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Rapid identification of *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* by species-specific PCR of large subunit ribosomal DNA

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A rapid PCR-based method for the identification of four *Candida* species is described. Primers to conserved sequences in the V3 region of large subunit rDNA were used to amplify DNA from *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. The sequences were aligned and areas of non-concordance were used to design four species-specific forward primers. In a blind study of 82 yeast strains, four PCRs based on these primers correctly identified nine *C. albicans*, 18 *C. glabrata*, 13 *C. parapsilosis* and 18 *C. krusei* strains after gel electrophoresis of amplified DNA. Furthermore, of 17 other *Candida* strains (10 species) and seven strains from other yeast genera (*Saccharomyces*, *Cryptococcus* and *Trichosporon*) only one false positive amplification product (with *C. dubliniensis*) was seen. These PCRs offer a rapid alternative to conventional techniques for the identification of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*.

Introduction

Members of the fungal genus *Candida* are amongst the most common hospital pathogens and are a particular problem in the management of immunocompromised patients [1]. Individuals who are infected with the human immunodeficiency virus, or undergoing bone marrow or solid organ transplantation, receiving corticosteroids or with indwelling catheters are especially at risk [2]. While *C. albicans* remains the most commonly encountered pathogen, a large number of other species, especially *C. glabrata*, *C. parapsilosis* and *C. krusei*, are emerging as important opportunist pathogens [3].

The antifungal susceptibility patterns exhibited by these different species are diverse [4]. Therefore, to initiate prompt antifungal therapy, a quick identification system is required. Currently, the identification of *Candida* spp. in the routine diagnostic laboratory relies primarily on culture of the organism and biochemical assimilation tests [5, 6]. The API 20C (bioMérieux, Basingstoke, Hampshire) is a reliable

and proven system in this context. Unfortunately, only 73.4% of yeasts are identifiable after incubation for 24 h. This rises to 99.3% after 48 h [5].

An alternative approach to the use of biochemical assimilation tests has been the development of differential culture media that facilitate the isolation and identification of clinically important *Candida* spp. Several of these, e.g., Nickerson's media, rely on the reduction of bismuth salts, but they often lack discrimination, especially in the presence of bacteria [7]. However, the recent evaluation of a commercially available chromogenic medium, CHROMagar *Candida* (CHROMagar Co., Paris, France), demonstrated its ability to identify presumptively members of three species: *C. albicans*, *C. krusei* and *C. tropicalis*. The sensitivities and specificities of >99% obtained were made on the basis of colony morphology and colour development [8]. Furthermore, this medium was able to differentiate these species directly from clinical specimens without the need for prior isolation. Unfortunately, the reported accuracy was achieved only after 48 h as 'colony colour development was inconsistent after 24 h of incubation' [8].

This delay in the identification of *Candida* spp. by biochemical and cultural procedures has led to a search for more rapid techniques and, recently,

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molecular methods have been applied to the epidemiology and identification of this genus [9, 10]. Southern analysis has been employed in the identification of many fungi and commercial kits for a number of species are now available [11].

The speed and sensitivity of PCR make it an ideal choice for the basis of a rapid identification system. Although several *Candida* genes have been developed as targets for PCR they have generally been used to detect only *C. albicans* [12–14]. More recently, reports have appeared which indicate that PCRs, based on variable sequences within the small subunit rRNA genes, can be used to discriminate between *C. albicans* and other pathogenic *Candida* spp. [15, 16]. Unfortunately, additional Southern hybridisation or restriction enzyme digestion, or both, are often required to identify yeasts to the species level [9, 17].

An alternative target that has been exploited for the development of *Candida*-specific PCRs is the cytochrome P-450-dependent lanosterol- α -demethylase gene [18]; portions of this gene from *C. glabrata* and *C. krusei* were cloned and sequenced. These sequences were compared with published data for *C. albicans* and *C. tropicalis*, resulting in the design of four apparently species-specific primers. Experimental testing of these four primers confirmed their specificity. However, these primers had a major drawback with regard to rapid identification; they all required different cycling conditions and, therefore, could not be performed simultaneously unless four PCR machines were available.

This report describes a series of PCRs, based on sequences in the large subunit rRNA gene, that can all be performed under the same cycling conditions. These PCRs allow the rapid (<3 h) identification of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. This represents a saving of up to 72 h on currently employed identification procedures.

Materials and methods

Organisms and growth conditions

The 82 strains used in this study were clinical isolates and strains from culture collections representing 14 *Candida* spp. and three other genera (Table 1). Fungi were obtained from: the National Collection of Pathogenic Fungi, PHLS Regional Mycology Laboratory, Bristol; the Department of Medical Microbiology, University of Wales College of Medicine, Cardiff; the Department of Infectious Diseases and Bacteriology, Royal Postgraduate Medical School, London; the Department of Infectious Diseases and Tropical Medicine, Monsall Hospital, Manchester and the Department of Microbiology, Trinity College, University of Dublin, Ireland.

The yeasts were grown routinely on glucose 1% w/v, yeast extract 1% w/v (YE) plates at 37°C or 30°C for 24–48 h. Long-term storage was in water cultures at room temperature.

Fungal DNA extraction

For DNA extraction, yeasts stored in water cultures were subcultured on to YE plates and incubated as above. A single colony was then subcultured and DNA was extracted from this culture.

A rapid DNA extraction procedure (<1 h) was developed to minimise the time required for species identification. A single yeast colony was inoculated into a 1.5-ml screw cap Eppendorf tube containing 500 μ l of lysis buffer (10 mM Tris-HCl, pH 7.5, 50 mM EDTA, pH 7.5, SDS 0.2% w/v), 250 μ l of lysis buffer equilibrated phenol, 250 μ l of chloroform:isoamylalcohol (24:1) and 250 μ l of 0.5-mm diameter glass ballontini beads. This mixture was disrupted at maximum speed on a mini-beadbeater (Biospec Products, Stratech Scientific, Luton) for 50 s and centrifuged at 9500 *g* for 5 min. The supernate was precipitated at room temperature for 5 min with 0.54 volumes of isopropanol and the DNA was pelleted at 9500 *g* for 10 min. The pellet was washed in ethanol 70% v/v, vacuum dried and resuspended in 200 μ l of distilled water.

Polymerase chain reaction

All oligonucleotide primers were obtained from the Advanced Biotechnology Centre, Charing Cross and Westminster Medical School, London and were produced on an Applied Biosystems Model 380B DNA synthesiser (Applied Biosystems, Warrington, Cheshire).

All PCR reactions were performed under the same reaction conditions and cycling times. Each 50- μ l reaction mix contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.0 mM MgCl₂, 20 μ M each of dATP, dCTP, dGTP dTTP, 2 μ M of each oligonucleotide primer, 0.5 units of Taq DNA polymerase and *c.* 25 ng of DNA. The reaction mixtures were amplified (Omnigene, Hybaid, London) as follows: 94°C $\times$ 4 min (1 cycle), 94°C $\times$ 30 s, 66°C $\times$ 90 s, 72°C $\times$ 15 s (30 cycles) followed by 72°C for 5 min [19]. After thermal cycling, 15 μ l of the amplified product was run on an agarose 1.2% w/v gel, with 1 $\times$ TBE as buffer, stained with ethidium bromide, destained with distilled water and visualised under UV light.

DNA sequencing and species-specific PCR primer design

PCR primers corresponding to nucleotides 40–63 (universal forward primer 5'-GCATATCAATAAGCG-GAGGAAAAG-3') and 637–654 (universal reverse

Table 1. Strains and isolates of yeast species used in this study

Yeast species	Strain and source	Yeast species	Strain and source
<i>C. albicans</i>	3153 (N) 3302 (N) 3303 (N) 3310 (N) 3363 (N) cgl [*] (D) 659/92 [*] (C) 4961 [*] (R) 4963 [*] (R)	<i>C. krusei</i>	1183 [*] (M) 1524 [*] (M) 1630 [*] (M) 105-1 [*] (Cx)
<i>C. glabrata</i>	3309 (N) 3605 (N) tcd5 [*] (D) tcd6 [*] (D) tcd7 [*] (D) tcd8 [*] (D) tcd9 [*] (D) tcd10 [*] (D) tcd11 [*] (D) tcd12 [*] (D) cob3 [*] (D) g503 [*] (D) afc3 [*] (D) ctb7 [*] (D) 571/92 [*] (C) 625/92 [*] (C) 4962 [*] (R) 11088A [*] (Cx)	<i>C. parapsilosis</i>	3104 (N) 3872 (N) tcd1 [*] (D) tcd2 [*] (D) tcd3 [*] (D) tcd4 [*] (D) sa17 [*] (D) sa19 [*] (D) sa23 [*] (D) sa25 [*] (D) cpap [*] (D) 674/92 [*] (C) 44 [*] (Cx)
<i>C. krusei</i>	3321 (N) 3599 (N) jmcga [*] (D) jmcgo [*] (D) ckr [*] (D) cdb [*] (D) 500/92 [*] (C) 207 [*] (M) 651 [*] (M) 807 [*] (M) 827 [*] (M) 919 [*] (M) 920 [*] (M) 933 [*] (M)	<i>C. tropicalis</i>	3111 (N) 3322 (N) edbb [*] (D) 558/92 [*] (C)
<i>C. famata</i>	1507 [*] (M)	<i>C. kefyr</i>	3106 (N) 3234 (N) 624/92 [*] (C)
<i>Saccharomyces cerevisiae</i>	3178 (N) 3275 (N)	<i>C. sake</i>	3860 (N) 448 [*] (M)
		<i>C. norvegensis</i>	3861 (N) 1589 [*] (M)
		<i>C. lipolytica</i>	717/92 [*] (C)
		<i>C. cutaneum</i>	136/92 [*] (C)
		<i>C. lambia</i>	130 [*] (M)
		<i>C. humicola</i>	659 [*] (M)
		<i>C. lusitaniae</i>	1372 [*] (M)
		<i>Trichosporon beigelli</i>	3853 (N)
		<i>Cryptococcus neoformans</i>	3003 (N) 3216 (N) 3218 (N) 3379 (N)

Letters in parentheses indicate source of each isolate as follows: N, National Collection of Pathogenic Fungi, MRL, Bristol; D, Department of Microbiology, Trinity College Dublin, Ireland; C, Department of Microbiology, University of Wales College of Medicine, Cardiff; R, Department of Infectious Diseases and Bacteriology, RPMS, London; M, Department of Infectious Diseases, Monsall Hospital, Manchester; Cx, Department of Medical Microbiology, CXWMS, London.

^{*}Clinical isolate.

primer 5'-GGTCCGTGTTTCAAGACG-3') of the *Saccharomyces cerevisiae* V3 region of large subunit rDNA have been reported previously [19]. These primers, which are universally conserved within the fungal kingdom, were used to generate PCR products from the following *Candida* strains: *C. albicans* NCPF 3302, *C. glabrata* 11088A, *C. parapsilosis* 44 and *C. krusei* 105-1. Amplified PCR products were purified on a Microcon 100 column (Amicon Inc., Beverly, USA) according to the manufacturer's instructions. Sequencing was performed on an Applied Biosystems 373A DNA sequencer with the PRISMTM Ready Reaction DyeDeoxyTM Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instruc-

tions. Universal forward or reverse primers at 0.2 µM were used as the sequencing primers and c. 600 ng of PCR product was included in the reaction. Sequence was determined in both directions and repeated on two PCR products obtained from separate amplifications.

Multiple sequence alignments were performed with Clustal V and areas of non-concordance were identified by manual observation [20]. All potential forward PCR primers from the various *Candida* spp. sequences were determined with MacVector v4.1.4 software (Eastman Kodak IBI, New Haven, CT, USA). Species-specificity of all potential forward primers was tested by the PCR simulation computer pro-

gramme, Amplify v1.0 (Bill Engels, University of Wisconsin, Genetics, USA).

The following primers that yielded specific products on the simulation programme were chosen for experimental study and were synthesised: *C. albicans* 5'-TTGGAGCGGCAGGATAATGG-3' (Ca-1); *C. glabrata* 5'-GTTTTGCGCCCCTTGCTCT-3' (Cg-1); *C. parapsilosis* 5'-GCGGTAGGATAAGTG-CAC-3' (Cpa-1) and *C. krusei* 5'-GACATGGGA-ATCGCGCACCG-3' (Ck-1).

Universal PCR products obtained from type strains *C. sake* NCPF 3860 and *C. norvegensis* NCPF 3861, plus those amplified from Manchester strains 448 and 1589 were also sequenced.

Results

Design of species-specific primers

Approximately 600 bp of DNA from the V3 region was amplified with the universal primers. In all cases the nucleotide sequence was determined in both directions and only overlapping regions were included.

Totals of 502, 509, 501, 518, 501, 516, 516 and 518 nucleotides were sequenced from the V3 regions of *C. albicans* NCPF 3302, *C. glabrata* 11088A, *C. parapsilosis* 44, *C. krusei* 105-1, *C. sake* type strain NCPF 3860, *C. norvegensis* type strain NCPF 3861, Manchester strain 448 and Manchester strain 1589 respectively. Comparison of the sequences obtained from *C. albicans* NCPF 3302, *C. glabrata* 11088A, *C. parapsilosis* 44 and *C. krusei* 105-1 revealed differences that were exploited to design primers for the amplification of homologous DNA only. The localisation of these primers within the V3 region of the large subunit rDNA is shown in Fig. 1.

Initially the specificity of the four PCRs was tested with DNA extracted from the four strains from which the primer sequences had been derived. Inclusion of one of the primers, Ca-1, Cg-1, Cpa-1 or Ck-1, in a PCR reaction with the universal reverse primer and homologous template DNA yielded the expected amplicons of 156, 217, 139 and 226 bp, respectively. No amplification was seen when heterologous DNA from the other three strains was included as target (Fig. 2). This confirmed the prediction that Ca-1, Cg-1, Cpa-1 and Ck-1 would be species-specific primers.

Identification of *Candida* spp. by PCR

The usefulness of these primers in the identification of various *Candida* spp. was then examined. DNA extracted from all the yeasts listed in Table 1 was randomly coded by a colleague not involved in this work. DNA from all 82 strains was amplified by each of the primer pairs and designated as *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* or none of these, i.e., no PCR product with any of the four PCRs. The code was then broken and comparison was made between conventional and PCR identification. The results obtained are summarised in Table 2.

All *C. albicans*, *C. glabrata*, *C. krusei* and *C. parapsilosis* strains were identified correctly. However, four of 18 of the *C. krusei* strain DNAs yielded weak bands with the *C. glabrata* PCR when 64°C was used as the annealing temperature. These bands disappeared when the temperature was increased to 66°C.

None of the DNA extracts obtained from other yeast genera (*Saccharomyces*, *Cryptococcus* or *Trichosporon*) produced a product with any of the four *Candida* PCRs. Similarly 15 of 17 other *Candida* strains (nine species) yielded no PCR products. However, amplification of DNA obtained from two Manchester isolates

Fig. 1. Partial sequence of the V3 region of large subunit rDNA from: Sc, *Saccharomyces cerevisiae* (sequence published by Gutell and Fox [25]); Ca, *C. albicans* NCPF 3302 (Z48339); Cg, *C. glabrata* 11088A (Z48341); Cp, *C. parapsilosis* 44 (Z48343) and Ck, *C. krusei* 105-1 (Z48342). Numbers in parentheses refer to the accession number for each sequence. Figures above the line indicate positions in the *S. cerevisiae* V3 region and the asterisks below indicate conserved nucleotides. Sequences in upper case and underlined are those used as species-specific primers in conjunction with a universal reverse primer [19].

Fig. 2. Agarose gel electrophoresis of PCR products obtained with the universal reverse primer and primers: Ca-1 (panel A); Cg-1 (B); Ca-1 (C) and Ck-1 (D). Target DNA was from *C. albicans* NCPF 3302 (lane 1), *C. glabrata* 11088A (2), *C. parapsilosis* 44 (3) and *C. krusei* 105-1 (4). Material shown in lane 5 was from amplification experiments without target DNA.

identified as *C. sake* 448 and *C. norvegensis* 1589 gave strong and reproducible PCR products of the expected sizes with the *C. albicans* and *C. krusei* PCRs, respectively (data not shown).

After the code had been broken each of the 82 strains was cultured again and DNA was extracted. Amplification of these second DNA extractions resulted in 100% concordance with the results of the blind PCR study.

Human or bacterial DNA purified from clinical isolates of *Staphylococcus epidermidis*, *Staph. aureus*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Escherichia coli* did not result in a product when included in three of the species-specific PCRs. A number of weak products were obtained with the *C. krusei*-specific PCR when DNA from *Ps. aeruginosa*, *Cit. freundii* or *Ent. faecalis* was included. However, when the amplifications were repeated no consistency in band pattern was seen and no amplicons of the expected size were seen.

Comparison of the Manchester strain 448, C. sake NCPF 3860 and *C. albicans* NCPF 3302 rDNA sequences

To determine if the amplification of Manchester strain 448 in the *C. albicans* PCR was a true false positive reaction, its V3 region sequence was determined and compared with those of the *C. sake* type strain (NCPF 3860) and *C. albicans* NCPF 3302 (the strain used to design the *C. albicans*-specific primer Ca-1). Seventy-five bp differences were detected in the V3 region between the Manchester strain 448 (Z48566) and *C. sake* NCPF 3860 (Z48580), whereas only 17 bp changes were seen with the sequence of *C. albicans* NCPF 3302.

Amplification of DNA extracted from the *C. sake* type strain NCPF 3860, by all four species-specific PCRs, yielded no products (data not shown).

Comparison of the Manchester strain 1589, C. norvegensis NCPF 3861 and *C. krusei* 105-1 rDNA sequences

Sequencing of the V3 region of Manchester strain 1589 (Z48567) revealed 143 bp changes compared with the *C. norvegensis* type strain NCPF 3861 (Z48568). However, no differences were seen in the sequences of *C. krusei* 105-1 and Manchester strain 1589.

Table 2. Identification of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* by species-specific PCR

Template DNA (number assayed)	Number of strains positive by species-specific PCR for			
	<i>C. albicans</i>	<i>C. glabrata</i>	<i>C. parapsilosis</i>	<i>C. krusei</i>
<i>C. albicans</i> (9)	9	0	0	0
<i>C. glabrata</i> (18)	0	18	0	0
<i>C. parapsilosis</i> (13)	0	0	13	0
<i>C. krusei</i> (18)	0	0*	0	18
Other <i>Candida</i> spp. (17)†	1‡	0	0	0§
Other yeast spp. (7)†	0	0	0	0

*Four of 18 *C. krusei* strains gave a weak band in the *C. glabrata* PCR when 64°C was the annealing temperature. These bands disappeared when the annealing temperature was increased to 66°C.

†See Table 1.

‡Product obtained with Manchester strain 448.

§Product obtained with Manchester strain 1589 which was originally identified as *C. norvegensis* but has been redesignated as *C. krusei*.

Amplification of DNA extracted from the *C. norvegensis* type strain NCPF 3861, by all four species-specific PCRs, yielded no products (data not shown).

Discussion

Candida spp. are now recognised as major pathogens in many different patient populations [21]. Primary culture from specimens takes a minimum of 24 h and often 48 h. As candidosis can spread rapidly, particularly in immunocompromised patients, prompt and appropriate therapy is required to reduce morbidity and mortality. To achieve this goal, a rapid identification system is required. Currently, biochemical identification systems, especially API 20C (bioMérieux) predominate. These require a minimum incubation of 24 h, and often 48 h, post-primary isolation to establish identity. This delay can often result in the species not being identified until 96 h after receipt of a specimen in the diagnostic laboratory. This may have serious consequences for the patient if inappropriate therapy has been initiated. Quick and simple identification procedures, e.g., germ-tube production, are available for *C. albicans* but not for other pathogenic *Candida* spp.

A series of PCRs was developed that differs from previously reported methods [9, 12–14, 16, 18] in utilising the large subunit rDNA gene as a target. These PCRs can be used to identify the four major pathogenic *Candida* spp.: *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. The forward primers for these four PCRs were designed from variable sequences contained within the V3 region of the large subunit rDNA of each species; the reverse primer used throughout was to a conserved sequence within the same region [19].

Only two discrepancies between conventional identification techniques and the PCR-based system were seen in a blind study of 82 yeast isolates. Manchester strains 448 (identified as *C. sake*) and 1589 (identified as *C. norvegensis*) yielded PCR products with the *C. albicans* and *C. krusei* PCRs, respectively. Comparison of the product from strain 448 with that of the *C. sake* type strain NCPF 3860 revealed 75 bp changes. Similarly, 143 bp differences were demonstrable between the sequences from strain 1589 and *C. norvegensis* type strain NCPF 3861. In a previous study the same V3 region from nine atypical *C. albicans* (*C. dubliniensis* sp. nov) [22] strains was sequenced and 100% concordance was obtained. Furthermore, five *C. albicans* sequences from the same region differed by only a single bp (D. J. Sullivan, T. J. Westerneng, K. A. Haynes, D. C. Coleman, unpublished results). These data strongly suggest that strains within a species vary little in this area of the V3 region and, therefore, both of these Manchester strains have been misidentified as *C. sake*

(strain 448) and *C. norvegensis* (strain 1589), respectively. This is not unlikely given the rarity with which they are encountered in the routine diagnostic laboratory. Further evidence for this was provided by the finding that the *C. sake* and *C. norvegensis* type strains, NCPF 3860 and NCPF 3861, did not yield products with any of the species-specific PCRs.

The sequences from strains 448 and 1589 were then compared with those obtained from *C. albicans* NCPF 3302 and *C. krusei* 105-1, respectively. No bp differences were identified between the sequences from Manchester strain 1589 and *C. krusei* 105-1. These results strongly suggest that Manchester strain 1589 should be redesignated as *C. krusei*. However, 17 bp differences were observed in the sequences from Manchester strain 448 and *C. albicans* NCPF 3302. Therefore, it is unlikely that strain 448 is *C. albicans*. However, the sequence was identical to that of the recently described *C. dubliniensis* [22] and strain 448 has subsequently been identified as such. These sequence comparisons indicate that only one of 82 yeast strains tested blind was misidentified, i.e., Manchester strain 448 (*C. dubliniensis*) as *C. albicans*.

Although the *C. krusei* PCR non-specifically amplified DNA from several bacteria, this would not interfere with the use of this procedure to identify *Candida* spp. rapidly from primary isolation plates. It is inconceivable that an experienced microbiologist would confuse colonies of *Ps. aeruginosa*, *Cit. freundii* or *Ent. faecalis* with those of *Candida* spp. No non-specific products were seen with the *C. krusei* PCR and heterologous yeast DNA (Table 2).

Unlike the cytochrome P-450 lanosterol- α -demethylase PCR protocols, the amplifications described here can all be performed under the same cycling conditions. Furthermore, in contrast to methods reported that utilise the small subunit rRNA genes as targets, they do not require subsequent hybridisation or restriction enzyme analysis for species identification. Hence, all four PCRs can be run simultaneously and identification, to the species level, can be achieved within 3 h of primary isolation – a saving of between 24 and 72 h on currently employed identification techniques. This reduction in the time required to identify an invading fungus should improve patient outcome, as appropriate antifungal therapy can be initiated earlier, e.g., if fluconazole therapy had been initiated and the yeast was subsequently identified as *C. krusei*, a species almost universally resistant to fluconazole, therapy could be adapted accordingly after only 24 h. This would have obvious benefits for patient management.

This approach could be extended to other pathogenic *Candida* spp., and other fungal species, by sequencing of their rDNA regions and the design of species-

specific primers [23] – an objective which may become a necessity given the increase in the number of fungi reported as pathogenic [24]. However, use of these PCRs in a diagnostic laboratory would require a significant investment in terms of specialised equipment, licencing agreements, staff training and labour costs.

This study demonstrates the utility of large subunit rDNA species-specific PCR for the rapid identification of *Candida* spp. isolated from clinical specimens. Of the 82 yeasts studied, 58 isolates belonged to the four major pathogenic species, i.e., *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei*. All of these 58 isolates were correctly identified by the species-specific PCR in the blinded study. Furthermore, identification was possible within 3 h of primary isolation, an improvement on current procedures of up to 72 h. Adoption of this technique should significantly improve on the service currently offered by diagnostic laboratories for identification of *Candida* spp.

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