriptive analysis performed using IBM SPSS Statistics 20. Multi-level logistic modelling was performed using software package MLwiN 2.30.

Descriptive Analysis

The descriptive outcomes I investigated included;

- Network characteristics (CRF)
- Patient Characteristics (CRF and diary)
- Presence and severity of (clinician reported) signs and symptoms (CRF)
- Use of diagnostic tests and other medical tests (i.e. if urine had an offensive smell, if urine was visibly turbid) (CRF)

- Antibiotic prescribing; yes/no, type, duration delayed prescribing, secondary prescriptions, concordance with relevant clinical guidelines (including antibiotic type, dose and duration) (CRF)
- Prescribing and advising paracetamol and ibuprofen (CRF)
- Further clinical advice, follow-up recommendations and routine consultation type (CRF)

The prevalence (numbers and percent), range, means, medians, standard deviations and inter-quartile ranges were described as appropriate for each outcome. The outcomes were described for the total study population and by network.

Grouping and Recoding Variables

Some variables were grouped or recoded for further analysis.

The 11 symptoms included; burning when passing urine; urgency; daytime frequency; night time frequency; fever; pain in the side; blood in the urine; smelly urine; tummy pain; restricted activities and feeling unwell. The seven point symptom severity grading score;

0 = Normal/not affected

1 = Very little problem

2 = Slight problem

3 = Moderately bad

4 = Bad

5 = Very bad

6 = As bad as it could be

The symptoms were recoded into present or absent; any symptom with severity score graded 1 – 6 was recoded as present and any symptom with a severity of 0 (equates to normal) recoded as normal. Burning when passing urine, urgency, daytime frequency and night-time frequency were also dichotomised into low and high symptom severity based on the histogram distribution (night time frequency scores 0-1= low and 2-6 = high; and for the remaining 3 symptoms 0-2 = low and 3-6 = high). The total symptom severity score was estimated for each patient by calculating the mean severity score of the 11

symptoms and scaled out of 100 so that it could be interpreted as a percentage of maximum symptom severity.

To investigate the prevalence of clinicians requesting urine for routine culture I added up the additional 'other' culture tests (including 'MSU' mid-stream urine, 'MCS' microscopy, culture and sensitivity, and 'culture and susceptibility' free text in the 'other tests' question 5 of the CRF) and urine dip-slides (as performed routinely in the Netherlands). If the data in the routine culture question of the CRF (question 6) was classed as missing, these were added to the total number to indicate at least one routine urine culture requested.

Oral temperature was grouped into low (<36.0°C); normal (≥36.0°C and ≤37.2°C); and high (>37.2°C); to look at the distribution of patients with or without fever. Fever is usually described as >38°C (17, 25) but as the measurement of temperature was not controlled (different methods may have been used at different sites) I decided to allow for a more generous cut-off for fever.

I based my analysis of the dipstick urinalysis results on the recommended urinalysis rules from the guidelines developed for each network, the rules included were;

- Positive nitrite (≥+); UK, Spain and the Netherlands guidelines suggest probable UTI
- Positive leucocyte (≥+); UK and Spain guidelines suggest equal probability UTI as other diagnosis
- Leucocyte(≥+), nitrite (≥+) and blood (trace) positive; UK, Spain and the Netherlands guidelines suggest probable UTI
- Leucocyte and blood positive; The Netherlands guidelines suggest performing a dip-slide/sediment test for confirmation of UTI
- Leucocyte, nitrite and blood negative; UK, Spain and the Netherlands guidelines suggest UTI unlikely
- Leucocyte and nitrite negative; UK and Spain guidelines suggest UTI unlikely

To determine if an antibiotic was prescribed I coded the missing data as 'no antibiotic prescribed'. Clinicians were asked to record details of all antibiotics prescribed; if they

did not indicate anything about prescribing I have assumed that this meant no antibiotic was prescribed (the CRF did not include a specific data collection question for this).

If an antibiotic had been prescribed I analysed the concordance of the prescription with the relevant network guidelines for first-line treatment for women with suspected uncomplicated UTI. This included the total dose and duration as well as antibiotic type. For the analysis the dose and times per day were multiplied with the duration to give the overall dose for each patient; this was done to avoid classing prescriptions as discordant if for example trimethoprim was prescribed as 50mg 4 times per day (QDS) for 3 days instead of 200mg twice per day (BD) for 3 days. This was then compared to the antibiotic type prescribed and the combination of the two compared to the networks guidelines. The recommended first-line antibiotic treatments (for women with uncomplicated UTI) for each network are as follows:

Cardiff and Southampton:

Trimethoprim 200mg BD 3 days; total dose 1200mg.

Nitrofurantoin 100mg BD 3 days; total dose 600mg.

Madrid/Catalonia:

Fosfomycin 3g OD 1 day; total dose 3g.

Nitrofurantoin 50mg QDS 7 days; total dose 1400mg.

Utrecht:

Nitrofurantoin 100mg BD or 50mg QDS 5 days; total dose 1000mg.

When categorising ‘any other advice’ I collated all the free-text advice and coded them into themes. For example, for the category ‘increase fluid uptake’ the free-text themes included (but not limited to): ‘drink more’;’ fluids ++’; ‘push fluids’; ‘plenty of water’. For the category ‘return/contact on worsening symptoms’ the free-text themes included (but not limited to); ‘to re-contact if pyrexial/blood in urine/abdo pain’; ‘to come again if not settling’; ‘see again if no better’; ‘advised to contact surgery if concerned’; ‘out of hours if necessary over the weekend’.

Factor analysis

Factor analysis of the 11 symptom variables was performed in SPSS to determine if there were any unobserved variables that could reduce the data into fewer number of factors. Participants with at least 1 missing symptoms score were removed from the analysis. I used the maximum likelihood extraction method with Eigenvalues >1 and oblimin rotation method with Kaiser normalisation. For any resulting factors the total symptom severity score was estimated for each patient by calculating the mean severity score of the symptoms included in each factor and scaled out of 100 so that it could be interpreted as a percentage of maximum symptom severity. These factored total symptom scores were included as variables in the multilevel model procedures.

Intra-Class Correlation (ICC)

To explore the importance of the two level sampling (patients within practices) the intra-class correlation (also known as intracluster correlation) has been calculated. This estimated the proportion of variability on the outcome attributable to the practice level sampling rather than the patient level sampling. The ICC is calculated by dividing the between-practice variance by the sum of the within- (between individuals) and between practice variances. The ICC has a value between 0 and 1; a higher ICC indicates more clustering and most of the variation in the outcome is due to practice-related factors over patient differences (89).

Multilevel Modelling

If the ICC shows a high level of clustering then the practice level variation will need to be accounted for (by partitioning random effects) using multilevel modelling in the analysis. I have used a two level random intercept model to include patients as the first (individual level with fixed effects) and practice as the second (group level with random effects) level.

As the response variables (described below) are binary, logistic regression analysis was used. This involves taking the $\log_e$ of the odds (iterative generalised least squares) and then applying a linear model to the transformed data (using quasi-likelihood methods);

the software package is programmed to do this. One and two level logit models were used to fit the univariate and multivariate data in the MLWiN 2.30 software package. University of Bristol Centre for Multilevel Modelling provided guidance on using this software and fitting the models(90).

Dependent/Response Variables

I performed three analyses that linked with this PhD: the use of routine culture in general practice; adherence to appropriate guidelines which included prescribing recommended first-line antibiotic treatment for suspected uncomplicated UTI in terms of antibiotic type only; and antibiotic type with recommended duration and total dose.

To investigate routine culture in practice I considered any patient with a 'yes' indicated for the questions:

- 'Would you under normal practice, send a sample for culture for this patient?' (CRF question 6) or;
- if 'Dip-slide', 'MSU', 'MCS' or 'culture and susceptibility' was written in the 'other tests' question (CRF question 5) ; as a yes answer.

If 'no' had been written;

- in question 6 and question 5 did not have any of the comments described above then the answer was considered to be 'no'.

If data was missing;

- from both questions or;
- just question 6 and question 5 did not have any of the comments above; this was excluded from the multilevel analysis.

To investigate adherence to guidelines when prescribing first-choice antibiotic for suspected uncomplicated UTI I only included patients who had been prescribed an antibiotic. The adherence to guidelines was analysed in the same way as the descriptive data described in the 'grouping and recoding variables' section above. I did not take into account other factors that may affect adherence to guidelines such as if the patient

should have been prescribed an antibiotic at all (with regard to signs, symptoms and dipstick/dip-slide results or the use of second or third-choice antibiotics).

Explanatory Variables and Univariate Analysis

I firstly explored the patient demographic, signs and symptom severity scores and management choices available from the study data collected as single explanatory variables in univariate (two level) logistic regression models. Patient demographics included: network (country); patient age; days with symptoms (≤28 days); months since last UTI; times treated for UTI in the past. All 11 symptoms were included: burning when passing urine; urgency; daytime frequency; night time frequency; fever; pain in the side; blood in the urine; smelly urine; tummy pain; restricted activities and feeling unwell. For all symptoms I entered the variables as binary; absence (grade 0) or presence (grade 1 -6). If, from the descriptive data, the symptom also had a normal distribution (over the grading scores) I checked for linearity against the two response variables using logistic regression. If there was linearity the symptoms were entered as continuous variables; if not the symptoms were entered as categorised/multinomial variables (0 – 6). The total symptom severity score (out of 100) was also included as an explanatory variable.

Of the management variables: oral temperature (both continuous and categorised into low, normal and high); under normal circumstances requesting a urine sample for routine culture and/or the use of dip-slides (for adherence to prescribing guidelines response variable only); cloudy urine; smelly urine; and antibiotic choice categorised into no antibiotic, trimethoprim, nitrofurantoin, fosfomycin or other (for routine culture response variable only) were included. The six dipstick rules described in the previous 'grouping and recoding variables' section were also analysed, these were all categorised into positive (or yes), negative (or no) and not done (if at least one of the dipstick tests in the rule was missing).

All multinomial variables need to have a reference variable chosen to which all other variables were compared in the analysis. My general rule when choosing the reference was the variable most distinct from the other variables: Utrecht for network; 0 times

treated for UTI in the past; 0 for symptom severity; normal for temperature; yes/positive for the dipstick rules.

Incorporated into the final two-level multivariate model were the explanatory variables that showed significance at 10% level. This 10% threshold was chosen to allow more variables to be included in the final model than usual (when using a more standard 5% significance threshold) and to avoid excluding variables that may change in significance once fitted into the multivariate model.

Multivariate Multilevel Model

Firstly a null (empty) 1 level (patient level only) logit model was fitted with the response variable and no explanatory variables. This was estimated using MQL1 (1^{st} order marginal quasi-likelihood), estimation methods are discussed briefly in the next section. From this the second level was added (practice level) to produce a two level null model, firstly estimated using MQL1 then PQL2 (2^{nd} order predictive quasi-likelihood). From this binary 2-level logit PQL2 estimated model the explanatory variables were added (only variables showing significance at the 10% threshold in the univariate analysis) to produce the final model. If variables were closely related (for example the dipstick rules) they were added to the model separately and only the most significant variable remained in the final model. This was to avoid collinearity which could cause erratic changes in the model due to the highly correlated nature of the explanatory variables.

Estimation procedures

Various methods in multilevel modelling can be used to produce the outcome estimates. First order Marginal Quasi-Likelihood (MQL 1) estimation is deemed to be the crudest method. Estimates may be biased, especially if sample sizes within higher level units (practices in this case) are small. Second order PQL is considered to be the best approximation, but convergence problems may be encountered. For this reason and as recommended by the programmers of MLwiN, first order MQL estimates were run initially to obtain starting values of second order PQL estimates(90).

A measure that can be used to determine the most appropriate model is the Deviance Information Criterion (DIC); this is used to determine the best fit for non-linear models such as binary logit models. This requires the model to be estimated using Markov chain Monte Carlo (MCMC) estimation rather than Quasi-likelihood estimation procedures. When comparing the DIC obtained from the MCMC estimation of the null 2 level model to the final 2-level model (with explanatory variables) the DIC should have reduced to show a better fit (90).

Alongside these estimates, standard errors (SE), odds ratios (OR) with associated 95% confidence intervals and p-values (calculated using chi-square methods) are also presented.

Measure of Variation

To quantify the amount of variation at each level the Variance Partitioning Coefficient (VPC) was calculated. In a two level model the VPC for level two (practice in this case) is the between practice variation divided by the sum of the between and within practice variation. The value can be interpreted as a percentage; the higher the VPC the more variance attributable to the second level (practice) which indicates a higher level of clustering. Logistic models do not include an individual error component (within practice variation/level 1 variance); the Linear Threshold Model is recommended which uses the variance of a standard logistic distribution $\pi^2/3 = 3.29$ as the within practice variation (level 1 variance) and I also used this method (90).

Median odds ratios were calculated to translate area level variance into odds ratio scale which is directly comparable to individual odds ratios. The calculation used was;

$MOR = \exp[\sqrt{(2 \times V_A)} \times 0.6745]$

$\approx \exp(0.95\sqrt{V_A})$

V_A is the area level variance and 0.6745 is the 75^{th} centile of the cumulative distribution function of the normal distribution with mean 0 and variance 1. If MOR is equal to 1 there would be no difference between areas (91).

Laboratory data analysis

The prevalence of microbiologically confirmed UTI and associated antibiotic resistance was determined for each network and overall.

Associations between GP reported visual turbidity, urinalysis dipsticks and requesting urines for routine culture (including dip-slides) and microbiologically proven UTI were investigated.

Results

Descriptive Analysis

Network and Patient Characteristics

A total of 795 women were recruited onto the POETIC observational study. This was reasonably equally distributed between three of the networks with Cardiff, Southampton and Madrid/Catalonia recruiting a total of 212, 245 and 205 respectively, and Utrecht recruiting a total of 133 patients. There were 49 participating general practices across the networks and the number of patients recruited per practice ranged from 2 to 45 with an average of 16.2 (Table 3.2.1).

Participants ranged from 16 to 91 years of age with a mean age of 45.7 years. The number of days with symptoms prior to consulting ranged from 0 to 325 (325 most likely a data error); after discussion with the trial management team and clinicians any record with >28 days symptoms is most likely not considered an acute uncomplicated UTI so were excluded from further analysis. The majority of women did not take any time off work although slightly more women took at least one day off in Southampton with 21.3% compared to 13.3% in total.

Table 3.2.1. Network Characteristics

Country	Site(s)	Patients	Practices (min - max, mean number

			patients per practice)
Wales	Cardiff	212[1] (26.7)	11 (5 – 45, 19.2)
England	Southampton	245[1] (30.8)	11 (9 – 33, 22.2)
Spain	Madrid	205 (25.8)	17 (2 – 28, 12.1)
	Catalonia		
The Netherlands	Utrecht	133 (16.7)	10 (5 – 28, 13.3)
Total		795 (100)	49 (2 – 45, 16.2)

1 – 1 missing baseline CRF

Proportion of diaries returned ranged from 61.8% in Cardiff to 78.9% in Utrecht with a total of 567/795 (71.3%) diaries returned. Overall 81.7% women recorded they had a UTI in the past and 27.7% of women indicated they had been treated for a UTI at least three times in the past year; this varied across countries with 16.1% in Utrecht to 39.6% in Madrid/Catalonia. The median number of months since last UTI was the same for each country at four months, and in total 93.2% women recorded they had been prescribed an antibiotic for their last UTI. As this was recorded in the patient diaries women that did not know or did not complete this question were not included in the calculation reducing the sample size to n=398 (Table 3.2.2).

Table 3.2.2. Patient Characteristics.

		Cardiff (n=212)	Southampton (n=245)	Madrid/ Catalonia (n=205)	Utrecht (n=133)	Total (n=795)
Mean Age (SD)[1]		42.1 (17.52)	48.0 (18.51)	45.88 (19.17)	45.68 (18.44)	45.68 (18.44)
Range of Days with symptoms[2]		1 - 90	1 - 45	0 - 60	0 – 325*	0 – 325*
Median Days with symptoms (IQR)[3]		3 (2, 6)	4 (2, 6)	2 (1, 5)	4 (3, 7)	3 (2, 6)
1 or more days off work		12/126 (9.5%)	29/136 (21.3%)	13/85 (15.3%)	3/80 (3.8%)	57/370 (13.3%)
Diaries Returned		131/212 (61.8%)	177/245 (72.2%)	154/205 (75.1%)	105/133 (78.9%)	567/795 (71.3%)
History of UTI		104/129 (80.6%)	153/176 (86.9%)	114/153 (74.5%)	88/104 (84.6%)	459/562 (81.7%)
Times Treated for UTI in past year[4]	**0**	38/99 (38.4%)	42/143 (29.4%)	22/111 (19.8%)	38/87 (43.7%)	140/440 (31.8%)
	1	15/99 (15.2%)	34/143 (23.8%)	22/111 (19.8%)	21/87 (24.1%)	92/440 (20.9%)
	2	24/99 (24.2%)	25/143 (17.5%)	23/111 (20.7%)	14/87 (16.1%)	86/440 (19.5%)
	≥3	22/99 (22.2%)	42/143 (29.4%)	44/111 (39.6%)	14/87 (16.1%)	122/440 (27.7%)
Median Months since last UTI (IQR)		4 (3, 6.25)	4 (2, 6.75)	4 (2, 8)	4 (2, 6)	4 (2, 7)
Patients Prescribed antibiotic treatment for last UTI[4]		84/95 (88.4%)	131/139 (94.2%)	101/106 (95.3%)	55/58 (94.8%)	371/398 (93.2%)

1 - total age range 16-91 years; 2 - clinician reported; 3 - >28 days excluded; 4 - don't know or missing results excluded; *likely data error

Clinician Reported Signs and Symptoms

UTI symptoms of daytime frequency and urgency were present in over 90% of the women recruited into the study and the median severity score for both these symptoms was 4 (bad). Burning when passing urine and night-time frequency were symptoms also present in the majority of women; 85.3% and 80.7% respectively with overall median severity scores of 3 (moderately bad).

Symptoms of tummy pain, feeling unwell, smelly urine and restricted activities were present overall (with median symptoms severity scores) in 64% (2; slight problem), 63.8% (1; very little problem), 58.8% (1) and 48.6% (0; normal) women respectively. Symptoms of pain in the side, blood in the urine and fever had median severity scores of 0 across all networks and were present in a total of 41.5%, 28.0% and 21.2% women respectively. The majority of symptoms had some variation across the four networks with fever having the widest variation with only 5.5% women in Utrecht reporting presence to the clinicians compared to 38.9% women in Southampton as shown in table 3.2.3.

The total symptom severity score is calculated from the mean severity scores of the 11 symptoms for each patient and then scaled to range between 0 – 100. The box and whisker plot in Figure 3.2.1 shows the median and inter-quartile ranges (IQR) of the total symptom severity scores for the four networks.

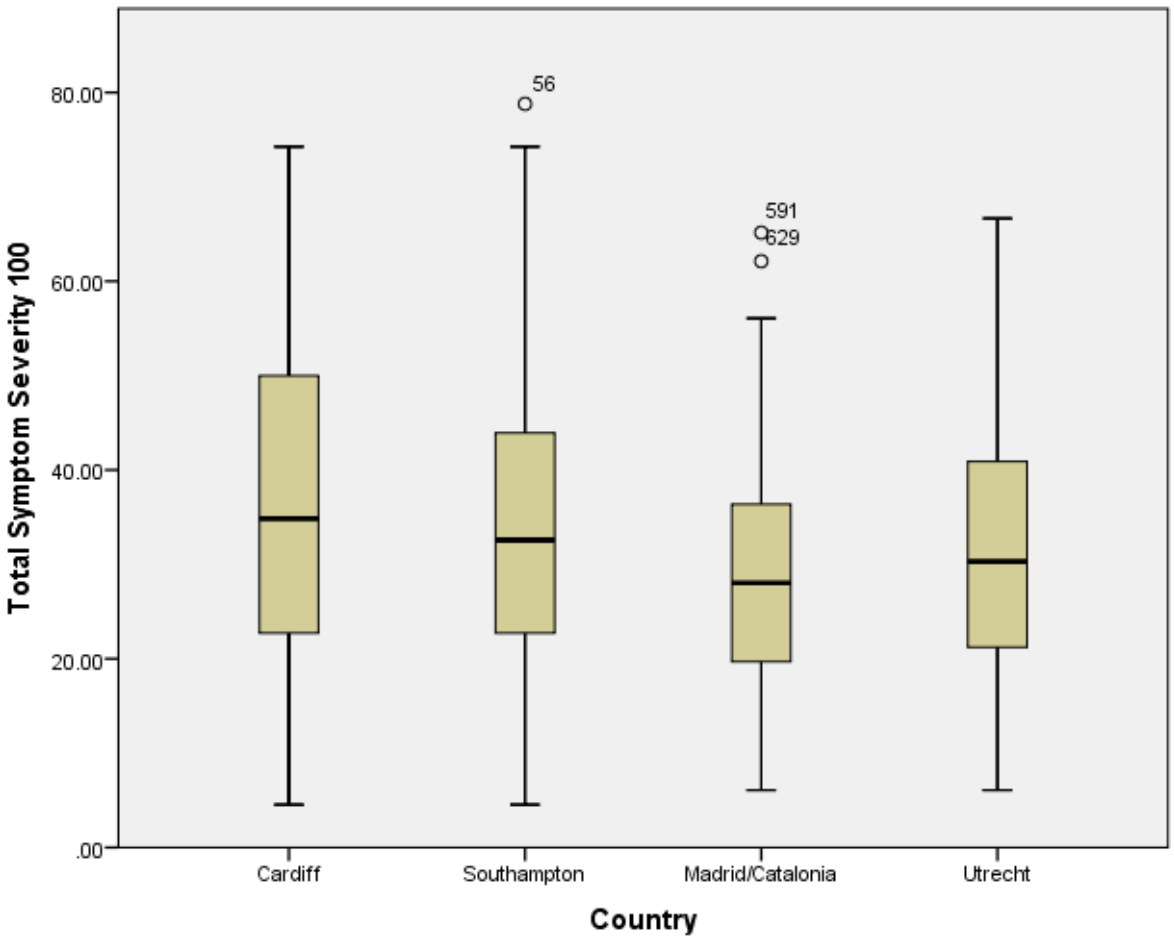
Table 3.2.3. Presence* of Clinician Reported Symptoms

	Cardiff (n=211)			Southampton (n=244)			Madrid/Catalonia (n=205)			Utrecht (n=133)			Total (n=793)		
	n	%	Median Grade	n	%	Median Grade	n	%	Median Grade	n	%	Median Grade	n	%	Median Grade
Fever	46/198	23.2	0	93/239	38.9	0	17/205	8.3	0	7/127	5.5	0	163/769	21.2	0
Pain in the Side	91/203	44.8	0	118/239	49.4	0	63/205	30.7	0	51/131	38.9	0	323/778	41.5	0
Blood in the Urine	61/202	30.2	0	71/238	29.8	0	63/205	30.7	0	22/131	16.8	0	217/776	28.0	0
Smelly Urine	126/203	62.1	2	150/239	62.8	1	106/205	51.7	1	75/130	57.7	2	457/777	58.8	1
Burning when passing urine	167/204	81.9	4	196/239	82.0	3	192/205	93.7	3	111/133	83.5	3	666/781	85.3	3
Urgency	181/204	88.7	4	226/239	94.6	4	183/205	89.3	3	115/133	86.5	3	705/781	90.3	4
Daytime Frequency	187/203	92.1	4	235/239	98.3	4	189/205	92.2	3	123/133	92.5	4	734/780	94.1	4
Night-time Frequency	168/202	83.2	3	205/239	85.8	3	155/205	75.6	2	100/132	75.8	3	628/778	80.7	3
Tummy Pain	122/204	59.8	2	171/239	71.5	2	114/205	55.6	1	91/130	70.0	2	498/778	64.0	2
Restricted Activities	107/202	53.0	1	120/239	50.2	1	69/205	33.7	0	82/132	62.1	1	378/778	48.6	0
Unwell	130/203	64.0	2	153/239	64.0	1	148/204	72.5	2	66/133	49.6	0	497/779	63.8	1

*present = very little problem (1) to as bad as it could (6); absent = normal/not affected (0)

The overall range for the symptom severity score was 4.55 to 78.99 with an overall mean of 32.7 (standard deviation 14.47). Madrid/Catalonia had the lowest median total symptom severity score and IQR (28.03; 19.7 – 36.4) and Cardiff had the highest (34.8; 22.7 – 50.0).

Figure 3.2.1. Total symptoms severity scores across the four networks (scaled out of 100).



Factor analysis of symptom severity scores

I decided to only accept factors that have an eigenvalue greater than 1, after extraction has taken place. This would therefore imply that only the first factor has a group of symptoms that cluster together strongly enough (Table 3.2.4). The scree plot (Figure 3.2.2) shows a steep drop between factor 1 and factor 2, and confirms that only factor one will be used for further analysis. Table 3.2.5 summarises the initial three factors with Eigenvalues >1.

Table 3.2.4. Total Variance Explained.

Factor	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings[a]
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total
1	3.296	29.959	29.959	2.726	24.779	24.779	2.350
2	1.476	13.421	43.380	.920	8.363	33.142	1.938
3	1.018	9.252	52.632	.328	2.985	36.128	1.288
4	.943	8.570	61.202				
5	.816	7.420	68.622				
6	.796	7.237	75.859				
7	.697	6.337	82.196				
8	.614	5.580	87.776				
9	.540	4.908	92.684				
10	.455	4.135	96.819				
11	.350	3.181	100.000				

Extraction Method: Maximum Likelihood.

a. When factors are correlated, sums of squared loadings cannot be added to obtain a total variance.

Figure 3.2.2. Scree plot of factors.

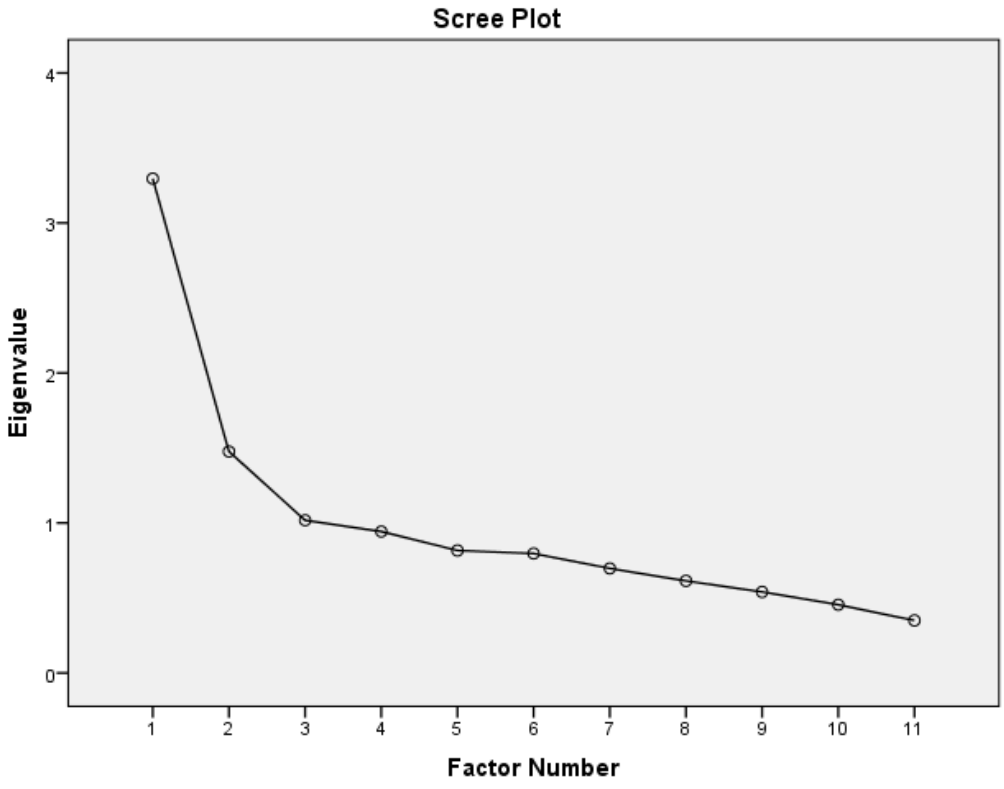
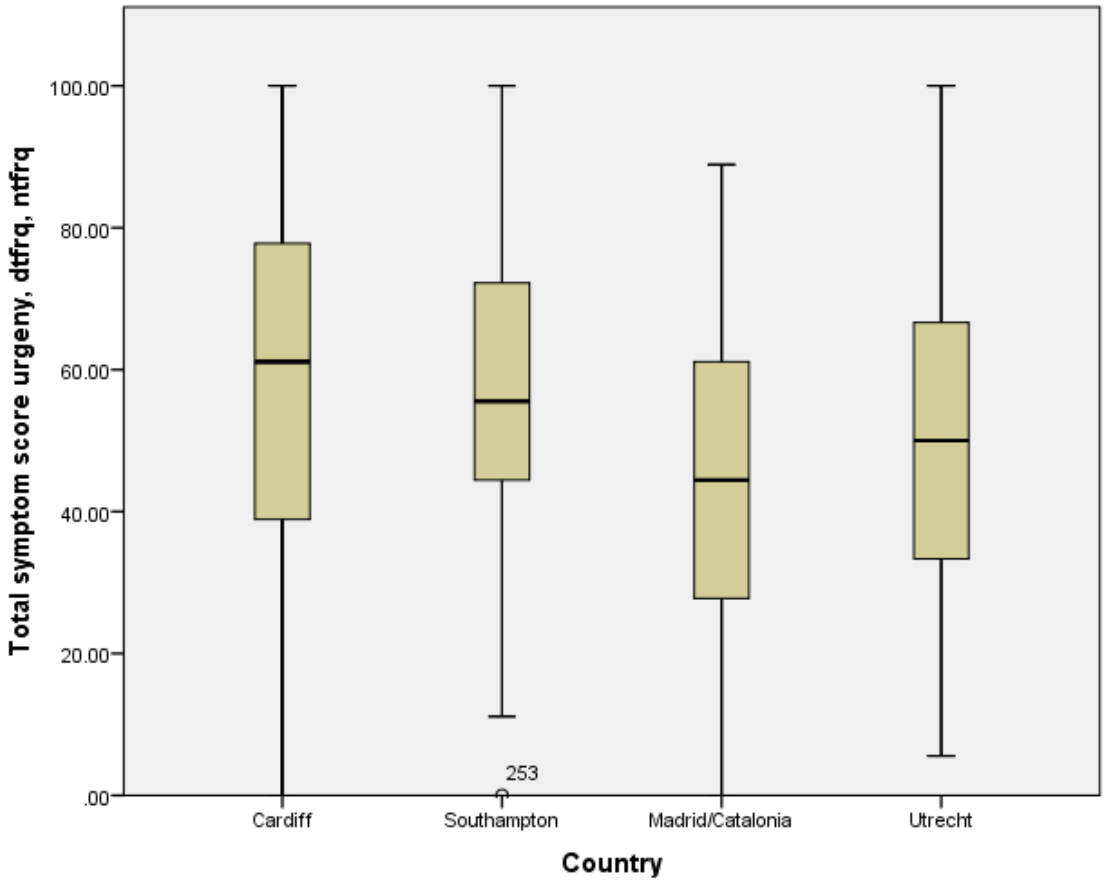


Table 3.2.5. Summary of Factor Analysis

Pattern matrix		
Factor 1 - accepted	**Factor 2 - rejected**	**Factor 3 - rejected**
S6. Urgency	S9. Tummy pain	S5. Burning
S7. Day time frequency	S11. Unwell	
S8. Night time frequency		

The overall range for the factor 1 symptom severity score was 0.00 to 100.00 with an overall mean of 52.89 (standard deviation 23.35). Madrid/Catalonia had the lowest median total symptom severity score and IQR for factor 1 (44.4; 27.8 – 61.1) and Cardiff had the highest (61.1; 38.9 – 79.2) (figure 3.2.3).

Figure 3.2.3. Total symptom severity scores for factor 1 (urgency, daytime frequency and night time frequency) across the four networks (scaled out of 100).



Patient Management: Diagnostic and other Tests

Dipsticks were the most commonly used test across all networks; the use was highest in Utrecht with 95.5% and lowest in Madrid/Catalonia at 68.8%, Cardiff and Southampton vary from 81.3% to 94.7% respectively. Of the urines checked for turbidity and smell a total of 42.5% were turbid and 29.8% had an offensive smell. There was some variation across the networks as shown in Table 3.2.6 with Madrid/Catalonia having the lowest proportion of urines with turbidity and smell. Overall 47.7% patients would under normal practice have had a routine culture requested. Cardiff and Southampton have the highest culture requests at about 61% for both networks, Madrid/Catalonia was 38.7% and Utrecht had the lowest culture requests at 16.5%. However, Utrecht are the only network in this study to perform dip-slides at the point of care; 37.6% patients had a dip-slide performed indicating 44% of the patients would have had at least one routine culture under normal circumstances (either dip-slide or routine laboratory culture).

The majority of women had a normal recorded temperature (84.2% with a temperature from 36°C to 37.2°C) and this was similar across the networks; a total of 6.8% women had an elevated temperature (>37.2°C) which could indicate fever, this was lowest in Madrid/Catalonia 1.5% and highest in Southampton 9.6%.

Regarding the individual dipstick urinalysis tests, nitrite was most commonly recorded for Madrid/Catalonia and Utrecht and leucocytes for Cardiff and Southampton networks, although the differences were minimal. Blood urinalysis results were less likely to be recorded across all networks (Table 2.2.5). Positive leucocytes (≥+) were the most common result with a total of 80.7% (61.2% in Utrecht and 81.2% to 89.2% in the other 3 networks); nitrite positive (≥+) was indicated in 35% cases; lowest in Cardiff 25% and highest in Utrecht 46.5%. Of the other dipstick rules investigated; 51.5% had positive leucocyte and blood; 18.4% had positive leucocyte, nitrite and blood; 16.4% had negative leucocyte and nitrite; and 11.9% had negative leucocyte, nitrite and blood urinalysis results. The results for these dipstick rules varies across the networks as shown in Table 3.2.7 and I do not know how these urinalysis results were used by the clinicians or nurses reading them.

Table 3.2.6. Use of diagnostic and other tests in the four networks.

		Cardiff (n=211)		Southampton (n=244)		Madrid/Catalonia (n=205)		Utrecht (n=133)		Total (n=793)	
		n	%	n	%	n	%	n	%	n	%
Dipstick Used		170/209	81.3	231/244	94.7	141/205	68.8	127/133	95.5	669/791	84.6
Urine Cloudy		88/170	51.8	96/225	42.7	42/141	29.8	56/127	44.1	282/663	42.5
Urine Smelly		50/170	29.4	80/225	35.6	28/141	19.9	40/128	31.3	198/664	29.8
Laboratory Culture routinely sent		121/201	60.2	135/219	61.6	79/204	38.7	20/121	16.5	355/745	47.7
Dip-slides		0	0	0	0	0	0	50/133	37.6		
Other culture indicated[1]		32/211	15.2	2/244	0.8	0	0	0	0		
At least 1 culture[2]		122/202	60.4	135/219	61.6	79/204	38.7	55/125	44.0	391/750	52.1
Other Tests		1 Blood Glucose; 1 pregnancy		1 Chlamydia		None		6 Sediment; 5 Microscopy; 2 Nitrate			
Oral Temperature (L,N,H)[3]	L	19/204	9.3	10/239	4.2	33/205	16.1	8/122	6.6	70/770	9.1
	N	170/204	83.3	206/239	86.2	169/205	82.4	103/122	84.4	648/770	84.2
	H	15/204	7.4	23/239	9.6	3/205	1.5	11/122	9.0	52/770	6.8

1 – recorded as 'MSU', 'MCS', 'culture and susceptibility' in 'other tests' section of CRF; 2 – calculated by adding additional 'other' culture tests and dip-slides to routine culture, if results were classed as 'missing' in original routine culture calculation (i.e. not included) these were added to total number;

3 – Oral Temperature: **L**: Low (<36.0°C), **N**: Normal (≥36°C and ≤37.2°C), **H**: High (> 37.2°C)

Table 3.2.7. Dipstick Urinalysis Results.

	Cardiff (n=170)		Southampton (n=231)		Madrid/Catalonia (n=141)		Utrecht (n=127)		Total (n=669)	
	n	%	n	%	n	%	n	%	n	%
POSITIVE Nitrite[1]	38/152	25.0	81/226	35.8	48/141	34.0	59/127	46.5	226/646	35.0
POSITIVE Leucocyte[2]	134/165	81.2	191/229	83.4	124/139	89.2	60/98	61.2	509/631	80.7
Leucocyte, nitrite AND blood POSITIVE[1]	16/109	14.7	27/184	14.7	22/101	21.8	20/69	29.0	85/463	18.4
Leucocyte AND blood POSITIVE[3]	68/114	59.6	87/186	46.8	59/101	58.4	26/69	37.7	240/470	51.1
Leucocyte, nitrite AND blood NEGATIVE[4]	15/109	13.8	20/184	10.9	4/101	4.0	16/69	23.2	55/463	11.9
Leucocyte AND nitrite NEGATIVE[5]	28/149	18.8	32/225	12.5	11/139	7.9	29/98	29.6	100/611	16.4

1 – UK, Spain, The Netherlands guidelines suggest probable UTI;

2 – UK and Spain guidelines suggest equal probability UTI as other diagnosis;

3 - The Netherlands guidelines suggest perform dip-slide/sediment test for confirmation;

4 - UK, Spain, The Netherlands guidelines suggest UTI unlikely;

5 – UK and Spain guidelines suggest UTI unlikely.

Patient Management: Antibiotic Prescribing

Participating clinicians in Cardiff, Southampton and Madrid/Catalonia prescribed antibiotics to over 90% of the patients in this study, whilst Utrecht clinicians prescribed to 59.4% of patients. The duration of the antibiotic course ranged from 1 to 28 days with Madrid/Catalonia having a median of 2 days (fosfomycin, a commonly used antibiotic in Spain is given as a single dose), Cardiff 3 days and Southampton and Utrecht 5 days as shown in Table 3.2.8. There was minimal delayed prescribing across the networks with Southampton having the highest delayed prescribing of 3.9% (9/232 patients). Additional antibiotics within the same consultation were prescribed to 5 patients; 3 in Cardiff and 2 in Madrid/Catalonia.

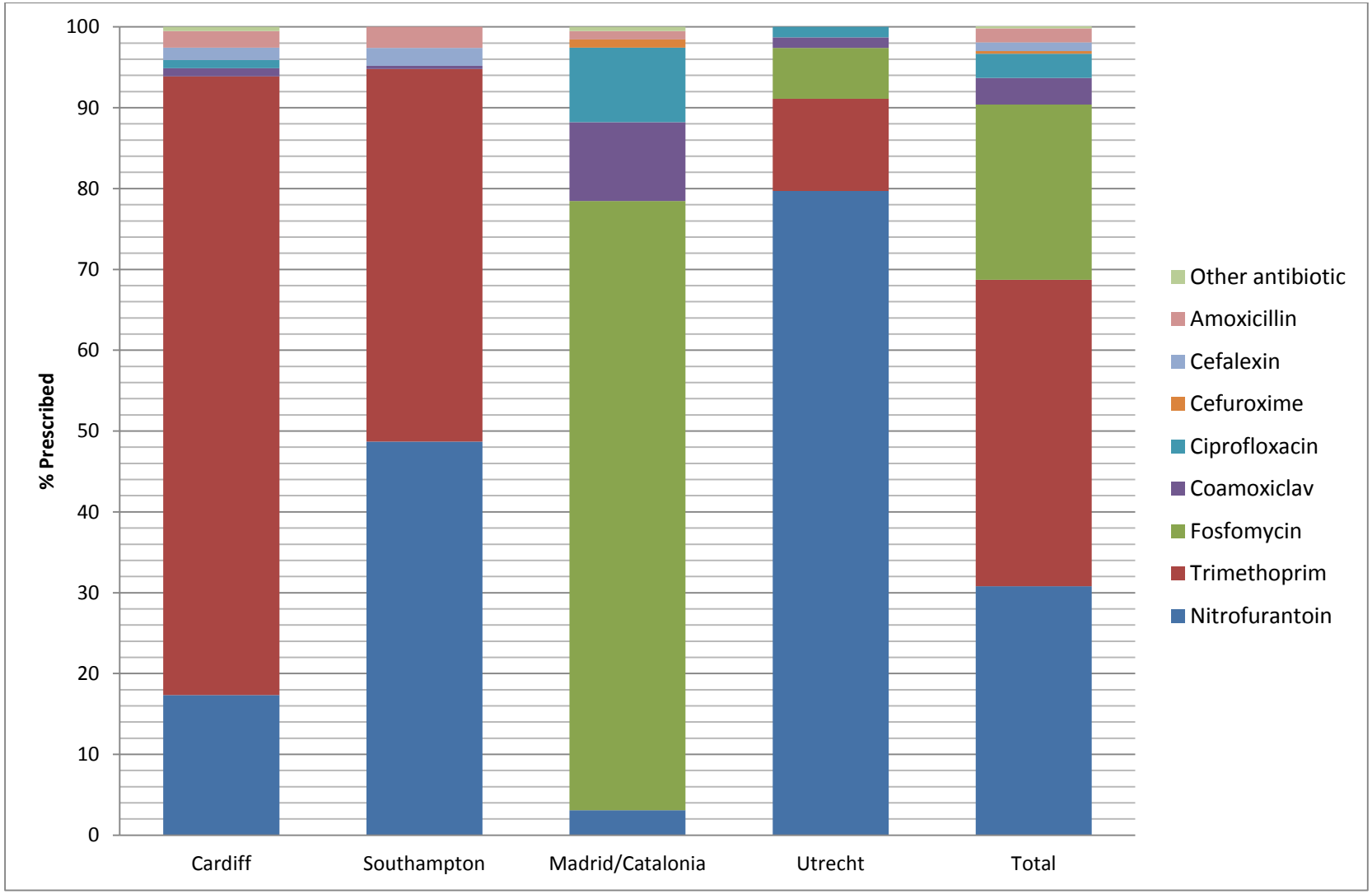
Table 3.2.8. Antibiotic prescribing across the networks.

	Cardiff (n=211)	**Southampton (n=244)**	**Madrid/ Catalonia (n=205)**	**Utrecht (n=133)**	**Total (n=793)**
At least one antibiotic prescribed	196/211 (92.9%)	232/244 (95.1%)	195/205 (95.1%)	79/133 (59.4%)	702/793 (88.5%)
Duration of Course (days)[1]	3.0 (2, 7)	5.0 (3, 28)	2.0 (1, 10)	5.0 (1, 28)	3.0 (1, 28)
Delay advised	2/196 (1.0%)	9/232 (3.9%)	1/195 (0.5%)	0/79 (0%)	12/702 (1.7%)
Additional antibiotic prescribed[2]	3/211 (1.4%)	0/232 (0%)	2/205 (1.0%)	0/79 (0%)	5/793 (0.6%)

1 – Median (Min, Max); 2 – within same consultation

Figure 3.2.4 shows the antibiotics prescribed by network. Trimethoprim was the principal antibiotic prescribed by the Cardiff network in 76.5% cases followed by nitrofurantoin 17.3% and minimal prescribing of amoxicillin (2%), cephalexin (1.5%), coamoxiclav (1%), ciprofloxacin (1%) and metronidazole (0.5%); in total 93.8% followed the recommended first-line treatment. Southampton prescribed trimethoprim and nitrofurantoin more equally at 46.1% and 48.7% respectively; other antibiotics prescribed by the Southampton network include amoxicillin (2.6%), cephalexin (2.2%) and coamoxiclav (0.4%); in total 94.8% followed the recommended first-line treatment. The Madrid/Catalonian network predominantly prescribed fosfomycin 75.4% followed by coamoxiclav (9.7%) and ciprofloxacin (9.2%) and minimal prescribing of nitrofurantoin (3.1%), cefuroxime (1%), amoxicillin (1%) and pipemidic acid (0.5%); in total 78.5% followed the recommended first-line treatment. In 79.7% cases the Utrecht network prescribed nitrofurantoin (recommended first-line treatment) followed by trimethoprim (11.4%), fosfomycin (6.3%), coamoxiclav (1.3%) and ciprofloxacin (1.3%).

Figure 3.2.4.Type of antibiotic prescribed per network.



Patient Management: Antibiotics prescribed following recommended type, dose and duration

Due to the high levels of prescribing in three of the networks, I wanted to compare the prescribing of antibiotics including type, dose and duration to the networks relevant country guidelines for first-line choice antibiotic treatment for uncomplicated UTI. Cardiff and Utrecht followed their respective guidelines in 75.0% and 73.7% of the cases, whilst Southampton and Madrid/Catalonia demonstrated congruence with their guidelines in only 32.6% and 28.6% of cases (Table 3.2.9).

Table 3.2.9. First-line antibiotic type, dose and duration prescribed as per relevant guidelines.

	Cardiff[1] (n = 211)		Southampton[1] (n=244		Madrid/ Catalonia[2] (n=205)		Utrecht[3] (n=133)		Total (n=793)	
	n	%	n	%	n	%	n	%	n	%
YES	147/196	75.0	75/230	32.6	55/192	28.6	56/76	73.7	333/694	48.0
NO	49/196	25.0	155/230	67.4	137/192	71.4	20/76	26.3	361/694	52.0

1 – Trimethoprim 200mg BD 3 days; Nitrofurantoin 100mg BD 3 days

2 – Fosfomycin 3g OD 1 day; Nitrofurantoin 50mg QDS 7 days

3 – Nitrofurantoin 5 days; 100mg BD or 50mg QDS

The main discordancy between the prescriptions and guidelines across the Cardiff and Southampton networks were due to the dose and duration of nitrofurantoin, as the Southampton network prescribed nitrofurantoin more frequently than the Cardiff network this was reflected in the lower level of overall concordance to guidelines. The trimethoprim prescriptions were generally in accordance with the guidelines although the Southampton network often prescribed for longer than the recommended 3 days (48.1% of all trimethoprim prescriptions). In 21.9% of cases the Madrid/Catalonia network prescribed antibiotics other than the recommended fosfomycin or nitrofurantoin. The major discordance when prescribing fosfomyicn was the duration; the majority of

prescriptions were for 2 days (55.6%) rather than 1 (38.9%) of 3g dose. The main reason for the Utrecht networks' discordancy was antibiotic type other than the recommended nitrofurantoin (21.1%); further details are included in summary box 3.2.5. This may not be surprising as the Netherlands only recommend one type of antibiotic as first-line treatment.

Figure 3.2.6 shows the distribution of concordant and discordant prescriptions by clinic in each network. Across all networks the majority of clinics/practices have a mix of concordant and discordant prescriptions; although Cardiff, Madrid/Catalonia and Utrecht have clinics (two per network) that are completely concordant and Southampton and Madrid/Catalonia have clinics that have not prescribed according to the guidelines for any of their patients (two and six clinics respectively).

Figure 3.2.5. Summary box detailing antibiotic type, dose, times per day and duration prescribed per network and in accordance with the relevant guidelines.

Cardiff

12/196 (6.1%) prescribed Antibiotics other than Trimethoprim or Nitrofurantoin

Trimethoprim (200mg BD 3 days) (n=150)

DOSE (100% in-line): **150/150 200mg (correct)**

TIMES per day (98.7% in-line): 1/150 OD; **148/150 BD (correct)**; 1/150 TDS.

DURATION (94.0% in-line): **141/150 3 days (correct)**; 2/150 5 days; 1/150 6 days; 6/150 7 days.

Nitrofurantoin (100 mg BD 3 days) (n=34)

DOSE (41.2% in-line): 20/34 50mg; **14/34 100mg (correct)**.

TIMES per day (0% in-line*): 34/34 QDS.

*% in-line increases if 50mg QDS (58.8% in-line)

DURATION (41.2% in-line): 1/34 2 days; **14/34 3 days (correct)**; 2/34 5 days; 17/34 7 days.

Southampton

12/230 (5.2%) prescribed Antibiotics other than Trimethoprim or Nitrofurantoin

Trimethoprim (200 mg BD 3 days) (n=106)

DOSE (100% in-line): **106/106 200mg (correct)**

TIMES per day (98.1% in-line): 1/106 OD; **104/106 BD (correct)**; 1/106 TDS

DURATION (51.9% in-line); **55/106 3 days (correct)**; 36/106 5 days; 13/106 7 days; 2/106 14 days.

Nitrofurantoin (100 mg BD 3 days) (n=112)

DOSE (24.1% in-line)*: 84/112 50mg; **27/112 100mg (correct)**; 1/112 200 mg

TIMES per day (17.0% in-line)*: **19/112 BD (correct)**; 93/112 QDS.

*% in-line increases if 50mg QDS or 100mg BD prescribed (~103/112 total; 92.0%)

DURATION (20.5% in-line): **23/112 3 days (correct)**; 51/112 5 days; 36/112 7 days; 2/112 28 days

Madrid/Catalonia

42/192 (21.9%) prescribed antibiotics other than Fosfomycin or Nitrofurantoin

Fosfomycin (3g OD 1 day) (n=144)

DOSE (91.7% in-line): 1/144 400mg; 7/144 500mg; 4/144 2g; **132/144 3g (correct)**.

TIMES per day: 136/144 OD; 1/144 BD; 7/144 TDS.

DURATION (38.9% in-line): **56/144 1 day (correct)**; 80/144 2 days; 1/144 5 days; 7/144 8 days.

Nitrofurantoin (50mg QDS 7 days) (n = 6)

DOSE (100% in-line): **6/6 50 mg (correct)**

TIMES per day (100% in line): 6/6 QDS

DURATION (0% in-line): 6/6 6 days

Utrecht

16/76 (21.1%) prescribed antibiotics other than Nitrofurantoin

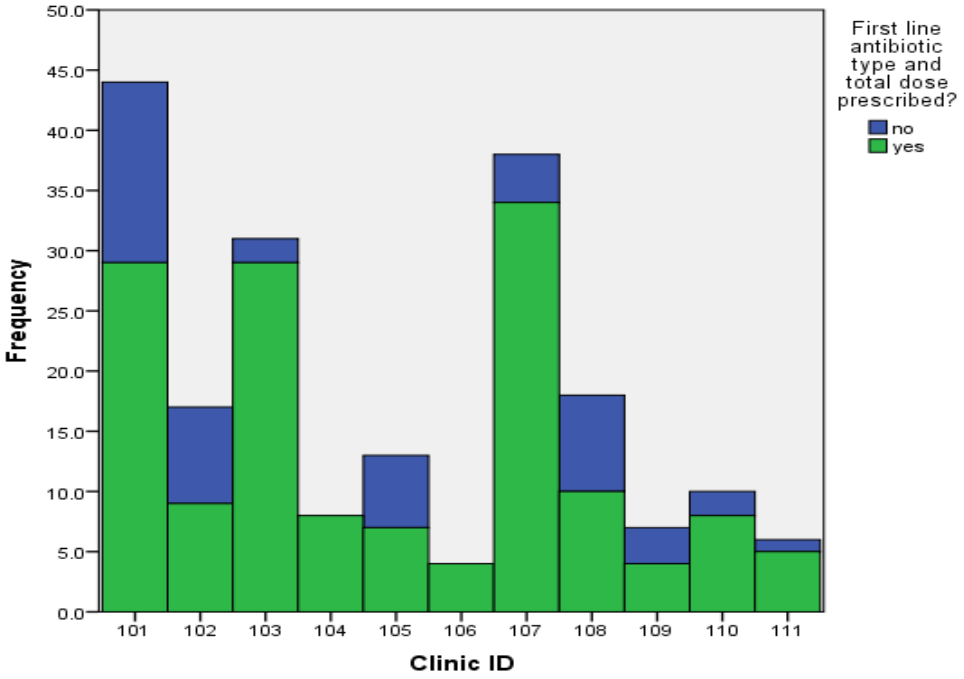
Nitrofurantoin (100mg BD 5 days OR 50mg QDS 5 days) (n = 60)

DOSE (100% in-line): **19/60 50 mg (correct); 41/60 100mg (correct).**

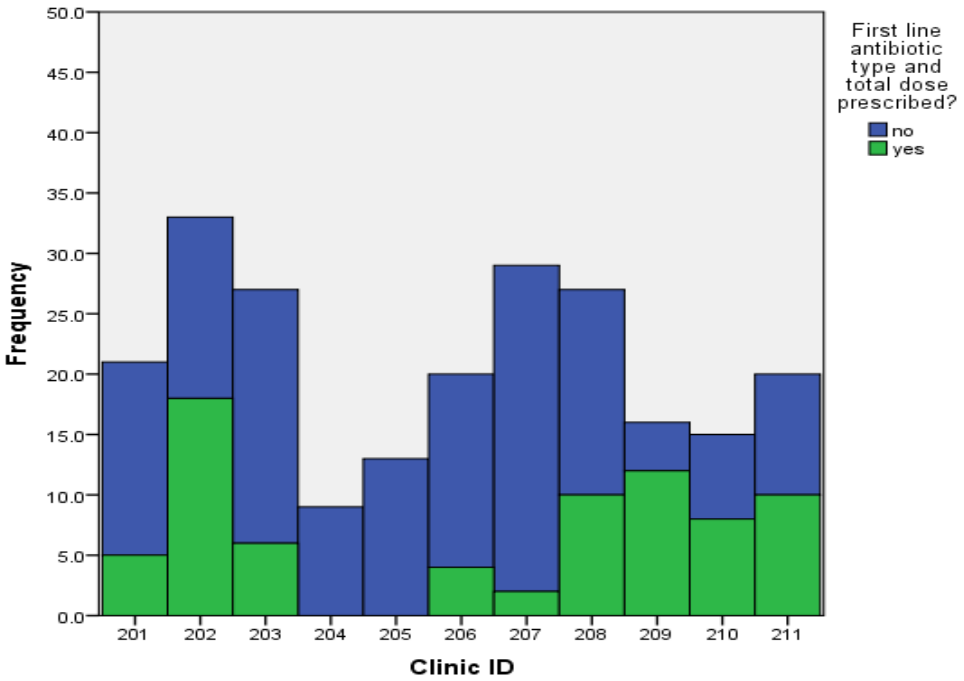
TIMES per day (96.7% in-line): **40/60 BD (correct)**; 1/60 TDS; **18/60 QDS (correct)**; 1/60 10dd (possible data error; most likely 1OD).

DURATION (95.0% in-line): 57**/60 5 days (correct)**; 1/60 7 days; 1/60 28 days.

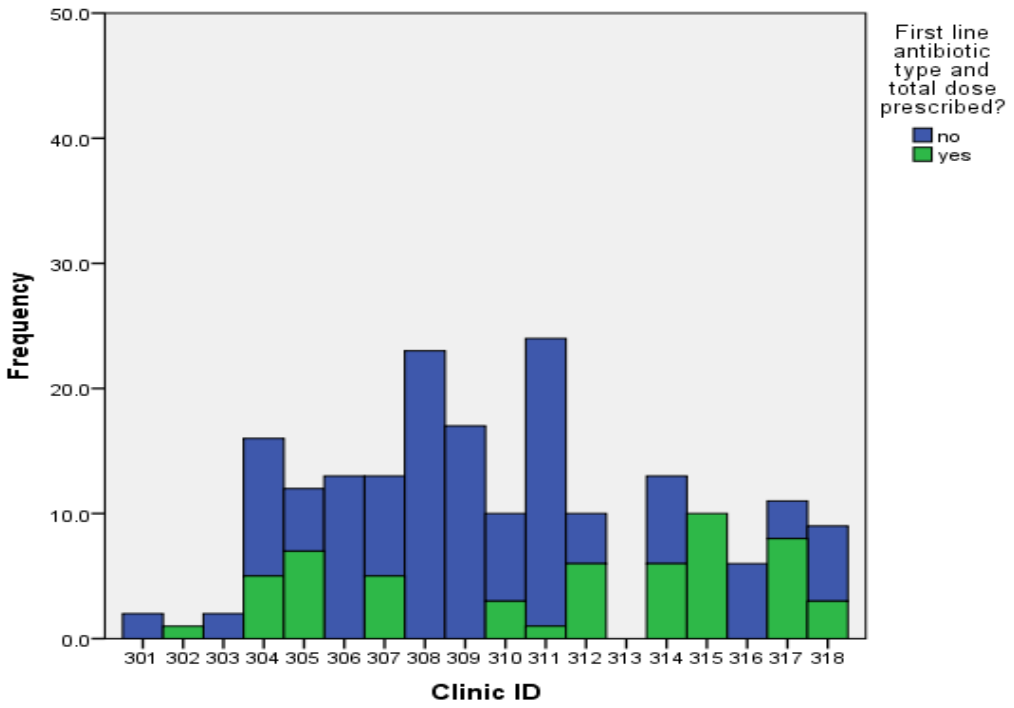
Figure 3.2.6. Antibiotic prescribing according to the relevant guidelines by country and participating clinics; A) Cardiff Clinics; B) Southampton Clinics; C) Madrid/Catalonia Clinics; D) Utrecht Clinics.



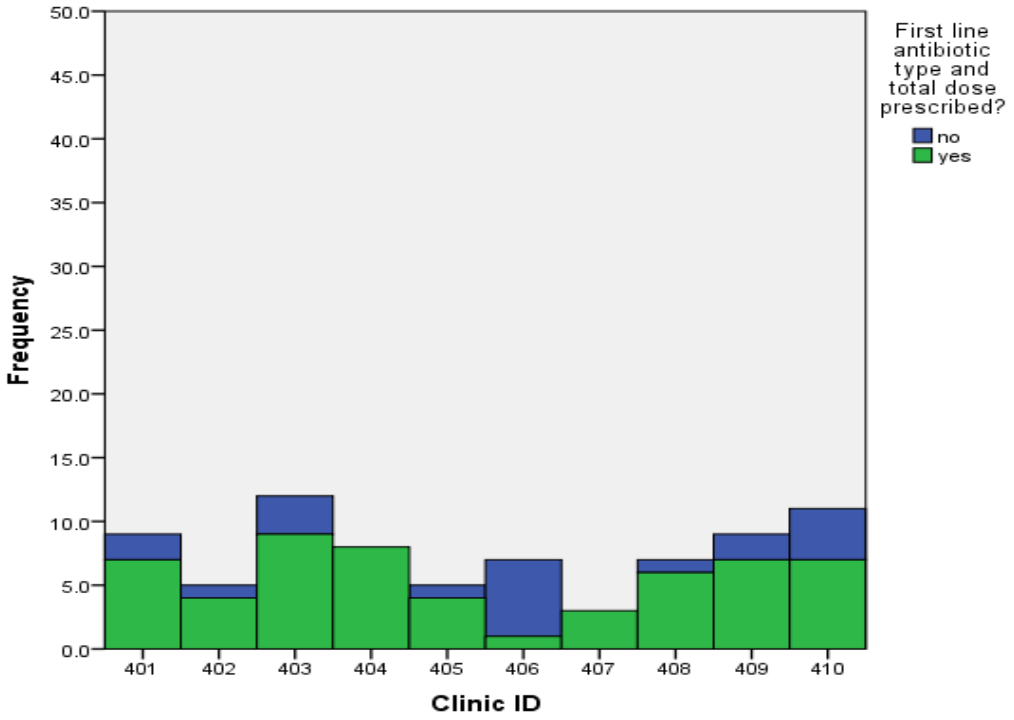
A) Cardiff



B) Southampton



C) Madrid/Catalonia



D) Utrecht

Patient management: other treatments

Only the Dutch guidelines mention the use of analgesics (if watchful waiting without antibiotics). Paracetamol was prescribed by the Madrid/Catalonia network in 20.5% of cases and advised by the Southampton network in 28.7% cases (Table 3.2.10). Additionally Ibuprofen was prescribed by the Madrid/Catalonia network (5.9%) and advised by the Southampton network (9.0%). The Utrecht network did not advise or prescribe either type of pain relief; the Cardiff network did but to a much lower level than Madrid/Catalonia and Southampton.

Table 3.2.10. Paracetamol and Ibuprofen prescribed or advised.

	Cardiff (n=211)	Southampton (n=244)	Madrid/ Catalonia (n=205)	Utrecht (n=133)	Total (n=793)
Paracetamol Prescribed	1/211 (0.5%)	2/244 (0.8%)	42/205 (20.5%)	0/133 (0%)	45/793 (5.7%)
Paracetamol Advised[1]	8/211 (3.8%)	70/244 (28.7%)	1/205 (0.5%)	0/133 (0%)	79/793 (10%)
Ibuprofen Prescribed	0/211 (0%)	0/244 (0%)	12/205 (5.9%)	0/133 (0%)	12/793 (1.5%)
Ibuprofen Advised[1]	2/211 (1.0%)	22/244 (9.0%)	1/205 (0.5%)	0/133 (0%)	25/793 (3.2%)

Patient management: advice and follow-up

All networks advised patients to increase fluid intake; this ranged from 54.6% in Madrid/Catalonia to 77% in Southampton. The Cardiff network also advised to return or contact their GP on worsening symptoms (10.4%) and use cranberry products (7.6%). The Southampton network also advised to return or contact their GP on worsening symptoms (18.9%); use pain relief (8.8%); rest (7.8%) and use cranberry products (7.4%). The Madrid/Catalonia network did not report giving as much advice as the other networks and other than increasing fluid intake recommend passing urine when needed

and emptying the bladder fully (3.4%). Clinicians in the Utrecht network also recommend passing urine when needed and emptying the bladder fully (21.8%) and using cranberry products (12%).

Other advice given by the networks is detailed in Table 3.2.11.

Table 3.2.11. Clinician Advice.

Baseline Clinical Advice (Free Text)		Cardiff (n=211)		Southampton (n=244)		Madrid/Catalonia (n=205)		Utrecht (n=133)		Total (n=793)	
		n	%	n	%	n	%	n	%	n	%
Fluids/ consumption	Increase fluid intake	137	64.9	188	77.0	112	54.6	74	55.6	511	64.4
	Cranberry product recommendations	16	7.6	18	7.4	0	0	16	12.0	50	6.3
	Reduce caffeine intake	3	1.4	3	1.2	0	0	0	0	6	0.8
	Avoid citrus fruit	0	0	2	0.8	0	0	0	0	2	0.3
	Avoid alcohol during treatment	0	0	0	0	0	0	1	0.8	1	0.1
Worsening symptoms	Return/contact on worsening symptoms	22	10.4	46	18.9	0	0	5	3.8	73	9.2
	Advice given on worsening symptoms	4	1.9	13	5.3	0	0	0	0	17	2.1
Over the counter (OTC) Medication/ Treatments	Pain relief recommendations (paracetamol, analgesics, hot pad)	1	0.5	20	8.2	0	0	3	2.3	24	3.0
	Cystitis powders and alkalising agents	3	1.4	9	3.7	0	0	0	0	12	1.5
	Take vitamin C	0	0	0	0	0	0	2	1.5	2	0.3

Table 3.2.11. Clinician Advice (continued).

Baseline Clinical Advice (Free Text)		Cardiff (n=211)		Southampton (n=244)		Madrid/Catalonia (n=205)		Utrecht (n=133)		Total (n=793)	
		n	%	n	%	n	%	n	%	n	%
Hygiene and Sexual Health	Pass urine when needed and empty bladder fully	0	0	12	4.9	7	3.4	29	21.8	48	6.1
	Pass urine before/after sex	1	0.5	4	1.6	0	0	7	5.3	12	1.5
	Use contraceptive precautions	2	0.9	0	0	0	0	0	0	2	0.3
	General hygiene advice	0	0	2	0.8	0	0	3	2.3	5	0.6
	Vulva tablet post sex	1	0.5	0	0.0	0	0	0	0	1	0.1
Antibiotic Use	Increase duration of antibiotic course if no resolution	0	0	2	0.8	0	0	0	0	2	0.3
	Decrease duration of antibiotic course if symptoms resolved	1	0.5	3	1.2	0	0	0	0	4	0.5
	Delay antibiotic prescription	1	0.5	1	0.4	0	0	0	0	2	0.3
	Complete antibiotic course	0	0	1	0.4	0	0	0	0	1	0.1
	Follow up with urine/culture results	7	3.3	3	1.2	0	0	2	1.5	12	1.5
Other advice	Rest	0	0	19	7.8	0	0	0	0	19	2.4
	Avoid perfumed bath products	0	0	1	0.4	0	0	0	0	1	0.1
	Use shower instead of bath	0	0	1	0.4	0	0	0	0	1	0.1
	Avoid swimming	0	0	1	0.4	0	0	0	0	1	0.1

Although the Madrid/Catalonia network did not report giving as much advice as the other networks they did arrange follow-up in 54.9% cases, more than any other network; the majority of patients (38.2%) were asked to follow-up with the GP/nurse in 8-14 days (Table 3.2.12). The Cardiff and Southampton networks only arranged follow-up in 18.7% and 12.4% cases respectively. Clinicians in Utrecht arranged follow-up in 34.6% cases; 23.8% had 'other' follow up arranged which included contacting the clinic for dip-slide results (14 cases) and having a follow-up urine test once the antibiotic course had been completed (6 cases).

The clinicians were also asked to indicate whether they would normally manage the case by phone; this was highest in the Southampton network at 64.5% followed by Utrecht at 62.2%. Madrid/Catalonian clinicians would have usually managed 40.2% cases by phone and clinicians in Cardiff 30.8% cases by phone (Table 3.2.12).

Table 3.2.12. Consultation and follow-up.

		Cardiff	Southampton	Madrid/ Catalonia	Utrecht	Overall
		n (%)	n (%)	n (%)	n (%)	n (%)
Follow-Up arranged with GP or Nurse?	None	165/203 (81.3)	212/242 (87.6)	92/204 (45.1)	85/130 (65.4)	554/779 (71.1)
	1-3 days	10/203 (4.9)	9/242 (3.7)	3/204 (1.5)	11/130 (8.5)	33/779 (4.2)
	4-7 days	7/203 (3.4)	14/242 (5.8)	28/204 (13.7)	1/130 (0.8)	50/779 (6.4)
	8-14 days	2/203 (1.0)	2/242 (0.8)	78/204 (38.2)	2/130 (1.5)	84/779 (10.8)
	Other*	19/203 (9.4)	5/242 (2.1)	3/204 (1.5)	31/130 (23.8)	58/779 (7.4)
Case usually managed by phone? (%)	No	139/201 (69.2)	83/234 (35.5)	122/204 (59.8)	48/127 (37.8)	392/766 (51.2)
	Yes	62/201 (30.8)	151/234 (64.5)	82/204 (40.2)	79/127 (62.2)	374/766 (48.8)

*Description of other follow-up recommendations included in appendix 3.2.7

Factors associated with routine culture

Intra-Class Correlation (ICC)

The ICC value for the response variable 'requesting a urine sample for routine culture/dip-slide' was calculated as 0.231 (23.1%). The ICC value for the response variable for 'antibiotics prescribed (type only) according to relevant guidelines indicating first-line treatment for women with uncomplicated UTI' was calculated as 0.161 (16.1%) and 'antibiotics prescribed (type, total dose and duration) according to relevant guidelines indicating first-line treatment for women with uncomplicated UTI' was calculated as 0.325 (32.5%). All ICC values indicate the need for multilevel modelling for these datasets.

Routine Culture: Univariate Multilevel Analysis

From the univariate analysis the only patient demographic that was significant at the 10% threshold was age. Network was also selected to go into the model as a fixed effect because of the differences in routine practice within each network although the p-value was 0.1320. Days with symptoms, months since last UTI and times treated for a UTI in the past did not show significance at this level. Of the signs and symptom severity scores only burning when passing urine and presence of increased night time frequency were significant. Daytime frequency, symptoms of fever, pain in the side, blood in the urine, smelly urine, tummy pain, restricted activities, feeling unwell, total symptom severity score (11 symptoms) and total symptom severity score for the factored variable (urgency, day time frequency and night time frequency) were not significantly associated ($p>0.1$) with requesting urine for routine culture. Of the management decisions such as dipstick urinalysis tests (including: nitrite positive; leucocyte positive; leucocyte, nitrite and blood positive; leucocyte and blood positive; leucocyte, nitrite and blood negative; leucocyte and nitrite negative; and leucocyte positive with nitrite negative) only nitrite positive and 'leucocyte positive with nitrite negative' were significant. As these variables are closely correlated (and to avoid collinearity) I introduced these variables into the model

separately and only the most significant variable was retained (nitrite). The only other management decision found to be significant in the univariate analysis was the choice of antibiotic prescribed. Oral temperature, cloudy urine and offensive smelling urine were not found to be significant.

Routine Culture: Multivariate Multilevel Analysis

The final model was generated to include network (Cardiff, Southampton, Madrid/Catalonia with Utrecht as the reference variable), patient age (continuous), symptom severity of burning when passing urine (continuous from 0 – normal to 6 - as bad as it could be), if night time frequency was present (no - reference), dipstick urinalysis – nitrite only (negative, not done and ≥+ as the reference) and type of antibiotic prescribed (trimethoprim, nitrofurantoin, fosfomycin, other and no antibiotic prescribed as the reference); the final model is shown in Table 3.2.13. The significance threshold for this final model was 5%.

Table 3.2.13. Two level logistic regression multivariate model for requesting urine samples for routine culture (under normal practice conditions) from women with suspected uncomplicated UTI.

		Estimate	Standard Error	Odds Ratio	95% CI		P-value
Empty Models	**1L null**	0.091	0.073	1.095	0.949	1.264	0.213
	2L null MQL1	0.036	0.160	1.037	0.758	1.419	0.822
	2L null PQL2 (n=750)	0.036	0.206	1.037	0.692	1.552	0.861
	2L null MCMC	0.066	0.229	1.068	0.682	1.673	0.773
Final Model	**Intercept (n=734)**	-0.662	0.689	0.516	0.134	1.991	0.337
Patient Demographic	**Network (Utrecht - Reference)**						
	Cardiff	1.927	0.645	6.869	1.940	24.318	0.003
	Southampton	1.627	0.622	5.089	1.504	17.221	0.009
	Madrid/Catalonia	0.966	0.703	2.627	0.662	10.422	0.169
	Age	0.016	0.005	1.016	1.006	1.026	0.001
Signs and Symptoms	**Symptom Severity: Burning when Urinating (high)**	-0.267	0.191	0.766	0.527	1.113	0.162
	Night Time Frequency (present)	0.417	0.396	1.517	0.698	3.297	0.292
Management	**Dipstick Nitrite (≥+ Reference)**						
	Negative	0.561	0.224	1.752	1.130	2.718	0.012
	Not done	0.160	0.319	1.174	0.628	2.193	0.616
	Antibiotic choice (No antibiotic - Reference)						
	Trimethoprim	-2.130	0.442	0.119	0.050	0.283	0.000
	Nitrofurantoin	-1.815	0.415	0.163	0.072	0.367	0.000
	Fosfomycin	-2.140	0.575	0.118	0.038	0.363	0.000
	Other	-1.636	0.514	0.195	0.071	0.533	0.001
Measures of Variation	**Variance (SE);**						
	Empty Model	1.582	(0.411)				
	Final Model	1.322	(0.366)				
	MOR (95% CI)						
	Empty Model	8.128	(4.058; 22.978)				
	Final Model	6.296	(3.616; 13.925)				
	VPC (empty model)	0.287 (0.325)					
	DIC;						
	Empty Model	890.025					
	Final Model	833.43					

From the two level null (empty) model (PQL2) the odds of requesting a urine sample for routine culture for a patient with suspected uncomplicated UTI picked at random from an average clinical practice was 1.037 (95% CI 0.692 – 1.552); indicating almost even odds with not requesting a sample and the difference was not significant. The network variable showed significance within this model (P<0.05) with individual networks Cardiff and Southampton showing significant differences to Utrecht. The odds of requesting a urine

sample in the Cardiff network compared to the Utrecht network was 6.869 (95% CI 1.94 – 24.32)); suggesting patients in Cardiff are approximately 7 times more likely than patients in Utrecht to have a urine sample for routine culture requested. Similarly patients in the Southampton networks are ~5 times more likely to have urine requested for culture than Utrecht. Madrid/Catalonia has an odds ratio of 2.627 but this was not significant.

Age was also significant in this model with an odds ratio of 1.016 (95% CI 1.006 – 1.026); the older the patient the more likely they are to be requested a urine sample for routine culture with, for example, women in their 60s are almost twice as likely to have urine requested for routine culture than women in their 20s[3].

Neither of the patient signs and symptoms (burning when urinating and night time frequency) remained significant in the model.

With regard to the management choices of clinicians both having a urinalysis dipstick positive for nitrite and deciding whether to prescribe antibiotics or not have a significant association with requesting urines for routine culture. If a clinician indicated that the urinalysis dipstick for a patient was nitrite negative this patient was 1.8 times more likely to have a routine culture requested than a patient with a dipstick positive (≥+) for nitrite or for a patient with no nitrite urinalysis performed (the OR for not using nitrite urinalysis is similar, 1.17, to a nitrite positive result with no significant difference).

Finally, not prescribing an antibiotic was significantly associated with an increase in requesting urines for routine culture. The type of antibiotic did not affect this; the odds of having a urine sample requested for routine culture if prescribed trimethoprim, nitrofurantoin, fosfomycin or other antibiotics are 0.12, 0.16, 0.12 and 0.19 respectively (all significant $p<0.05$) so depending on the antibiotic between 5.25 – 8.33 times less likely than no antibiotic.

The variance partition coefficient (VPC) in the final model was 0.287; this indicates that 28.7% of the remaining variation was due to level 2 variation and the inter-cluster effect

[3] (estimate 0.016 x 40 years = exp0.64 = 1.896 OR)

(difference between practices) remains relatively large (the empty model with no explanatory variables had a VPC of 32.5%).

The residual heterogeneity between GP practices (MOR=6.3) indicates that a patient can be 6 times more likely to have a routine culture requested depending on the practice visited even after taking into account network, patient age, dipstick urinalysis (nitrites) and antibiotic prescribing.

The DIC (deviance information criterion) is smaller in the final model (833.43) compared to the empty model (890.025) suggesting a better fit after the explanatory variables have been added.

Factors associated with prescribing antibiotics for uncomplicated UTI according to network relevant guidelines

Prescribing first-line antibiotic type: Univariate multilevel analysis

The first response variable evaluated here was of the patients prescribed antibiotics (88.5%) what were the associations with prescribing following the networks' relevant guidelines for first-line antibiotic type for women with suspected uncomplicated UTI.

From the univariate analysis the patient demographics that were found to be significant at the 10% threshold included network and history of treatment. Those found not to be significant included age, days with symptoms and months since last UTI. Of the patient signs and symptoms; presence of increased daytime frequency, presence of symptoms of fever, presence of pain in the side, presence of feeling unwell and high level of burning when passing urine were all significantly associated. Urgency, night time frequency, blood in the urine, smelly urine, tummy pain, restricted activity and total symptom severity scores (all symptoms and factored variable) were not significantly associated. From the management decisions analysed oral temperature was significantly associated with prescribing antibiotics according to first-line treatment type in the relevant guidelines. All dipstick analysis tests/rules, requesting urine for routine culture, cloudy urine and offensive smelling urine were not significantly associated.

Prescribing first-line antibiotic type: Multivariate multilevel analysis

The final model was generated to include network (Cardiff, Southampton, Madrid/Catalonia with Utrecht as the reference variable), if day time frequency was present (no - reference), symptoms of fever present (no –reference), pain in the side (no – reference), feeling unwell (no – reference), high level of burning when passing urine (low – reference) and oral temperature (normal – reference); the final model is shown in Table 3.2.14. The significance threshold for this final model was 5%. Although history of treatment (1, 2, 3 or more times, do not know and none as reference) was significant in the univariate model the sample size decreased substantially to n=236 and could not be included in the final multi-level model.

From the two level null (empty) model (PQL2) the odds of prescribing first-line treatment type according to the network relevant guidelines for a patient with suspected uncomplicated UTI picked at random from an average clinic/practice is 9.88 (95% CI 6.15 – 15.87); the patient is 10 times more likely to be prescribed a recommended first-line treatment than not (highly significant difference).

The network association was highly significant ($P<0.05$); the odds of the Cardiff and Southampton networks prescribing following guidelines compared to Utrecht were 6.06 (95% CI 1.51 – 24.48) and 10.48 (95% CI 2.53 – 43.38) respectively indicating that patients in these networks are 6 and 10 times more likely to get an antibiotic according to their networks first-line treatment guidelines for uncomplicated UTI. The difference between Madrid/Catalonia and Utrecht was not significant. Clinicians in Cardiff and Southampton (as part of the UK) have access to the same guidelines however clinicians in Cardiff are 42% less likely to prescribe first-line antibiotic treatment type according to the guidelines than Southampton.

Table 3.2.14. Two level logistic regression model of antibiotics prescribed (type only) according to relevant networks guidelines for first-line treatment for women with uncomplicated UTI.

		Estimate	Standard Error	Odds Ratio	95% CI		P value
Empty Models	**1L null**	2.023	0.117	7.561	6.012	9.510	0.000
	2L null MQL1	1.849	0.194	6.353	4.344	9.293	0.000
	2L null PQL2 (n=694)	2.290	0.242	9.875	6.145	15.868	0.000
	2L null MCMC	2.277	0.238	9.747	6.114	15.541	0.000
Final Model	**Model Intercept (n=662)**	0.976	0.753	2.654	0.607	11.610	0.195
Patient Demographics	**Network (Utrecht - Reference)**						0.000
	Cardiff	1.802	0.710	6.062	1.507	24.376	0.011
	Southampton	2.349	0.725	10.475	2.529	43.380	0.001
	Madrid/Catalonia	-0.148	0.600	0.862	0.266	2.795	0.805
Signs and Symptoms	**Daytime Time Frequency (present)**	0.884	0.543	2.421	0.835	7.017	0.104
	Symptoms of Fever (present)	-0.512	0.398	0.599	0.275	1.307	0.198
	Pain in the Side (present)	-0.339	0.303	0.712	0.393	1.290	0.263
	Feeling Unwell (present)	-0.886	0.392	0.412	0.191	0.889	0.024
	Burning when passing urine (high)	0.793	0.302	2.210	1.223	3.995	0.009
Management	**Oral Temperature (normal – Reference)**						0.002
	Low	0.980	0.566	2.664	0.879	0.083	0.049
	High	-1.579	0.515	0.206	0.075	0.002	0.003
Measures of Variation	**Variance (SE);**						
	Empty Model	1.529	0.545				
	Final Model	0.912	0.403				
	MOR (95% CI)						
	Empty Model	7.695	(3.307; 32.491)				
	Final Model	4.477	(2.745; 9.251)				
	VPC (empty model)	0.217 (0.328)					
	DIC Empty Model	452.25					
	DIC Final Model	408.87					

The only variables in the signs and symptoms that remained significant at the 5% threshold when put into the final model were feeling unwell and burning when passing urine. Feeling unwell had an odds ratio of 0.41 (95% CI 0.19 – 0.89) so patients that were feeling unwell were 59% less likely to have a recommended first-line antibiotic type prescribed. Patients that experienced high levels of burning when passing urine

(symptom score ≥3) were over twice as likely to be prescribed an antibiotic that was recommended as first-line than patients with milder symptoms.

Oral temperature remained significant in the final model and patients with a high temperature were 80% less likely to likely to have a recommended first-line antibiotic type prescribed than those with a normal temperature.

The variance partition coefficient (VPC) in the final model was 0.217; this indicates that 21.7% of the remaining variation is due to level 2 variation and the inter-cluster effect (difference between practices) remains relatively large. However, by adding the explanatory variables the VPC reduced by about 10% from the empty model.

The median odds ratio of 4.48 (95% CI 2.75 – 9.25) in the final model suggests that patients are almost 5 times more likely to be prescribed an antibiotic type according the networks guidelines depending on which GP practice is visited.

The DIC (deviance information criterion) is smaller in the final model (408.87) compared to the empty model (452.25) suggesting a better fit after the explanatory variables have been added.

To investigate antibiotic prescribing according to relevant network guidelines further I ran another model with antibiotic prescribing according to the guidelines to include type, total dose and duration. The final model is included in appendix 3.2.8. From the two level null (empty) model (PQL2) the odds of prescribing first-line treatment (type, total dose and duration) according to the network relevant guidelines for a patient with suspected uncomplicated UTI picked at random from an average clinic/practice was 0.968 (95% CI 0.551 – 1.698); indicating almost even odds not prescribing according to the guidelines with no significant difference.

The variables that remained significant in the final model ($P<0.05$) included the network (Southampton and Madrid/Catalonia compared to Utrecht), history of treatment (3 or more times compared to no history of treatment) and having a urinalysis dipstick for leucocytes performed. The only variable that was significant for both models was network. However in this model GP practices in the Southampton and Madrid/Catalonia networks were 91% (95% C 0.019 – 0.421) and 96% (95% CI 0.009 – 0.199) less likely

to prescribe according to their networks recommended guidelines for antibiotic type, total dose and duration compared to the Utrecht network respectively. In the previous model GP practices in the Southampton network were more likely to prescribe the recommended antibiotic type according to the guidelines than Cardiff. However in this model Cardiff is 9 times more likely than Southampton to prescribe according to the recommended first-line antibiotic type with total dose and duration.

The median odds ratio for this model reduced from 97.3 (95% CI 8.12 – 22172.72) to 7.53 (95% CI 3.22 – 32.47) when the individual level explanatory variables were added. However patients are still 3 to 32 times likely to be prescribed an antibiotic according to the guidelines for type, total dose and duration depending on which GP practice they consult at.

When comparing the two prescribing models the individual effects were relatively more important on prescribing antibiotic type over prescribing antibiotic type with total dose and duration; this is reflected in the final MOR of 4.5 compared to 7.5. Likewise the remaining unexplained level 2 variance for both models was 21.7% for antibiotic type and 31.4% for antibiotic type, dose and duration.

Laboratory data and use of point of care tests

UTI prevalence and uropathogen identification

Cardiff and Southampton had a very similar prevalence of microbiologically proven UTI at 28.4 % (95% CI 22.6 – 35.1%) and 28.2% (95% CI 22.7 – 34.6%) respectively. The prevalence in Spanish and Dutch networks were much higher at 49.8% (95% CI 35.6 – 49.8%) and 64.6% (95% CI 55.9 – 72.4%) respectively (Table 3.2.15).

Table 3.2.15. Prevalence of proven uncomplicated UTI

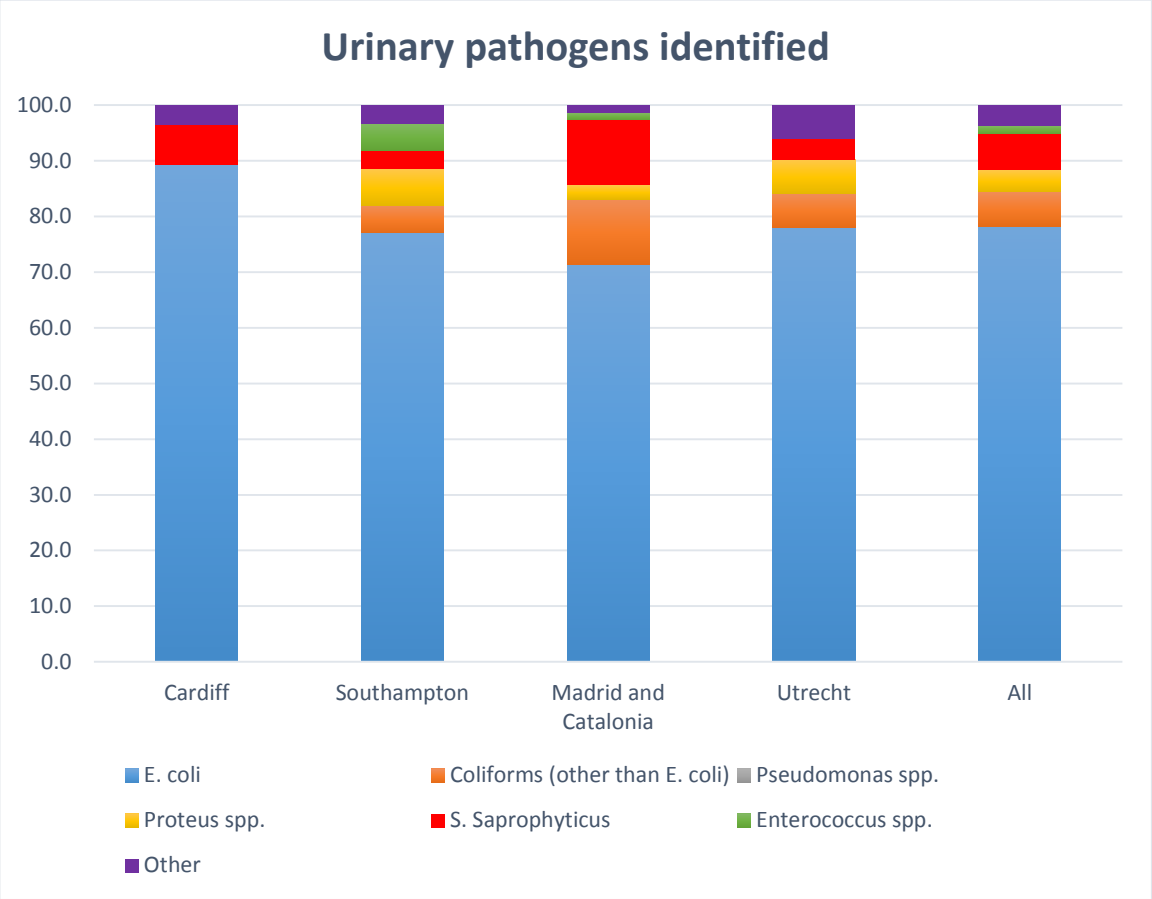
	N	UTI Negative	UTI Positive	Prevalence of UTI (%)	95% CI	
Cardiff	197	141	56	28.4	22.6	35.1
Southampton	216	155	61	28.2	22.7	34.6
Madrid/ Catalonia	181	104	77	42.5	35.6	49.8
Utrecht	127	45	82	64.6	55.9	72.4

The most common uropathogen isolated across all networks from the microbiologically positive urine samples was *E. coli* ranging from 71.4% in the Spanish network to 89.3% in the Cardiff network (Figure 3.2.7).

Use of point of care tests and routine culture

The use of diagnostic and other tests has already been described in table 3.2.6. Figure 3.2.8 compares the use of dipstick urinalysis, prevalence of turbid (cloudy) urine and request for routine culture with the prevalence of microbiologically proven UTI across the four networks.

Figure 3.2.7. Prevalence of urinary pathogens isolated from microbiologically UTI positive urine samples.



Dipstick urinalysis is the most commonly used test across the networks regardless of the prevalence of UTI. Routine culture requests are more frequent in the Cardiff and Southampton networks even though the prevalence of UTI is lower than in Madrid/Catalonia or Utrecht networks.

Figure 3.2.8. Use of diagnostic and other tests and prevalence of microbiologically proven UTI.

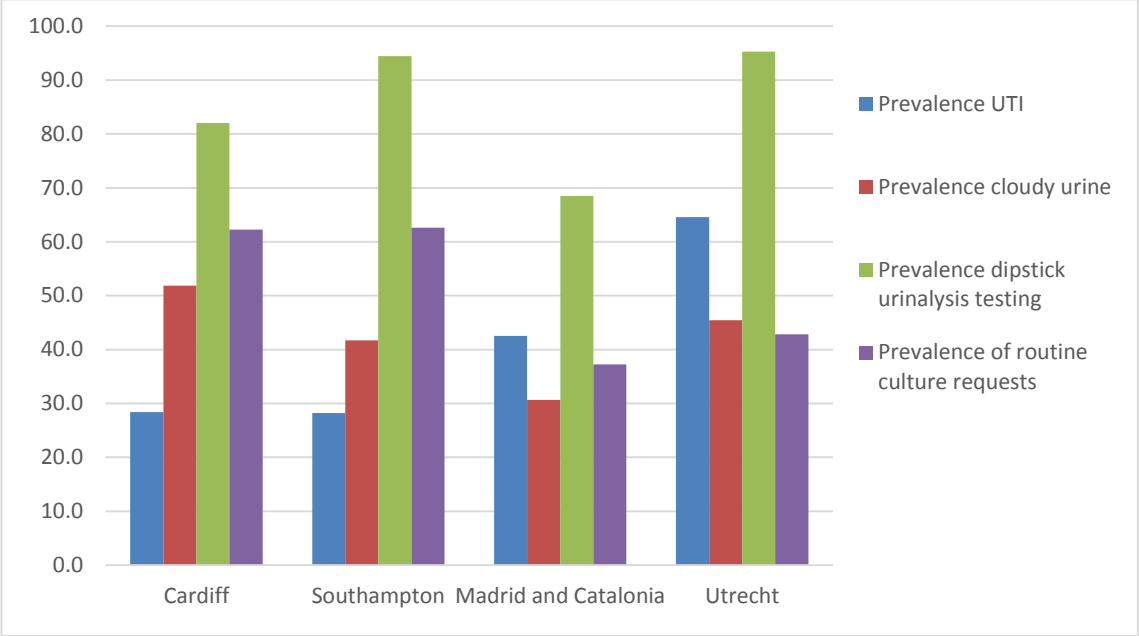
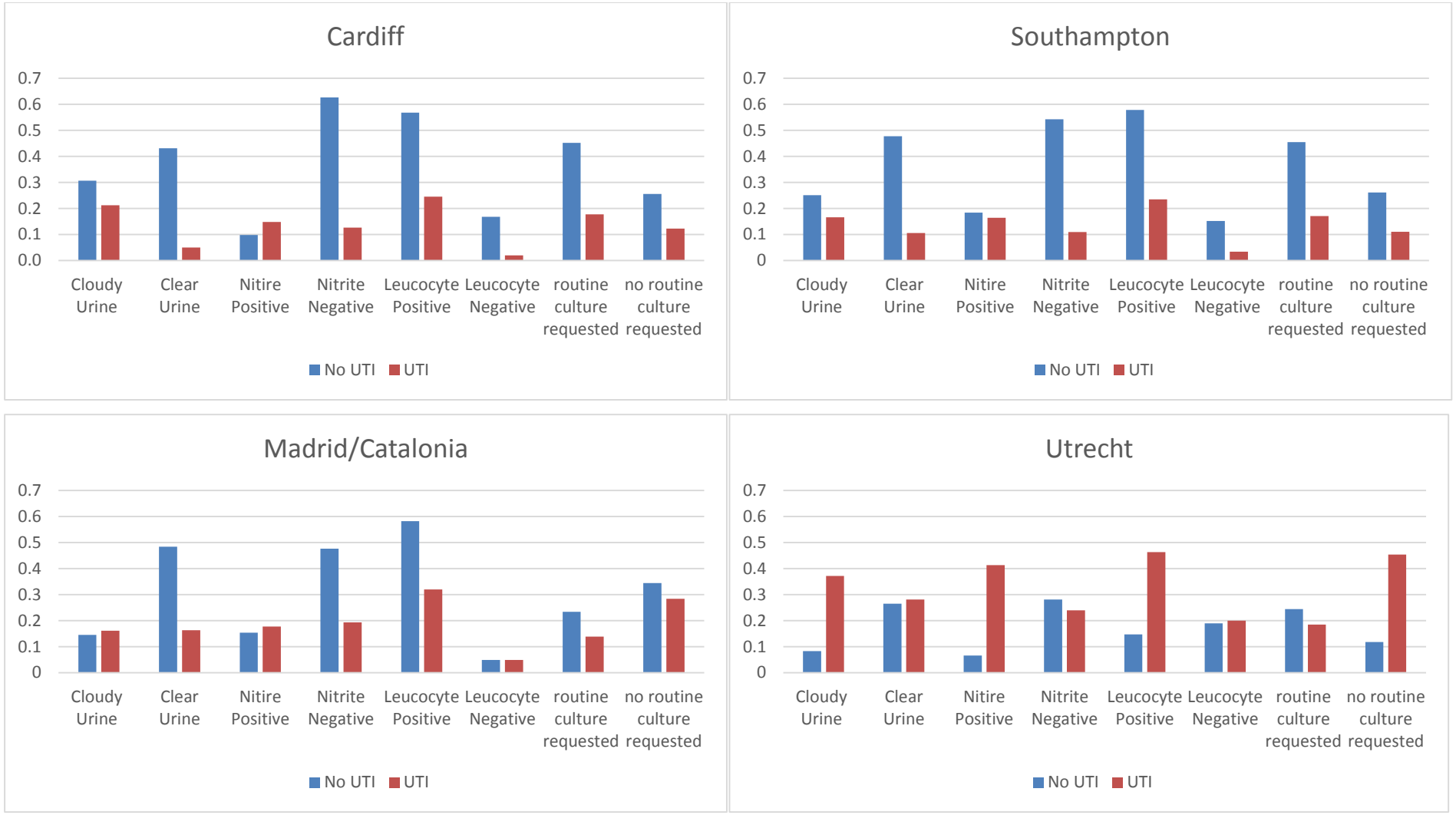


Figure 3.2.9 illustrates each diagnostic test result with the proportion of corresponding positive and negative UTI samples. For the UK networks and to a lesser extent the Spanish network the most useful tests may be clear urines or nitrite negative results as predictors of not having a UTI. For the UK networks leucocyte negative results may also be a good predictor of not having a UTI but the proportion of negative leucocyte results was much lower. In Utrecht cloudy urine, positive nitrite and positive leucocyte results may be useful predictors of having a UTI. There doesn't seem to be any difference between requesting routine culture results and a patient having a UTI or not, except in the Utrecht network where patients with a microbiologically proven UTI are less likely to have a routine culture requested by their GP.

Figure 3.2.9. Diagnostic test outcomes and proportion of positive and negative UTI.



As the prevalence of UTI is much higher in the Spanish and Dutch networks compared to the UK networks I have chosen to only include the diagnostic accuracy analyses for urine turbidity, nitrite and leucocyte dipstick urinalysis for the UK networks. The results for the other networks are included in appendix 3.2.9.

The negative predictive values for urine turbidity, nitrite urinalysis and leucocyte urinalysis were 89.6% (95% CI 80.8 – 89.6), 83.2 % (95% CI 75.0 – 89.1) and 89.7% (95% CI 73.6 – 96.4) respectively for the Cardiff network. The Southampton network showed similar results with 81.9% (95% CI 73.9 – 87.8), 83.2% (95% CI 75.9 – 88.6) and 81.6% (95% CI 66.6 – 90.8) NPV for urine turbidity, nitrite urinalysis and leucocyte urinalysis respectively. None of the tests showed high positive predictive values but nitrite urinalysis had the highest association with an odds ratio of 5.4 (95% CI 4.73 – 5.75) for the combined UK networks.

The laboratory evaluation study (section 2; chapter 4) showed that the best prediction rule was that if the urine was visually turbid AND nitrite OR leucocyte positive, it was likely to be positive for a UTI. I analysed the same rule on the observational data for the UK networks and found the results as follows; sensitivity 67.4% (95% CI 57.4 – 76.0); specificity 65.3% (95% CI 59.3 – 70.8); PPV 41.3% (95% CI 33.8 – 49.2) and NPV 84.7% (95% CI 79.0 – 89.0). Table 3.2.16 shows the raw data with likelihood ratios and odds ratios for each test.

Table 3.2.16. Diagnostic accuracy of visually assessing urine turbidity, nitrite and leucocyte dipstick urinalysis in determining microbiologically positive UTI.

Network	Test	True Positives	True Negatives	False Positives	False Negatives	% correctly classified	Likelihood Ratio (+ / -)	Odds Ratio
Cardiff	**Turbid urine**	34	69	49	8	64.4	1.94 / 0.32	5.98
	Nitrite positive	21	89	14	18	68.8	3.96 / 0.53	7.42
	Leucocyte positive	38	26	88	3	40.0	1.20 / 0.32	3.74
	Urine cloudy and nitrite or leucocyte positive	33	75	42	8	68.4	2.24 / 0.30	7.37
Southampton	**Turbid urine**	33	95	50	21	64.3	1.77 / 0.59	2.99
	Nitrite positive	33	109	37	22	71.4	2.37 / 0.54	4.42
	Leucocyte positive	48	31	118	7	32.2	1.10 / 0.61	1.80
	Urine cloudy and nitrite or leucocyte positive	31	96	49	23	63.8	1.70 / 0.64	2.64
UK	**Turbid urine**	67	164	99	29	64.3	1.85 / 0.48	3.83
	Nitrite positive	54	198	51	40	70.2	2.80 / 0.54	5.24
	Leucocyte positive	86	57	206	10	39.8	1.14 / 0.48	2.38
	Urine cloudy and nitrite or leucocyte positive	64	171	91	31	65.8	1.94 / 0.50	3.88

Discussion

There are a limited number of studies observing the routine management of UTI in primary care; and comparing clinical practice between local GPs as well as between European countries. The POETIC observational study, from which I analysed and included the management data in this thesis achieved this. Unique aspects of this pragmatic study include a sample size of 793 evaluable patients from four different European countries collecting data using the same CRF to allow for adjustment for case mix.

Recruitment

Recruitment into the study was limited in the Utrecht network compared to the other networks. The Netherlands have a different management system for uncomplicated UTI compared to the other countries whereby patients drop a urine sample off to the practice without seeing a nurse or GP, the urine sample is tested using dipstick urinalysis and/or dip-slides and the woman is telephoned with the result and asked to collect a prescription if necessary (confirmed from communication with the Netherlands POETIC study team clinicians). If the study site staff were unable to see the patients to discuss the study and/or gain consent this will have influenced recruitment potential within this network. The presented results may be less generalizable for this network than the other three networks with higher recruitment figures. The number of participants recruited per practice was highly variable for all the countries. The high intra-class correlations could be partly a result of this (for example within practice variation may be limited with only two participants) although this does not affect the sample size as there is no comparison group and as the main outcome of antibiotic prescribing had a conservative estimate of 50% this will result in narrower 95% confidence intervals.

The number of diaries return ranged from 61.8% in Cardiff to 78.9% in Utrecht. The number of diaries returned overall and in the Cardiff network is similar to another

prospective observational study in a multi-European primary care setting (80). Other than telephoning the participants to request the return of the diary there were no other mechanisms to improve diary return at this stage, for example, telephone interview requesting essential information from the diary. Another multi-European, investigator-driven prospective observational study of patients with acute cough (GRACE-01) suggested the following to improve diary response rate for similar trials: sending a letter when the patient is unable to be contacted by phone; registering a preferred contact number and time to call; flow diagram at the beginning of the patient diary detailing completion process; using different coloured paper for general questions that distinguish from the daily questions; advertise and hold a lottery for all patients who have returned diary as an incentive to complete and return the diary; shortened version of patient diary with selected key main outcome questions when no patient diary has been returned (92).

Patient Characteristics

The mean age of women presenting with uncomplicated UTI was similar across the networks with the lowest in Cardiff and highest in Southampton; the overall mean age is similar to the mean age of women (44 years; range 16 to 99 years) presenting to an observational study of uncomplicated UTI in Canada (93) . Days with symptoms ranged hugely from zero to 325 days; I expect the latter to be a data error however there were still women recorded as having >28 days and up to 3 months symptoms. This suggests that some GPs interpret women with chronic symptoms to have an uncomplicated (acute) UTI. I did not find a definition for the number of days with symptoms to be considered acute in the literature, however the Utrecht networks guidelines (based on guidelines from the Netherlands) in this study suggest <7 days as uncomplicated (appendix 3.2.6) and another review suggests an average of 6 days (94). The systematic review included studies that described women consulting with a mean or median of 3 days symptoms (26, 28, 81, 82). Time off work was minimal suggesting generally uncomplicated UTI symptoms were not debilitating.

The majority of women had a history of UTI and this was similar across the networks; a study in Canada reported that 86% women recruited onto their study had also had least one episode of UTI previously (93). Number of treatments for UTI in the past year generally reflect the prevalence of antibiotic prescribing during this study (discussed later) although Cardiff had the highest proportion of women in the no treatment bracket (in the past year) within their network. This may reflect the slightly younger age of the women recruited onto the study.

The number of months since last UTI episode was similar across the networks as is the high percentage of women having been prescribed an antibiotic for their last UTI episode. The latter results are in-line with having a history of UTI but slightly discordant with times treated for UTI in the past year, although this may be down to women having had antibiotic treatment longer than a year ago. The proportion of antibiotic prescribing for last UTI episode is in-line with the studies current prescribing for UTI in the Cardiff, Southampton and Madrid/Catalonia networks. The Utrecht network has a much higher figure for antibiotic prescribing for last UTI than the current UTI although this may be due to recall bias of the patient (previous antibiotic treatment was captured in the patient diary whereas current practice was recorded by the GP in the CRF at the time of consultation).

Clinician Reported Signs and Symptoms

Daytime frequency and urgency were both the most prevalent and severely graded (as 'bad') symptoms across all networks. This is not surprising considering the inclusion criteria specified that women participating in the study had to have at least one symptom of dysuria, urgency including nocturia, and frequency. Dysuria and nocturia were also highly prevalent and with a median severity grading of 'moderately bad'. Frequency and dysuria were also the most prevalent symptoms reported in the systematic review although urgency was reported by fewer studies and had a lower prevalence (26, 81-84). None of the other symptoms had a (median) severity grading greater than 'slight problem'. Blood in the urine and symptoms of fever had low prevalence. The Utrecht network had much lower (half) reported blood in the urine than the other networks but

was much more in line with the reported prevalence from the systematic review (29, 81-84). A qualitative study reported women with blood in their urine were more worried about their symptoms and this formed a strong driver for seeking help (95); in this study the Utrecht network did also have the lowest recruitment figures. Southampton had much higher reporting (at least double) of symptoms of fever which may also be linked to the slightly higher reported number of days off work for women in this network. Only two studies in the systematic review reported on the symptoms of fever and the prevalence was minimal (1.9 – 9.3%) (83, 84) and another study based in Canada reported minimal presence of fever (6.6%) (93).

Women were recruited with suspected uncomplicated UTI and as such may have had fewer and less severe symptoms than seen in routine practice for all suspected urinary tract infections. The total symptom severity score had a wide range; some women had very low symptom severity prior to consulting their GP while others had much more severe symptoms. The low median total symptom severity scores across the networks could be associated with the women on the study having had a prior UTI and recognising the symptoms quickly and consulting earlier. For women with higher severity scores it could be that some women wait longer to see their GP or see if the symptoms resolve prior to seeing their GP for treatment. In a qualitative study of women's experiences of self-care for symptoms of UTI and their journey to the GP failure to alleviate symptoms through lay remedies was the commonest cited rationale for consulting and regardless of prior experience, fear of the consequences of continued symptoms – particularly potential for kidney infection (95).

The Canadian study by McIsaac et al. found 48% of women recruited had moderately severe symptoms; 28% mild; and 24% severe. It is difficult to compare severity of symptoms when grading scales are different and severity is subjective to the patient (93). Little et al. found that patients with more somatic symptoms and more severe symptoms at baseline were likely to have longer lasting symptoms; may be more in need of antibiotic treatment but that if doctors took a positive attitude about their patients diagnosis duration of symptoms could be reduced (82).

The factor analysis resulted in one unobserved variable which included the symptoms of urgency, daytime frequency and night time frequency. This factor had a higher mean total symptom severity score than with all the symptom variables included, however, this is because the these three factors had higher individual scores than the other variables. This factor was not associated with increased likelihood of prescribing antibiotics according to the guidelines with regard to antibiotic type or type, total dose and duration.

Diagnostic management choices and prevalence of UTI

Requesting urines for laboratory culture was higher than expected and is not recommended in UK and Spanish networks' guidelines for women with uncomplicated UTI for every patient on a routine basis. This could be due to the influence of participating in the study or the way this data was collected. Requesting urines for culture was not associated with increased likelihood of prescribing antibiotics according to the guidelines with regard to antibiotic type or type, total dose and duration. Even when laboratory culture results are available the management of the patient does not necessarily change accordingly (86) and laboratory culture has been suggested to be more useful in resistance surveillance to inform empirical antibiotic choice (96). A limitation of this study for this question is that all GPs had to send a urine sample for culture as part of the study procedures. This may result in false positive answers rather than actual routine practice. Not prescribing antibiotics was significantly associated with sending samples for culture. The Netherlands can choose to use dip-slides at the point of care and if used will show (after 24h) if there is bacterial growth or not which can guide the clinician in deciding whether or not a bacterial UTI is present and whether to prescribe. However, dip-slides are unable to test for antibiotic susceptibility and cannot guide most appropriate antibiotic choice. From the systematic review I also found that studies which requested urines as part of the study procedures (and therefore would have had culture results available for the participating GPs after a few days) tended to have lower empirical prescribing levels (29, 32, 86). However, reverse causality where both outcomes are also predictors could be an issue with regard to requesting urines for routine culture and antibiotic prescribing. Antibiotics could be prescribed or not depending on whether a culture was requested or

conversely a culture could have been requested depending on whether antibiotics were prescribed or not. There is no way to know from my study data which is the true outcome or predictor.

Other factors associated with sending urines for routine culture include increasing patient age and nocturia. Indicators of pyelonephritis and recurrent UTI may warrant culture as indicated by the UK's HPA guidelines but presence of loin pain (pain in the side), fever, times treated for UTI in the past and months since last UTI were not significantly associated with sending urines for culture in my model. Exclusion criteria for the study included history of pyelonephritis and antibiotic treatment for UTI in the past 4 weeks; clinicians would therefore not consider their patient as having pyelonephritis or recent recurrent UTI if recruited into the study which fits with my results.

Urinalysis dipsticks were the most commonly used tests across all four networks. This is similar to studies in Spain, Sweden, Germany and Canada where use of dipstick urinalysis ranged from 84% to 93% (26, 28, 29, 93). Nitrite and leucocyte tests were the most commonly recorded in fairly equal measure across the networks except the Utrecht network where the leucocyte test was recorded less than nitrite. The CRF does not ask the clinician to record if the individual urinalysis test result was used in the management of the patient. However, from the multilevel analysis I found that negative nitrite results are associated with an increase in the (indicated) use of routine culture and dip-slides (in Utrecht); this may be due to the clinicians' uncertainty of an infection when obtaining this result. All networks' guidelines indicate a positive nitrite result as probable UTI. However, only 35% of clinicians recorded positive nitrite tests from this study. Having a positive leucocyte result was significantly associated with prescribing an antibiotic that was different to that recommended by the guidelines (for type, total dose and duration). Leucocytes (white blood cells) are an indication of infection and as such may cause the clinician more concern and therefore to prescribe a different antibiotic to avoid treatment failure due to a concern about potential resistance (for example in the case of trimethoprim) or for a longer duration to avoid recurrence of the UTI. The UK and Spain guidelines indicate positive leucocyte tests as having equal probability of UTI as

compared to other diagnosis. In total 80% recorded a positive leucocyte test across all networks suggesting increased uncertainty and it being largely a redundant test in practice.

Over half of all women had clear urine (or non-cloudy as indicated by the clinician). Previous studies have shown that clear urine is a good indicator of no microbiological infection and the HPA guidelines recommend another diagnosis with clear urine (if less than 3 symptoms or not severe).

All women were requested to provide a urine sample for microbiological confirmation of UTI as part of the study procedures. The prevalence of microbiologically confirmed UTI in this study ranged from 28.3% in the UK networks to 64.6% in the Utrecht network with *E. coli* being the most common uropathogen isolated across all network. The higher prevalence of microbiologically confirmed UTI in Utrecht and Madrid/Catalonia could be due to using different laboratories to culture the urine samples (the UK networks used the same laboratory) or recruitment of women with higher incidence of UTI in different networks. The higher prevalence of UTI particularly in the Utrecht network shows that the usefulness of different diagnostic tests is variable depending on the network it is used in. For example in the Cardiff network, clear urines may be useful in identifying patients without a microbiologically confirmed UTI where as in the Utrecht network almost equal numbers of patients with and without microbiologically confirmed UTI had a clear urine sample. Likewise negative nitrite results may be an indicator of no UTI in Cardiff patients but in Utrecht a positive nitrite result is more useful in identifying patients with a UTI.

This study was purely observational and not set up for the purpose of diagnostic evaluation. However, looking at only the UK networks (as they had the same prevalence of microbiologically confirmed UTI) if clear urines were used as in indication of a diagnosis other than UTI 54% (193/359) patients could be screened out; with 8% (29/359) of patients with a true UTI missed. If patients showed a nitrite negative result, 69% (238/343) could be excluded from empirical treatment or further diagnostic tests but 12% (40/343) of patients with a true UTI would be missed. If the patients showed a leucocyte negative result, 18.7% (67/359) could be excluded from empirical treatment or

further diagnostic tests with fewer patients with a true UTI missed (2.8%). For the UK networks none of the urinalysis dipstick tests or evaluation of urine turbidity perform well at confirming a microbiologically positive UTI.

Patient Management: Antibiotic Prescribing and other Treatments

The UK and Spain networks prescribed antibiotics in over 90% of cases; the Netherlands network prescribed in just under 60% of cases. Delayed prescribing and prescription of second antibiotics were minimal across all networks.

The systematic review shows similar levels of prescribing in the nine included European countries; with just under 60% prescribing in Ireland and Germany and up to 99% in Turkey. The two more recent English studies reported in the review (81, 82) show high levels of prescribing similar to the Southampton network in this study, likewise the Spanish study by Llor C et al. (26) included in the review reported the same prescribing levels as the Spanish network in this study. The Welsh study included in the review showed a much lower level of prescribing than this study although this may be due to the fact that the study by O'Brien et al. relied on patient recall of antibiotic prescription rather than GPs indicating this at the time of consultation (32). Another Welsh UTI study found patients' recall of antibiotics used (in the previous 12 months) was too difficult for many patients and had to rely on prescription data from medical records instead (11).

This study involved the recruitment of women with suspected uncomplicated UTI with at least one of three key urinary tract symptoms (dysuria, urgency including nocturia, and frequency); current HPA (UK) and Spanish guidelines recommend empirical antibiotic treatment for women with severe or ≥3 symptoms of UTI. I did not analyse the appropriateness of prescribing according to this guideline. Instead I allowed (from the study protocol) for the fact that clinicians suspected the patient had uncomplicated UTI (as they were recruited onto the study) and unless there were other factors of concern (such as recurrence or previous treatment failure and age) empirical first-line antibiotic prescribing may be deemed appropriate. For this reason I chose to analyse factors

associated with prescribing first-line antibiotics following the networks' guidelines firstly with regard to antibiotic type and secondly with regard to antibiotic type with recommended total dose and duration. Not prescribing according to the first-line antibiotic type included the network the patient was in (UK networks associated with prescribing according to recommended type), the feeling of being unwell and having a high temperature and no/low burning sensation when passing urine. This may suggest that if a GP suspects the patient is more generally unwell or has fever a second line/alternative treatment is preferable. The main discordancy I found with the Cardiff and Southampton (and Madrid/Catalonia) networks were that they prescribed the correct antibiotics but for a longer duration than recommended. Other than the network the patient was recruited in, having 3 or more treatments for UTI in the past and having a leucocyte positive urinalysis dipstick test are associated with discordant antibiotic prescribing choices for type as well as total dose and duration in the relevant guidelines; this could be related to fear of recurrent infection or more serious infection. The systematic review included five studies in England, Germany, Spain, Sweden, and Turkey that also observed longer antibiotic treatments than recommended for uncomplicated UTI (26, 28, 29, 81, 84). A three day course of antibiotic treatment should suffice for most women with lower urinary tract infection, including elderly patients. Single dose treatment is less effective but has fewer side effects (19). A study in Wales investigating prior antibiotics use and the risk of antibiotic-resistant community-acquired urinary tract infection found there was significant increased risk of resistance associated with length of course of trimethoprim compared with no prescription with odds ratios (ORs) 4.62 >=7days and 1.60<= 7 days. The ratios of these ORs was 2.89 showing that the OR for the longer course was significantly greater than that for the short course prescription. The risk of trimethoprim resistance was also significantly associated with the number of prescriptions in the previous 12 months. Prescriptions of seven or more days showed significance within the last three months; for prescriptions of <7days the association was significant only in the following month (11).

It may be that clinicians consider antibiotic resistance to be generally unaffected by their practice and that some clinicians prescribe antibiotics for longer to give their patients the best chance of recovery. Other UTI studies in European primary care have shown similar findings in that antibiotics are often prescribed for longer duration than recommended and are not compliant with the guidelines (28, 97). Over –prescribing either when the patient does not need an antibiotic at all or for longer duration and total dose may lead to risks of side-effects without achieving more meaningful rapid recovery. Another concern is generation of resistance in the community from over-exposure of antibiotics in the individual. Previous UK studies evaluating the effects of trimethoprim prescribing in primary care on bacterial resistance have shown higher rates of resistance with increasing duration, dose and recent exposure (11, 24, 98). A study looking at factors associated with the probability of antibiotic resistance of uropathogenic *E. coli* found previous antibiotic use and the general practice visited as risk factors for a patient with a UTI being diagnosed with *E. coli* resistant to this agent (the study evaluated resistance to ciprofloxacin and trimethoprim) (99). There was limited data collected on the general practice or clinician level factors so I was unable to evaluate if these factors were associated with antibiotic prescribing. I also could not account for any changes in antibiotic prescriptions either by the GP or pharmacist which were not captured in the study CRF at baseline nor adherence of the patient in taking the prescribed antibiotics (antibiotic consumption) for the duration of treatment. However, a study on LRTI in primary care found that patient reported levels of adherence to study medication (amoxicillin or placebo; two 500mg tablets three times a day for 7 days) was very high (88%) (100). Although I did not capture data on changes in prescriptions prior to dispensing the medication the aim of this study is to look at how patients are managed by the GP at the time of consultation; any changes to the prescriptions after the consultation are likely to be minimal (personal discussion with the POETIC study Investigators/GPs).

The guidelines I used for comparison were developed by the POETIC study team principal investigators and clinicians for each network based on each countries

guidelines (appendix 3.2.4 – 3.2.6). There are other guidelines that the GPs may prefer to follow (for example the British National Formulary for UK GPs). Other barriers described in a systematic review of the literature include lack of familiarity and awareness (volume of information, time needed to stay informed, guideline accessibility); lack of agreement with specific guidelines and guidelines in general (interpretation of evidence, applicability to the patient, not cost-beneficial, too rigid to apply, not practical, lack of confidence in guidelines developer); lack of outcome expectancy; lack of self-efficacy; lack of motivation/inertia of previous practice; external barriers (patient preferences, lack of time, lack of resources, lack of reimbursement, presence of contradictory guidelines) (101). The Cardiff and Southampton networks have the same guidelines but the Cardiff network showed a much higher accordance in first-line prescription duration than Southampton. This may be down to the fact that Southampton prescribed nitrofurantoin much more than Cardiff which mainly prescribed trimethoprim. Both are recommended first-line treatments but when nitrofurantoin is prescribed the discordancy in total dose/duration increases. The BNF (British National Formulary) agrees with the UK networks guidelines on the daily dose of nitrofurantoin with a recommendation of 3 -7 days; the BNF also agrees with the daily dose of trimethoprim but has no recommendation on the duration (102). If the study clinicians are using this instead then the empirical prescriptions may still be considered appropriate.

Another multi-European observational study (GRACE) describing antibiotic prescribing for adults with acute cough/lower respiratory tract infection found that Cardiff, Southampton and the Netherlands networks prescribed the preferred antibiotic choice (type) in over 75% cases. The Spain networks in this GRACE study (Barcelona and Mataro) had much lower preferred antibiotic choice and tended to prescribe alternative antibiotic options (103). This agrees with the results in this study that the Cardiff, Southampton and Utrecht networks prescribe first-choice antibiotic type and the Madrid/Catalonia networks are better at prescribing first choice antibiotics for UTI than acute cough although this did not include dose and duration.

The CRF captured data on other medications both prescribed and advised; I have only presented data on paracetamol and ibuprofen as other medications were minimal. The Madrid/Catalonia network was the only network to prescribe paracetamol (Cardiff and Southampton prescribed to one and two patients respectively). Paracetamol is an over the counter common medication which does not need a prescription in the UK. It may help to reduce fever and alleviate mild to moderate pain; the Southampton network had by far the highest advice on paracetamol and much higher than the Cardiff network (as both part of the UK). The Southampton network did have the highest prevalence of symptoms of fever and time off work which may explain this advice. The Madrid/Catalonia network was also the only network to prescribe ibuprofen although this was minimal and the Southampton network once again advised most on ibuprofen.

Patient Management: Advice and Follow-Up

The most common advice across all networks was to drink more fluids, water in particular.

There were a lot of various pieces of advice given broadly covering fluids and consumption; worsening symptoms; treatments; hygiene and sexual health; and antibiotic use. Some of the less common advice included ‘using a shower instead of a bath’ and to ‘avoid citrus fruit’. A review of the literature by Car J., summarised that randomised trials indicate that drinking cranberry or taking cranberry concentrate tablets reduces the risk of symptomatic, recurrent infection by 10% to 20%; post-coital voiding does not prevent cystitis; there is no evidence that poor urinary hygiene predisposes women to recurrent infections; and there is no rational for giving women specific instructions regarding the frequency of urination, the timing of voiding, wiping patterns, douching, the use of hot tubs, or earing of pantyhose (19).

It may be interesting to look at advice given by practice and/or clinician to see if the advice is specific to a practice/person or more generalised. The Southampton network offered the most and most varied advice whereas the Spain network only advised on increasing fluids and passing urine/emptying bladder fully.

Routine follow up is not recommended for lower urinary tract infections but is recommended for upper urinary tract infections after the treatment is completed (19). The UK networks had limited follow-up, Spanish GPs requested most follow-up with patients 8-14 days after the consultation and the Utrecht network generally requested patients follow up for urine test results.

A report from the UK Department of Health's Standing Medical Advisory Committee (SMAC) Sub-Group on Antimicrobial Resistance in 1998 advised that prescribing antibiotics after telephone consultation should be limited to exceptional cases, however, under normal conditions both the Southampton and Utrecht networks in this study would have managed more cases by phone than face to face. As the guidelines recommend prescribing empirically for patients with obvious symptoms of uncomplicated UTI then telephone consultations may be acceptable for those patients. The Utrecht network had problems recruiting because of this system although the Southampton did not and actually recruited the most patients overall. After personal communication with one of the Southampton's networks Principal Investigators (Dr M Moore), I learnt that the Southampton trial sites changed their usual practice to be able to recruit for the trial. As an observational study this may have caused some selection bias but was unavoidable if recruitment was to be achieved.

Limitations

Patient outcomes were not evaluated as they were not part of my study aims and objectives.

Although the CRFs were completed for almost all patients and diary returns was reasonable across the networks, individual questions were not always completed so the overall numbers for certain data points were reduced. This was particularly the case when developing the multilevel model for antibiotic prescribing as only patients with data for all response and explanatory variables could be included. As one of the variables

reduced the sample size significantly (history of treatment) this variable had to be excluded from this model.

There was large variation in the number of practices recruiting per network and the number of patients recruited to each practice. This led to more clustering; the lower the number of patients recruited to a practice the less likely there will be within practice variation (although only cluster sizes of 5 or more were included in the models). The original sample size calculation was based on an assumption ICC of 5.7% however this was up to four fold higher in the multilevel analyses performed by myself. The sample size calculation was not generated with my research questions in mind but as part of the larger POETIC observational study as a result confidence intervals are wider than initially specified. For example the ICC value for the response variable for 'antibiotics prescribed (type only) according to relevant guidelines indicating first-line treatment for women with uncomplicated UTI' was calculated as 0.16 (n=662) resulting in a design effect of 4.059 and effective sample size of 163, +/- 8% precision margin. In order to have obtained the +/- 5% margin initially proposed, in a population with an ICC of 0.16 and cluster size of 20, the number of completed CRFs would have had to be 1563.

There was also limited data collected on the general practices such as the information about the clinicians taking part (who consulted with the patient, sex, age, years in practice), whether the practice was in a rural/city or affluent/deprived area, size of the practice in terms of staff and patients registered. This could not be used in the multilevel modelling to look at level 2 effects; which were shown to be important by the large VPC and MOR values. Likewise there was limited data collected on the patient demographics such as ethnicity, social status, education, marital status that may have had associations with the outcomes.

Sources of Bias

As this was a prospective observational study under the management of four established European primary care research networks there may have been some selection bias. Firstly the selection of the participating practices was not random or selected from the

whole of each country; therefore, the generalizability of the findings may not be appropriate to each country which the network represents. For example, practices agreeing to participate may have clinicians or nurses that are more interested in research or UTI than other general practices. Networks may have recruited practices that are research experienced and that have good recruitment potential. However, a random sample of practices across the whole of each country would not have been feasible and the networks should still represent the diversity and influence of contextual, health service and cultural variations across clinical outcomes.

Participating clinicians were asked to register sequential patients with symptoms of UTI and assess their eligibility for the study. There may have selection bias by the recruiting clinicians; they may have selected women they thought were more likely to participate fully by completing diaries and providing urine samples; women who were less ill may have been easier to recruit; patients not fluent in the networks native language may have not been selected; consultation type could have led to selection bias i.e. patients that usually consult via telephone may not have been recruited and therefore only patients that were available for a face to face consultation were selected. I did not look at any differences between women who agreed to participate and those that declined or were not invited to participate as part of my research.

The interpretation of what constitutes an uncomplicated UTI may be very clinician/GP specific and variable between participating clinicians and/or practices. The inclusion criteria was broad which avoids influences from the study team over the clinicians in determining which patients have an uncomplicated UTI.

Although this was an observational study of routine practice, influences of the research networks study teams were unavoidable in that data had to be collected through study designed CRFs, patient diaries and direct contact during follow-up for the return of patient diaries. Clinicians and patients were aware they were participating in a research study and this may have influenced them in some way.

Systematic non-response to the CRF and/or diary (either by the patient or clinician) could lead to bias; ideally all data should have been collected for every patient recruited into the study, however this was not the case.

Some of the questions in the diary used for my analyses included ‘history of UTI and treatment’ which may have led to recall bias.

Section 5: Overall Discussion

In this chapter I will summarise what is already known in this area, discuss the main findings and implications of the research that make up this PhD thesis, how my research can contribute to the antibiotic resistance public health problem, the main strengths and limitations of my research, and provide an overall conclusion.

How the thesis developed

This PhD originally focused on the development of chromatic sensing as a diagnostic test at the point of care for common infections. The first aim was to decide which common infection this test had the potential to diagnose; chronic obstructive pulmonary disease (COPD) and UTI were originally chosen but I quickly realised access to and working with sputum samples was problematic (working inside a containment level 3 laboratory and sputum samples are often small and very mucoid making them difficult to manipulate) and so the PhD focused on the diagnosis and management of UTI alone. In parallel the POETIC study was initiating and I joined this team with the aim of contributing to the UK Flexicult^{TM} SSI-urinary kit evaluation and the analysis of the observational management data. Within the first year of my PhD my supervisors and I concluded that the chromatic sensing test was not at a stage for further clinical evaluation and required more research on the physical and mathematical development which was not the aim of my research. However, as I was evaluating the UK Flexicult kit in a laboratory analytic performance study for POETIC I decided to include the chromatic sensing test at this stage to get some preliminary data on using this test with clinical samples (urine) which may contribute to future development of the test. My contribution to the optimisation of the set-up of chromatic sensing technique for the evaluation of urine samples has led to a publication (70) which may help direct future research in this area and the further development of this technique as a point of care test. Describing UTI management in primary care is essential step to minimising unwarranted clinical variation and the

systematic review and POETIC observational data enabled me to evaluate this. I hope that this thesis not only reflects the individual studies but also brings the studies together into one comprehensive piece of research that contributes to the initiative to improve appropriate antibiotic prescribing for uncomplicated UTI in primary care.

What is already known on this subject?

UTI is one of the most common bacterial infections affecting humans. Many women presenting to their clinician with classic (uncomplicated) UTI symptoms and/or history of UTI are prescribed an antibiotic agent empirically (prior to determination of an etiological diagnosis) by their GP without any additional testing to confirm if bacteria are present and if antibiotics are actually necessary; however, clinical score alone has been shown to have limited value (40, 44) and women of all ages are at increased risk of UTI after antibiotic use, exacerbating the problem (16, 21).

Further investigations at the point of care predominantly include dipstick urinalysis; but its use is limited by low negative predictive value – it is not useful in ruling out infection (44, 50) and therefore its use for improving appropriate antibiotic prescribing for uncomplicated UTI is questionable. Laboratory culture is not recommended for uncomplicated UTI (25) and if requested by GPs does not necessarily result in a change in antibiotic prescribing (86). Additionally it has been shown that patients symptomatic for UTI are often not found to be microbiologically positive for a uropathogen (32); although this may be due to additional factors such as poor sampling, transportation and storage of urine leading to cell death, the semi-quantitative and subjective nature of microbiological culture, and the (varying) definition of positive UTI (38).

Most countries have national or international guidelines for the management of (uncomplicated) UTI in primary care. Cultural factors play a substantial role in the production of guidelines, both in the selection of literature and in the formulation of recommendations. There are noticeable differences in selection of evidence between guidelines. For example Christiaens et al. compared four European guidelines and

showed that of 205 literature citations, only seven were shared between three guidelines and only one by all four guidelines (Germany, Netherlands, Norway, Belgium) (104). More references were shared in the therapeutic sections than in the diagnostic sections; this may be partly due to lack of diagnostic trials in general practice. Most diagnostic data are from laboratory based studies which cannot be extrapolated to general practice; even the definition of UTI in a clinical setting is controversial (104). National antibiotic recommendations depend on local resistance rates and national drug licencing regulations. Even so, the current recommended treatment for uncomplicated UTI in most countries involves a limited number of first-choice options (one or two antibiotics) with higher dose, short duration antibiotic therapy (11, 22, 25, 105). The selection of mutational antibiotic resistance in bacteria is often promoted by prolonged antibiotic therapy, by infection sites where it is difficult to achieve high drug concentrations and by under-dosage (sub-lethal level for bacteria). It is generally accepted that the duration of antibiotic therapy influences the impact on gut flora, which may act as a reservoir of future opportunistic pathogens (6). There is also evidence that treatment with a variety of antibiotics causes a transient increase in both the proportion of *E. coli* from the normal flora that are drug resistant, and in the proportion of subjects who carry drug-resistant *E. coli* (106). National guidelines are often not adhered to and patient management including antibiotic prescribing varies between practices as well as countries. In a large study including a representative sample of general practices in the Netherlands, it was found that GPs followed UTI treatment guidelines in 42% cases and that level of adherence varied widely (0-95%) between practices (31). Likewise in the UK, whilst there was a 21% fall in the number of antibiotic prescriptions between 1995 and 2000 in general practice there was also a six-fold variation in the number of prescriptions between different general practices (1).

Surveillance of antibiotic usage is an important component of effective antibiotic stewardship but the data currently available often has significant limitations. In particular, although information is available on the overall levels of antibiotic prescribing in the community, information on the clinical conditions for which the drugs were prescribed is

limited, so it is not always possible to assess whether treatment guidelines are being followed (8). Nevertheless a recent UK survey has reported the proportion of women aged 16-74 years with selected UTI diagnoses linked to an antibiotic prescription who were prescribed trimethoprim was 53.5% in 2011 down from 62.1% in 1995 and the proportion prescribed a short course of antibiotic increased from 8.4% to 49.5% in 2011. There was increased prescribing of nitrofurantoin from 4.8% to 24.0%; and the proportion that was short course increased from 5.9% to 20.1%. Yet again the variation between practices was particularly marked for treatment duration; with an IQR for the proportion of trimethoprim course that were short course of 16 – 71% in 2011(8).

Reducing unnecessary antibiotic prescribing is undisputedly important in minimising the selection of new resistance. Equally critical, though, is the choice of which antibiotic is used and at what dose and duration, for there are marked difference in selectivity both between and within drug classes. There is also a great need to improve and accelerate the development of suitable diagnostic tests for common infections and bacterial resistance, suitable for use at the point of care, thereby reducing or removing the need for protracted empirical treatment with broad-spectrum agents (107).

Study Outcomes

UK Flexicult SSI Urinary Kit

Main findings

The UK version of the Flexicult SSI-Urinary kit had never been evaluated for its analytic performance compared to the current UK reference standard prior to my study. As a result of this study I found;

- Cephalothin and co-amoxiclav antibiotic sections of the UK Flexicult plates fail to inhibit the growth of a sensitive strain of *E. coli* within their recommended 8 week shelf-life;
- The UK flexicult plate showed a high level of false positive results when compared to NHS routine microscopy and culture, after evaluating the plates for

quantification, identification, and predominance. The sensitivity of the test markedly improves when the plates are evaluated for quantification only so that any visible growth ≥10^5 cfu/mL (innumerable discreet colonies to growth covering the plate) may be a UTI but growth <10^5 cfu/mL is likely to be another diagnosis;

- Diluting turbid urines prior to inoculation on the UK Flexicult plates did not improve the diagnostic accuracy when evaluating the plates for quantification, identification, and predominance;
- Other than trimethoprim and 1st generation cephalosporins the UK Flexicult plates showed higher levels of resistance than routine NHS susceptibility testing;
- The plates are highly subjective; after training the GPs and providing both the manufacturers manual and access to a website with guidance on reading the plates the GPs only showed moderate strength of agreement with myself compared to the more experienced biomedical scientists who showed substantial strength of agreement.

Implications

Implications and recommendations based on the UK Flexicult evaluation study include;

- Informing the design and management of the POETIC RCT by:
    - developing the Flexicult CRF for the RCT;
    - the manufacturers' revised the UK Flexicult brochure based on my experience of using the kits;
    - developing the POETIC Flexicult training website;
    - shortening the shelf-life of the UK Flexicult plates from 8 weeks to 6 weeks prior to using the kits in the RCT.
- Recommending the use of the plate as a screening test rather than a diagnostic test in primary care. Not only does the sensitivity of the test improve, the plates are much simpler to read when quantification is the only criteria.
- By diluting urines 1 in 1000 the detection limit of the plates is >10^5 cfu/mL; if this test is used for screening out negative samples only this could be a simple way

to differentiate between positive and negative samples i.e. if there is any bacterial growth after inoculating the plate with urine diluted 1 in 1000 the patient may have a UTI and further testing and/or empirical prescribing of antibiotics is recommended.

- Co-amoxiclav and cephalothin should not be included in a point of care culture based test such as Flexicult that requires extended shelf-life for transport and storage.
- The plates could be changed to remove the antibiotic sections altogether if just used as a screening test. Alternatively the plates could be used to test for susceptibility to recommended first-line antibiotics only i.e. in the UK this would include trimethoprim and nitrofurantoin. This would increase the surface area for inoculation and bacterial growth and should improve interpretation.
- To use the UK Flexicult plates to their best performance there needs to be appropriate user training and guidance; this may be in the form of an interactive web based system or workshops. As the kits are used more frequently the understanding and performance of the user should increase.

Chromatic sensing

Main findings

There was no published evidence that a novel chromatic sensing technique had ever been applied to identify bacterial infection of urine prior to my research, and my findings have shaped the research agenda regarding using chromatic sensing as a POCT for diagnosis of common infections in primary care. Chromatic sensing had a similar analytic performance to visually assessing urine with a sensitivity of 100%. However the system needs to be evaluated based on various users, equipment and locations, particularly as the fundamental process of this system uses ambient light and screen illumination.

Implications

Chromatic sensing removes the subjectivity or uncertainty of assessing the turbidity of urine by the assessor and may be useful in practice if GPs unwilling to use their own perception for screening out negative samples.

Further development of the chromatic sensing system is required to formalise the equipment set-up, automate the results for instant output and ideally improve the ability to identify true positive samples before further clinical and economic evaluations.

Visual turbidity and dipstick urinalysis

Main Findings

The laboratory evaluation study showed that visually assessing urine as clear (non-turbid) had a 100% sensitivity (no false negative results). From the observational study I found that half the urines from patients were assessed as clear by the study clinicians; so unless symptoms or patient history are highly indicative of UTI, antibiotic prescribing could possibly be reduced by 50% using this simple method. However, when I analysed the observational turbidity data with the associated microbiology results 8% of the samples had a false negative result.

Urinalysis dipsticks are the most commonly used POCT for uncomplicated UTI across the four networks evaluated in the observational study and from the studies evaluated in the systematic review.

Analysing the data from the UK networks only; recording a nitrite negative result could screen out more patients than recording a leucocyte negative result (69% versus 19%), however, more women with true UTI would be missed using the nitrite negative urinalysis result (12% versus 3%). The laboratory evaluation of dipsticks showed a similar trend in that negative nitrite results were more prevalent but negative leucocyte results had lower numbers of false negative results. All networks' guidelines indicate a positive nitrite result as probable UTI. From the laboratory evaluation study, I found the most accurate prediction rule to be 'visually turbid urine with either positive nitrite or leucocyte urinalysis dipstick result' as indicative of a bacterial UTI. However this is not replicated when

analysing the data from the observational study. None of the urinalysis dipstick tests or evaluation of urine turbidity perform well at confirming a microbiologically positive UTI.

Although urinalysis dipsticks are the most commonly used POCT for uncomplicated UTI this does not have an impact on antibiotic prescribing as in the UK as >92% patients were prescribed at least one antibiotic suggesting there may be different reasons for their use. I found that negative nitrite results are associated with an increase in routine culture requests and the use of dip-slides (in Utrecht). Having a positive leucocyte result was significantly associated with prescribing an antibiotic that was different to that recommended by the guidelines (for type, total dose and duration).

Implications

Current UK guidelines indicate that if a urine is not cloudy to consider another diagnosis; further diagnostic testing and/or empirical treatment is not recommended. Even though almost half the urines in the UK networks were assessed as clear the majority of patients still had a urinalysis dipstick test performed and were prescribed an antibiotic. This suggests that GPs are not confident in using this recommendation in practice.

Although the visual assessment of turbidity was not as sensitive when evaluating the POETIC data as the laboratory evaluation data this study was not set up as a diagnostic evaluation study. In the laboratory evaluation study I was the only assessor of whether the urine was turbid or not and I had a set of criteria that helped to assess the degree of turbidity in the urine.

I believe there is importance in evaluating the visual assessment of urine as a simple screening test for UTI in a pragmatic diagnostic study. The use of set criteria for turbidity, a visual comparator/aid (similar to McFarlands standards) or the use of a turbidimeter may improve the sensitivity and reduce subjectivity in practice. Positive results from such a study may improve the adherence to HPA guidelines in practice.

The results of my studies suggest that urinalysis dipsticks are not useful in guiding GPs management of UTIs. Negative nitrite results have too high a risk to patients with a true infection being missed and the proportion of patients with a leucocyte negative result are too small to make the test particularly useful.

Routine Culture and dip-slides

Main findings

Routine culture requests for patients with suspected uncomplicated UTI in the UK (60% in the Cardiff and Southampton networks) are much higher than should be expected if the management guidelines for UTI in primary care were being followed. Routine culture requests were significantly associated with increasing age, night-time frequency and a nitrite negative dipstick test results which could be causing the GP uncertainty in the diagnosis.

In the POETIC study Utrecht was the only network to use dip-slides as a POCT. Utrecht is also the network with the lowest level of antibiotic prescribing although I am unable to say if this directly a result of using the dip-slides.

However, I did find that not prescribing antibiotics was significantly associated with requesting samples for routine culture/or the use of dip-slides. There could be reverse causality involved and it is difficult to know if not prescribing an antibiotic results in a request for urine culture or if requesting samples for culture stops antibiotic prescribing.

The prevalence of microbiologically confirmed UTI particularly in the Utrecht and to a lesser extent the Spanish networks was much higher than in the UK networks. The prevalence of microbiologically confirmed UTI in the UK networks of the POETIC study and the Flexicult laboratory evaluation study were similar.

Implications

GPs in the UK rely heavily on the NHS for routine urine culture even when it is not recommended and this could be due to diagnostic uncertainty. This could be alleviated by the introduction of a test such as Flexicult that could screen out negative samples at the point of care reducing the burden on the NHS laboratories and supporting the GPs diagnosis and management decisions associated with suspected UTI or not.

Antibiotic prescribing

Main findings

- Antibiotic prescribing for uncomplicated UTI in the UK and Spanish networks was found to be over 90%. The Dutch network was lower at just under 60%.
- The UK networks prescribed first-line antibiotics according to the country specific guideline recommendations in the majority of cases.
- The Spanish and Dutch networks also prescribed antibiotics according to their country specific guidelines in over 80% cases.
- Prescribing an antibiotic that was not recommended as first-line was associated with the feeling of being unwell, having a high temperature and having no or limited burning sensation when passing urine. History of UTI was also associated (but I was unable to include this in the multi-level model as the sample size was too small).
- The duration of the prescribed antibiotic course was often longer than recommended.
- Discordant treatment regimens including dose and duration were associated with network (Southampton or Spain), three or more prior antibiotic treatments for UTI in the past and having a leucocyte positive dipstick which could cause the GP to consider a more complicated/resistant infection.
- Variation in prescribing according to the guidelines between practices was shown and unexplained level 2 variation from the multilevel models was large.

Implications

- Concern over diagnostic uncertainty of more complicated UTI is associated with prescribing alternative antibiotics to recommended first-line or for longer than recommended – removing this diagnostic uncertainty may help GPs follow the guidelines.

- For currently available antibiotics manufacturers could be asked to develop infection based, user friendly packaging/blister packs for various treatment regimens for community acquired infections, for example, uncomplicated UTI blister pack of trimethoprim would contain 6 doses of 100mg to be taken twice a day for three days. This would have cost implications that would need to be evaluated.
- Research into antibiotics that are currently unlicensed in the UK such as fosfomycin and pivmecillinam would be worthwhile as resistance levels should be low (but this will need to be studied) and patient management and compliance should be more plausible (for example fosfomycin is a single dose).
- Pharmacist led quality control checks of prescriptions could improve concordance with the guidelines.
- Future studies would benefit from collecting data on the GPs, GP surgeries and other level 2 variables which may explain the difference between practices.

What does my research add to fight against antibiotic resistance?

The current recommendation is to prescribe empirically for suspected uncomplicated UTI; the systematic review and observational study show that antibiotics are being prescribed empirically when a GP suspects uncomplicated UTI. The systematic review showed that within published studies definitions of uncomplicated UTI vary greatly. Results from the POETIC observational study also suggest that diagnostic uncertainty leads to antibiotic prescribing outside of the recommended guidelines. In primary care, tests at the point of care tend be more useful in screening out samples rather than confirming a diagnosis (76). With further pragmatic evidence visually assessing urine for turbidity and the UK Flexicult kits could both be useful in screening out large proportions of patients from empirical prescribing. This could remove diagnostic uncertainty for those GPs and patients but also reduce empirical antibiotic prescribing by half. Where GPs do want a definite diagnosis, routine NHS culture and susceptibility testing is the reference

standard; however by screening out a large proportion of samples that do not need to be processed the NHS laboratories can focus more on uncertain or complicated UTIs.

There was a recommendation by the UK Department of Health's Standing Medical Advisory Committee (SMAC) Sub-Group on Antimicrobial Resistance in 1998 limiting prescribing for uncomplicated cystitis to 3 days in otherwise well women which this study has shown is still not being consistently adhered to in 2014. Of the remaining patients that do require empirical prescriptions GPs may be more assured in adhering to guidelines of short course high dose therapy which will limit the collateral effect of resistance.

Strengths and weaknesses

The limitations and sources of potential bias have been discussed previously for each study included in this thesis. This section will discuss what I consider to be main the strengths and weaknesses of my overall research.

The laboratory evaluation study had a limited sample size of 200 samples; this was greatly reduced for the susceptibility analysis. There may be concern that the samples were from a highly selective population, however, samples were not selected based on any information other than suspected UTI. The proportion of microbiologically confirmed UTI positive samples in this study was within the approximate usual proportion given by Dr Robin Howe, Director and National Lead for Microbiology Services, Public Health Wales, University Hospital of Wales. It was also similar to the proportion of positive samples in the POETIC observational study (UK only). Although not all samples were derived from the target population where the test may be used i.e. patients consulting in primary care with suspected uncomplicated UTI, the laboratory based studies were to evaluate the analytical performance compared to the reference standards which are currently and routinely used in NHS practice. The prevalence of infection from the hospital based samples used in the performance evaluations most likely over-estimated the prevalence of infection and prevalence of resistance in the target population as these

samples were from in-patients and the samples from out-patients may well have been selected for referral to routine culture due to complications or concerns of a resistant infection by the GP. Likewise there were probably more contaminants from these laboratory samples than there would be in practice (requesting urine directly from the patient, possibly using a MSU collection device and performing the POCT within a few hours; no transport or storage/time delay effects on the samples) but this should result in the tests performing better at the point of care than in the laboratory.

If the diagnostic tests were found acceptable at this stage the next step would be to test with the target populations and at the point of practice in a pragmatic randomised study. These laboratory studies also provide information on the clinical practicalities such as potential difficulties for naïve users; and flaws in the design and quality control issues; which can go on to inform further research.

Using the defined search criteria the systematic review found there are limited published studies evaluating the routine management of uncomplicated UTI in primary care in Europe. Even within these studies the source populations vary due to different recruitment and enrolment processes. This has been highlighted as a major limitation and studies that had completely different recruitment processes were excluded from the synthesis of results.

The POETIC observational data provides evidence of the actual routine management of patients consulting primary care with uncomplicated UTI. The main limitation for this part of my thesis was that the sample size was not generated with my research questions in mind and the maximum potential level of clustering accounted for was 5.70%. There were much higher levels of clustering for prescribing antibiotic type according to the guidelines, requesting a routine urine culture and prescribing antibiotic type, dose and duration according to the guidelines (ICC=0.16; 0.23; 0.33 respectively) at practitioner-level within the sample than initially predicted. This has resulted in a larger design effect and smaller effective sample size. As I am unable to increase the sample size the precision around the confidence intervals are wider than initially specified. Because this study was underpowered, Type II errors, incorrect failure to reject a false null hypothesis

(false negatives), may have occurred. In order to be confident in future studies that no similar errors will occur, the sample size should be specifically calculated with the modelling of such predictors in mind. Additionally, the univariate analyses used to determine which predictors should go into the models involved multiple testing and may have caused Type I errors (false positives); the p-values and associations described within this preliminary analysis need to be interpreted within the context of the multilevel models framework.

Much research data is routinely acquired, that is, it is derived from data entered into routine databases or samples received in hospital laboratories, with little knowledge of why it was referred and how representative it is to the general population. In practice, firstly, a symptomatic patient has to make the decision to consult a clinician, then the general practice system determines whether this complaint warrants consultation, then upon consultation (which may be by telephone), a clinician diagnoses the patient and decides whether to prescribe or not based on clinical symptoms or history alone or to use additional diagnostic strategies. Changes in antibiotic use can be due to changes in any of these decisions and procedures – simply comparing prescribing data at the level of clinician may not provide comparable data (9). Observational studies provide data at the individual level and provide information about clinical infections in large populations of representative patients but are highly vulnerable to bias and confounding. The POETIC study used broad patient inclusion criteria and a data collection protocol was used by all networks. However, there was limited data collected on secondary level effects such as clinician gender, age, experience, practice location (rural/city/affluent/poor) and practice size, which considering the level of clustering may have been useful to explain some of the between practice variance. The fact that both the POETIC study and systematic review have demonstrated similar findings (e.g. high levels of empirical prescribing and not following short term treatment) is convincing evidence that the associations are real and has not been produced by chance, confounding or bias. Consistency with other studies means that the most persuasive evidence to support a judgement of a cause-effect relationship arises when ‘a number of

studies, conducted by different investigators at various time using alternative methodology in a variety of geographic or cultural settings and among different populations, all show similar results' (108).

Final Conclusions

I have met the overall research aim of my thesis. Firstly, I have evaluated the analytic performance of various POCTs for uncomplicated UTI and provided recommendations for future research and use in clinical practice. Secondly, I have described the management of uncomplicated UTI in primary care in Europe both through a systematic review of the literature and from the analysis of observational data from a multi-centre, multi-national primary care based study.

This thesis continues to support the evidence that antibiotics are being prescribed empirically for uncomplicated UTI. First-line antibiotics are generally being prescribed according to relevant guidelines, however, duration of the prescribed antibiotic course was often longer than recommended. Concern over diagnostic uncertainty of more complicated UTI is associated with prescribing alternative antibiotics to recommended first-line or for a longer course than recommended – removing this diagnostic uncertainty would help GPs follow the guidelines.

POC tests in primary care are most useful in their clinical reasoning by ruling out UTI particularly in cases of ambiguous clinical signs and symptoms; confirming the need for further testing in more complicated cases; or empirical prescribing with a test of treatment in uncomplicated cases (109). This is apparent in two of the POC test evaluated in my study. By using quantification only, irrespective of predominance or identification, the sensitivity of UK Flexicult was increased to 100%; indicating this POC culture test is most useful in screening out negative urine samples prior to antibiotic prescribing and/or sending samples for diagnostic culture and susceptibility testing. Likewise my laboratory study showed a sensitivity of 100% for the visual assessment of urine turbidity again indicating its potential as a highly effective screening tool in primary care. Current UK

guidelines recommend that if a urine is not cloudy to consider another diagnosis to UTI and that further diagnostic testing and/or empirical treatment is not recommended. Even though almost half the urines in the UK POETIC networks were assessed as clear the majority of patients still had a urinalysis dipstick test performed and were also prescribed an antibiotic. This suggests that GPs are not confident in using this recommendation in practice. The use of set criteria for turbidity (such as the criteria used in my study), a visual comparator/aid (similar to McFarlands standards or the BronkoTest for sputum) or the use of a turbidimeter may improve the sensitivity and reduce subjectivity in practice. Positive results from pragmatic studies of these tests may also improve the adherence to guidelines in practice.

I have been able to describe the variations in management between countries and to a limited extent between practices. The variations did not prove to be warranted with respect to patient demographic or symptom severity when consulting and the management processes employed could be more efficient between practices and countries. Variations included lower empirical prescribing in the Netherlands network compared to the UK and Spanish networks. Additionally variations were seen in prescribing first-line antibiotics and duration of the course of prescriptions across the networks. Guidelines do vary from network to network, for example, the Netherlands only have one recommended first-line antibiotic so the higher level of prescribing an alternative antibiotic to first-line may not be surprising. Interestingly the UK networks in Wales and England which do use the same guidelines did still show differences in the adherence to prescribing the correct duration of treatment. It's imperative that future studies collect data on the secondary level factors to do with the GPs and practices that can then be evaluated when looking at these variations which were not possible in my analyses. Having a standardised guideline for the management of uncomplicated UTI between regions and countries may not be straightforward or appropriate. As one example, analysing the POETIC data has shown that countries such the Netherlands and Spain have a much higher prevalence of microbiologically confirmed UTI which in

turn shows the value of different diagnostic tests is variable depending on the region it is used in.

Overall I found the most interesting outcomes from my PhD research to be the continued prescribing of antibiotics for a duration longer than recommended even though over 10 years ago there was a report from the UK Department of Health's Standing Medical Advisory Committee (SMAC) Sub-Group on Antimicrobial Resistance that recommended limiting prescribing for uncomplicated cystitis to three days in otherwise fit women (8). POC tests in primary care could be incredibly useful in helping clinicians decide whether to prescribe an antibiotic or not, however, these tests need to be simple, non-subjective and accurate, which currently they are not. For example, from both the systematic review and observational study urinalysis dipsticks were shown to be the most commonly used POCT for uncomplicated UTI, however their use did not show any associated reduction in antibiotic prescribing. Alternatively my studies have shown greater potential for both a basic visual assessment of turbidity and the use of a simple culture based test for screening out patients with uncomplicated UTI, however, pragmatic evaluations are needed to support and ultimately encourage their use in practice.

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Appendices

2.1.1 Flexicult laboratory evaluation ethical and research and development approval letters

NHS
Health Research Authority

NRES Committee North East - County Durham & Tees Valley
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TEDCO Business Centre
Viking Industrial Park
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13 August 2012

Prof Christopher Butler
Cardiff University
School of Medicine
5th Floor
Neuadd Meirionnydd
Heath Park
Cardiff
CF14 4XN

Dear Prof Butler

Study title:	**Laboratory Evaluation and Development of Rapid Diagnostic Tests for Urinary Tract Infection**
REC reference:	**12/NE/0306**
Protocol number:	**SPON 1142-12**

The Proportionate Review Sub-committee of the NRES Committee North East - County Durham & Tees Valley reviewed the above application on 13 August 2012.

Ethical opinion

The sub-committee noted the following ethical issues and Miss Emily Bongard (Research Student) responded as follows:

1. Members noted that on the filter page of the IRAS form question 2c appeared to contradict the answer given to 2a and asked the research team to resolve this. It was further noted that the statements made in question A6-2 of the IRAS form, and elsewhere within the application, indicated that 2c was correct and 2a was not; the Committee therefore requested that if that was the case the research team should confirm this.

   Miss Bongard explained that filter question 2a point a) was completed incorrectly and confirmed that they would not be taking new samples at any stage during this study and therefore should be completed as 'no'. 2a point c) was correct - yes they would only be using surplus tissue or existing stored samples not identifiable to the researcher.

2. The Committee noted that the answer to B4-15 of the IRAS form stated that samples would be returned to the current holder but also that non relevant materials would be disposed off. Given that human urine is a relevant material under the Human Tissue Act the Committee asked the researcher team to resolve the apparent contradiction and conflicting plans, and in doing so to specify what would be returned to the

sample holder and what would be disposed of. If it was intended to return the sample, Members requested clarification what the purpose is; as the researchers would be unable to link the sample back to the person from whom it was obtained it was unclear why they would want it back or what they would do with it once they got it back.

Miss Bongard explained that the researchers will get varying amounts of anonymised urine (anything above 5 ml) from the laboratory staff, depending on the quantity left over from the routine testing. She confirmed that the researchers would use approximately 3 - 5 ml for the flexicult test and 2 -3 ml for the dipsticks and chromatic sensing testing, and therefore would take approximately 5 - 8 ml of urine. The remaining urine (unused) would be returned to the custody of the PHW Laboratory staff (after which she commented that she did not know what would happen to it), and any 'used' urine would be disposed of after each test was completed. However, Miss Bongard commented that the researchers could dispose of the unused urine as well, as like the Committee mentioned it would be anonymised and unable to be linked to the patient.

The Sub-Committee were satisfied with Miss Bongard's response and agreed that it would make sense for the research team to correctly dispose of both the used and unused urine providing the PHW laboratory was in agreement.

3. Members noted that section B5 of the IRAS form referred to different storage and disposal arrangements and asked the research team to resolve this apparent contradiction and conflicting plans. If the latter is an error, related to the possible error in completing screening question 2 - i.e. the study does not involve any new sampling – the Committee requested that the research team clarify this.

   Miss Bongard replied that Part B section 5 should not have been completed - this was an error due to the incorrect completion of filter question 2a and requested that Committee disregard this section.

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Confirmation should also be provided to host organisations together with relevant documentation.

Approved documents

The documents reviewed and approved were:

Document	*Version*	*Date*
Covering Letter	1.0 - 030812	03 August 2012
Evidence of insurance or indemnity	1.0	22 June 2012
Investigator CV	Chris Butler,	29 May 2012
Investigator CV	BongardEJ	04 May 2012
Letter from Sponsor	SPON1142-12ButlerC, v1.0	02 August 2012
Other: CV - GaIM		31 May 2012
Other: Peer Review Frank Dunstan	v1.0	03 August 2012
Other: Peer Review InaamHaq	1.0	13 April 2012
Other: Peer Review Robert Newcombe	1.0	13 April 2012
Other: Study Peer Review Investigator Comments	1.2	27 April 2012
Protocol	2.1	26 July 2012
REC application	1.0 060812	06 August 2012
Summary/Synopsis	Flow Diagram Evaluation, 1.0	03 August 2012

Membership of the Proportionate Review Sub-Committee

The members of the Sub-Committee who took part in the review are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/NE/0306	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

pp
Dr A MacSween
Chair

Email: sarah.grimshaw@sotw.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

"After ethical review – guidance for researchers" SL-AR2

Copy to: *Miss Helen Falconer, Cardiff University*
Dr Edward Guy, Public Health Wales Trust

NRES Committee North East - County Durham & Tees Valley

Attendance at PRS Sub-Committee of the REC meeting on 13 August 2012

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr EA Baker	Clinical Research Scientist	Yes	
Dr A MacSween	Principal Lecturer in Research Governance	Yes	
Mrs Sophie Welch	Research Governance & Policy Officer	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Sarah Grimshaw	Assistant Co-ordinator
Miss Hayley Jeffries	REC Co-ordinator

GIG CYMRU NHS WALES | Iechyd Cyhoeddus Cymru Public Health Wales

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Institute of Primary Care and Public Health
Cardiff University
School of Medicine
5th Floor, Neuadd merionnydd
Hath Park
Cardiff
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Wednesday 5th September 2012

Dear Professor Christopher Butler,

Re: 2012PHW0018 Laboratory Evaluation and Development of Rapid Tests for UTI

The above project was assessed by the Research Risk Review Committee held on Tuesday 4th September 2012 at which the following Committee members were present:

Prof Gareth Williams (Committee Chair), Cardiff University
Dr Jim Fitzgibbon, Lay Member
Mrs Nicola Heales, Lay Member
Anthony Arcari, Professional lead for Health and Safety, Public Health Wales
Dr Edward Guy, Head of R&D, Public Health Wales
Kathryn Ashton, R&D Coordinator, Public Health Wales
Catherine Turpin, R&D Secretary, Public Health Wales

I am pleased to confirm that the study has been **approved** by the Committee and may commence within Public Health Wales.

Approval lapses if the project does not commence within 12 months of Trust approval. The Committee reserves the right to be provided with information on the progress of the project at any time.

Random audits may be carried out to ensure that the project complies with the relevant Research Governance Framework standards. If any amendments to the project are required, amended versions of all documentation should be forwarded to the R&D Office for consideration and approval prior to any changes taking place. Any serious adverse incidents relating to the project must be reported to the R&D office.

Please inform the R&D office when the project is completed. A final written report is required at this time before the project can be closed.

If you require any further information please contact Catherine Turpin Public Health Wales R&D Office, Unit 1 Charnwood Court, Heol Billingsley, Parc Nantgarw, Cardiff CF15 7QZ.

Yours sincerely

Dr Edward Guy
Head of Research and Development, Public Health Wales

2.1.2 Flexicult data worksheet

Laboratory Evaluation and Development of Rapid UTI Diagnostic Tests

Worksheet 1: Flexicult™ SS Urinary Kit

Processing Details:

Study Number	Date Inoculated	Time Inoculated	Initials	Date Read	Time Read	Initials	Culture Time

Processing Comments:

Quantification and Identification:

	FLEXICULT Lot No:			Expiry Date:		Colorex UTI Media Lot No:			Expiry Date:	
Organism	Colony Count*	CFU/mL	Colour	Size (mm)	Bacterial ID	Colony Count*	CFU/mL	Colour	Size (mm)	Bacterial ID
1										
2										
3										

*0 = no growth, ≤20 = record number, 21 -100 = +, semi-confluent = ++, confluent = +++, contaminated = contaminated

Quantification and Identification Comments:

Susceptibility:

Plate section	Antibiotic	Organism 1 ID:		Organism 2 ID:		Organism 3 ID:	
		Colony Count*	S/R	Colony Count*	S/R	Colony Count*	S/R
1	Cephalothin						
2	Ciprofloxacin						
3	Amoxicillin/Clavulanate						
4	Nitrofurantoin						
5	Trimethoprim						

Susceptibility Comments:

Image Captured (Y/N)		Date		Time	

Final Report	

Staff Name: Staff Signature: Date:

Checked By: *Signature:* *Date:*

Version: 0.4 Date: 24/09/12

2.1.3 Diagnostic Performance Measures

Sensitivity and specificity assess the tests diagnostic accuracy. Sensitivity shows the likelihood of a positive test result if an individual were to truly have the disease. The specificity shows the likelihood of having a negative test result if an individual does not have the disease. The sensitivity and specificity are characteristics of the test itself and are independent of the disease.

Disease prevalence is combined with sensitivity and specificity to create the **positive and negative predictive values**. The positive predictive value (PPV) is the probability that the patient has the disease when the test result is positive. The negative predictive value (NPV) is the probability that the patient does not have the disease if the test result is negative. The PPV and NPV are post-test probabilities, that is, they are the updated probabilities given the information provided by the positive and negative test results, respectively.

The error rate is a weighted average of errors among persons with the disease (the false negatives) and among those without the disease (the false positives).

The likelihood ratio combines into one number the sensitivity and specificity of the test. The likelihood ratio for a positive test is the sensitivity divided by one minus the specificity (Likelihood of a positive result in patients with the disease/likelihood of a positive result in patients without the disease). The likelihood ratio for a negative test gives the chances that a negative result will be expected in a patient who does not have the disease, as opposed to one who does. If test A has a higher positive likelihood ratio (PLR) and higher negative likelihood ratio (NLR) than test B, test A should be regarded as superior to test B; if one test has higher PLR but lower NLR, we cannot say unequivocally which is superior.

The receiver operating characteristics (ROC) methodology was developed specifically to enable display of the trade-off between sensitivity and specificity for any ordinal or continuous variable as the dichotomy point is altered. It is technically possible

to do the same thing for a binary predictor variable, but this doesn't add any value as there is just one point on the curve. The other important landmark on the plot is the diagonal line (at 45° if equal scales are used) from (0, 0) to (1, 1), which represents what a useless test would look like. The ROC curve is normally convex upwards. The most accurate ROC curve is one that arches up to the upper left-hand corner of the graph before moving to the upper right hand corner of the graph.

The area under the receiver operating curve (AUROC) is the same as U/mn, the Mann-Whitney U-statistic divided by the product of the sizes of the positive and negative groups. It serves as a very general measure of discrimination. The AUROC can range from 0 to 1, with 0.5 corresponding to the null hypothesis of no difference between positives and negatives.

2.1.4 Reproducibility data form

*Included are pages 1, 2 and 6; pages 3 – 5 are the same as page 2 with space for the remaining plate readings

POETIC
point of care testing for urinary tract infection in primary care

UK Flexicult™ SSI-Urinary Kit: Inter-Observer Reproducibility Study

Instructions:

1) Please take any time necessary to read and familiarise yourself with the Flexicult brochure; record the time taken in the table below.
2) Complete the table below prior to starting the evaluation and record the start and finish times for the evaluation of 60 test plates.
3) There are 60 Flexicult tests to read, numbered 1 -60, in the corresponding row complete the details of each test plate.
4) For test plates with 'no growth' OR 'mixed growth with no predominant organism' only complete the columns with the headings highlighted in white. For tests with pure or predominant growth (that is 10x greater growth than any other organism) complete the columns highlighted in grey as well.
5) The first 3 lines of the table (xxx) show worked examples of how to complete the table.
6) There are additional rows at the end of table (without test numbers) to use if needed (please enter the test number) and a comments table at the end of the document if you would like to record more detailed information for any test plate (e.g. difficult to read, unsure of the identification).
7) Once the evaluation is complete please provide your overall thoughts on reading/interpreting the Flexicult Tests in the box on the final page.
8) Please initial each page and record the time each page was started.

Name			
Initials			
Job Title			
Date			
Time Taken to read Flexicult Brochure	Prior to Today: Today:	Did you view the POETIC/Flexicult Website Prior to this Evaluation? ☐ Yes ☐ No Was it helpful? ☐ Yes ☐ No	
Time Evaluation Started		**Time Evaluation Finished**	

1/6

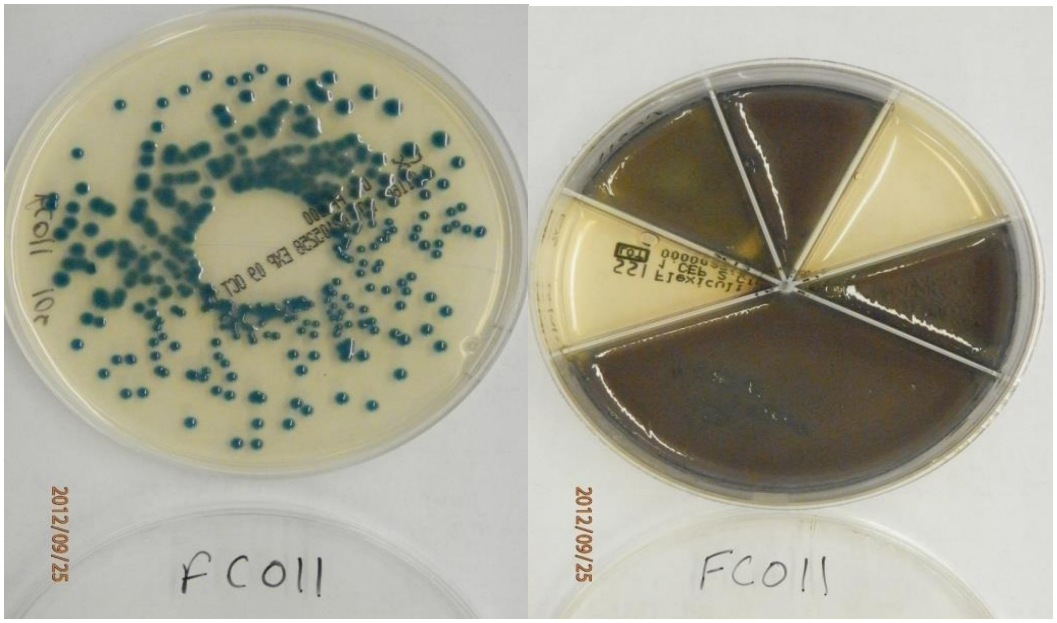
Initials: ..... Time: ..... Test No.	All Tests				If Pure or Predominant Organism (>10x) Present; Complete this Section						Susceptibility Profiles				
	Growth (✓)				Quantification (✓) cfu/mL			Colour of Pure/Predominant Colonies	Identification of Pure/Predominant Colonies (Bacteria) *if unsure, write 'unsure'	Did you read Antibiotic Sections? (Y/N)	Please record; S- susceptible; R- resistant; U - unsure				
	No growth	Pure	Mixed Predominant Organism (>10x) Present	Mixed - No Predominant Organism Present	$<10^3$	$\geq 10^3$	$\geq 10^5$				1. Ceptha-lothin	2. Cipro-floxacin	3. Co-amoxiclav	4. Nitro-furantoin	5. Tri-methoprim
xxx			✓				✓	Pink	E. coli	Y	S	S	R	S	U
xxx				✓											
xxx		✓			✓			Dark Blue	Klebsiella	N					
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															

Initials: ..... Time: ..... Test No.	All Tests				If Pure or Predominant Organism (>10x) Present; Complete this Section					Susceptibility Profiles				
	Growth (✓)				Quantification (✓) cfu/mL			Colour of Pure/Predominant Colonies	Identification of Pure/Predominant Colonies (Bacteria) *if unsure, write 'unsure'	Did you read Antibiotic Sections? (Y/N)	Please record; S- susceptible; R- resistant; U - unsure				
	No growth	Pure	Mixed Predominant Organism (>10x) Present	Mixed - No Predominant Organism Present	$<10^3$	$\geq 10^3$	$\geq 10^5$				1. Ceptha-lothin	2. Cipro-floxacin	3. Co-amoxiclav	4. Nitro-furantoin	5. Tri-methoprim

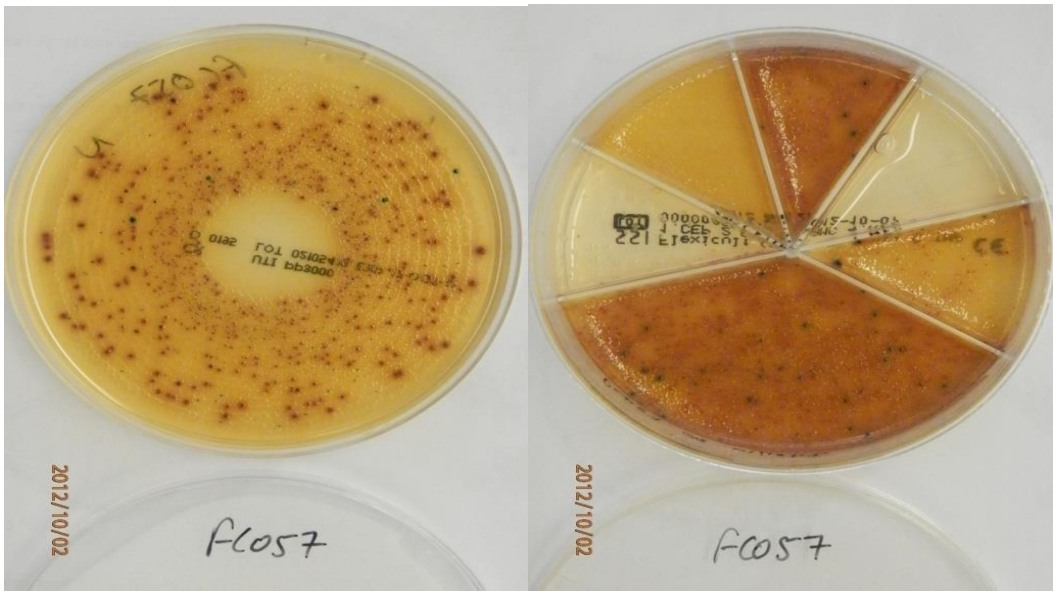
Test Number	Additional Comments

Overall Comments on reading/interpreting Flexicult tests	

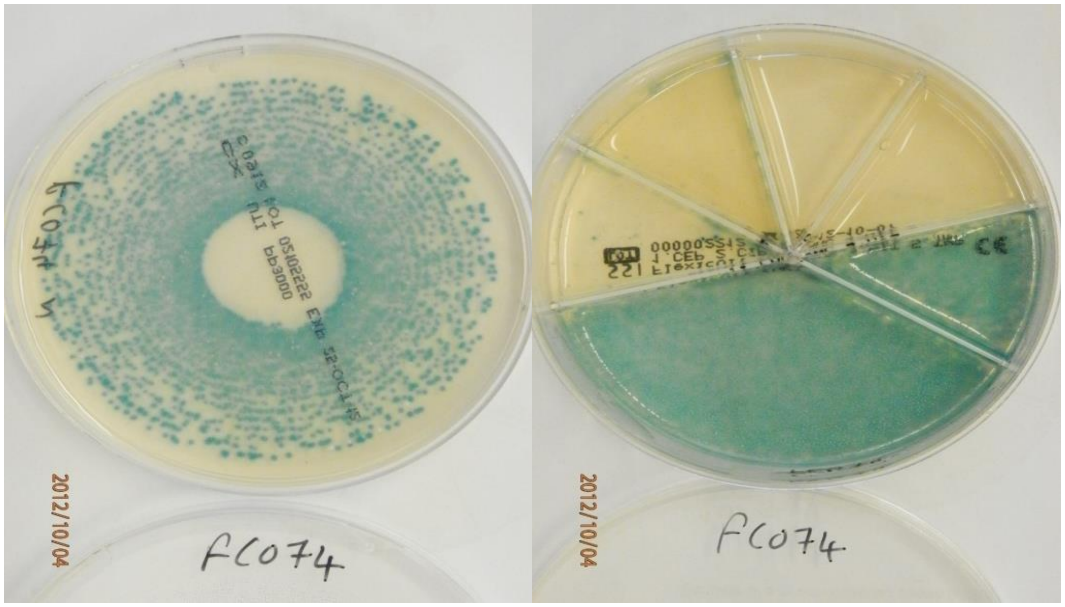
2.1.5 Images of spiral plate cultures and corresponding Flexicult plates for four samples evaluated in this study



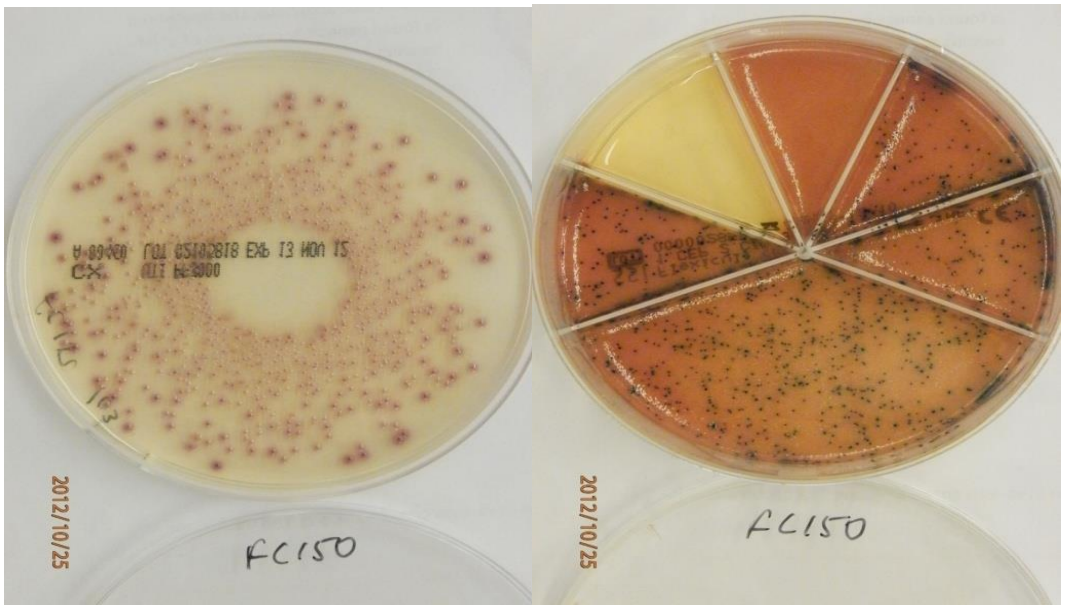
FC011	
Spiral Plating (Left picture) Quantification = $>10^5$ cfu/mL Identification = Coliform	**Flexicult (Right picture)** Quantification = $>10^5$ cfu/mL Identification = Mix, No predominant organism



FC057	
Spiral Plating (Left picture) Quantification = $>10^5$ cfu/mL Identification = Proteus predominant (>10x)	**Flexicult (Right picture)** Quantification = $>10^5$ cfu/mL Identification = Mix, Proteus probable predominant organism due to agar colour change



FC074	
Spiral Plating (Left picture)	**Flexicult (Right picture)**
Quantification = 10^4 - 10^5 cfu/mL	Quantification = >10^5 cfu/mL
Identification = Enterococcus sp.	Identification = *Enterococcus* spp.



FC150	
Spiral Plating (Left picture)	**Flexicult (Right picture)**
Quantification = >10^5 cfu/mL	Quantification = >10^5 cfu/mL
Identification = E. coli predominant (>10x)	Identification = *E. coli* predominant (>10x)

2.3.1 Development of chromatic sensing system set-up and preliminary analyses

Study aim

The aim of this study is to explore chromatic sensing as a potential point-of-care test to help GPs in their decision to prescribe antibiotics to patients with suspected urinary tract infection. This study represents two steps in the process: determining the feasibility (this section); and evaluating the analytic performance of this novel diagnostic test intended for clinical use (section 2 chapter 3 of the main thesis).

Section objectives

1. Refine and optimise the chromatic sensing technology set-up for capturing images of complex liquids including urine;
2. Refine and optimise the chromatic analysis of complex liquids and urine samples;
3. Explore the potential of chromatic sensing in determining the presence of bacteria in urine.

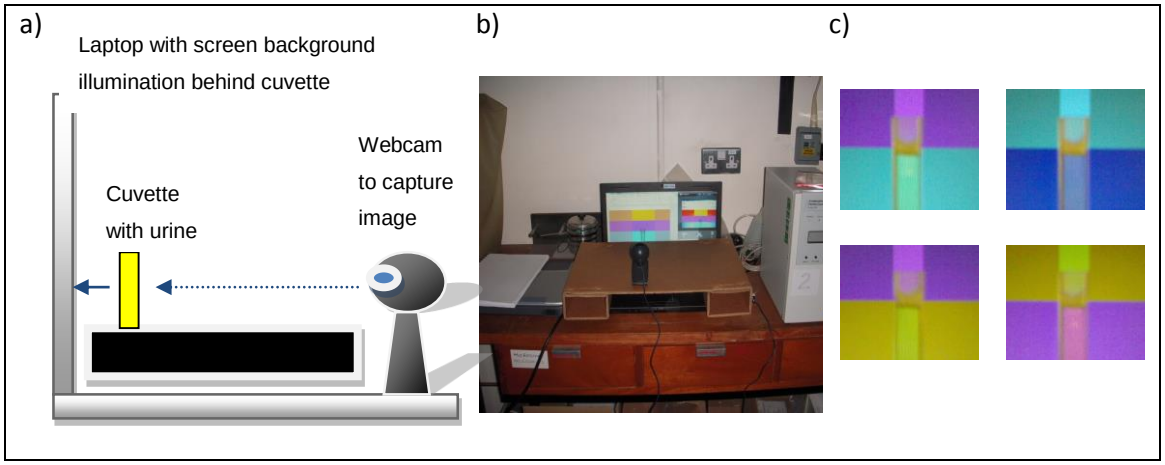
Methods

System Set-Up

The set-up consisted of a portable computer (Toshiba Portege R500 Laptop), whose visual display unit (VDU) provided the main illumination and a web-cam connected to the computer which captured images of the plastic cuvette containing the liquid (Sigma polystyrene cuvette with stopper #C5677-100EA) . The chromatic information was extracted via software installed in the computer. A stand holding the cuvette(s) was placed approximately 2 cm in front of the laptop screen, with the required background (Microsoft Office PowerPoint template on screen, developed and provided by Liverpool

University), and the webcam positioned so that the screen was visualised through the liquid in the cuvette (see Fig. 1 below).

Figure 1. Set-up and image capture of liquid samples using a laptop and webcam: a) diagram of set-up; (b) photograph of an example of the laptop set-up; (c) example of images captured using this set-up.

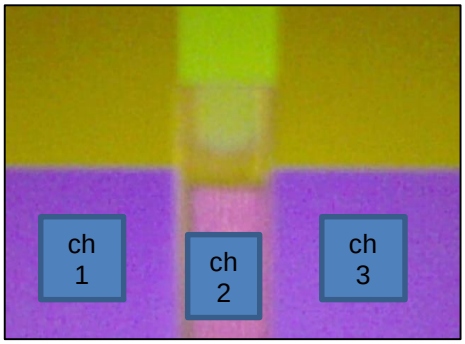


Various cuvette positions, screen templates and lighting conditions were assessed to obtain the most appropriate image for chromatic analysis.

Once the image had been captured red, green and blue outputs from three positions on the image were calculated using specific software developed by Liverpool University (Figure 2). Two areas captured either side of the cuvette and this was used to control for extraneous effects such as room lighting and shadows. The third area was of the actual liquid being analysed.

Figure 2; An example of the JPEG images captured for chromatic analysis: a) three areas of RGB analysis including the sample (ch2) and two comparator areas (ch1+ ch3) for quality control; (b) example of the RGB outs for each area on the image.

a) b)



Chromatic outputs RGB:

ch 1: R: 252 G: 252 B: 187

ch 2: R: 166 G: 161 B: 212

ch 3: R: 212 G: 215 B: 183

Initial urine analysis

The screen of the laptop had a Microsoft Power-point template slide containing different colours (purple, light blue, yellow, dark blue) to be used as a background for the initial urine samples.

Six culture positive (> 1 x 10^5 cfu/mL) and six microscopy negative anonymised patient urine samples were provided by the Microbiology Laboratory, University Hospital Wales. Approximately 3 ml of urine was transferred to a plastic cuvette. The cuvette containing the sample was placed on a stand above the laptop keyboard, ~2cm in front of the coloured screen.

Images were captured in the Microbiology Laboratory, University Hospital Wales by myself. Specifically developed software for RGB data extraction installed on the laptop was used at the time of image capture and both the JPEG images and RGB outputs were sent to Liverpool University via email. In Liverpool the R, G and B outputs of each positive and negative urine sample with each coloured background were analysed and mapped using different chromatic parameters (based on mathematical models) to determine the optimum differentiation between the known bacterially positive and negative samples.

A further six culture positive (> 1 x 10^5 cfu/mL) and six microscopy negative urine samples were added to the previous 12 urine samples for further chromatic analysis. The chromatic parameters used for the total 24 samples varied from the initial 12 urines to look more specifically at the dominant wavelength between Blue (B) and Red (R) under blue (b) background illumination ((B/R)b); and the difference between dominant

wavelengths (B or R) under blue and purple (p) background illuminations ((B/R)b – (B/R)p).

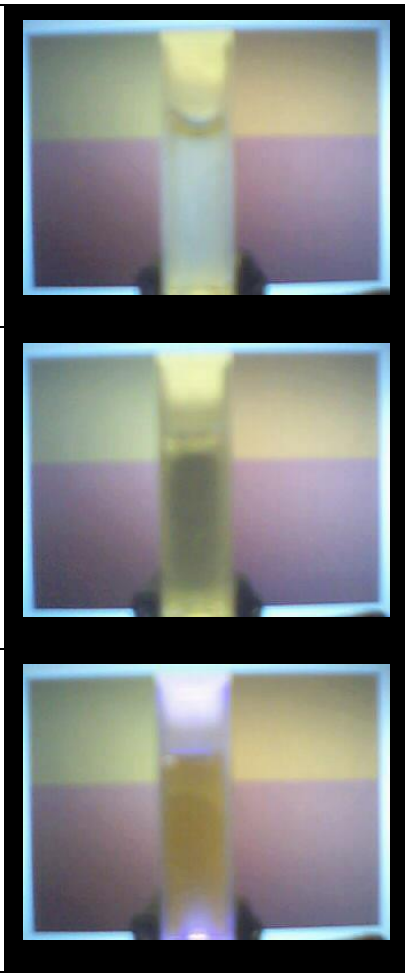
Assessing complex liquids: Turbidity

To indicate the form and features of chromatic cluster maps and to address the issue of the effect of complex liquid mixtures on chromatic signatures, preliminary tests were performed on controlled mixtures of some common clear and turbid liquids plus some sterile filtered urine spiked with known quantities of *E. coli* bacteria. Chromatic analysis assessed transmission (absorption) of light through the various liquids, reflection of light and fluorescence as shown in Figure 3.

Assessing turbidity: Tea and milk mixtures

Milk was chosen for the assessment of turbidity to form mixtures with water and tea (colour). The water – tea mixtures are representative of optical variations produced by changes in relative concentrations of two clear liquids with different polychromatic optical transmission properties. Tests with water – milk are representative of optical variations produced by changes in relative concentrations of components, one of which (milk) produces turbid conditions in an otherwise clear liquid. Tests with tea – water – milk represent the further complex condition of optically different clear liquids (water and tea) with a turbid medium (milk). The water/tea mixtures were made up as; water only – 1/3 tea – 2/3 tea – 3/3 tea. Skimmed milk was added to the water or tea as drops from a 1 mL Pasteur pipette (0, 5, 10, 30 drops). For each liquid 2.5mL was placed into a spectrophotometer cuvette ready for image analysis.

Figure 3. A description of the three light sources; screen, ambient and UV with examples of the final images captured under each light source.

Screen Light Source (Transmitted Light - Impurity)	• Attenuated white screen behind cuvette, absorption of light transmitted through the liquid	
Ambient Light Source (Reflection/Scattered Light - Turbidity)	• Black card stuck to the back of the cuvette, measure of the reflection of ambient light by the liquid	
Ultra-Violet Light (Fluorescence in Sample)	• UV light source shone through the bottom of the cuvettes upwards, • Black card as background to measure the fluorescence	

Urine samples spiked with *E. coli*

A pool of human urine was sterile filtered through 0.2µm filter (Millipore) to remove any commensal bacteria and larger particles. An inoculum of *E. coli* (ATCC #25922) was prepared in sterile water at 0.5 McFarland standard; this involved visually comparing the turbidity of the *E. coli* spiked urine with the McFarland standard until they are the same turbidity (this is a standardised microbiological process for determining bacterial suspensions). This 0.5 McFarland standard equates to approximately 1 x 10^8 cfu/mL *E. coli* inoculum. From this inoculum dilutions of 1:10; 1:100 and 1:1000 into the sterile urine were made to have additional inoculums of 1 x 10^7; 1 x 10^6 and 1 x 10^5 cfu/mL respectively. For each urine *E. coli* inoculum 2.5mL was placed into a spectrophotometer cuvette ready for image capture and analysis.

Transmission (Absorption of light through the liquid)

Tests were performed with polychromatic light from the VDU screen (attenuated white background) transmitted through the cuvette containing the sample in the presence of ambient light. The outputs from the three wavelength-dependent pixel elements (R, G, B) of the selected image regions were then extracted for further processing by the team at Liverpool University.

For the water-tea-milk liquids chromatic cluster maps were used to examine various trends produced by the physically different forms of liquid mixtures: two clear liquids (water and tea) with different transmission spectra; turbid component in an otherwise colourless mixture (water and milk); and turbid component (milk) with two clear liquids of different concentrations (tea and water). The chromatic cluster maps were based upon the camera outputs R (long wavelengths) and B (short wavelengths), these were corrected (compared to the reference background colours to make sure each image was the same), normalised with the water result (0–1) and plotted against each other. Ambient light was mathematically, rather than physically, excluded from the transmission by means of subtracting the reflected ambient light from the combined result of VDU illumination plus reflected light: Rtrans = R(VDU illumination) – Rrefl; Btrans = B(VDU illumination) – Brefl.

Reflection (scatter of ambient light from the liquid)

To assess the reflection/scatter of ambient light caused by the turbidity of the various liquids I replaced the VDU illumination (attenuated white screen) with a piece of black card stuck to the back-side of the cuvette, allowing only ambient light to be captured and assessed from the liquid.

Fluorescence

E. coli has been reported to have a peak wavelength optical spectra of ~260nm (75) which is in the ultraviolet spectrum with an overall wavelength range of 200-800nm; human vision and camera optics have a range of 400-650nm. To assess the lower end

of the spectrum for *E. coli* (200-400 nm) Liverpool university developed a self-contained ultra-violet (UV) light with cuvette holder that shone the UV light through the base of the cuvette containing the urine (inoculated with various amounts of *E. coli* as above), a black card was stuck to the back-side of the cuvette and the fluorescence captured through the image from the webcam. Unlike the transmission and reflection of light the analysis focused on the G output; it may be expected that G covers a more suitable range at which output fluorescence may occur and it provided the most stable output of RGB (as advised by Liverpool University).

Results

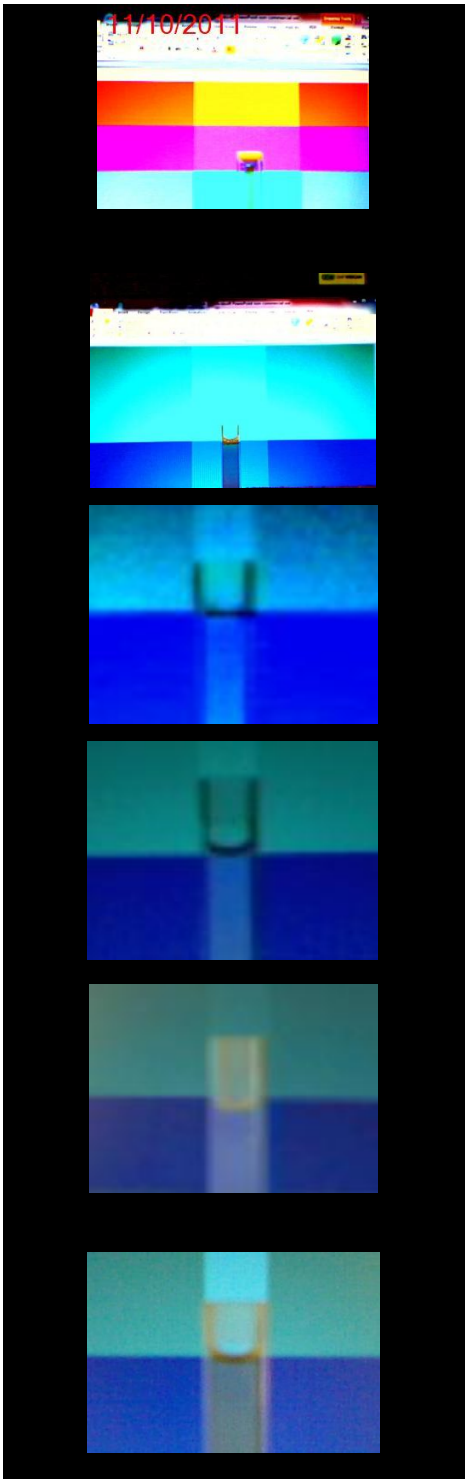
System set-up

Optimisation of the images from the laptop/webcam was based on trial and error and communication with the team in Liverpool, the steps are shown in Figure 4.

The background template was developed by Liverpool University and varied in colours and formats; varying the colours increased or decreased the R, G or B outputs. To allow the camera to automatically adjust itself correctly for each test, the cuvette was positioned in the middle of the screen with the dividing horizontal line between the two back-ground colours half-way up the screen and the liquid meniscus close to this line. External light sources such as that falling from windows or artificial light were unavoidable as this study was performed in a busy microbiology laboratory. However I avoided placing the laptop in direct sunlight or directly below artificial lights to minimise glare and extraneous effects.

Final camera settings were set to' Auto': Exposure 67; Brightness 20; Contrast 20; Saturation 50; Sharpness 3; and Gamma 3.

Figure 4. Optimisation of cuvette image through repositioning, zoom, lighting and background template.



Problem: The area of urine to be analysed was very small and colours too bright
Action: Changed cuvette, new Power Point template produced

Problem: Image better with only two background colours on each image
Action: Zoom into Image

Problem: Image too bright, halo effect
Action: Reduced screen brightness, covered laptop

Problem: Background colours too dark
Action: Screen brightness set to auto, uncovered laptop and new Power Point template produced

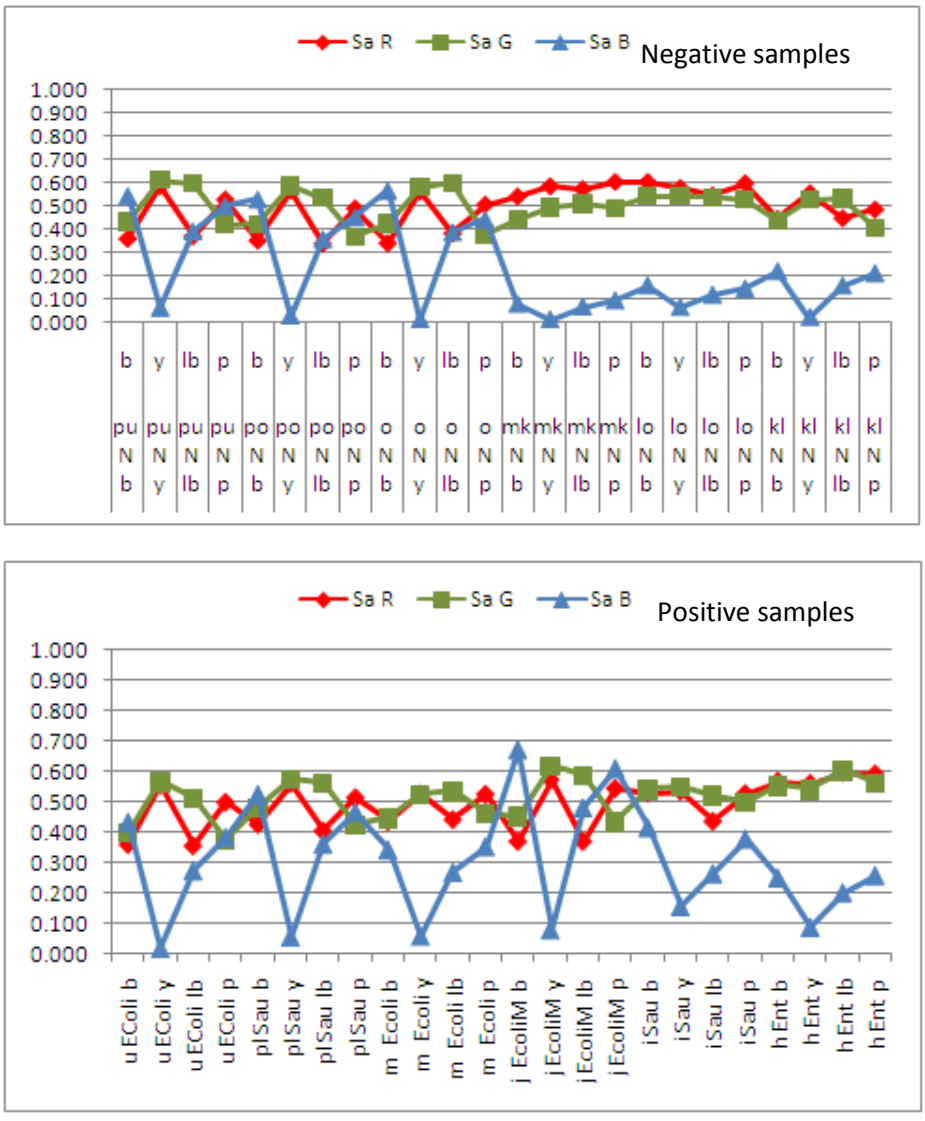
Problem: Background still too dark, resolution poor
Action: New Power Point template produced, Image resolution increased by using manual focus of webcam

Acceptable Image for Initial Urine Analysis

Initial urine analysis results

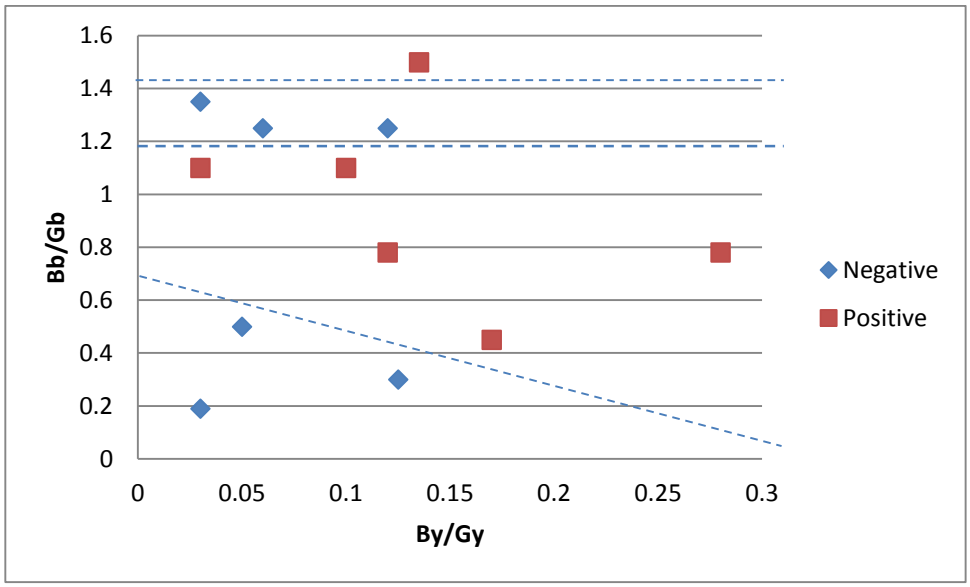
The results showed similar values for the R and G under all illuminations but the B outputs showed more variation. The output for B was approximately 0 for all negative and most positive samples under yellow screen illumination (Figure 5) indicating limited or no Blue wavelength with this set-up. This raw data indicated that the best chromatic parameters to follow up on were to analyse B and R or B and G outputs rather than R, G and B as the outputs for R and G are the same.

Figure 5. Graphs showing camera outputs of Red, Green and Blue (Sa R, Sa G, Sa B) for 6 bacterial negative urine samples (pu, po, o, mk, lo and kl) and 6 bacterial culture positive urine samples (uEColi, plSau, mEcoli, jEcoliM, iSau, hEnt) each sample having four background illuminations (b – blue, y – yellow, lb – light blue, p – purple)



Further chromatic mapping using the parameters of dominating wavelength B or G with blue versus yellow background illuminations (Bb/Gb and By/Gy respectively) showed distinctive clustering of positive and negative samples (Figure 6). This analysis also showed that two distinct clusters of negative samples and one positive sample were located separately from the other positive samples. The reasons for this required further investigation; although the turbidity of the urine for both the differences in negative clusters and the single outlying positive urine was of key interest.

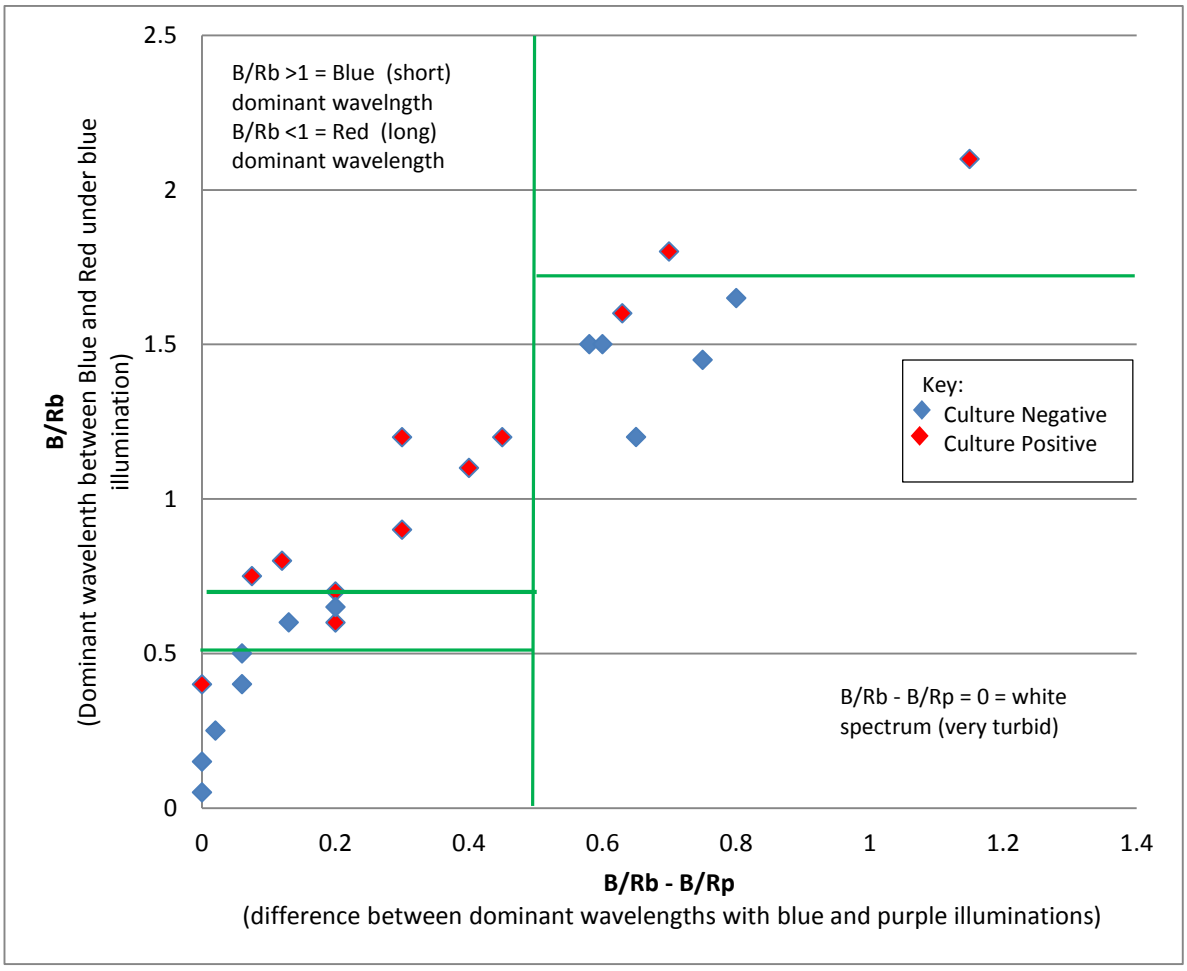
Figure 6. Chromatic Map of the Blue (B) to Green (G) dominant wavelength under blue illumination (Bb/Gb) versus the amount of B to G dominant wavelength under yellow illumination (By/Gy) in bacterial positive and negative urine samples



When the additional 12 urines samples were analysed with the initial 12 urines samples the results indicated a weak short wavelength component (B) compared to more dominant long wavelength (R) when B/Rb<1 under blue illumination. B/Rb – B/Rp → 0 indicates little difference between the dominating wavelengths under blue and purple illumination i.e. results in a broad spectrum (~ white) and this could be an important indication of turbidity (Figure 7). Taking this into account the graph was grouped into cut-offs to show clustering of chromatically turbid and non-turbid samples using B/Rb - B/Rp < 0.5 as turbid. When looking at the samples in this section the negative urines showed a dominant long wavelength (R) compared to the positive urine samples. Likewise when

looking at non-turbid samples (B/Rb – B/Rp > 0.5) the positive urine samples showed a stronger dominant short wavelength (B) compared to the negative samples. The turbidity of the urine warranted further evaluations.

Figure 7. Chromatic Map showing the dominant wavelength between Blue (B) and Red (R) under blue illumination (B/Rb) and the difference between dominant wavelengths under blue and purple illuminations (B/Rb – B/Rp) for bacterial positive and negative urine samples



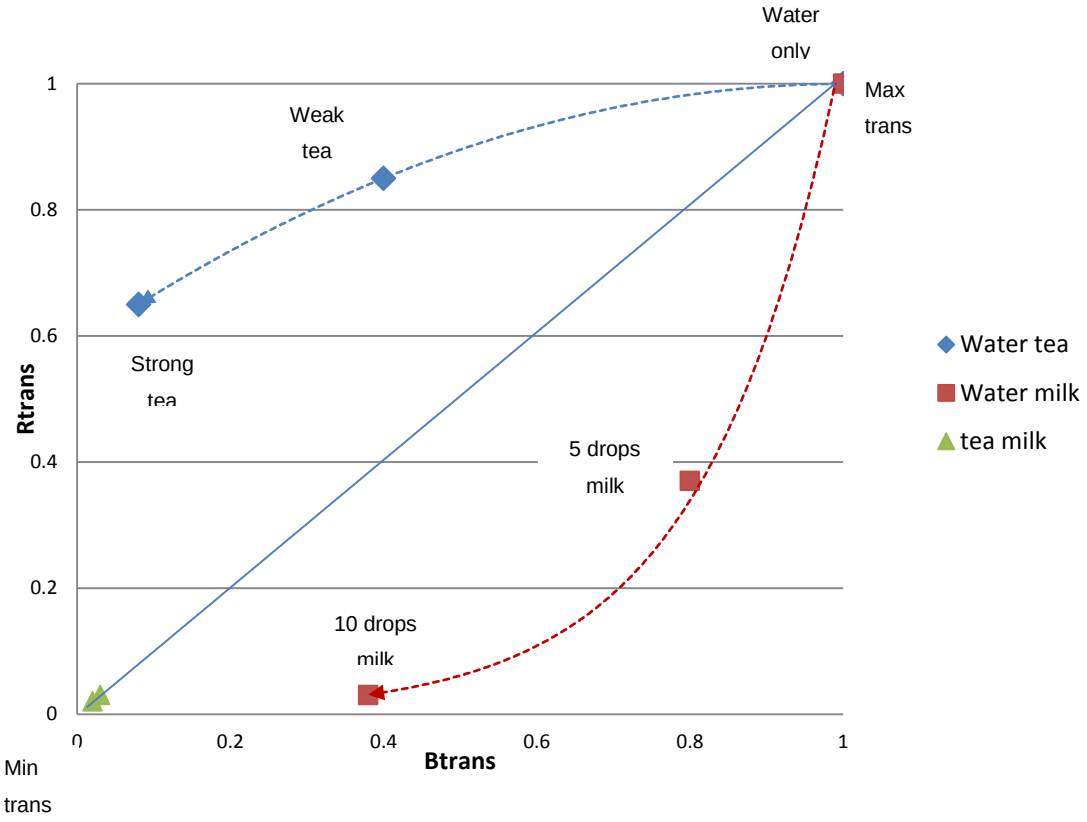
Assessing complex liquids: Turbidity

Transmission of light

Milk and tea mixtures

Figure 8 shows an example of an Rtrans : Btrans cluster map for optical transmission (trans) of the VDU illumination for tea diluted with water, water with a few drops of milk (5 and 10 drops) and water, tea and milk mixtures (milk 5, 10, 30 drops).

Figure 8. Chromatic Cluster Map of R:B light transmission through various water-tea-milk liquids.



The Rtrans : Btrans results for mixtures of water and tea (clear non-turbid liquid mixtures) show that the short wavelengths (B) reduce in strength with increasing tea concentration more rapidly than the long wavelengths (R). As such the Rtrans:Btrans locus for this liquid mixture lies **above** the equal strength locus Rtrans = Btrans.

The Rtrans : Btrans results for mixtures of water and milk (turbid mixtures) show that the long wavelengths (R) reduce in strength with increasing milk concentration more rapidly than the short wavelengths (B) and may be associated with polychromatic light

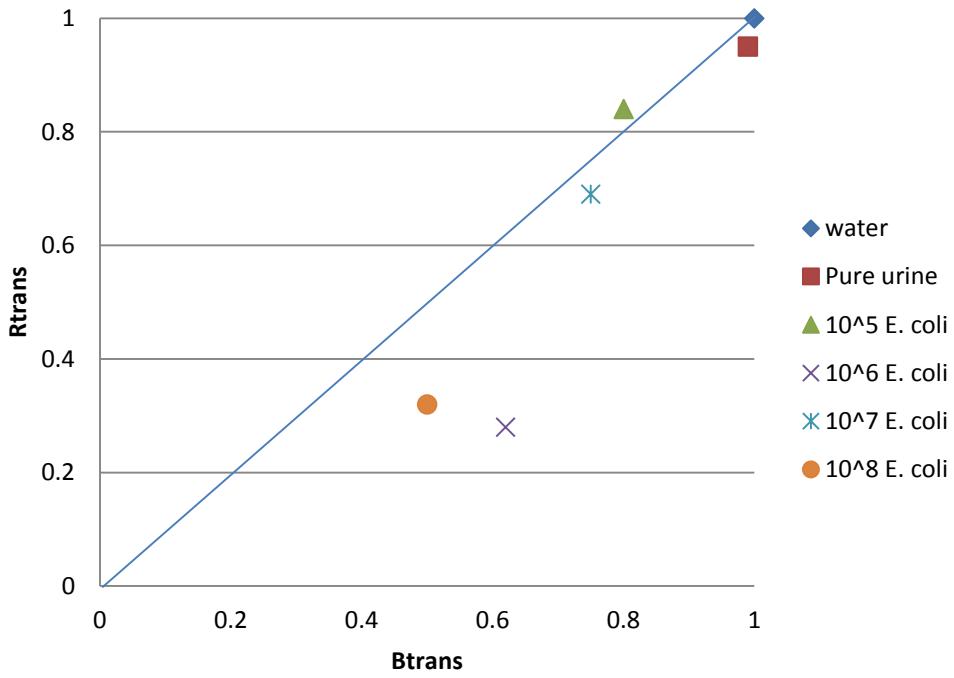
scattering by the turbidity. The Rtrans : Btrans locus lies well **below** the equal strength locus Rtrans = Btrans for this liquid mixture.

Results for polychromatic light transmission through the more complex mixtures of tea – milk show how the Rtrans and Btrans strengths have both been substantially reduced from the water - tea values by the same milk concentrations (5 and 10 drops) used for the water – milk tests. They lie close to the origin i.e. Rtrans ~ Btrans→0.

Urine samples spiked with E. coli

The Rtrans:Btrans transmission cluster maps were also applied to the sterile urine spiked with various concentrations of *E. coli* as shown in Figure 9. This showed that both Rtrans and Btrans decrease as the *E. coli* concentration in the urine increases (although there was an anomalous result with 10^6 cfu/mL).

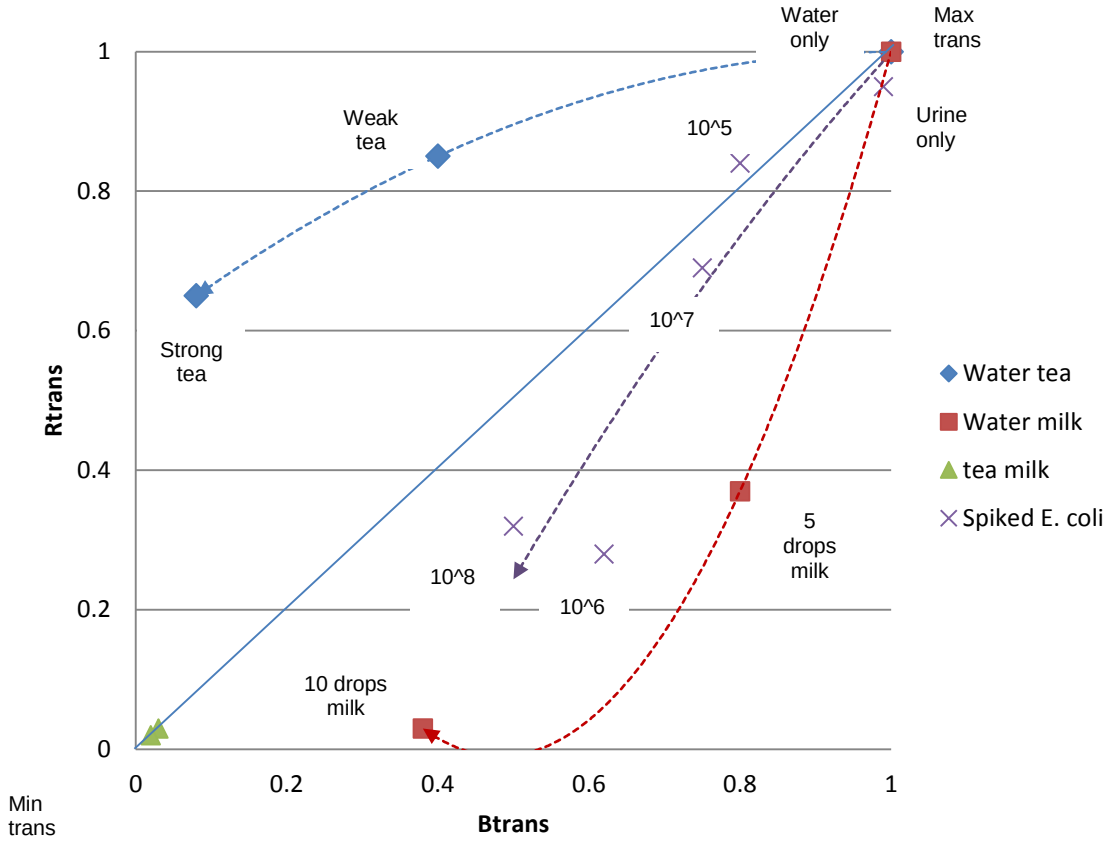
Figure 9. Chromatic Cluster Map of R:B light transmission through various urine *E.coli* inoculums.



The urine-*E. coli* >10^5 cfu/mL results lie below the equal strength locus (Rtrans=Btrans) implying that the changes in Rtrans are greater than the changes in Btrans. These results are similar to the water-milk trends rather than the water-tea trends (Figure 10)

suggesting the urine *E. coli* liquids have a complex mixture of pure liquid and turbidity effects, the former dominating for low levels of *E. coli* and the latter becoming increasingly significant at higher *E. coli* levels, but not to the same extent as the tea-milk mixtures where Rtrans ~ Btrans→0. Figure 4.1.11 illustrates the Rtrans:Btrans chromatic maps of all these liquids.

Figure 1. Rtrans:Btrans chromatic map of various complex liquid mixtures.

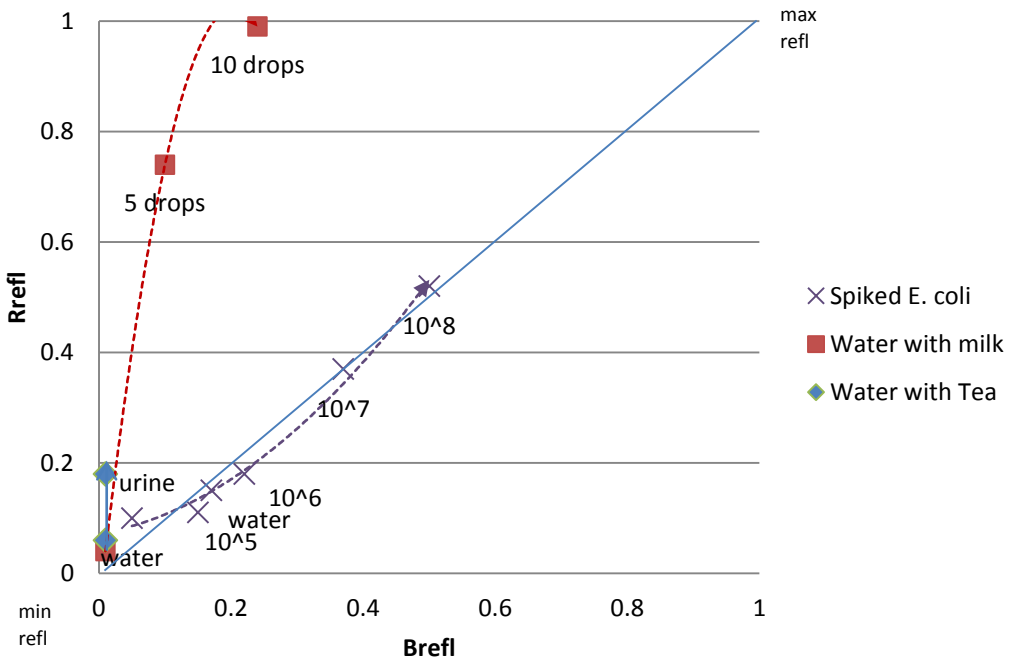


Reflection (scatter of ambient light from the liquid)

The Rrefl and Brefl values for water were ~0.04 and 0.01 respectively indicating only low levels of ambient light scattered. For water- tea liquid the Brefl was of a similar low value to water although the Rrefl values increased with tea concentration, leading to the water-tea locus being close to the Rrefl axis. For water with increasing milk concentration (increasing turbidity) the Rrefl, Brefl chromatic map shows that the longer wavelengths (R) increase in strength with increasing milk/turbidity more rapidly than the shorter (B)

wavelengths. The Rrefl:Brefl locus lies well above the equal strength locus Rrefl:Brefl (Figure 11).

Figure 11. Chromatic Map of Rrefl : Brefl for various complex liquids.

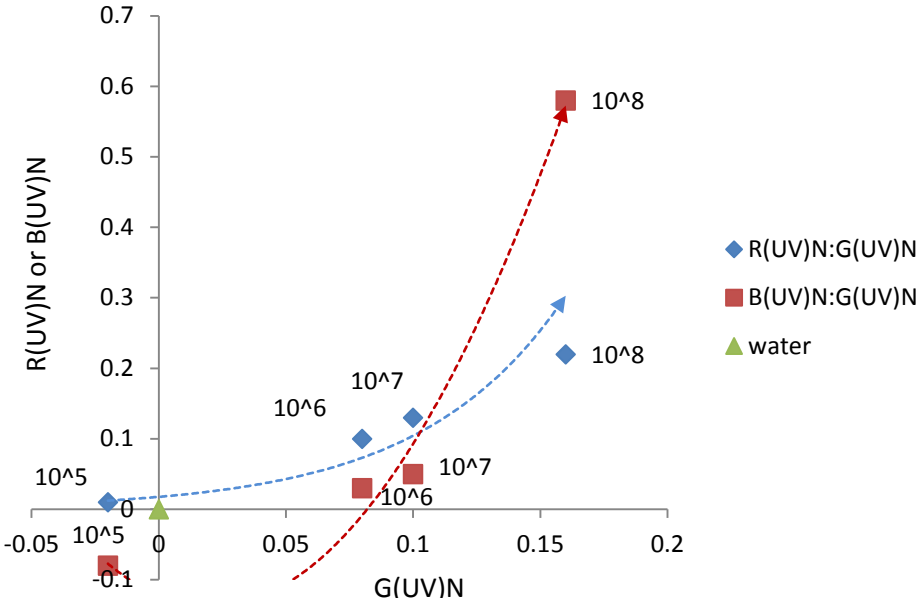


For the urine inoculated with various concentrations of *E. coli* the results for the Rrefl:Brefl showed that higher levels of *E. coli* (>10^5 cfu/mL) followed the Rrefl=Brefl locus. For *E. coli* at 10^5 cfu/mL the result was not much different to the urine alone (without *E. coli*) and the water value, indicating little scatter effect due to the *E. coli* contamination at this inoculum concentration.

Fluorescence

The outputs for R:G and B:G UV light source with water and the four inoculums of *E. coli* in sterile filtered urine are shown in Figure 12. There was reasonable discrimination between all the *E. coli* concentrations with both R:G and B:G (although 10^6 and 10^7 cfu/mL similar) however urine with *E. coli* at 10^5 cfu/mL had similar properties to water suggesting this detection limit in practice may not be useful using fluorescence.

Figure 12. Chromatic cluster map of R:G and B:G UV fluorescence through various urine *E.coli* inoculums.

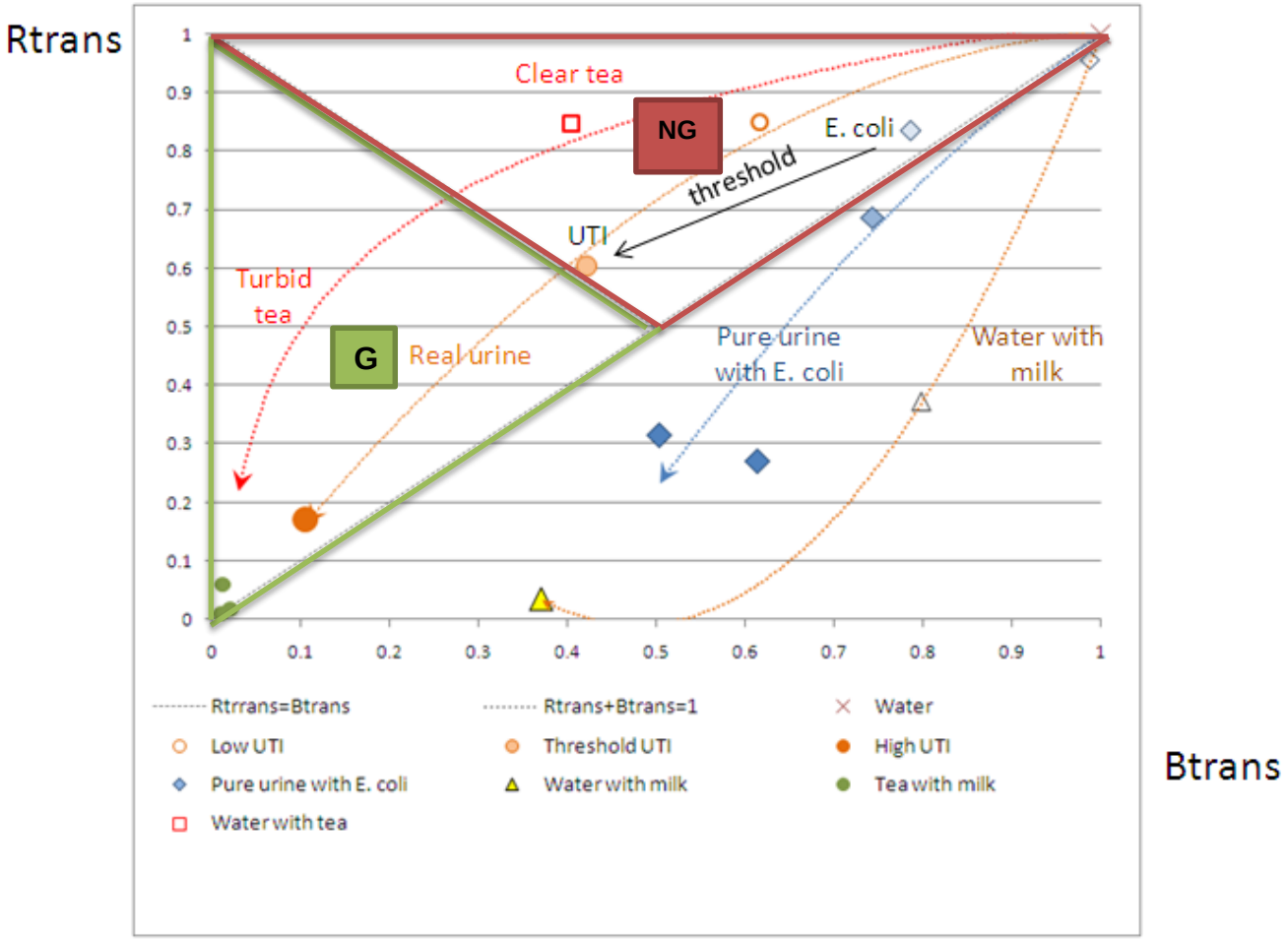


Final chromatic analysis maps and cut-off values for analytic performance evaluation

The chromatic methods used for analysing the urine samples were based upon the transmission chromatic parameters Rtrans, Btrans and reflection chromatic parameters Rrefl, Brefl plotted against each other to form chromatic cluster maps.

Rtrans, Btrans are the long and short optical wavelength components respectively, recorded by the web-camera, of the light transmitted from a white VDU screen through the urine samples with correction for ambient light. [Rtrans, Btrans are also corrected for camera and screen variations (via screen reference areas) and are normalised to accommodate ambient light variations → scale 0 - 1 (via black + white cards)].

Figure 13. R:B Transmission chromatic cluster map comparing sterile filtered urine inoculated with known quantities of *E. coli*; water-tea-milk liquids and preliminary clinical urine samples; Area indicated by G denotes clinical urine samples with outputs in this are considered UTI positive, area indicated by NG denotes clinical urine samples with outputs in this area considered UTI negative.



On the Rtrans : Btrans chromatic map (Figure 13), light having equal magnitude of components Rtrans and Btrans lie on the line Rtrans = Btrans; since urine samples tend to be yellow – orange in colour are expected to lie above this line on the map (Rtrans > Btrans). The map is subdivided into two further sectors by the line Rtrans + Btrans = 1. The earlier tests showed that the regions;

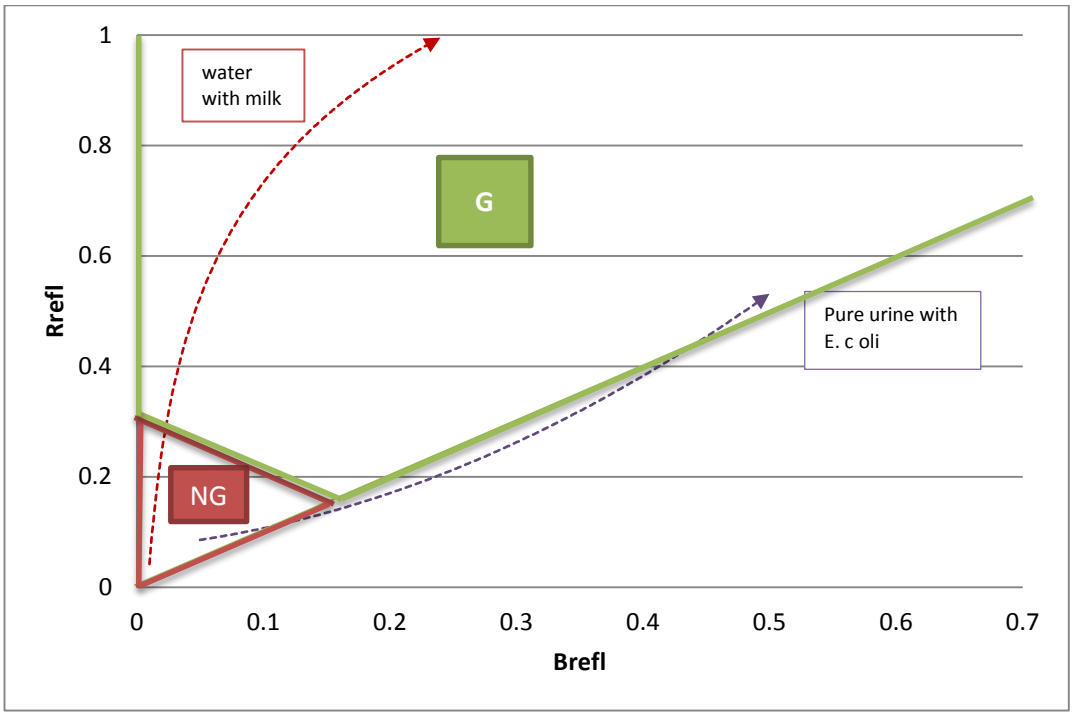
- Rtrans, Btrans → 1 correspond to the transmitting liquid being clear (high optical transmission);
- Rtrans, Btrans → 0 correspond to the transmitting liquid being turbid (optical absorption);
- The line Rtrans + Btrans = 1 forms the boundary between these two regions.

The urine samples that have Rtrans:Btrans outputs that fall within the section of the graph indicated by a green triangle and letter G ($0<(Rtrans=Btrans)<0.5$ and $0.5 < Rtrans, Btrans < 0.5$) were considered positive for a UTI at $>10^5$ cfu/mL. Urine samples that had Rtrans:Btrans outputs that fall within the section of the graph indicated by a red triangle and letters NG ($0.5<(Rtrans=Btrans)<1$ and $Rtrans + Btrans \geq 1$) were considered negative for a UTI at $\leq 10^5$ cfu/mL.

Similarly for the reflection (scatter) chromaticity Rrefl:Brefl based on the *E. coli* inoculated urine results and some preliminary clinical urine samples the following criteria has been set (Figure 14);

- No significant bacterial growth samples ($<10^5$ cfu/mL) fall in the red NG sector $Rrefl + Brefl < 0.3$; $Brefl \leq Rrefl$; $Brefl \leq 0.15$
- Significant bacterial growth ($>10^5$ cfu/mL) samples fall in the green G sector $Rrefl + Brefl > 0.3$; $Brefl \leq Rrefl$; $0.15 < Brefl \leq 1$
- The threshold bacterial growth is in the range $Rrefl + Brefl = 0.3$; $Brefl \leq 0.15$.

Figure 14. R:B Reflection chromatic cluster map comparing sterile filtered urine inoculated with known quantities of *E. coli* and water -milk liquids; Area indicated by G denotes clinical urine samples with outputs in this area considered bacterially UTI positive, area indicated with NG denotes clinical urine samples with outputs in this area considered bacterially UTI negative.



Both types of chromatic analysis were used in the analytic performance evaluation (laboratory evaluation study). During the laboratory evaluation study which is described in the Section 2 Chapter 3 the first 67 urines (out of 195) were chromatically analysed un-blinded to the NHS microbiology urine culture results (quantification cfu/mL and bacterial identification); this was to allow for changes in chromatic analysis algorithms or cut-offs, although no changes were eventually made.

The transmission data is referred to as **Chromatic Indication** and the reflection data as **Chromatic Turbidity** in this thesis.

2.3.2 Chromatic sensing power point template for VDU screen

2.3.3 Sensitivity, specificity, positive predictive value and negative predictive value of various urinalysis prediction rules in determining UTI

Urinalysis Test	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
Nitrite	36.6 (26.4 – 48.2)	87.2 (80.2 – 92.0)	61.9 (46.8 – 75.0)	70.8 (63.2 – 77.4)
Leucocyte (positive ≥ trace)	71.8 (60.5 – 81.0)	73.6 (65.3 – 80.5)	60.7 (50.0 – 70.5)	82.1 (74.02 – 88.1)
Leucocyte (positive ≥ +)	62.0 (50.3 – 72.4)	80.8 (73.0 – 86.7)	64.7 (52.8 – 75.0)	78.9 (71.1 – 85.1)
Nitrite OR leucocyte (≥+) positive	77.1 (66.1 – 85.4)	69.6 (61.1 – 77.0)	58.7 (48.5 – 68.2)	84.5 (76.3 – 90.2)
Blood (H ≥ trace)	54.9 (43.4 – 66.0)	68.0 (59.4 – 75.5)	49.4 (38.6 – 60.2)	72.6 (64.0 – 79.9)
Blood (NH ≥ trace)	22.5 (14.4 – 33.5)	85.6 (78.4 – 90.7)	47.1 (31.5 – 63.3)	66.0 (58.5 – 72.9)
Blood H or NH (≥trace)	64.8 (53.2 – 74.9)	56.8 (48.0 – 65.2)	46.0 (36.6 – 55.7)	74.0 (64.4 – 81.7)
Protein (≥trace)	50.7 (39.3 – 62.0)	58.4 (49.6 – 66.7)	40.9 (31.2 – 51.4)	67.6 (58.3 – 75.7)
Nitrite, Leucocyte (≥+) AND Blood (Trace) positive	12.7 (6.8 – 22.4)	98.4 (94.4 – 99.6)	81.8 (52.3 – 94.9)	66.5 (59.4 – 72.9)
Nitrite AND Leucocyte (≥+) OR Blood (Trace) positive	26.8 (17.9 – 38.1)	95.2 (89.9 – 97.8)	76.0 (56.6 – 88.5)	69.6 (62.3 – 76.0)
Nitrite OR Leucocyte (≥+) AND Blood (Trace) Positive	67.6 (56.1 – 77.3)	80.8 (73.0 – 86.7)	66.7 (55.2 – 76.5)	81.5 (73.7 – 87.3)
Nitrite, Leucocyte AND Blood (H) negative	83.1 (72.7 – 90.1)	48.8 (40.2 – 57.5)	48.0 (39.3 – 56.7)	83.6 (73.4 – 90.3)
Nitrite AND Leucocyte negative	67.6 (56.1 – 77.3)	80.8 (73.0 – 86.7)	66.7 (55.2 – 76.5)	81.5 (73.7 – 87.3)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	36-37
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	36-37
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	38
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	38
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	38
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	39
Test methods	7	The reference standard and its rationale.	36, 39-40
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	39-41
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	39-41
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	38
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	38-39
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	43-44
	13	Methods for calculating test reproducibility, if done.	46-50
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	51
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	51
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	39
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	39, 91
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	52
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	54
	20	Any adverse events from performing the index tests or the reference standard.	NA
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	54
	22	How indeterminate results, missing data and outliers of the index tests were handled.	52
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	NA

	24	Estimates of test reproducibility, if done.	59-62
DISCUSSION	25	Discuss the clinical applicability of the study findings.	83-86, 93-94

2.3.4 STARD checklist for reporting of studies of diagnostic accuracy

3.1.1 Critical appraisal and data extraction form

Literature Review: Relevance to Review

Citation:														
Questions	**Does the paper address a clearly focused issue relevant to the review (UTI)?**					**Is the study population appropriate? (study may be mixed but results/outcomes must be for defined and review relevant population)**						**Is the Setting appropriate?**		**Is the choice of study method appropriate for observation of <u>routine clinical UTI management</u> in primary care (exclude qualitative, vignettes, case studies, RCT's)**
						Inclusion			**Exclusion**			**Primary Care**	**Europe**	
	1° Questions			**2° Questions**										
	Clinical signs/symptoms	Diagnostics (POCT/culture)	Therapy – yes/no, type, duration	Symptomatic recovery	Congruence to relevant guidelines	Female	>15 years	Suspected uncomplicated	Non- Pregnant	Non-catheter	No co-morbidities			
Yes														
Can't tell														
No														
N/A														
Comment														
Accepted for Critical Appraisal?														

Critical Appraisal

Citation:																	
Questions	**Recruitment/Selection (relevance to general population)**						**Performance**			**Incomplete data**		**Outcomes/Results**					**Other Bias or confounding factors (describe in comments)**
	Recruiters (e.g. GPs)	Eligibility of Participants	Setting/ Location	Method of Recruitment	Period (Time/Date) of Recruitment	Sample size	GPs blinded to study conditions	Participants blinded to study	Influence of outcome assessors	Exclusions/ withdrawals	Unavailable/ missing data	Clearly defined outcomes	Selective outcome reporting	Unit of assignment match unit of	1° analysis	2° analysis (e.g. subgroup and adjusted	
Low Risk Bias																	
Can't Tell																	
High Risk Bias																	
N/A																	
Comments																	
Accepted for Data Extraction ?																	

Literature Review: Data Extraction Table

Study Details	Population and Setting	Research Parameters	Methods of analysis Results	Key Outcomes/Findings (relevant to the review)	Notes
Authors:	**What setting(s) (country; location; setting):**	**What was/were the research questions:**	**Brief description of method and process of analysis:**	**Primary Outcomes:**	**Limitations identified by author:**
Year: **Citation:**	**What population were the sample recruited from:** **How were they recruited:**				
Aim of Study:	**How many participants were recruited:** **Response Rate:**	**How were the data collected** **Unit of data collection (individual, group, community):**		**econdary Outcomes:**	**Limitations identified by review team:**
Study Design:	**Were there specific exclusion criteria:** **Were there specific inclusion criteria:** **Population demographic (age, sex, ethnicity, occupation, education, socioeconomic position – if applicable)** **Differences between responders and non-responders:**	**What method (s):** **By whom:** **When:**	**Statistical methods (if applicable):**	**Overall conclusions:**	**Evidence gaps and/or recommendations for future research:** **Source of funding:**

3.2.2 POETIC study CRF

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POETIC
point of care testing for urinary tract infection in primary care

SEVENTH FRAMEWORK PROGRAMME

Baseline Assessment - To be filled in by the Doctor or Nurse **CRF** 0 3

Today's Date ☐☐ / ☐☐ / ☐☐☐☐ (d d / m m / y y y y) Centre ID ☐ Patient ID ☐☐☐☐ Site ID ☐☐

Current symptoms

1. Please rate the patient's current symptoms

Using the scoring system below, please tick a number for each symptom

SCORE	SEVERITY OF SYMPTOM/PROBLEM
0	Normal/not affected
1	Very little problem
2	Slight problem
3	Moderately bad
4	Bad
5	Very bad
6	As bad as it could be

SEVERITY OF SYMPTOM	0	1	2	3	4	5	6
Fever	☐	☐	☐	☐	☐	☐	☐
Pain in the side	☐	☐	☐	☐	☐	☐	☐
Blood in urine	☐	☐	☐	☐	☐	☐	☐
Smelly urine	☐	☐	☐	☐	☐	☐	☐
Burning (Burning or pain when passing urine)	☐	☐	☐	☐	☐	☐	☐
Urgency (Having to go in a hurry)	☐	☐	☐	☐	☐	☐	☐
Daytime frequency (Having to go more often than usual during the day)	☐	☐	☐	☐	☐	☐	☐
Night time frequency (Having to go more often than usual during the night)	☐	☐	☐	☐	☐	☐	☐
Tummy pain (When not passing urine)	☐	☐	☐	☐	☐	☐	☐
Restricted activities	☐	☐	☐	☐	☐	☐	☐
Unwell	☐	☐	☐	☐	☐	☐	☐

How many days has this patient been off work because of this illness prior to today? ☐☐

If the patient does not work please tick here. ☐

How many days has the patient had symptoms? ☐☐

POETIC
point of care testing for urinary tract infection in primary care

SEVENTH FRAMEWORK PROGRAMME

Patient management

CRF 03

2. Was a dipstick test performed? ☐ Yes ☐ No

If 'YES', what were the results? If NO please skip to Q5

Leukocytes	☐ Negative	☐ +	☐ ++	☐ +++		
Nitrites	☐ Negative	☐ +	☐ ++	☐ +++		
Protien	☐ Negative	☐ +	☐ ++	☐ +++	☐ ++++	
Ketones	☐ Negative	☐ +	☐ ++	☐ +++	☐ ++++	
Glucose	☐ Negative	☐ +	☐ ++	☐ +++	☐ ++++	
pH	☐ 5.0	☐ 6.0	☐ 6.5	☐ 7.0	☐ 7.5	☐ 8.0 ☐ 8.5
Blood	☐ Negative	☐ Non-haem Trace	☐ Non-haem ++	☐ Haem Trace		
	☐ Haem +	☐ Haem ++	☐ Haem +++			

3. Did the urine appear cloudy? ☐ Yes ☐ No

4. Did the urine have an offensive smell? ☐ Yes ☐ No

5. Were any other tests peformed? (e.g microscopy, Dipslide Tests)

6. Would you under normal practice, send a sample for culture for this patient? ☐ Yes ☐ No

7. Please record the patient's temperature ☐☐.☐ ^{0}C

Treatment

8. Please record **all antibiotics** prescribed at this visit

Name	Dose per unit	Times per day	Duration (days)	Delayed?*

* Please tick the delayed box if the patient was given an antibiotic prescription in the event that their condition did not get any better.

9 Please record **all other medication** prescribed at this visit

Name	Dose per unit	Times per day	Duration (days)

10. Please record **any other** medication **advised** at this visit eg NSAID, Alkaline Agents

Name

11. Please give details of any **other advice** given? eg - drink plenty of fluid

12. At this first visit was a follow-up contact arranged with a GP or Nurse?

- ☐ No **If NO please skip to Q14**
- ☐ In 1-3 days time
- ☐ In 4-7 days time
- ☐ In 8-14 days time
- ☐ Other: Please specify

13. Is this **follow up** contact by:

- Phone ☐
- Face to face ☐

14. Would this case usually have been managed over the phone?

- Yes ☐
- No ☐

Once completed this form should be sent to:

POETIC, SEWTU, 7th floor Neuadd Meirionnydd, Cardiff University, Heath Park, Cardiff, CF14 4YS.

For SEWTU use only:	Received	Received by:	Entered onto database	Entered by:
	d d / m m / y y		d d / m m / y y	

3.2.3 POETIC study Diary (page 2 only)

13904

POETIC
point of care testing for urinary tract infection in primary care

SEVENTH FRAMEWORK PROGRAMME

SECTION 1: About you (please complete this section **on the day you saw your doctor**)

Part A.

1. Date of birth:

☐☐ / ☐☐ / ☐☐☐☐
d d m m y y y y

Part B. History of Urine Infections

1. Have you ever had a urine infection diagnosed by a doctor, at any point in the past (**Not counting this current episode**)? (Please tick **one box**)

☐ Yes ☐ No ☐ Do not know

If No or Do not know then go to **Part C**

2. How many times have you been treated for a urine infection in the past year? (Please tick **one box**)

☐ 0
☐ 1
☐ 2
☐ 3 or more
☐ Do not know

3. If you have had a urine infection in the past year, how many months since your last one? ☐☐

How was your last urine infection treated? (Please tick **one box**)

☐ Antibiotic Name of Antibiotic (if known) ☐
☐ Other If Other, please specify ☐
☐ Do not remember
☐ No treatment

3.2.4 UK guidelines: management of uncomplicated UTI in primary care

Diagnosis and Management of uncomplicated UTI in Adult Women in Primary Care

Adapted for the POETIC Study from the UTI Guideline Developed by the Health Protection Agency

KEY MESSAGES

- **The diagnosis of UTI is based primarily on symptoms and signs. Empirical antibiotics without any further diagnostic tests are indicated with a clinical diagnosis of urinary infection and 3 or more from dysuria, frequency, urgency, suprapubic tenderness and visible haematuria.**
- **Asymptomatic bacteriuria is rarely an indication for antibiotic treatment**
- **In sexually active young women with urinary symptoms consider *Chlamydia trachomatis***

DIAGNOSIS

Severe[2C] or ≥ 3 symptoms of UTI[4B+]
- **Dysuria**
- **Frequency**
- **Suprapubic tenderness**
- **Urgency**
- **Haematuria**

AND → NO vaginal discharge or irritation[2, 5C] → 90% culture positive → Give empirical antibiotic treatment (see below)

Mild or ≤ 2 symptoms of UTI (as above) → Obtain urine specimen → Urine NOT cloudy 97% NPV[7-9B-] → Consider other diagnosis

URINE CLOUDY
Perform urine dipstick test with nitrite
When reading test WAIT for the time recommended by the manufacturer

Positive nitrite, and leucocytes and blood 92% PPV[4B+]
Or
Positive nitrite alone[4B+]
→ Probable UTI
→ Treat with first line agents (see below)

Negative nitrite
Positive leucocyte[12]
→ UTI or other diagnosis equally likely
→ Review time of specimen *(morning is most reliable)*
Treat if severe symptoms or consider delayed antibiotic prescription and **send urine for culture**

Negative nitrite, leucocytes and blood 76% NPV[4B+]
Or
Negative nitrite and leucocyte
Positive blood[6] or protein[12]
→ Laboratory microscopy for red cells is less sensitive than dipstick
UTI Unlikely
→ Consider other diagnosis
Reassure and give advice on management of symptoms
Safety net in case of worsening symptoms

Predictive value of urinary dipstick testing

Negative Predictive Value				Positive Predictive Value		
-	-	-	Nitrite	+	+	-
+	-	-	Leuc	+	One or neither positive	+
-	+	-	Bld	+		+
57%	57%	76%		92%	81%	81%

URINE CULTURE IN WOMEN > 65 YEARS

- **Do not send urine for culture in asymptomatic elderly with positive dipsticks**
- Only send urine for culture if two or more signs of infection, especially dysuria, fever > 38 ° or new incontinence.[4, 5C]
- Do not treat asymptomatic bacteriuria in the elderly as it is very common.[1B+]
- Treating does not reduce mortality or prevent symptomatic episodes, but increases side effects & antibiotic resistance.[2, 3B+]

WHEN ELSE SHOULD I SEND A URINE FOR CULTURE?

- **Pregnancy:** If symptomatic, for investigation of possible UTI.[1B+]
  In all at 1st antenatal visit - as asymptomatic bacteriuria is associated with pyelonephritis & premature delivery.[1, 2B+]
- Suspected **pyelonephritis** [3C] *(loin pain and fever).*
- **Failed antibiotic treatment** or **persistent symptoms**.[5A+, 6B-]
  E. coli with Extended-spectrum Beta-lactamase enzymes are increasing in the community.
  ESBLs are multi-resistant but usually remain sensitive to nitrofurantoin or fosfomycin.[7, 8B+, 9A+]
- **Recurrent UTI, abnormalities of genitourinary tract, renal impairment** — more likely to have a resistant strain.

TREATMENT

First Line:

- Trimethoprim[12B+] (200 mg BD) or nitrofurantoin[13B+, 14C, 15B+] (100mg m/r BD[16C]; Do not use if eGFR < 60)
- 3 days[11, 17, 18A+]

Second Line: perform culture in all treatment failures[2B]

- Amoxicillin resistance is common; only use if susceptible[19B+]
- Community multi-resistant Extended-spectrum Beta-lactamase E. coli are increasing: consider nitrofurantoin (or fosfomycin 3g stat in women[20, 21B, 22A] plus 2nd 3g dose in men 3 days later[23]), on advice of microbiologist.
- Advise women with LUTI, who are prescribed nitrofurantoin, not to take alkalinising agents (such as potassium citrate).

Grading of Guidance Recommendations

In the development of this guidance a full Medline search for recent articles since the last review in 2008 was undertaken, other searches were undertaken at the discretion of the experts and development team. The guidance has been reviewed by members of CKS, The BIA, BSAC, RCGP and The Department of Health Antimicrobial Resistance and Health Care Associated Infections Advisory Group. It is in line with **CKS**, **SIGN** & **NICE**.

The strength of each recommendation is qualified by a letter in parenthesis.

Study design	Recommendation grade
Good recent systematic review of studies	A+
One or more rigorous studies, not combined	A-
One or more prospective studies	B+
One or more retrospective studies	B-
Formal combination of expert opinion	C
Informal opinion, other information	D

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3.2.5 Spain guidelines: management of uncomplicated UTI in primary care

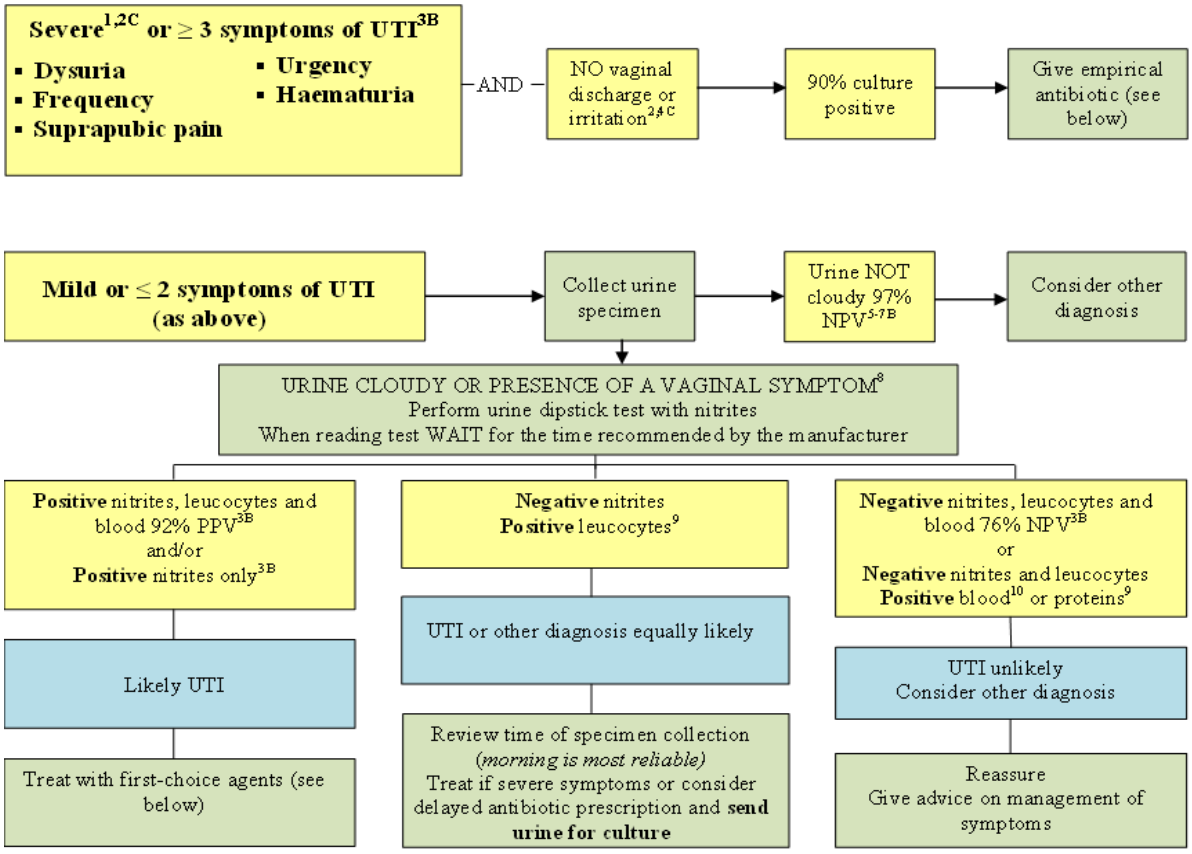
Diagnosis and management of uncomplicated UTI in adult women in primary care

Adapted for the POETIC study for the general practitioners in Spain

KEY MESSAGES

- **The diagnosis of UTI is based mainly on symptoms and signs. Empirical treatment without any further diagnostic tests are indicated with a clinical diagnosis of urinary tract infection and 3 or more form the following criteria: dysuria, frequency, urgency, suprapubic pain, and/or visible haematuria**
- **Asymptomatic bacteriuria is rarely an indication for antibiotic treatment**
- **Vaginitis should be considered in women with symptoms of UTI and vaginal discharge**
- **Infection caused by Chlamydia trachomatis should be considered in promiscual people, without stable partner, prostitutes and/or parenteral drug users**

DIAGNOSIS



Predictive value of urinary dipstick testing

Negative predictive value				Positive predictive value		
-	-	-	Nitrites	+	+	-
+	-	-	Leucocytes	+	One or neither positive	+
-	+	-	Blood	+		+
57%	57%	76%		92%	81%	81%

URINE CULTURE IN WOMEN > 65 YEARS

- **Do not send urine for culture in elderly women with typical clinical symptoms of UTI with positive dipsticks**
- Only send urine for culture if ≥2 signs of infection, especially recurrence, fever>38°C or incontinence[4C]
- O not treat asymptomatic bacteriuria in the elderly as it is very common[1B]
- Treating does not reduce mortality or prevent symptomatic episodes, but increases side effects and antibiotic resistance[2,11B]

WHEN ELSE SHOULD I COLLECT URINE FOR CULTURE?

- **Pregnancy:** If symptoms, investigate if there is UTI[1B]
  In all the visits – as asymptomatic bacteriuria is associated with pyelonephritis and premature delivery[1,2B]
- Suspected **pyelonephritis**[11C] *(loin pain and fever).*
- **Failed antibiotic treatment or persistent symptoms**[4A,10B]
- Extended-spectrum β-lactamase-producing *E. coli* and *K. pneumoniae* strains (ESBL) are increasing in the community. ESBLs are multiresistant but usually remain sensitive to nitrofurantoin or fosfomycin[5,6B,7A] Remember that bacteriemia occurs in 15-20% of the cases of UTI caused by ESBL-producing *E. coli* strains[12]
  The resistance rates of *E. coli* in Spain currently range from 8.7% and 29% towards amoxicillin and clavulanate and from 23.4% and 42.9% towards ciprofloxacin[13,14]
- **Recurrent UTIs**
- **Suspected subclinical pyelonephritis**: diabetes, anomalies of the genitourinary tract, renal function impairment, immunosuppression, episodes of UTI during childhood, episodes of pyelonephritis in the previous year, recurrences, catheterisation of the urinary tract, hospitalised UTI — more likely caused by a resistant strain

TREATMENT

First choice:

- Fosfomycina trometamol 3 g 1 day[1A]; or,
- Nitrofurantoin[1B] (50 mg q.i.d; do not use if clearance < 60 ml/min), for 7 days

Second choice (reserved antibiotics for uncomplicated urinary tract infections): order urine cultures in all the therapy failures[2B]

- Ciprofloxacin 250 mg (or ofloxacin 200 mg or norfloxacin 400 mg) b.i.d. for 3 days[1]
- Amoxicillin and clavulanate 500 mg t.i.d. for 5 days; use if the germ is susceptible[15B]
- Extended-spectrum β-lactamase-producing *E. coli* strains are increasing: consider nitrofurantoin (or fosfomycin 3 g in women[16,17B,18A]), or microbiologic counselling
- Give advice to women with cystitis who are prescribed nitrofurantoin not to take alkalinising products (such as potassium citrate)

Grading of Recommendations

In the development of this guideline a full Medline search until 2013 was undertaken and other searches were undertaken at the discretion of the experts and development team of POETIC study. The strength of each recommendation is qualified by a letter in superscript.

Study design	Recommendation grade
Good systematic review or one or more rigorous studies	A
One or more prospective or retrospective studies	B
Formal combination of expert opinion	C
Informal opinion, other information	D

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3.2.6 The Netherlands guidelines: management of uncomplicated UTI in primary care

Diagnosis and management of uncomplicated UTI in adult women in primary care

Adapted for the POETIC study from the Dutch UTI Guideline

Definitions

UTI: bacteriuria with clinical symptoms

Bacteriuria: indicated by a positive nitrite, dipslide showing at least 10^4 colony forming units (cfu) per ml or a culture showing at least 10^5 cfu/ml.

Guidelines for diagnosis

Anamnesis

Ask for complaints en symtoms indicating:

- cystitis: micturition symptoms such as painful or burning urination, increased urinary frequency, urgency, hematuria
- signs of tissue invasion (pyelonephritis): fever, chills, malaise, pain in the side, pain in the perineum, delirium

If symptoms of an UTI are present, also ask for:

- severity of symptoms/pain, previous episodes of similar complaints, new of changes the appearance of vaginal discharge, pain in the back or abdomen
- recent invasive bladder research
- in women with 3 or more UTIs per year: sexual activity, relationship with intercourse, use of condoms and/or spermicidal agents;

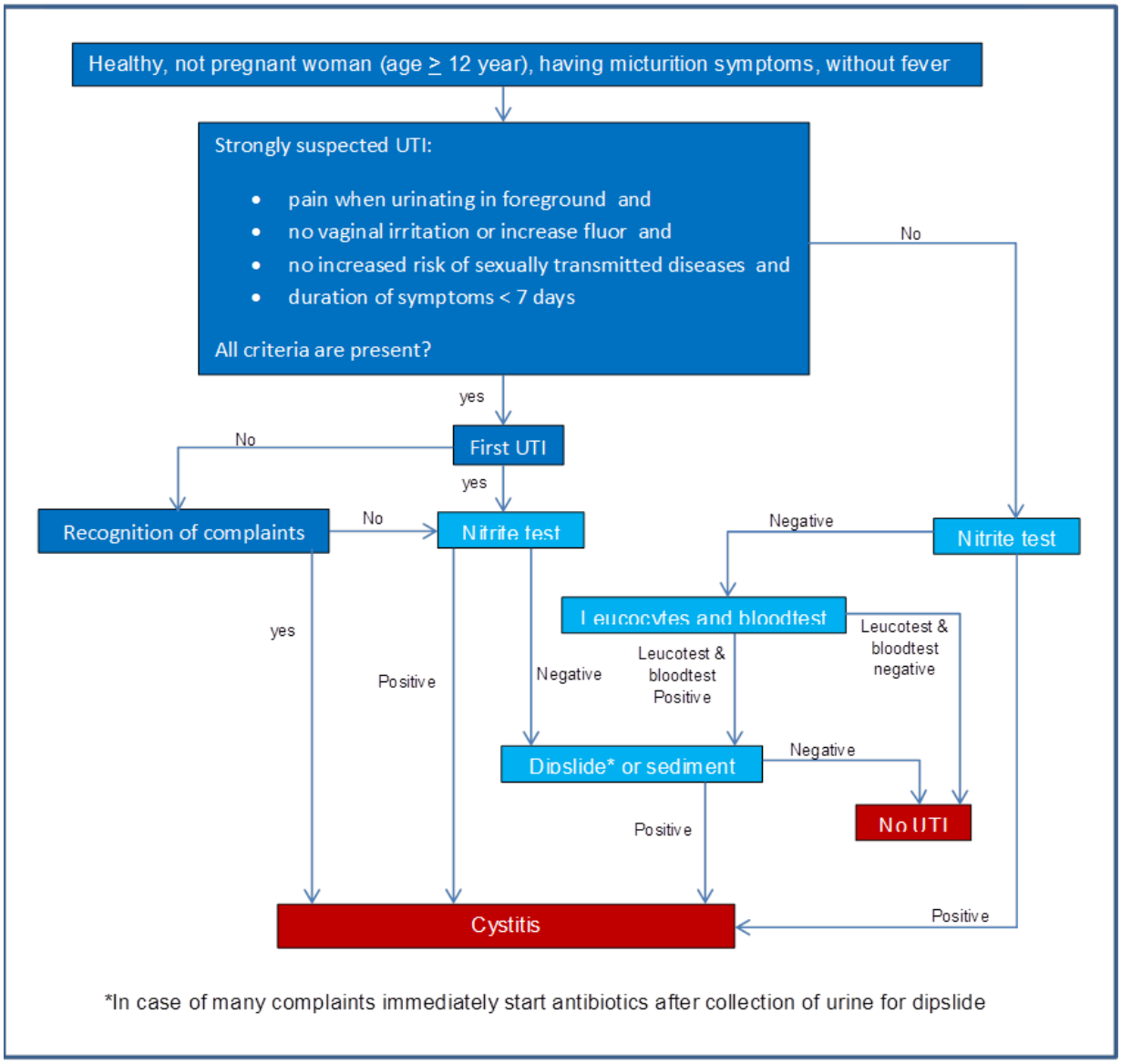
physical examination

Perform physical examination in case of:

- in women with 3 or more UTIs per year: inspection genital area, vaginal examination
- signs of tissue invasion: degree of illness, temperature, blood pressure, pulse, dehydration, delirium symptoms, abdominal examination (peritoneal irritation, pain in the side, urinary retention);

Analysis of urine

See flow-chart



Urine analysis for healthy, not pregnant women

Urine analysis is not needed if:

Strongly suspected UTI in healthy, not pregnant woman, who previously had an UTI and clearly recognizes the symptoms (see the flow-chart);
patients for whom an alternative diagnosis is more likely;

Guidelines for management of healthy, not pregnant women

Information and non-medication treatment

Cystitis is common, is not contagious, it can heal by itself and can occasionally come back.
Discuss the possibility of watchful waiting (while drinking a lot and, if necessary, use of analgesia) and prescription of delayed antibiotics.
In case of recurrent cystitis: drink a lot, do not postpone micturition, immediate micturition after intercourse, reconsider use of condoms / diaphragms with spermicidal lubricants.

Medicinal treatment

1st choice: nitrofurantoin 5 days, 2 dd 100 mg (regulated release) or 4 dd 50 mg
2nd choice: fosfomycin 1x 3g, 2 hours after finishing meal, preferably before going to sleep
3rd choice: trimethoprim 3 days, 1dd 300 mg before going to sleep

Medicinal treatment; options in case of recurrent cystitis

Self treatment: provide the woman with a prescription for nitrofurantoin or fosfomycin (as described above), which can be taken as soon as she recognizes the signs of an UTI.
Prophylaxis (in case of three or more urinary tract infections within a year). Choose from:
cranberry tablets (2 dd 500 mg) or liquid (according to instructions on the package);
continuous antibiotic prophylaxis for 6-12 months: nitrofurantoin (50-100 mg) or trimethoprim (100 mg), 1 dd before going to sleep;
post-coïtum prophylaxis, during 6-12 months: nitrofurantoin (50-100 mg) or 100 mg trimethoprim, within 2 hours after each coitus, 1 dd maximum;
For postmenopausal women: estriol vaginal ovules or cream (0.5 mg once daily, after 2 weeks reduce to 0.5 mg twice a week maximum) for a period up to maximal 6 months.

Control and referral

In case of cystitis: Analysis of urine if symptoms do not reduce sufficiently 3-5 days after starting treatment with antibiotics; In case of persistent symptoms after a second course: culture (via dipslide) and resistance determination.

In case of cystitis despite prophylaxis: Consider another method of prophylaxis after the patient is cured. Refer patient if UTIs recur frequently despite prophylaxis.

3.2.7 'Other' follow-up recommendations from POETIC observational study data

Follow Up Advice		Cardiff (n=20)		Southampton (n=5)		Spain (n=3)		The Netherlands (n=31)		Total (n=59)	
		n	%	n	%	n	%	n	%	n	%
Follow Up for Test Results	Patient to visit if MSU negative	1	5.0	0	0.0	0	0.0	1	3.2	2	3.4
	Follow up for blood test result	0	0.0	1	20.0	0	0.0	0	0.0	1	1.7
	Contact for dip-slide result	0	0.0	0	0.0	0	0.0	14	45.2	14	23.7
	Contact for urine test results	2	10.0	0	0.0	0	0.0	0	0.0	2	3.4
	Follow up for STI test results	0	0.0	0	0.0	0	0.0	1	3.2	1	1.7
	Follow up for Bladder USS with results	0	0.0	1	20.0	0	0.0	0	0.0	1	1.7
Further Testing	Follow up urine test after finishing antibiotics	1	5.0	0	0.0	0	0.0	6	19.4	7	11.9
	Repeat urine test if symptoms persist	0	0.0	1	20.0	0	0.0	0	0.0	1	1.7
	Repeat urine sample requested; day 2 morning sample	0	0.0	0	0.0	0	0.0	1	3.2	1	1.7
Symptomatic Follow Up	Follow up if symptoms worsen or persist	15	75.0	0	0.0	3	100.0	5	16.1	23	39.0
Other	Telephone back (in 4 days)	0	0.0	1	20.0	0	0.0	0	0.0	1	1.7
	Same day appointment made	1	5.0	0	0.0	0	0.0	2	6.5	3	5.1
	Follow up if any problems	0	0.0	1	20.0	0	0.0	0	0.0	1	1.7
	Follow up in 3 weeks	0	0.0	0	0.0	0	0.0	1	3.2	1	1.7

3.2.8 Multilevel Model: Antibiotics prescribed according to recommended network guidelines for type, total dose and duration

	Estimate	SE	OR	95% CI		Z ratio	Wald statistic/ Chi sq	P-value
Model Intercep	2.053	0.776	7.791	1.702	35.658	2.646	6.999	0.009
Country (reference Utrecht)								
Cardiff	-0.199	0.795	0.820	0.173	3.893	-0.250	0.063	0.802
Southampton	-2.420	0.793	0.089	0.019	0.421	-3.052	9.313	0.002
Madrid/Catalo	-3.179	0.797	0.042	0.009	0.199	-3.989	15.910	0.000
History of Treatment (reference 0)								
1	-0.164	0.415	0.849	0.376	1.914	-0.395	0.156	0.693
2	-0.556	0.411	0.573	0.256	1.283	-1.353	1.830	0.176
3 or more	-1.119	0.423	0.327	0.143	0.748	-2.645	6.998	0.008
Do not know	-0.447	0.787	0.640	0.137	2.991	-0.568	0.323	0.570
Night time frec	0.019	0.411	1.019	0.455	2.281	0.046	0.002	0.964
Fsymptoms of	0.035	0.426	1.036	0.449	2.387	0.082	0.007	0.933
Pain in the side	-0.376	0.317	0.687	0.369	1.278	-1.186	1.407	0.236
Restricted activ	-0.418	0.344	0.658	0.335	1.292	-1.215	1.477	0.224
Feeling Unwell	-0.173	0.357	0.841	0.418	1.693	-0.485	0.235	0.628
Urgency (high)	-0.168	0.371	0.845	0.409	1.749	-0.453	0.205	0.651
Dipstick leucocytes (reference ≥+)								
negative	0.933	0.507	2.542	0.941	6.867	1.840	3.386	0.066
Not done	1.298	0.488	3.662	1.407	9.530	2.660	7.075	0.008
Routine culture	-0.212	0.339	0.809	0.416	1.572	-0.625	0.391	0.532
empty variance	3.145	0.798	23.220	4.859	110.950	3.941	15.532	0.000
final variance	1.507	0.556	4.513	1.518	13.420	2.710	7.346	0.007
DIC empty:	714							
DIC final:	374.68							
Units: CSID	48							
Units: PID	364							
	Variance	SE	OR	95% CI		MOR	95% CI	
empty	3.145	0.798	23.220	4.859	110.950	97.295	8.119	22172.727
final	1.507	0.556	4.513	1.518	13.420	7.525	3.223	32.466

3.2.9 POETIC POCT Data

Cloudy Urine					
	Total	Cardiff	Southampton	Madrid and Catalonia	Utrecht
TP	132	34	33	20	45
TN	256	69	95	60	32
FP	127	49	50	18	10
FN	89	8	21	26	34
Sensitivity	0.597285	0.809524	0.611111	0.434783	0.56962
Specificity	0.668407	0.584746	0.655172	0.769231	0.761905
PPV	0.509653	0.409639	0.39759	0.526316	0.818182
NPV	0.742029	0.896104	0.818966	0.697674	0.484848
LR+	1.801261	1.949466	1.772222	1.884058	2.392405
LR-	0.602499	0.325742	0.593567	0.734783	0.564873
Odds ratio	2.989649	5.984694	2.985714	2.564103	4.235294

Dipstick Urinalysis					
	Total	Cardiff	Southampton	Madrid and Catalonia	Utrecht
TP	222	42	55	46	79
TN	56	21	6	26	3
FP	387	118	149	78	42
FN	54	14	6	31	3
Sensitivity	0.804348	0.75	0.901639	0.597403	0.963415
Specificity	0.126411	0.151079	0.03871	0.25	0.066667
PPV	0.364532	0.2625	0.269608	0.370968	0.652893
NPV	0.509091	0.6	0.5	0.45614	0.5
LR+	0.920739	0.883475	0.937947	0.796537	1.03223
LR-	1.547748	1.654762	2.540984	1.61039	0.54878
Odds ratio	0.594889	0.533898	0.369128	0.494624	1.880952

Dipstick Nitrite					
	Total	Cardiff	Southampton	Madrid and Catalonia	Utrecht
TP	126	21	33	22	50
TN	291	89	109	59	34
FP	78	14	37	19	8
FN	93	18	22	24	29
Sensitivity	0.575342	0.538462	0.6	0.478261	0.632911
Specificity	0.788618	0.864078	0.746575	0.75641	0.809524
PPV	0.617647	0.6	0.471429	0.536585	0.862069
NPV	0.757813	0.831776	0.832061	0.710843	0.539683
LR+	2.721812	3.961538	2.367568	1.963387	3.322785
LR-	0.538483	0.53414	0.53578	0.689757	0.453462

Odds ratio	5.054591	7.416667	4.418919	2.846491	7.327586
Dipstick Leucocyte					
	Total	Cardiff	Southampton	Madrid and Catalonia	Utrecht
TP	169	38	48	39	44
TN	81	26	31	6	18
FP	291	88	118	71	14
FN	35	3	7	6	19
Sensitivity	0.828431	0.926829	0.872727	0.866667	0.698413
Specificity	0.217742	0.22807	0.208054	0.077922	0.5625
PPV	0.367391	0.301587	0.289157	0.354545	0.758621
NPV	0.698276	0.896552	0.815789	0.5	0.486486
LR+	1.059026	1.200665	1.102003	0.939906	1.596372
LR-	0.787945	0.320826	0.61173	1.711111	0.536155
Odds ratio	1.344035	3.742424	1.801453	0.549296	2.977444
Requesting routine culture					
	Total	Cardiff	Southampton	Madrid and Catalonia	Utrecht
TP	113	32	34	25	22
TN	176	48	52	62	14
FP	246	85	90	42	29
FN	150	23	22	51	54
Sensitivity	0.429658	0.581818	0.607143	0.328947	0.289474
Specificity	0.417062	0.360902	0.366197	0.596154	0.325581
PPV	0.314763	0.273504	0.274194	0.373134	0.431373
NPV	0.539877	0.676056	0.702703	0.548673	0.205882
LR+	0.737055	0.910374	0.957937	0.814536	0.42922
LR-	1.367525	1.158712	1.072802	1.125637	2.182331
Odds ratio	0.53897	0.785678	0.892929	0.723623	0.196679