'Nappy pad' urine samples for guiding investigation and treatment of urinary tract infection (UTI) in young children: Findings from the 'DUTY' prospective diagnostic cohort study

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Abstract

Background

Although sampling urine using nappy pads is preferred by parents and recommended when a clean catch sample can't be obtained, we do not know the added diagnostic utility of 'nappy pad' urine samples, nor the proportion that are contaminated.

Setting

Acutely unwell children <5 years presenting to 233 primary care sites in England and Wales.

Method

Logistic regression to identify independent associations of symptoms, signs and urine dipstick test results with UTI; diagnostic utility quantified as area under the receiver operator curves (AUROC). Nappy pad rule characteristics, AUROC, and contamination compared to findings from clean catch samples.

Results

Nappy pad samples were obtained from 3205 children (82% <2 years; 48% female), and culture results available for 2277 (71.0%). 30 (1.3%) met our laboratory definition of UTI. Female gender, smelly urine, darker urine, and absence of nappy rash were independently associated with UTI, with an internally validated, coefficient model AUROC of 0.81 (0.87 for clean catch) that increased to 0.87 (0.90 for clean catch) with the addition of dipstick results. GPs' 'working diagnosis' had an AUROC 0.63 (95% CI 0.53 to 0.72). 12.2% of nappy pad and 1.8% of clean-catch samples were 'frankly contaminated' (risk ratio 6.66; 95% CI 4.95 to 8.96; p <0.001).

Conclusion

Nappy pad urine culture results, with features that can be reported by parents and dipstick tests, can be clinically useful, but are less accurate and more often contaminated compared to clean catch urine culture results, which should be prioritised. Dipstick testing adds diagnostic accuracy.

Key words

Urinary Tract Infections; Paediatrics; Diagnosis; Anti-Bacterial Agents;

How this fits in

Up to 80% of urinary tract infections (UTI) in young children presenting to primary care are missed. Timely and accurate diagnosis is essential to avoid over- and under- treatment and investigation. This is especially difficult in pre-verbal children who are not toilet trained, and present with undifferentiated symptoms. General practitioners use, and parents prefer, nappy pads for collecting urine from children who are still in nappies, but the clinical utility of data derived from nappy pad samples, the added value of dipstick testing, and the proportion of contaminated samples is not known. We found that culture results from urine obtained using nappy pads, together with features that can be reported by parents, can be clinically useful in identifying acutely unwell pre-school children presenting to primary care who have a UTI, but with less accuracy compared to clean catch sampling. However, contamination rates are nearly seven times higher in nappy pad than in clean catch samples. Clean catch urine sampling in children in primary care should therefore be prioritised over the nappy pad method, but if urine sampling is done using nappy pads, then the addition of dipstick testing significantly improves diagnostic accuracy.

Introduction

Urinary tract infection (UTI) may be missed in up to 80% of children presenting to primary care.(1, 2) Accurate diagnosis of UTI is essential to avoid over or under treatment with antibiotics and to appropriately target burdensome and expensive investigations.(3) This is especially important in younger, pre-verbal children who are not yet toilet trained and who often present with non-specific symptoms, making the decision about which children to investigate for UTI difficult.(3) Obtaining a urine sample can be time consuming and especially challenging in primary care where most children first present.(4) The nappy pad sampling method has been recommended by National Institute for Health and Care Excellence (NICE) in young children in nappies (diapers) when a clean catch sample cannot be obtained.(3) Urine sampling needs to be simple, relaible and acceptable, and parents find nappy pads easy to use, comfortable for their children, and prefer them to the clean catch method.(5) Nappy pad sampling is used in everyday care(1), and general practitioners report using nappy pad urine collection in over 40% of infants.(6) Many parents feel that the clean catch method is messy and time consuming and give up trying.(3, 5) However, the clinical utility of the information obtained from the nappy pad method of urine sampling is unclear, contamination rates may be higher than other sampling methods, and children in nappies present differently to older children who are more able to describe symptoms and in whom clean catch sampling is easier. Obtaining urine samples by more invasive methods such as supra-pubic aspiration or catheterisation is neither feasible nor acceptable in most primary care settings. We therefore aimed to develop a clinical prediction rule for the diagnosis of UTI based on sampling using the nappy pad method, and compare its diagnostic utility to a similar rule based on 'clean catch' urine samples (to be reported elsewhere in full).(7) In addition, we estimated the added diagnostic value of dipstick testing once a nappy pad sample was obtained and compared contamination rates by sampling method.

Methods

Participants

The Diagnosis of Urinary Tract infection in Young children (DUTY) study was a multicentre, prospective, diagnostic cohort study that recruited children aged less than five years in primary care.(8) Children were eligible if presenting with any acute (<28 days), undifferentiated illness (even when the clinician was confident of the diagnosis, such as a child with bronchiolitis) and/or new urinary symptoms.

Index tests and urine collection

Following consent, 107 index test items were recorded. Parent reported items included the child's medical history and symptoms. Clinician assessed items came from a full clinical examination, including their global impression of illness severity (rated 0 to 10), their rating of the likelihood of UTI, and urine dipstick results

(performed after rating UTI likelihood). Index test items were derived from literature review and input from our co-investigator group.

We used the NICE recommended 'Newcastle Nappy Pads' for those children who wore nappies (diapers) and for those in whom the parent/guardian did not think clean catch would be successful.(3, 8, 9) First, the parent was asked to clean the nappy area using water or wipes (the wipes being supplied by the study). A nappy pad was inserted inside a clean nappy, and the nappy refastened. The nappy pad was removed as soon as the child urinated in order to reduce the risk of contamination. The perineum was cleaned again and a fresh pad inserted every 30 minutes until micturition or immediately if the pad became contaminated with faeces. Once the child had urinated, and wearing disposable gloves, the research nurse or clinical study officer (RN/CSO) removed the pad and urine was extracted into a sterile container as per the manufacturer's instructions. If it was not possible to obtain a sample prior to the child leaving the primary care site, the parent was given the necessary equipment and advice on obtaining a urine sample at home. The parent was advised to store the sample in the fridge and return it to their primary care site as soon as possible, ideally within 24 hours. The RN/CSO telephoned parents the next day to remind them to return the sample. Where feasible, the RN/CSO offered to collect the urine sample from the child's home.

At the primary care site, urine samples were dipstick tested (using Siemans/Bayer multistix 8SG) for blood, protein, glucose, ketones, nitrite, leukocyte esterase, pH and specific gravity (eight dipstick index tests). All index tests and the clinician's working diagnosis ('clinical diagnosis') were measured blind to the reference standard.

When there was at least 1ml of urine leftover urine after a priority sample was sent to a National Health Service (NHS) laboratory, it was sent using first class Royal Mail Safeboxes^{TM} in boric acid monovettes to the Public Health Wales Microbiology Laboratory in Cardiff ("research laboratory"). Results from the research laboratory are used in the current analyses.

Reference standard

The research laboratory spiral plated (Don Whitley, UK) 50uL onto chromogenic agar and standard blood agar. A full description of the methods used in the research laboratory, and how these differed from the range of standard operating procedures in local NHS labs, is forthcoming.(10) Quantitative total counts were recorded for up to six organisms and the presence of antimicrobial substances measured. Samples were processed using a single, standardised procedure. We defined uropathogens as members of the *Enterobacteriaceae* group. Our microbiological definition of UTI was the presence of $\geq 10^5$ Colony Forming Units (CFU)/mL of a single uropathogen ('pure growth') or $\geq 10^5$ CFU/mL of a uropathogen with ≥ 3 $\log_{10}$

(1000-fold) difference between the growth of this and the next species ('predominant growth'). Aside from children's dates of birth, laboratory staff were blind to index tests. As there is no single accepted definition of contamination, we considered three definitions: growth of more than two organisms of more than $>10^5$ (frank contamination) (11); growth of two or more organisms at more than 10^5 (12, 13); and growth of more than two organisms at greater than 10^4 >2 organisms (probable contamination according to Jackson(11) or frank contamination according to Bekeris(14)).

Statistical analysis

We examined the frequency of symptom and sign categories, blind to their associations with urine culture results, and merged the least frequent categories prior to analyses. We used logistic regression to estimate associations of index tests with urine culture positivity. P-values were derived using likelihood ratio tests. For ordinal variables, both heterogeneity and trend p-values were derived. Continuous variables were divided into quintiles and trend p-values were derived using the median within categories. We examined plots of the log odds of culture positivity against the median within quintiles for evidence of non-linearity. We used two methods for dealing with missing data, including the response "don't know" to questions about the presence of symptoms such as pain, or crying when passing urine. In both univariable and multivariable analyses missing data were coded as the modal value, usually absence of the symptom. We repeated multivariable analyses using the chained equations approach to multiple imputation: estimates and Wald p-values (15) based on 50 imputed datasets derived using Rubin's rules.(16)

We derived prediction rules in three stages. First, we selected symptoms and signs with either trend or heterogeneity univariable p values <0.01. Second, we derived models from selected symptoms and signs using backwards stepwise selection and an exclusion criterion of heterogeneity p value >0.1. Third, we used backwards stepwise selection with the same exclusion p value for models in which dipstick results were added. We investigated the effect of using more liberal p value thresholds of 0.1 and 0.2 at the first stage, and found no important differences in the final models (results available from the authors on request).

We quantified diagnostic accuracy as the Area Under the Receiver Operating Characteristic (AUROC) curve (also known as the c-statistic). We also estimated AUROC values for clinical judgement of UTI. We conducted internal validation of the models using the bootstrap procedure described by Steyerberg (17) and calculated a calibration slope (shrinkage factor) by which we multiplied model coefficients in order to derive internally validated odds ratios. For each model, we selected cut-points corresponding to a range of values for sensitivity, and then calculated the corresponding specificity, negative and positive predictive values, and the proportion of children classified positive. These were compared against 'clinical diagnosis'

of UTI (where clinicians considered UTI to be 'fairly' or 'very' certain). We re-ran models leaving out predictive features that might lack face validity.

Sample size calculation

Our sample size calculation assumed a candidate predictor with 10% prevalence and UTI prevalence of 2%. With 80% power and a two-sided alpha of 5%, 3000 urine sample results were required to detect an odds ratio of 2.4 while 3100 results would give a 95% CI with width 10% for an algorithm with 80% sensitivity. We originally proposed to recruit 4000 children, combining analyses for children with both clean catch and nappy pad samples, anticipating we would recover urines from 77.5% for algorithm derivation, and a further 2000 children for external validation. However, we did not anticipate the need to stratify and report separate analyses by urine collection method. For the present analysis, we therefore decided to use all available nappy pad results to derive the model, and to conduct internal bootstrap validation instead of external validation.

Results

Participants

We recruited participants from 233 primary care sites (225 GP practices, four Walk-in Centres and four paediatric EDs) across England and Wales between April 2010 and April 2012. Of 10138 children screened eligible, 1276 (12.6%) declined participation, 1684 (16.6%) could not be recruited for other reasons and 15 (0.15%) withdrew.

Of the 234 primary care sites taking part, 198 sites (85%) completed and returned at least one screening log to the study centres. These show that 7350 children were screened but not recruited because they declined (1276), were not eligible (4390), or for other reasons (1684) which included: left the primary care site prior to invitation (811); did not give consent (214) or there was a language barrier (112) and an appropriate translator was not available at the time of recruitment. There were slightly more boys (mean difference of 5.2%; 95% CI 2.2% to 8.2%) among those for whom participation was declined (n = 1276) compared to those who did agree to participate in the DUTY Study (n = 7163). The mean age in the declined sample was 24.06 months vs. 26.88 months among participants (mean difference 2.04 months, 95% CI 1.08 to 3.00 months). We did not collect clinical information on those who declined.

Urine was collected from a total of 6241 children, 3205 using nappy pads. 3164 (98.7%) of nappy pad samples were sent to NHS labs and 2363 (73.7%) to the research laboratory. The number of reference standard results available from the research laboratory (our final analytic sample) was 2277 (71.0%). 82% of children providing nappy pad samples were aged <2 years (mean 1.3, SD 0.8), and the mean illness severity score was 2.3 points (SD 1.5). 48% of children were girls; and overall, 1.3% had a UTI (Table 1). The clean

catch sample (n=2740) had mean age 3.5 years (SD 1.0), mean illness severity score 2.2 points (SD 1.6) and 53.8% were girls.

2102 (92.3%) samples were provided within 24 hours of index test measurement and there was no relationship between UTI and time from urine collection to laboratory arrival. Antimicrobial substances, which can arise from the use of both systemic antibiotics and locally applied cleaning agents, were found in 6.6% of nappy pad samples, and were more likely to be present in children with than without UTI. GPs' 'working diagnosis' would have correctly identified 4 (13.3%) of the 30 UTIs with 97.0% specificity and an AUROC 0.63 (95% CI 0.53 to 0.72).

Nappy pad model

Table 2 shows adjusted odds ratios for the index tests (marked* in Table 1) selected for the nappy pad model. Parent-reported smelly urine, darker urine, female gender and the absence of a nappy rash were independently associated with UTI: for the first two there was evidence of graded associations. No clinical examination findings were independently associated with UTI. The presence of leukocytes and nitrites from dipstick urine testing were independently associated with UTI. The symptoms and signs model had reasonable diagnostic accuracy (validated AUROC for the multiple imputed model was 0.78 and diagnostic accuracy increased (p=0.036) with addition of dipstick findings (validated AUROC 0.82)). Figure 2 shows the multiple imputed ROC curves for the models with and without dipstick urinalysis.

We re-ran the multiple imputation analysis excluding the nappy rash variable, and found that there was a reduction of 0.07 in validated AUROC in the symptom and sign model (from 0.78 to 0.71) and a reduction of 0.03 in the validated AUROC in the symptom, sign and dipstick model (from 0.82 to 0.80). We checked the association between antimicrobial substances in the urine and nappy rash, and found no association (p=0.82).

Comparison of findings using nappy pads and catch samples

The validated AUROC for the nappy pad model was inferior to the model derived using clean catch samples, which was 0.87 for symptoms and signs, increasing to 0.90 with dipstick results (to be reported fully in a future publication). Table 3 provides the proportion of nappy pad and clean catch samples considered to be contaminated according to three published definitions. (11-14) 'Frankly contaminated' urine was found in 12.2% of nappy pad and 1.8% of clean-catch samples, risk ratio 6.66 (95% CI 4.95 to 8.96; p <0.001).

Discussion

Summary of findings

Four features (female gender, smelly urine, darker urine, and absence of nappy rash) that could be reported by parents, and no clinical signs, were associated with a microbiological diagnosis of UTI in children sampled using nappy pads. These features were substantially more predictive of a microbiological diagnosis of a UTI than clinicians' 'working' or clinical diagnoses, but less predictive than data obtained for older children sampled using the clean catch methods. More than 10% of samples obtained by nappy pads were 'frankly' contaminated, compared to less than 2% of samples obtained by clean catch from predominantly older children. The addition of dipstick testing improved diagnostic accuracy of nappy pad samples.

Strengths and limitations

The DUTY study is the largest primary care diagnostic accuracy study of clinical symptoms, signs and dipstick tests for diagnosing UTI in young children, and it achieved high levels of data completeness. We asked clinicians to obtain a clean catch urine sample whenever possible, but ultimately the decision whether to sample by clean catch or the nappy pad method was up to the parents, who generally used the nappy pad method in younger children. Children sampled using the nappy pad method were therefore younger and may have differed in other ways as well, for example being more unwell or distressed. We asked parents to replace pads at regular intervals until a sample was obtained. However, we did not use alarms to trigger scheduled replacement of pad, and this may have led to increased contamination rates.(12)

There were a relatively small number of UTI diagnoses, and we found that overall NHS laboratories have identified higher numbers of UTIs in this and in previous studies.(2, 18) It is plausible that the nappy pad contamination masked the presence of UTI leading to under-diagnosis in comparison to clean catch samples, in which lower contamination and higher UTI rates were observed. Our conservative criteria for a microbiological UTI diagnosis may have also contributed.

Our study reference standard defined uropathogens as members of the *Enterobacteriaceae* family at the UK guidelines'(19, 20) threshold of a pure/predominant growth of $\geq 10^5$ CFU/ml. We chose not to use a lower threshold as this carries an increased risk of false positives, although there are recommendations that a lower threshold should be used.(3, 21, 22) The diagnosis of UTI is a clinical one taking microbiological analysis into account, and a lower microbiological threshold in the presence of high clinical suspicion would be acceptable for the purposes of clinical care as opposed to this diagnostic study. We used a rigorous criterion (minimum 3-log difference between the predominant and next most concentrated organism) for defining predominance. This definition could have reduced estimated prevalence if some UTIs were

incorrectly classified as contamination. In addition, a small proportion of the positive cultures may have been false positives due to asymptomatic bacteriuria or contamination.

Collecting an uncontaminated urine specimen is most difficult in the youngest children, and no method has yet been found to reliably distinguish pathogen from contaminant, especially when they co-exist. Our definition of UTI excluded atypical bacteria causing UTIs, which are also thought to be more common in younger children, potentially reducing our estimated UTI prevalence.(19) (23)

Results in context with other studies

The NICE guidelines found "insufficient data to draw conclusions about urine collection bags and urine collection pads," but recommended their use when a clean catch sample cannot be obtained.(3) We have not been able to identify further studies addressing nappy pads since the NICE recommendations were published.(24) A systematic review of the accuracy of specimens obtained from nappy pads included three studies that compared sampling by nappy pad to sampling by urine bag, and one study that compared nappy pad specimens to specimens obtained by suprapubic aspiration. The latter study found 100% sensitivity and 94% specificity between the two methods. A randomised trial found that replacing pads every 30 minutes until a sample was obtained reduced contamination.(12)

We found a 1.3% prevalence of microbiological diagnoses of UTI. The only other UK primary care study found a 6% prevalence when urines were analyzed in National Health Service (NHS) laboratories.(2) We found a similar UTI prevalence of 5.6% for the DUTY study urines overall in NHS laboratories.(18) Fever was not an inclusion criterion in that study or in ours. However, a systematic review of 10 studies, eight of which were conducted in hospital emergency department, with one in a clinic and emergency department setting, and one in a clinic setting, and all conducted in the USA apart from a clinic study In Taiwan, found a UTI prevalence of 7% among infants presenting with fever,(25). An Australian study (with incomplete urine sampling) found a prevalence of 3.4% children presenting with fever to emergency departments. (26)

Our systematic search identified one systematic review that included eight primary studies of 7892 children aged less than 5 year(24) and three further primary studies (26-28) that included 17462 children, that assessed associations between clinical features symptoms and signs and a UTI diagnosis. The data we found show that no individual symptom or sign or combination of symptoms or signs was sufficient to rule in a diagnosis of UTI. Among the remaining studies, largely conducted in hospital emergency departments, abdominal pain, back pain, dysuria, frequency, and new-onset urinary incontinence increased the likelihood of a UTI.(29) Stridor, audible wheeze, circumcision, temperature <39°C with a source, abnormal chest sounds, chest crackles, age 3 years or less, not feeling hot, and breathing difficulty decreased the likelihood

of UTI. The largest study, which included almost 16,000 children aged less than five years presenting to emergency departments in Australia,(26) derived a diagnostic model based on a combination of 27 symptoms and signs. However, this study did not involve systematic urine sampling, and most children did not have urine sampled. This model was found to have an AUROC of 0.80 (95% CI 0.78 to 0.82), which is similar to findings from our DUTY study for children sampled with nappy pads.

Previous investigation of malodorous urine has shown conflicting results,(30) but our study strongly supports its diagnostic value. We investigated, but did not find evidence for, a number of non-specific symptoms (including fever, vomiting, lethargy, irritability and poor feeding) previously found to be associated with UTI (24) and recommended for clinical use by NICE.(3) It remains possible that such symptoms are of use in the secondary care settings in which studies reporting their utility were conducted, or in children with a different illness spectrum. This finding underlines the importance of including a wide range of illness presentation in studies of predictors of diagnoses, especially when symptoms and signs are notoriously non-specific. Studies that include only children with symptoms and signs previously found to be associated with the diagnosis risks missing previously unidentified predictors and 'research circularity' (looking for, and finding, symptoms and signs in children included in studies if they have those symptoms and signs).

The reduction in the risk of UTI associated with presence of a nappy rash should be interpreted with caution. The inverse association may arise through lower likelihood of a UTI when there is a plausible alternative diagnosis (conditioning on the common effect of primary care attendance). (31) Alternative explanations are that rash may be a risk factor for contamination of urine, and this masks the presence of a UTI, or that skin products used to treat nappy rash could render the urine sterile. However, we found no evidence of an association between nappy rash and contamination, nor the presence of antimicrobial substances in the urine and nappy rash. An increased likelihood of contamination of nappy pad samples might also explain the more modest associations of symptoms and dipstick test results with UTI than were found in clean catch samples.(10) We re-ran the models excluding nappy rash and found modest reductions in the symptoms and signs, and symptoms, signs and dipstick model AUROCs (respectively 0.07 and 0.03).

Conclusions

Nappy pad urine sample culture results together with symptoms that can be reported by parents can be clinically useful in identifying acutely unwell pre-school children presenting to primary care who have a UTI, but with less accuracy, and with increased contamination compared to clean catch sampling. Clean catch urine sampling in children in primary care should be prioritised, especially in children with nappy rash.

However, if sampling is done using nappy pads, then the addition of dipstick testing significantly improves diagnostic accuracy.

Further research is needed to distinguish pathogens from contaminants, and to establish the cost effectiveness of different sensitivity/specificity cut points using routine health service laboratory results. We do not know precisely how results from clean catch sampling would compare to nappy pad sampling in younger children: contamination may vary by age as well as by sampling method. Randomising children to sampling method would shed light on this.

Ethical approval and the role of the funder

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Competing interest statement

All authors declare they have no conflict of interest and have signed the conflict of interest form.

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Table 1. Nappy pad samples: children's characteristics and crude odds ratios for index tests* associated with UTI

Demographics/index tests	**N (%)[a]**	**UTI (%)[b]**	**Crude OR (95% CI)[e]**
Total	2277	30 (1.3%)	
Age of child			
< 6 months	369 (16.2%)	5 (1.4%)	1.72 (0.54,5.46)
6 - < 12 months	603 (26.5%)	11 (1.8%)	2.33 (0.90,6.04)
1 - < 2 years	884 (38.8%)	7 (0.8%)	1 (ref)
2 - <3 years	353 (15.5%)	7 (2.0%)	2.53 (0.88, 7.28)
3 - <4 years	58 (2.5%)	0 (0.0%)	n/a
4 years plus	10 (0.4%)	0 (0.0%)	n/a
Time from index tests to taking urine sample			
Sample before recruitment	120 (5.3%)	2 (1.7%)	1.33 (0.31,5.67)
< 24 hours	1982 (87.0%)	25 (1.3%)	1 (ref)
24 hours to < 48 hours	109 (4.8%)	3 (2.8%)	2.22 (0.66,7.45)
48 hours to < 72 hours	18 (0.8%)	0 (0.0%)	n/a
72 hours plus	48 (2.1%)	0 (0.0%)	n/a
Clinician diagnosis prior to dipstick			
No UTI certain to very certain	1033 (45.4%)	8 (0.8%)	0.52 (0.22,1.19)
Uncertain or No UTI fairly certain	1201 (52.7%)	18 (1.5%)	1(ref)
UTI fairly to very certain	38 (1.7%)	4 (10.5%)	7.76 (2.49, 24.18)
Missing	5 (0.2%)	0 (0.0%)	
Gender			
Male	1183 (52.0%)	9 (0.8%)	1 (ref)
Female	1094 (48.0%)	21 (1.9%)	2.55 (1.16,5.60)
Smelly urine			
No problem	1518 (66.7%)	12 (0.8%)	1 (ref)
Slight problem	206 (9.0%)	4 (1.9%)	2.20 (0.73,6.61)
Moderate problem	138 (6.1%)	5 (3.6%)	4.18 (1.52,11.50)
Severe problem	26 (1.1%)	4 (15.4%)	20.21 (6.29,64.97)
Missing/not known	389 (17.1%)	5 (1.3%)	
Darker urine			
No problem	1764 (77.5%)	19 (1.1%)	1 (ref)
Slight problem	83 (3.6%)	2 (2.4%)	2.19 (0.51,9.43)
Moderate or severe problem	41 (1.8%)	4 (9.8%)	9.59 (3.17,29.02)

Demographics/index tests	**N (%)[a]**	**UTI (%)[b]**	**Crude OR (95% CI)[e]**
Missing/not known	389 (17.1%)	5 (1.3%)	
Nappy rash[d]			
No problem	1715 (75.3%)	29 (1.7%)	1 (ref)
Slight to severe problem	560 (24.6%)	1 (0.2%)	0.10 (0.01,0.77)
Missing	2 (0.1%)	0 (0.0%)	
Dipstick: leucocytes			
Negative	1759 (77.3%)	13 (0.7%)	1 (ref)
Trace	125 (5.5%)	1 (0.8%)	1.09 (0.14,8.38)
+	119 (5.2%)	4 (3.4%)	4.69 (1.50,14.61)
++	177 (7.8%)	4 (2.3%)	3.12 (1.01,9.66)
+++	91 (4.0%)	8 (8.8%)	12.99 (5.24,32.20)
Missing	6 (0.3%)	0 (0.0%)	
Dipstick: nitrites			
Negative	1916 (84.1%)	13 (0.7%)	1 (ref)
Positive	355 (15.6%)	17 (4.8%)	7.39 (3.55,15.35)
Missing	6 (0.3%)	0 (0.0%)	

[a] Percentage relative to the total number of observations (i.e. 2277)

[b] Percentage relative to the total number of observations within that category

[c] Crude ORs calculated using modal imputation

Table 2. Nappy pad samples: models based on symptoms and signs; and on symptoms, signs and dipstick results, including results based on multiple imputation. Odds ratios calculated using shrunken estimates from the bootstrap internal validation calibration slope

	Symptom and sign model		**Symptom sign and dipstick model**	
Index tests	**Adjusted OR (95 % CI)[a]**	**MI[b] adjusted OR (95% CI)**	**Adjusted OR (95 % CI)[a]**	**MI[b] adjusted OR (95% CI)**
Gender				
Male	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Female	1.95 (1.11,3.41)	1.96 (1.06,3.61)	1.41 (0.80,2.48)	1.45 (0.78,2.72)
Smelly urine				
No problem	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Slight problem	1.61 (0.73,3.54)	1.97 (0.82,4.71)	1.44 (0.67,3.11)	1.79 (0.76,4.23)
Moderate problem	2.51 (1.14,5.51)	3.39 (1.46,7.89)	2.15 (0.98,4.68)	2.96 (1.26,6.97)
Severe problem	7.40 (2.98,18.36)	10.14 (3.85,26.69)	3.97 (1.58,9.96)	6.13 (2.28,16.47)
Darker urine				
No problem	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Slight problem	1.89 (0.66,5.46)	1.99 (0.65,6.12)	1.81 (0.65,5.07)	1.92 (0.63,5.88)
Moderate or severe problem	2.46 (0.98,6.21)	2.26 (0.85,6.01)	2.29 (0.93,5.62)	2.27 (0.87,5.93)
Nappy rash				
No problem	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Slight to severe problem	0.16 (0.04,0.66)	0.13 (0.03,0.61)	0.19 (0.05,0.71)	0.16 (0.04,0.66)
Dipstick: leukocytes				
Negative			1 (ref)	1 (ref)
Trace			0.87 (0.23,3.31)	0.81 (0.18,3.61)
+			2.06 (0.92,4.63)	2.18 (0.88,5.43)
++			1.63 (0.73,3.62)	1.78 (0.73,4.30)
+++			3.27 (1.66,6.41)	3.35 (1.57,7.15)
Dipstick: nitrites				
Negative			1 (ref)	1 (ref)
Positive			3.16 (1.91,5.24)	3.70 (2.10,6.52)
ROC (95% CI)	0.769 (0.68, 0.85)	0.805 (0.72, 0.89)	0.858 (0.79, 0.93)	0.870 (0.80, 0.94)
Validated ROC[c]	0.744	0.778	0.799	0.821
Δ ROC[d] (95% CI)			0.089 (0.02,0.16)	0.065 (0.00,0.13)
Δ ROC[d] p-value			0.012	0.036

Calibration slope	0.695	0.749	0.647	0.708

[a] Missing values coded to modal category [b]MI: multiple imputation [c] Internal validation using the bootstrap procedure [d] The difference in ROC between symptom and sign model and symptom, sign and dipstick model

Figure 2. Nappy pad ROC curve from multiple imputation symptoms and signs only (solid line) and symptoms, signs and dipstick (dotted line)

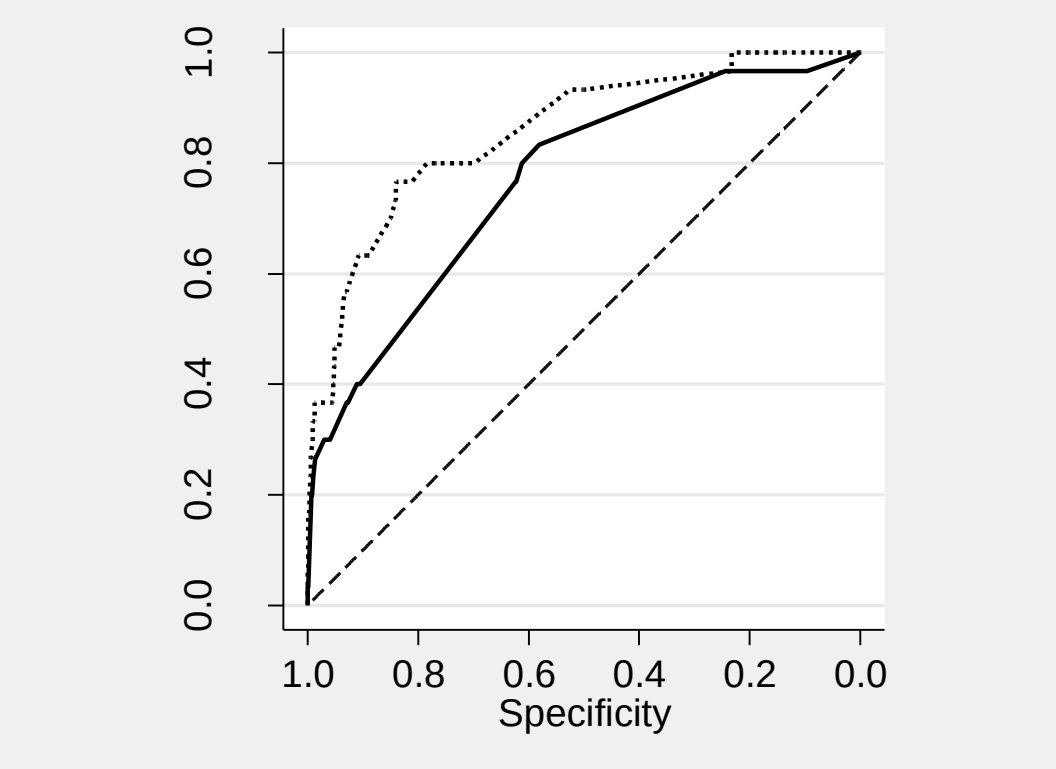

Table 3. The numbers of contaminated samples using different definitions of contamination. The highlighted row is the definition of contamination that we use in our subsequent analyses

Paper	**Contamination definition**	**Clean catch contaminated N(%)**	**Nappy pad contaminated N(%)**	**Risk difference (95% CI)**	**Risk ratio (95% CI)**	**P value**
Jackson (11), frank contamination	>10e5 >2 organisms	50/2740 (1.8%)	277/2277 (12.2%)	0.103 (0.089,0.118)	6.666 (4.959,8.963)	<0.001
Feasey (13)or Rao(12)	>10e5 ≥2 organisms	78/2740 (2.8%)	426/2277 (18.7%)	0.159 (0.141,0.176)	6.572 (5.196,8.312)	<0.001
Jackson(11) probable or frank contamination or Bekeris(14)	>10e4 >2 organisms	175/2740 (6.4%)	599/2277 (26.3%)	0.199 (0.179,0.219)	4.119 (3.513,4.829)	<0.001

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