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Detection of *Candida* in Concentrated Oral Rinse Cultures by Real-Time PCR

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The incidence of oral candidosis has increased in recent years, largely as a result of the emergence of human immunodeficiency virus infection and the more widespread use of immunosuppressive chemotherapy. This development has been associated with a need for more reliable methods for the detection of *Candida*. The present study assessed the performance of a real-time PCR and two block-based PCRs for the detection of *Candida* in 193 concentrated oral rinse culture (CRC) specimens. A total of 102 CRC specimens were positive by culture for *Candida*; and 96, 90, and 75 of these were also positive by real-time, N18-specific, and internal transcribed spacer (ITS)-specific PCRs, respectively. The five false-negative results by the real-time PCR were all non-*Candida albicans* positive by culture. Of the 91 culture-negative CRC specimens, 20, 41, and 44 were positive by the real-time PCR and the N18- and ITS-specific PCRs, respectively. All three PCRs detected fungal DNA in 8 culture-negative CRC specimens, with a further 30 being positive by two of the three PCRs. A total of 32 CRC specimens were *Candida* free by all methods. In summary, a real-time PCR that provides a sensitive, specific, and rapid alternative technique for detection of *Candida* in the mouth is described.

The predominant fungi isolated from the human mouth belong to the genus *Candida*, and while there are more than 350 *Candida* species, approximately 10 of these colonize the oral cavity. *Candida albicans* is recognized as the most prevalent species (~70 to 75% of isolates), followed by *C. glabrata* and *C. tropicalis* (7% of isolates) (9, 13). The reported rate of oral carriage of *Candida* yeasts among healthy individuals has been variable but ranges from 35 to 80% (1, 4) and is dependent on the population studied and the sampling methods used.

Debilitation of an individual can result in the occurrence of clinical oral candidosis, which may be defined as acute or chronic pseudomembranous candidosis (oral thrush), acute erythematous candidosis (*Candida*-associated denture stomatitis), chronic erythematous candidosis, or chronic hyperplastic candidosis (CHC) (3). Previously, host factors that were implicated in the onset of these infections received greater attention than the virulence factors of *Candida*. This was reflected in many diagnostic microbiological laboratories, where the ability to identify yeast isolates was limited. In recent years there has been increased interest in oral candidosis, partly due to the escalation of human immunodeficiency virus infection and AIDS and the more widespread use of immunosuppressive chemotherapy. As a consequence, there has been an associated increased demand for reliable sampling and identification methods.

Methods for sampling of the mouth for *Candida* include the taking of a smear (16), swab (15), or imprint specimen for culture (2); culture of whole saliva (24); and concentrated oral rinse culture (CRC) (10, 15). CRC is widely used, and although it does not directly target specific mucosal lesions, it provides a measure of the candidal load and can permit detection of other microbes. *Candida* levels of 600 CFU/ml of CRC specimen have been reported for healthy commensal carriage (10, 11), with higher numbers (~2×10^3 to 3×10^3 CFU/ml) evident in individuals with conditions that predispose them to infection (15). Samaranayake et al. (15) compared CRC with imprint culture for detection of oral microbes and found that CRC was superior for yeast detection. They subsequently recommended the use of CRC as the screening approach for detecting oral yeast carriage.

One drawback of detection methods based on culture is the delay before a diagnostic report can be made available to the clinicians. Primary isolation media are routinely incubated for 48 h at 37°C, followed by a possible 72-h incubation for physiological or biochemical identification (20). The advent of differential primary agars, such as CHROMagar *Candida* (CHROMagar Ltd., Paris, France) and Albicans ID (BioMérieux, Basingstoke, United Kingdom), has enabled the presumptive identification of certain species (14), although physiological identification is still recommended and is a necessity for the majority of species. More recently, the introduction of molecular PCR-based techniques has resulted in the development of tests that can potentially detect *Candida* directly in oral samples. One such approach, namely, real-time PCR with the LightCycler instrument (Idaho Technologies, Idaho Falls), was recently developed for the sensitive (1 to 5 CFU/ml) and rapid (1-day) detection of seven clinically relevant *Candida* species in blood (18).

The aim of the present study was to assess the performance of this real-time PCR for the detection of *Candida* species directly in CRC samples. Originally, the real-time assay was

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TABLE 1. Agreement between culture and PCR for detection of *Candida* in CRC samples[a]

Clinical sign(s)	No. of samples	No. of samples positive by culture	% Agreement with culture (no. of samples PCR positive)					
			Real-time PCR		ITS-specific PCR		N18-specific PCR	
			Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Lichen planus	19	10	80 (8)	78 (2)	67 (6[b])	44 (5)	80 (8)	44 (5)
BMS, sore mouth	48	24	96 (23)	63 (9)	71 (17)	50 (11[c])	79 (19)	46 (13)
Angular cheilitis	5	3	100 (3)	50 (1)	67 (2)	0 (2)	100 (3)	0 (2)
Coated tongue	1	0	ND	0 (1)	ND	100 (0)	ND	0 (1)
Dry mouth	14	11	100 (11)	67 (1)	91 (10)	67 (1)	100 (11)	67 (1)
Leukoplakia	4	4	75 (3)	ND	100 (4)	ND	100 (4)	ND
Ulceration	2	0	ND	100 (0)	ND	50 (1)	ND	50 (1)
Pemphigoid	2	1	100 (1)	50 (1)	100 (1)	0 (1)	100 (1)	0 (1)
Fissured tongue	1	0	ND	100 (0)	ND	0 (1)	ND	0 (1)
Perioral dermatitis	1	1	100 (1)	ND	100 (1)	ND	100 (1)	ND
CHC	7	6	100 (6)	100 (0)	83 (5)	100 (0)	67 (4)	0 (1)
PC	14	8	100 (8)	67 (2)	75 (6)	50 (3)	75 (6)	50 (3)
CEC	19	12	83 (10)	100 (0)	50 (6)	57 (3)	100 (12)	57 (3)
AEC	5	4	100 (4)	100 (0)	100 (4)	0 (1)	100 (4)	0 (1)
Previous candidosis	3	1	100 (1)	50 (1)	100 (1)	100 (0)	100 (1)	100 (0)
Healthy mouths[d]	48	17	100 (17)	94 (2)	71 (12)	52 (15)	94 (16)	74 (8)
Total	193	102	94 (96)	78 (20)	74 (75)	51 (44)	88 (90)	55 (41)

[a] Abbreviations: ND, not determined; BMS, burning mouth syndrome; CHC, chronic hyperplastic candidosis; CEC, chronic erythematous candidosis; PC, pseudomembranous candidosis; AEC, acute erythematous candidosis.
[b] One isolate not tested.
[c] Two isolates not tested.
[d] Clinically healthy oral mucosa with no history of oral candidosis.

developed to detect candidemia, and so it was necessary to determine the detection limit of the real-time PCR for *Candida* in spiked CRC samples rather than blood. It was also essential to establish how effective the real-time assay was for detection of clinical and commensal populations by direct comparisons to the "gold standard" culture and other molecular approaches.

MATERIALS AND METHODS

Collection of CRC samples and culture. A total of 145 samples for CRC (Table 1) were taken from patients (49 males, 96 females) attending the Oral Medicine Clinic of the Dental School, University of Wales College of Medicine, Cardiff, United Kingdom. As the patients were sampled as part of the routine mycological diagnostic service, they were suspected of being clinically colonized with *Candida*. In addition, a further 48 samples for CRC were obtained from healthy volunteers (20 males, 28 females) who had no clinical signs of oral candidosis. The procedure used for CRC sample collection involved rinsing of the mouth with 10 ml of sterile phosphate-buffered saline (PBS; 0.1 M; pH 7.2), which was held in the mouth for 1 min prior to collection in a sterile container. Each rinse was centrifuged (2,000 × *g*; 10 min), the supernatant was removed, and the deposit was resuspended in 1 ml of PBS. A portion (50 μl) of the concentrate was inoculated onto Sabouraud dextrose agar (Lab M, Bury, United Kingdom) with a spiral plater system (Don Whitley Scientific, Shipley, United Kingdom) prior to incubation at 37°C for 48 h. Suspected *Candida* colonies were subcultured onto plates of CHROMagar Candida, which were incubated for 48 h at 37°C for isolation of multiple yeast species. The remaining 950 μl of the sample for CRC was stored at −20°C prior to DNA extraction. Confirmation of yeast identity was achieved with the API 32C system (BioMérieux).

Detection limits of PCR with CRC samples. Ten control strains of *Candida* (Table 2) were cultured in yeast nitrogen base medium (Becton Dickinson, Oxford, United Kingdom) supplemented with 0.5% (wt/vol) glucose for 24 h at 37°C. The resulting yeast growth was harvested by centrifugation, washed three times in PBS, and enumerated with a hemocytometer counting chamber. Serial decimal dilutions were prepared in PBS to provide an estimated 10^6 to 10^0 yeasts/ml, and these were used to evaluate the sensitivities and the specificities of the PCRs.

Extraction of DNA from concentrated rinse samples. DNA was extracted from 950 μl of CRC concentrate and spiked control samples by use of the QIAamp DNA Mini kit (Qiagen, Crawley, United Kingdom), as described by White et al. (18). The extracted DNA was resuspended in 50 μl of Tris-EDTA buffer. Portions of this template DNA were then used for PCR (Table 3). Negative controls of uninoculated PBS were also included for DNA extraction and PCR.

PCR detection of *Candida*. The real-time PCR assay (18) used panfungal primers (primers L18F and L18R) to amplify a variable region within the 18S rRNA gene. The resulting 140-bp product was detected by SYBR Green incorporation and hybridization of a cyanine 5-labeled *Candida*-specific probe (18) (Fig. 1). One of the block-based PCRs used primers and reaction conditions previously described for fungal DNA amplification (23); however, the second block-based PCR used new primers N18F and N18R, which were designed to be *Candida* specific (Table 3). The *Candida*-specific block-based PCR (with primers N18F and N18R) targeted the 18S rRNA gene and amplified a 620-bp product spanning the region from 238 to 857 bp in the 18S rRNA gene of *C. albicans* (GenBank accession no. M60302.1). The amplicons generated by both block-based systems were detected by standard agarose gel electrophoresis and ethidium bromide staining.

TABLE 2. Detection of 10 *Candida* species in CRC samples by PCR

Candida species	Isolate reference	Lower detection limit (no. of yeasts/ml) of[a]:		
		Real-time PCR	ITS-specific PCR	N18-specific PCR
C. albicans	GDH 2346	10^0–10^1	10^4–10^5	10^1–10^2
C. dubliniensis	CD36 NCPF 3949	10^1–10^2	10^4–10^5	10^1–10^2
C. krusei	Lab strain	10^1–10^2	10^4–10^5	10^1–10^2
C. viswanathii	Lab strain	10^0–10^1	10^5–10^6	10^0–10^1
C. guilliermondii	Y02.02	ND	10^5–10^6	10^0–10^1
C. glabrata	Y33.149	10^2–10^3	10^4–10^5	10^1–10^2
C. famata	Lab strain	ND	10^3–10^4	10^0–10^1
C. inconspicua	Lab strain	10^4–10^5	10^3–10^4	10^1–10^2
C. parapsilosis	NCPF 3104	10^0–10^1	10^5–10^6	10^1–10^2
C. tropicalis	Lab strain	10^0–10^1	10^4–10^5	10^1–10^2

[a] The sensitivity of culture is 1 colony in 50 μl or 20 CFU/ml of concentrate. ND, not detected.

RESULTS

Detection limits of PCR with CRC samples. The limits of detection of the PCRs were initially assessed to confirm that the previous methodology used with blood samples was transferable to CRC samples. Table 2 presents the limits of detection for the real-time and block-based PCR systems for 10 *Candida* species. Real-time PCR detected all species except *C. guilliermondii* and *C. famata*. The *Candida*-specific (N18) block-based PCR showed good levels of detection that actually matched those of the real-time PCR for certain species (Table 2 and Fig. 2). The internal transcribed spacer (ITS) block-based PCR detected a wide range of *Candida* species but had consistently lower levels of detection (Table 2).

Culture and PCR detection of *Candida* in CRC samples. To compare the performance of the gold standard culture with those of the molecular methods, a clinical study of samples from 145 people with from various oral conditions was performed. In addition to this population, samples from a further 48 healthy control people with no symptoms of oral candidosis were tested to determine the degree of false-positive, clinically insignificant results generated by each assay.

Candida was detected by culture in 85 (59%) of the 145 clinical CRC samples and in 17 (38%) of the 48 healthy control CRC samples. Of the 85 clinical CRC samples that were culture positive, *C. albicans* was evident in 72 (85%). Other yeasts detected by culture, either in combination with *C. albicans* or alone, were *C. glabrata* ($n = 4$), *C. krusei* ($n = 2$), *Candida inconspicua* ($n = 1$), *Candida* spp. ($n = 15$), and *Saccharomyces cerevisiae* ($n = 1$). More than one *Candida* species was detected in 10 of the 85 samples positive by culture. *C. albicans* was the only species detected by culture in the 17 positive healthy control CRC samples.

Real-time PCR demonstrated that 97 (67%) of the 145 clinical CRC samples and 19 (40%) of the 48 healthy control CRC samples were positive for *Candida*. The N18-specific block-based PCR was positive for 109 (75%) clinical CRC samples and 22 (46%) of the healthy control CRC samples. In comparison, the ITS-specific PCR gave positive results for 93 (64%) clinical CRC samples and 25 (52%) healthy control CRC samples.

Table 1 and Fig. 3 present the agreement between *Candida* culture and PCR detection of *Candida* in the CRC samples. By using culture as the gold standard, the overall sensitivity and specificity of the real-time PCR were 94 and 78%, respectively, with only six culture-positive clinical CRC samples being negative by the real-time PCR. All six were culture negative for *C. albicans*. All culture-positive healthy control CRC samples were positive by real-time PCR.

Compared with the real-time PCR, a lower correlation with CRC positivity was evident by the block-based PCRs. The N18-specific PCR achieved a sensitivity and a specificity of 88 and 55%, respectively. By using 95% confidence intervals (12) it was decided that the difference in sensitivity between the real-time PCR and the N18-specific PCR was not significant (95% confidence intervals, 14 to −2%). However, by using the same statistical method it was shown that the real-time PCR assay was 11 to 34% (95% confidence intervals) more specific than the N18-specific PCR. The sensitivity and specificity of the ITS-specific PCR were 74 and 51%, respectively (Fig. 3),

TABLE 3. Primers and conditions of specific PCRs used in the study

Primer or probe (sequence)	Components of PCRs[a]	No. of cycles	DNA denaturation conditions	Primer annealing conditions	Primer extension conditions
ITS 1 (5′-TTC GTA GGT GAA CCT GCG G-3′) and ITS 2 (5′-GCT GCG TTC TTC ATC GAT GC-3′)	1× *Taq* buffer, 0.2 mM each dNTP, 0.5 μM each primer, 1.5 mM $MgCl_2$, 2.5 U of *Taq* polymerase, 5 μl of extracted DNA (equivalent to 100 μl of rinse concentrate)[b]	30	95°C for 1 min	55°C for 1 min	72°C for 2 min
N18F (5′-TTT GAT GAT TCA TAA TAA CTT TT) and N18R (5′-CGT CCC TAT TAA TCA TTA CGA T)	1× PCR buffer (1.5 mM $MgCl_2$), 0.2 mM each dNTP, 0.75 μM each primer, 2.5 U of *Taq* polymerase, 10 μl of DNA template (equivalent to 200 μl of rinse concentrate)[b]	35	Initial denaturation at 94°C for 5 min 94°C for 1 min	55°C for 1 min	72°C for 1.5 min and final cycle at 72°C for 7 min
L18F (5′-CTC GTA GTT GAA CCT TGG), L18R (5′-GCC TGC TTT GAA CAC TCT), and Cyanine 5-labeled probe (TTTTGATGCGTACTGGACCCTGT)	5 μl Light Cycler master mixture (Biogene), 0.5 μl of each primer (final concentration, 0.6 μM), 0.5 μl of SYBR Green (diluted 1:1,000), 1 μl of *Candida* probe (final concentration, 300 nM), 1 μl of template DNA (equivalent to 20 μl of rinse concentrate)[b]	60	Initial denaturation at 95°C for 15 s and then 95°C for 0 s	62°C for 2 s	74°C for 2 s

[a] The reaction mixtures were 50 μl for the ITS- and N18-specific PCRs and 10 μl for the LightCycler PCR. *Taq* buffer components, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100 (pH 9.0). dNTP, deoxynucleoside triphosphate.

[b] Assuming 100% DNA extraction efficiency.

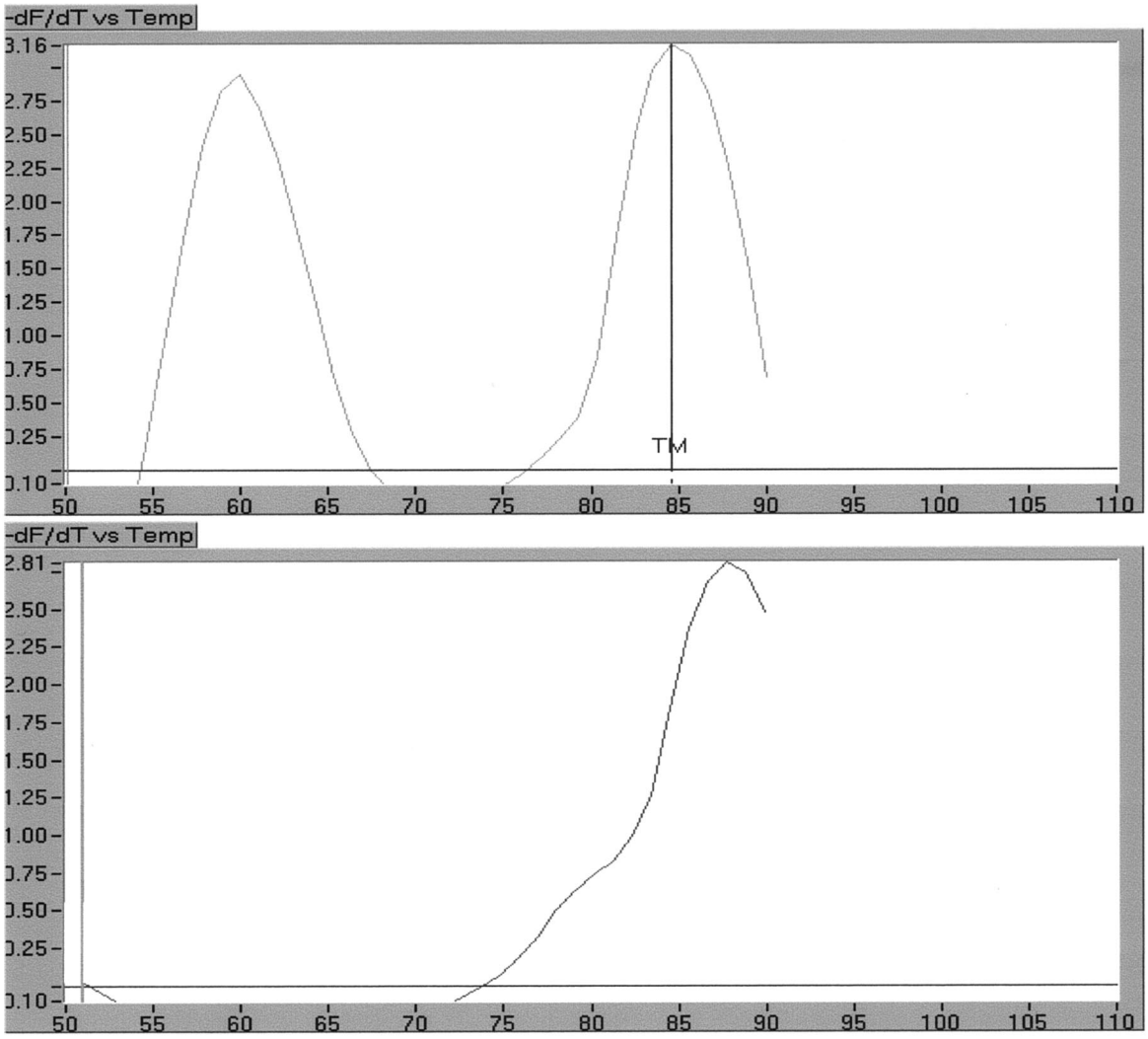

FIG. 1. Real-time PCR melting curve showing a positive sample (top) and a negative sample (bottom). The first peak indicates probe melting, and the second peak indicates amplicon melting.

with both of these values being statistically significantly inferior to those of the real-time assay (results not included). The positive and negative predictive values were 83 and 92%, 69 and 81%, and 63 and 63% for the real-time PCR, the N18-specific PCR, and the ITS-specific PCR, respectively.

Among the 91 culture-negative CRC samples, the real-time PCR revealed that 20 were positive; this compares with 41 and 44 positive samples by the N18- and ITS-specific PCRs, respectively. Thirty-two culture-negative CRC samples were negative by all three PCRs. All three PCRs were positive for eight culture-negative CRC samples, and a further 30 culture-negative CRC samples were positive by two of the three PCRs (Fig. 4).

Categorization of the culture-negative CRC samples into clinical (n = 60) and healthy control (n = 31) groups shows that only two (6%) samples from healthy controls that were culture negative by CRC were positive by the real-time PCR. In comparison, 18 (30%) culture-negative clinical CRC samples were positive by the real-time PCR. Eight (26%) culture-negative CRC samples from healthy controls were N18-specific PCR positive, and 33 (55%) of the culture-negative clinical CRC samples were also N18-specific PCR positive. Analysis of the ITS-specific PCR data revealed that 15 (48%) culture-negative healthy control CRC samples were PCR positive, and similar results were obtained for the culture-negative clinical CRC samples, 29 (48%) of which were ITS-specific PCR positive (Fig. 3 and 4).

DISCUSSION

PCR for fungal detection has primarily been limited to research laboratories but has recently been introduced into certain diagnostic mycology laboratories, especially in cases of possible fungemia, where rapid diagnosis is a necessity and culture from blood has proven problematic (5). Since PCRs are gradually being integrated alongside standard laboratory methods, it was thought that it would be of benefit to assess specific PCRs for candidal detection in CRC samples.

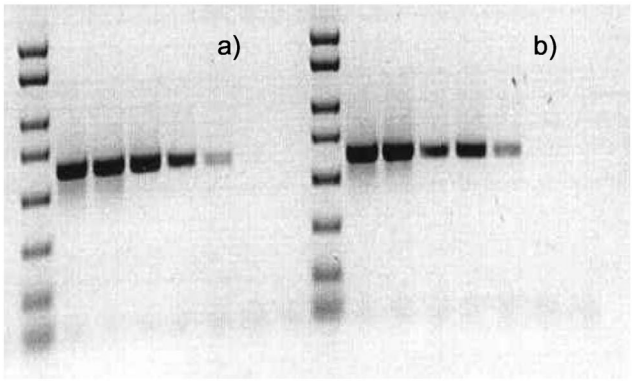

FIG. 2. Products obtained by N18-specific PCR for diluted suspensions with 10^0 to 10^6 *C. albicans* (a) and *C. dubiniensis* (b) yeasts/ml.

Initial studies with spiked CRC samples indicated a real-time PCR detection sensitivity of 1 to 10 yeasts/ml for *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. viswanathii*. The detection levels were lower for other *Candida* species (*C. dubliniensis*, *C. glabrata*, *C. kefyr*, and *C. inconspicua*). The *Candida*-specific probe did not hybridize to amplicons from *C. guilliermondii* or *C. famata*, a finding that was in agreement with our previous assessment of the PCR with blood samples (18). The *Candida*-specific probe had originally been designed for the detection of *Candida* in patients with candidemia, a condition that is primarily attributed to *C. albicans* and, to a lesser extent, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, and *C. krusei* (18). Modification of the probe to enable detection of *C. guil-*

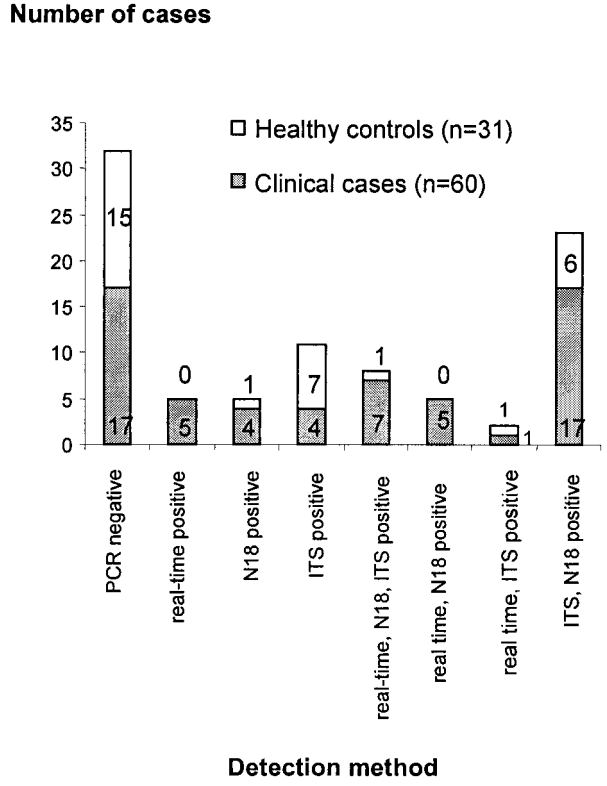

FIG. 4. Agreement between negative culture and PCR results.

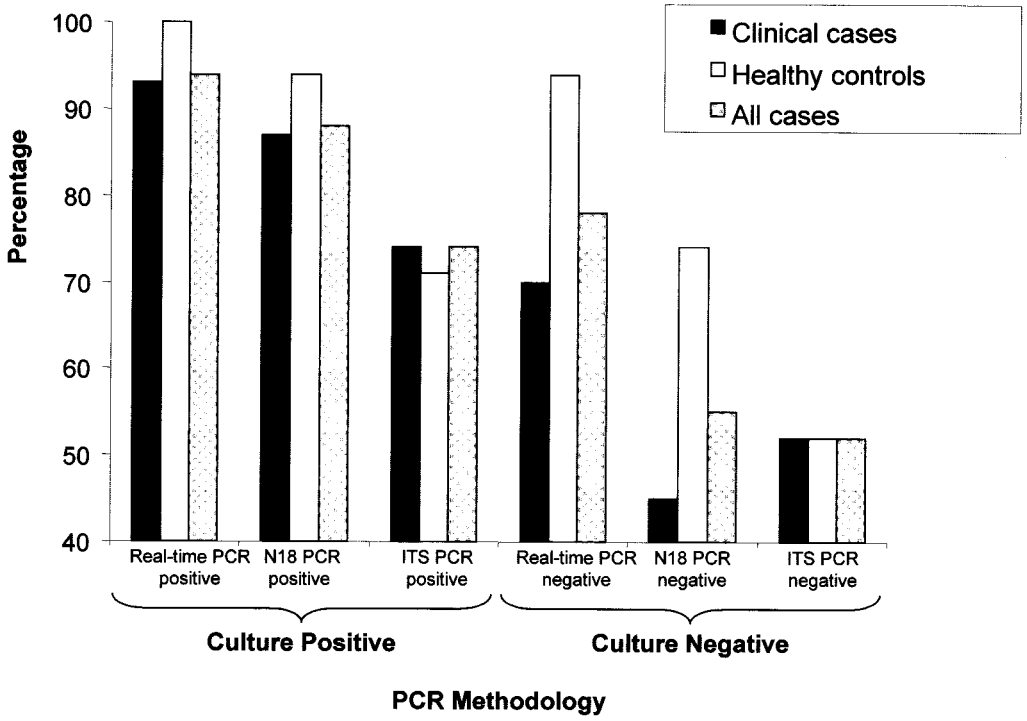

FIG. 3. Percent agreement of PCR and culture.

liermondii and *C. famata* is possible, although the significance of these two species in oral candidosis remains uncertain. Having established that the real-time PCR methodology was applicable to these spiked control oral samples, the performance of the PCR was determined with 193 CRC samples. Forty-eight of these CRC samples were from individuals considered free of oral candidosis. These samples were included to ensure that an increased sensitivity of detection by real-time PCR would not result in the production of large numbers of false-positive (clinically insignificant) results. This situation would have negated the value of the PCR as a suitable clinical test; however, the results show that the real-time PCR and the gold standard culture produced comparable results for our healthy population.

Culture of the 145 clinical CRC samples from patients with a variety of oral signs and symptoms revealed that 85 were positive for *Candida*, representing a colonization rate of 59%. This value was noticeably higher than the 35% carriage rate for the 48 CRC samples from healthy mouths (17 positive samples), which compares favorably with the 36.8% rate published by Abu-Elteen and Abu-Alteen (1). In the case of candidal presence from patients with various disease states, the higher incidence observed was expected, although comparison with other studies is problematic due to the diversity of patient groups. The overall incidence of *C. albicans* in the 102 culture-positive CRC samples was 87%. This high incidence of *C. albicans* relative to those of the other species concurs with most studies of the candidal epidemiology of the mouth (6, 8). An interesting observation from the culture data was the apparent high incidence (compared with that in the healthy mouths) of *Candida* in oral conditions not primarily associated with the yeasts. This observation was particularly evident for patients with lichen planus, burning mouth syndrome, and dry mouth. The significance of *Candida* in these cases is uncertain, but the altered oral state in these patients may, in part, offer an environment more favorable to candidal colonization. Similar high incidences of *Candida* from the mouths of individuals with leukoplakia (82%), lichen planus (47%), and burning mouth syndrome have been reported (6, 7).

Overall, the real-time PCR compared favorably with culture, with the results of the real-time PCR achieving 94% agreement with those of culture for the culture-positive samples. The six samples with false-negative results were all non-*C. albicans* by culture, and therefore, the results probably relate to the failure of the probe to hybridize to the amplicon. Conversely, 20 real-time PCR-positive samples (18 clinical CRC samples and 2 healthy control CRC samples) were detected among the 91 culture-negative CRC samples, possibly highlighting the higher sensitivity of real-time PCR (1 to 10 yeasts/ml) compared with that of CRC (>20 CFU/ml of concentrate). In addition, the PCR approach was not reliant on the presence of viable organisms, thereby further enhancing its relative sensitivity over culture. Fifteen of the 20 real-time PCR-positive, culture-negative samples were positive by at least one other PCR, and 8 culture-negative CRC samples were positive by all PCR methods. These findings accentuate the failure of culture to detect *Candida* in certain oral samples.

Both block-based PCRs produced more than double the number of culture-negative, PCR-positive results for CRC samples than real-time PCR, although the clinical significance of these results is uncertain. Dividing the culture-negative CRC samples into clinical and healthy control CRC samples reveals that the percentage of PCR-positive samples among the healthy control samples was much higher for the block-based PCRs (N18-specific PCR, 26%; ITS-specific PCR, 48%) than for the real-time PCR (6%). If culture is considered the gold standard, these findings could raise a question over the clinical relevance of block-based PCR-positive results for culture-negative samples.

As the number of N18-specific PCR-positive samples was greater than the number of culture-positive samples for both the healthy control and clinical populations but the 95% confidence intervals were approximately the same for both populations (2 to 26% for healthy controls, 4 to 24% for the clinical cases), the clinical significance of the additional N18-specific PCR-positive clinical samples is ambiguous. Conversely, as the real-time PCR detected *Candida* in only 6% of the culture-negative healthy control CRC samples (i.e., it did not produce large numbers of false-positive, clinically irrelevant results), the rise to a 30% rate of detection for the clinical (symptomatic) group is of interest. This is confirmed by the 95% confidence intervals, which determined that the number of healthy control samples positive by real-time PCR was not statistically different from the number of the healthy control samples positive by culture (95% confidence interval, 11 to −3%), whereas the number of additional clinical samples positive by real-time PCR was 2 to 15% greater (95% confidence intervals) than the number of clinical samples positive by culture, and as such, the results for the samples could be considered true positive.

The lower specificities of the block-based PCR methods over that of real-time PCR could, in part, account for the increased incidence of positive results by the block-based PCRs. Since our experiments with spiked samples confirmed the overall greater sensitivity of real-time PCR than other molecular methods, it was likely that specificity largely contributed to the increased numbers of block-based PCR-positive samples. However, different PCR conditions and template volumes may also have had an effect. Determination of whether the results for block-based PCR-positive, culture-negative CRC samples were true positive or false positive would require additional testing, such as sequencing or restriction fragment length polymorphism analysis (21, 22, 23), thus increasing the complexity, cost, and time required to obtain an assay result.

The question about the significance of culture-negative CRC samples and clinical relevance needs to be raised, particularly when positive results are evident by real-time PCR. It has been suggested that the presence of elevated numbers of *Candida* organisms indicates infection rather then normal commensal carriage (9). However, *Candida* levels up to 9×10^3 CFU/ml were evident in healthy controls in our studies, and on occasion, these levels were higher than those in patients with oral candidosis (data not shown). In patients with CHC, hyphal penetration of the oral mucosa occurs, and in these patients low numbers of yeasts may be recoverable from the mucosal surface (17). The value of culture in patients with CHC may therefore be limited, particularly if CRC is used for yeast isolation. Furthermore, when patients have received antifungal therapy the candidal load may be significantly reduced but not eradicated, partly explaining the high recurrence of oral candidosis among certain patients (19). In these circum-

stances the improved sensitivity of real-time PCR would be advantageous.

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