MAJOR ARTICLE

A Prospective Clinical Trial of a Real-Time Polymerase Chain Reaction Assay for the Diagnosis of Candidemia in Nonneutropenic, Critically Ill Adults

R. McMullan,[1] L. Metwally,[1] P. V. Coyle,[1] S. Hedderwick,[2] B. McCloskey,[3] H. J. O'Neill,[1] C. C. Patterson,[4] G. Thompson,[1,3] C. H. Webb,[1] and R. J. Hay[4]

Departments of [1]Medical Microbiology and [2]Infectious Diseases, [3]Regional Intensive Care Unit, The Royal Hospitals, and [4]School of Medicine and Dentistry, The Queen's University of Belfast, Belfast, Northern Ireland

(See the editorial commentary by Bennett on pages 897–8)

Background. Invasive *Candida* infection among nonneutropenic, critically ill adults is a clinical problem that has received increasing attention in recent years. Poor performance of extant diagnostic modalities has promoted risk-based, preemptive prescribing in view of the poor outcomes associated with inadequate or delayed antifungal therapy; this risks unnecessary overtreatment. A rapid, reliable diagnostic test could have a substantial impact on therapeutic practice in this patient population.

Methods. Three TaqMan-based real-time polymerase chain reaction assays were developed that are capable of detecting the main medically important *Candida* species, categorized according to the likelihood of fluconazole susceptibility. Assay 1 detected *Candida albicans, Candida parapsilosis, Candida tropicalis,* and *Candida dubliniensis.* Assays 2 and 3 detected *Candida glabrata* and *Candida krusei,* respectively. The clinical performance of these assays, applied to serum, was evaluated in a prospective trial of nonneutropenic adults in a single intensive care unit.

Results. In all, 527 specimens were obtained from 157 participants. All 3 assays were run in parallel for each specimen; they could be completed within 1 working day. Of these, 23 specimens were obtained from 23 participants categorized as having proven *Candida* infection at the time of sampling. If a single episode of *Candida famata* candidemia was excluded, the estimated clinical sensitivity, specificity, and positive and negative predictive values of the assays in this trial were 90.9%, 100%, 100% and 99.8%, respectively.

Conclusions. These data suggest that the described assays perform well in this population for enhancing the diagnosis of candidemia. The extent to which they may affect clinical outcomes, prescribing practice, and cost-effectiveness of care remains to be ascertained.

Failure to adequately treat invasive *Candida* infection—or, indeed, failure to initiate therapy in a timely manner—has been associated with poor outcomes, including increased mortality [1–3]. This, combined with low physician confidence in routine diagnostic tests with lengthy turnaround time, has promoted the practice of risk-based prescribing [4–7]. Such preemptive drug prescription, based on extensive anatomic colonization in high-risk patients, reduces the likelihood of inadequate antifungal therapy, but it comes at the cost of probable overtreatment, with its attendant risks of unnecessary toxicity, resource consumption, and selection in favor of resistant organisms. Consequently, the development of a high-performance diagnostic tool, in which physicians could have confidence, may facilitate a more targeted approach to antifungal prescribing.

Although advances in blood culture systems have increased detection sensitivity to ~70% for invasive candidiasis, not all laboratories have adopted all available optimization measures; culture and subsequent identification may require 48–96 h, and 5–7 days must pass before a blood culture may yield negative results [4, 8, 9]. Nonculture techniques with shorter time to

Received 13 September 2007; accepted 3 November 2007; electronically published 7 February 2008.

Reprints or correspondence: Dr. R. McMullan, Dept. of Medical Microbiology, Kelvin Laboratories, The Royal Hospitals, Belfast, Northern Ireland BT12 6BA (Ronan.Mcmullan@belfasttrust.hscni.net).

Clinical Infectious Diseases 2008; 46:890–6

1058-4838/2008/4606-0017$15.00
DOI: 10.1086/528690

result, such as antigen, antibody, and metabolite detection, have been investigated. None has achieved acceptance as an ideal diagnostic tool, and only a few have been produced commercially [10–19]. Some reported nucleic acid detection systems have produced promising initial results in preliminary clinical trials; several have limitations such as cumbersome methodology and lack of robust clinical verification [20–31].

The purpose of this trial was to thoroughly evaluate the performance of 3 real-time PCR assays for detecting common *Candida* species directly from clinical specimens. The critically ill, nonneutropenic population targeted is one in which the burden of disease is substantial, and molecular assay performance data have thus far been lacking.

METHODS

Oligonucleotide Design and PCR Assays

Primer design. Primers used in assay 1 to amplify 4 *Candida* species *(Candida albicans, Candida tropicalis, Candida parapsilosis,* and *Candida dubliniensis)* and a probe for amplicon detection have been published previously [20, 32]. Primers for *Candida glabrata* and *Candida krusei* were designed in-house using the Lasergene software program, version 5 (DNAstar), for assays 2 and 3, respectively (table 1); probes for these assays were previously published [32]. The assays were based on a set of single-round TaqMan-based assays reported previously by our group [33]; these were modified into a nested format for improved sensitivity in this trial. The 3 assays were developed such that assay 1 detected the characteristically fluconazole-susceptible group of species *(C. albicans, C. tropicalis, C. parapsilosis,* and *C. dubliniensis),* whereas assays 2 and 3 detected *C. glabrata* and *C. krusei,* respectively.

Real-time PCR amplification of* Candida *DNA. First-round amplification was performed on 5 μL of extract added to 20 μL of the first-round master mix, containing 1× Taq polymerase buffer (Promega), 3 mmol/L $MgCl_2$, 0.2 mmol/L each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP; Promega), 0.2 μmol/L each primer, and 0.5 U/mL of Taq DNA polymerase (Promega). Amplification was performed as follows: 1 cycle of 94°C for 3 min; 30 cycles of 94°C for 10 s, 58°C for 10 s, and 72°C for 30 s; followed by 1 cycle of 72°C for 5 min.

An aliquot (1 μL) of first-round products was added to 19 μL of second-round master mix and was handled as previously described [33] in a second round of amplification to achieve nested real-time PCR amplification of *Candida* DNA. Specimens that were positive in only 1 aliquot were repeated and, if reactive, were considered to be PCR positive.

Specimen Processing and Analysis

Serum specimens were stored at –20°C until the assays were performed. Next, 3 aliquots (400 μL each) of each serum sample were extracted with the QIAamp DNA mini kit (Qiagen) with 2 modifications. First, carrier RNA (10 μg/μL; Sigma-Aldrich) was added to lysis buffer at a concentration of 5.6 μg of carrier RNA/sample immediately prior to extraction. Second, after

Table 1. Oligonucleotide structure of primers used.

Assay, primer	Sequence 5′→3′	Product size, bp	Target gene
Four-species assay			
Can 1A	F1, GAGGGCAAGTCTGGTG	210	18S
Can 1B	R1, CCTGCTTTGAACACTCTAA	…	…
Can 1C	F2, CTCGTAGTTGAACCTTGG	140	18S
Can 1D	R2, GCCTGCTTTGAACACTCT	…	…
Can P1	6FAM-TTTTGATGCGTACTGGACCC-BHQ1	…	…
Candida glabrata assay			
Gla 1A	F1, CGTAGGTGAACCTGCGGAAGGATC	853	ITS1, 5.8S, ITS2
Gla 1B	R1, GTTCAGCGGGTAATCCTACCTG	…	…
Gla 1C	F2, CCTGTTTGAGCGTCATTTCC	229	ITS1, 5.8S, ITS2
Gla 1D	R2, AGCACGCACAAAACACTCACTTAT	…	…
Gla P1	6FAM-TAGGTTTTACCAACTCGGTGTTGAT-TAMRA	…	…
Candida krusei assay[a]			
Kru 1A	F1, CGTAGGTGAACCTGCGGAAGGATC	488	ITS1, 5.8S, ITS2
Kru 1B	R1, CTTAAGTTCAGCGGGTATTC	…	…
Kru 1C	F2, CCTGTTTGAGCGTCATTTCC	219	ITS1, 5.8S, ITS2
Kru 1D	R2, CTTAAGTTCAGCGGGTATTC	…	…
Kru P1	6FAM-AGCTGGCCGAGCGAACTAGACTTTT-TAMRA	…	…

NOTE. F, forward primer, R, reverse primer.

[a] The *C. krusei* assay was seminested.

DNA elution, YM-100 micro concentrators (Millipore) were used to increase the DNA concentration.

Clinical Trial Design and Setting

Design. A prospective observational diagnostic trial was conducted to verify these assays in the Northern Ireland Regional Intensive Care Unit. This is a 24-bed adult intensive care and high-dependency unit (ICU) that receives ~700 patients per year. This trial received prospective approval from both the local research ethics committee and the hospital research governance committee.

The trial was conducted in 2 stages. Stage 1 was intended to evaluate the specificity, positive predictive value (PPV), and negative predictive value (NPV) of the assays in this critically ill group by testing all disease-negative and disease-positive persons meeting the entry criteria. Stage 2 was designed to add only disease-positive persons to the cohort by selecting patients with proven candidemia; this was intended to make the clinical sensitivity estimate more robust.

Stage 1 participant recruitment and specimen categorization. Consecutive patients admitted to the ICU who remained there for >72 h were eligible for inclusion in this trial until ~500 specimens had been collected. Consent was sought from either the patient or their legal representative. Occurrence of any of the following was considered to be a valid reason to exclude potential participants from recruitment: anticipated discharge from ICU or withdrawal of active treatment within 24 h of recruitment, neutropenia, or receipt of systemic antifungal drugs at the time of recruitment or within the preceding 14 days.

During stage 1, serum samples were collected prospectively from all recruited participants twice weekly. Each specimen was categorized according to the likelihood of invasive *Candida* infection in the participant from whom it was obtained, as evaluated on the day of specimen collection. Retrospective reclassification based on microbiological culture results was permitted only for contemporaneously obtained specimens. The categories were based on clinical and laboratory parameters using adapted definitions published by the European Organization for Research and Treatment of Cancer's (EORTC) invasive fungal infection group [34], modified such that they could be applied with greater relevance to nonneutropenic adults (table 2). Allocation to these categories was agreed by both a microbiologist and intensivist responsible for routine clinical care of the participants on each day of specimen collection. Clinical teams were encouraged to highlight any "borderline" participants, defined as those for whom the most suitable category of infection was unclear.

Stage 2 participant recruitment and specimen categorization. Consecutive adult patients with laboratory-proven candidemia after completion of stage 1 of the trial were eligible for inclusion in stage 2. Again, consent was sought from either the patient or their legal representative. All specimens were categorized as proven infection.

Table 2. Definitions of invasive *Candida* infection categories.

Definition
Proven disease: any 1 of the following
Histologic evidence of yeast cells or hyphae or pseudohyphae from normally sterile site
Blood culture positive for *Candida* species; positive culture of blood obtained via a central venous catheter were not accepted if specimens contemporaneously taken either via an arterial catheter or from a peripheral vein yielded negative results
Positive culture result for sample from any other normally sterile site (excludes urine, sputum, bronchoalveolar lavage, mucous membrane swabs, and specimens from skin sites)
Probable disease
1 of the following
Fever (temperature, >38°C) persisting despite receipt of broad-spectrum antibacterial therapy for >96 h
Temperature <36°C or >38°C, plus
Receipt of immunosuppressive therapy for ⩾7 of the preceding 30 days (excluding corticosteroids)
Symptomatic AIDS
Receipt of corticosteroid therapy for ⩾21 days in past 60 days
Plus 1 of the following
Cultures positive for the same *Candida* species from at least 2 noncontiguous (including nonsterile) sites
Bulls-eye lesions in liver and/or spleen on ultrasound or CT imaging accompanied by an elevation in serum alkaline phosphatase level
Unlikely disease: neither of the above categories met

Data analysis. Performance of the group of *Candida* assays was evaluated on a per-specimen basis with respect to the reference standard applied; parameters assessed were clinical sensitivity, specificity, PPV, and NPV. The clinical sensitivity was also evaluated on a per-patient basis. Statistical analysis was performed using the Stata statistical software package, release 8.0 (Stata). PPV and NPV were evaluated based on stage 1 data only; clinical sensitivity and specificity were evaluated based on pooled data from both stages.

On the basis of achievement of assay clinical sensitivity of ⩾80%, accepting confidence limits about this sensitivity of ⩽10% and confidence limits about the specificity of ⩽5%, it was estimated that collection of 500 specimens from disease-negative participants and 60 specimens from disease-positive participants would be required. Consequently, it was decided that when the number of specimens from disease-negative participants reached ~500 (designated stage 1 of the trial) only specimens from disease-positive participants would be collected; this period was designated stage 2 and ran from the end of stage 1 until the end of month 20.

RESULTS

PCR Assays

The probe for assay 1 hybridized appropriately with *C. albicans, C. tropicalis, C. parapsilosis,* and *C. dubliniensis,* but not with *Candida famata, Candida kefyr, Candida guillermondii, Candida sphaerica, Candida lusitaniae, C. krusei,* and *C. glabrata,* nor with other bacteria or fungi tested. The *C. krusei* probe hybridized only with *C. krusei* and the *C. glabrata* probe only with *C. glabrata.*

The analytical sensitivity of the real-time PCR assays were determined as previously described [33]. The detection end point was 10^{-10} for the fluconazole-susceptible *Candida* assay and 10^{-11} for the *C. glabrata* and *C. krusei* assays. The end point copy number for each of the 3 assays was as follows: assay 1 (fluconazole-susceptible *Candida* assay), ~2.3 copies/mL; assay 2 (*C. glabrata* assay), ~0.56 copies/mL; and assay 3 (*C. krusei* assay), ~0.22 copies/mL.

Clinical Trial Results

During the first stage, 145 participants were recruited, resulting in collection of 515 specimens. During the second stage, 12 participants were recruited, resulting in collection of 12 specimens. No participants were categorized as "borderline."

Demographic and outcome data for stage 1 participants. Of the 145 participants recruited, 97 (67%) were male. Of the Intensive Care National Audit and Research Centre diagnostic categories for underlying illness on admission to the ICU, the 3 most frequently encountered categories were trauma (37%), respiratory disease (23%), and gastrointestinal disease (14%). The median APACHE II score at admission was 22 (range, 7–37). Participants during stage 1 accounted for a total of 2473 ICU bed-days. The median duration of ICU stay was 11 days (range, 2–230 days). One hundred nineteen participants (82%) survived ICU admission.

Fungal infection data for stage 1 participants. Of the 145 participants, 11 (7.6%) developed proven invasive *Candida* infection, and 6 (4.1%) developed probable infection prior to withdrawal from the study; the other 128 participants (88.3%) remained in the category of unlikely infection throughout. The 11 participants with proven infection resulted in allocation of 11 specimens to this category, and the participants with probable infection resulted in allocation of 13 specimens to this category; the remaining 491 specimens collected were allocated to the unlikely infection category. All participants who developed proven invasive *Candida* infection in stage 1 of the trial had documented candidemia: 9 with *C. albicans,* 1 with *C. glabrata,* and 1 with *C. famata.*

PCR assay results from stage 1. None of the 491 specimens allocated to the unlikely category were PCR positive. Three (23%) of the 13 specimens allocated to the probable category were PCR positive; all 3 were obtained from 1 participant who subsequently developed documented candidemia. All 11 specimens allocated to the proven group were from participants with contemporaneous laboratory-proven candidemia. Nine (82%) were PCR positive; 1 PCR-negative specimen had been obtained from a participant with *C. albicans* candidemia, and 1 had been obtained from a patient with *C. famata* candidemia. The timing of the PCR sampling with reference to the blood sampling that resulted in positive culture results in the proven group ranged from −1 day to +5 days. The 2 negative PCR specimens were both taken at +1 day.

Fungal infection data for stage 2 participants. All 12 specimens collected were from 12 participants who had documented candidemia. Of these, 9 had candidemia due to *C. albicans,* 2 had candidemia due to *C. glabrata,* and 1 had mixed candidemia with both *C. albicans* and *C. glabrata.*

PCR assay results from stage 2. Eleven (91.7%) of the 12 specimens were PCR positive; however, the participant with mixed candidemia was positive only with assay 1 (the *C. albicans* group assay) but not the *C. glabrata* assay. The specimen that tested PCR negative was for a participant with *C. albicans* candidemia. The timing of the PCR sampling with reference to the blood sampling that resulted in positive culture results ranged from 0 days to +7 days. The negative PCR specimen was obtained on the same day as the positive blood culture specimen.

Assay Performance

On the basis of a per-specimen analysis of proven and unlikely infection episodes only, the clinical sensitivity of the group of PCR assays was 87% (90.9% if the *C. famata* episode was excluded); 20 of 23 specimens from participants in the proven infection category tested positive (table 3). On the basis of per-patient analysis, the clinical sensitivity estimate was identical. The clinical specificity was 100%; no specimens from participants in the unlikely infection category tested positive. The PPV, based on stage 1 data, was 100%. The NPV was 99.6%; if the *C. famata* infection is excluded, this increases to 99.8%.

If participants in the probable infection category were assumed to be truly disease positive and incorporated in the per-specimen analysis, the sensitivity and NPV of the assays would decrease to 64% and 98%, respectively, although the specificity and PPV would be unaffected. Including these 6 participants in the per-patient analysis would reduce the assay sensitivity estimate to 72%.

DISCUSSION

The assays developed in this study for direct detection of candidemia performed well, with observed clinical sensitivity and specificity of 87% and 100%, respectively. However, if the 1 episode of candidemia attributed to *C. famata* is excluded, the clinical sensitivity increases to 91%. This was identical in both

Table 3. Summary of assay performance analyses.

	Overall performance		Excluding participant with *Candida famata* infection	
Performance measure	Proportion (%)	95% CI, %	Proportion (%)	95% CI, %
Sensitivity	20/23 (87)	66.4–97.2	20/22 (90.9)	70.8–98.9
Specificity	491/491 (100)	99.3–100	491/491 (100)	99.3–100
Positive predictive value	20/20 (100)	83.2–100	20/20 (100)	83.2–100
Negative predictive value	491/493 (99.6)	98.5–100	491/492 (99.8)	98.9–100

NOTE. Analysis excludes the specimens from the 6 participants in the probable category.

the per-specimen and per-patient analyses, because each disease-positive patient produced only 1 specimen allocated to the proven category. *C. famata* is an exceptionally rare fungal pathogen, and its exclusion from the analysis is logical.

The time required to produce a result using the assays is <6 h, and, conveniently, this result categorizes species according to risk of fluconazole resistance, which is of immense therapeutic value. Such rapid methods offer the opportunity to allow infection diagnosis to have a real-time impact on empirical antifungal therapy.

Although these data are encouraging, they must be interpreted cautiously, because the relevance of such performance parameters depends on the context of testing. In this trial, the assays were adopted as a screening tool on a cohort of participants, which was skewed toward disease-negative persons; therefore, PPVs and NPVs are useful only when applied in a similar screening model. Moreover, it is unlikely that the assays would be used in this way in clinical practice; therefore, although this approach is useful for assay validation, their performance may be different if adopted into diagnostic algorithms.

The present trial was designed to overcome some of the deficiencies of previous reported work; nonetheless, the results should be interpreted with care. For example, fewer specimens in the proven category were collected than had been expected; this reduced the precision of the estimates of sensitivity and PPV. Second, the work was based in a single center, although the APACHE II scores and survival rates in the cohort were similar to those reported from a United Kingdom–wide audit (http://www.icnarc.org).

Disease definitions were based on consensus definitions endorsed by the EORTC for use in clinical trials, with adaptations to make these more applicable to nonneutropenic adults (table 2). The lack of available disease definitions previously validated for nonneutropenic adults somewhat limits these data; however, reproducible definitions were used that varied only modestly from the EORTC definitions. Although the definition of proven infection is not disputed, the definition of probable infection in this population presents difficulty. It is almost impossible to judge whether participants assigned to this category should be regarded as truly disease positive, because the definitions were based on commonly used therapeutic decision models, which are biased toward overtreatment. For this reason, and because only 6 participants were allocated to this category, the analysis was conducted both with and without this group. Moreover, because candidemia in effect became the reference standard, interpretation of data from the probable infection group becomes even more uncertain.

In view of the size of the disease-positive sample recruited in this trial, the spectrum of species of *Candida* implicated in observed disease was limited. Also, only 1 episode of mixed candidemia occurred in this trial; this involved *C. glabrata* and *C. albicans.* Only *C. albicans* was detected by the assays in this specimen. The explanation for this is unclear; it is possible that the *C. glabrata* load was lower than the detection threshold of the assay and that the *C. albicans* load was higher, but an alternative explanation may exist.

During the past decade, several trials have reported direct detection of fungal DNA from clinical specimens. Moreira-Oliveira et al. [24] evaluated a PCR assay in a heterogeneous group of 225 high-risk hospitalized patients. When blood culture was used as the reference standard, the performance was disappointing, with sensitivity of 72%, specificity of 91%, PPV of 66%, and NPV of 93%; of note, our main analysis effectively used blood cultures as the reference standard.

A further *C. albicans*–specific real-time assay that adopted TaqMan technology was described by Maaroufi et al. [29]. Unfortunately, the clinical specificity of the assay could not be determined. Furthermore, this assay could not detect species of *Candida* other than *C. albicans.* The study of Klingspor and Jalal [23] included a large patient sample, but, again, the data analysis was grossly compromised by the paucity of clinical information and lack of a clear reference standard.

The trial described herein is unique in many respects and, therefore, reliable data comparisons are difficult; however, when compared with available published data in which laboratory-proven candidemia was used as the reference standard, these assays have demonstrated very good performance.

Nonetheless, the precision with which the performance measures were estimated in this trial would be improved by eval-

uation with larger sample size of disease-positive persons. Moreover, evidence of multicenter applicability would add weight to the clinical utility of the assays. Furthermore, given that all patients with proven infection in this data set had candidemia, these data do not assess assay performance in non-candidemic invasive *Candida* infection. The exclusion criteria adopted may explain the underrepresentation of the latter; these patients are likely to have received preemptive antifungal therapy and were thus excluded from the trial prior to meeting the case definition of proven infection.

Some major health economic questions also remain to be addressed before widespread adoption of such molecular tools in routine diagnosis. The cost-effectiveness of this and similar techniques has not been adequately estimated. It will be increasingly difficult to justify adding sequential tests to the routine diagnostic pathway if molecular diagnostic methods do not replace conventional techniques. Moreover, the applicability of molecular testing to a given laboratory without "batching" specimens may affect its incorporation into routine use.

In summary, these data suggest that the described assays may perform well for the rapid diagnosis of candidemia in non-neutropenic adults. The assays show high sensitivity, excellent specificity, and results potentially available on the same day.

Acknowledgments

Financial support. This work was supported by the Northern Ireland Health and Personal Social Services Research and Development Office as part of commissioned research under the antimicrobial resistance action plan.

Potential conflicts of interest. All authors: no conflicts.

References

1. Morrell M, Fraser VJ, Kollef MH. Delaying the empiric treatment of candida bloodstream infection until positive blood culture results are obtained: a potential risk factor for hospital mortality. Antimicrob Agents Chemother **2005**; 49:3640–5.
2. Harbarth S, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med **2003**; 115:529–35.
3. Ibrahim EH, Sherman G, Ward S, Fraser VJ, Kollef MH. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest **2000**; 118:146–55.
4. Ostrosky-Zeichner L, Rex JH, Bennett J, Kullberg BJ. Deeply invasive candidiasis. Infect Dis Clin North Am **2002**; 16:821–35.
5. Eggimann P, Garbino J, Pittet D. Management of *Candida* species infections in critically ill patients. Lancet Infect Dis **2003**; 3:772–85.
6. Leon C, Ruiz-Santana S, Saavedra P, et al. A bedside scoring system ("Candida score") for early antifungal treatment in nonneutropenic critically ill patients with *Candida* colonization. Crit Care Med **2006**; 34:730–7.
7. Jacobs S, Price Evans DA, Tariq M, Al Omar NF. Fluconazole improves survival in septic shock: a randomized double-blind prospective study. Crit Care Med **2003**; 31:1938–46.
8. Berenguer J, Buck M, Witebsky F, Stock F, Pizzo PA, Walsh TJ. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis: disseminated versus single-organ infection. Diagn Microbiol Infect Dis **1993**; 17:103–9.
9. Maaroufi Y, De Bruyne JM, Duchateau V, Georgala A, Crokaert F. Early detection and identification of commonly encountered *Candida* species from simulated blood cultures by using a real-time PCR-based assay. J Mol Diagn **2004**; 6:108–14.
10. Hui M, Cheung SW, Chin ML, Chu KC, Chan RC, Cheng AF. Development and application of a rapid diagnostic method for invasive candidiasis by the detection of D-/L-arabinitol using gas chromatography/mass spectrometry. Diagn Microbiol Infect Dis **2004**; 49:117–23.
11. van Deventer AJ, van Vliet HJ, Hop WC, Goessens WH. Diagnostic value of anti-*Candida* enolase antibodies. J Clin Microbiol **1994**; 32: 17–23.
12. Iruretagoyena JR, Regulez P, Quindos G, Ponton J. Antibodies to *Candida albicans* germ tubes in two intensive care patients with invasive candidiasis. Rev Iberoam Micol **2000**; 17:93–6.
13. Sendid B, Jouault T, Coudriau R, et al. Increased sensitivity of mannanemia detection tests by joint detection of alpha- and beta-linked oligomannosides during experimental and human systemic candidiasis. J Clin Microbiol **2004**; 42:164–71.
14. Sendid B, Poirot JL, Tabouret M, et al. Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic Candida species. J Med Microbiol **2002**; 51:433–42.
15. Garcia-Ruiz JC, del Carmen Arilla M, Regulez P, Quindos G, Alvarez A, Ponton J. Detection of antibodies to *Candida albicans* germ tubes for diagnosis and therapeutic monitoring of invasive candidiasis in patients with hematologic malignancies. J Clin Microbiol **1997**; 35: 3284–7.
16. Takesue Y, Kakehashi M, Ohge H, et al. Combined assessment of beta-D-glucan and degree of candida colonization before starting empiric therapy for candidiasis in surgical patients. World J Surg **2004**; 28: 625–30.
17. Odabasi Z, Mattiuzzi G, Estey E, et al. β-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. Clin Infect Dis **2004**; 39:199–205.
18. Digby J, Kalbfleisch J, Glenn A, Larsen A, Browder W, Williams D. Serum glucan levels are not specific for presence of fungal infections in intensive care unit patients. Clin Diagn Lab Immunol **2003**; 10: 882–5.
19. Yeo SF, Huie S, Sofair AN, Campbell S, Durante A, Wong B. Measurement of serum D-arabinitol/creatinine ratios for initial diagnosis and for predicting outcome in an unselected, population-based sample of patients with *Candida* fungemia. J Clin Microbiol **2006**; 44:3894–9.
20. White PL, Shetty A, Barnes RA. Detection of seven *Candida* species using the Light-Cycler system. J Med Microbiol **2003**; 52:229–38.
21. Wahyuningsih R, Freisleben HJ, Sonntag HG, Schnitzler P. Simple and rapid detection of *Candida albicans* DNA in serum by PCR for diagnosis of invasive candidiasis. J Clin Microbiol **2000**; 38:3016–21.
22. Ahmad S, Khan Z, Mustafa AS, Khan ZU. Seminested PCR for diagnosis of candidemia: comparison with culture, antigen detection, and biochemical methods for species identification. J Clin Microbiol **2002**; 40:2483–9.
23. Klingspor L, Jalal S. Molecular detection and identification of Candida and Aspergillus spp. from clinical samples using real-time PCR. Clin Microbiol Infect **2006**; 12:745–53.
24. Moreira-Oliveira MS, Mikami Y, Miyaji M, Imai T, Schreiber AZ, Moretti ML. Diagnosis of candidemia by polymerase chain reaction and blood culture: prospective study in a high-risk population and identification of variables associated with development of candidemia. Eur J Clin Microbiol Infect Dis **2005**; 24:721–6.
25. Lin MT, Lu HC, Chen WL. Improving efficacy of antifungal therapy by polymerase chain reaction-based strategy among febrile patients with neutropenia and cancer. Clin Infect Dis **2001**; 33:1621–7.
26. Morace G, Pagano L, Sanguinetti M, et al. PCR-restriction enzyme analysis for detection of *Candida* DNA in blood from febrile patients with hematological malignancies. J Clin Microbiol **1999**; 37:1871–5.
27. Morace G, Sanguinetti M, Posteraro B, Lo Cascio G, Fadda G. Iden-

tification of various medically important *Candida* species in clinical specimens by PCR-restriction enzyme analysis. J Clin Microbiol **1997**; 35:667–72.
28. Pryce TM, Kay ID, Palladino S, Heath CH. Real-time automated polymerase chain reaction (PCR) to detect *Candida albicans* and *Aspergillus fumigatus* DNA in whole blood from high-risk patients. Diagn Microbiol Infect Dis **2003**; 47:487–96.
29. Maaroufi Y, Heymans C, De Bruyne JM, et al. Rapid detection of *Candida albicans* in clinical blood samples by using a TaqMan-based PCR assay. J Clin Microbiol **2003**; 41:3293–8.
30. Loeffler J, Dorn C, Hebart H, Cox P, Magga S, Einsele H. Development and evaluation of the nuclisens basic kit NASBA for the detection of RNA from *Candida* species frequently resistant to antifungal drugs. Diagn Microbiol Infect Dis **2003**; 45:217–20.
31. White PL, Archer AE, Barnes RA. Comparison of non-culture-based methods for detection of systemic fungal infections, with an emphasis on invasive *Candida* infections. J Clin Microbiol **2005**; 43:2181–7.
32. Shin JH, Nolte FS, Holloway BP, Morrison CJ. Rapid identification of up to three *Candida* species in a single reaction tube by a 5′ exonuclease assay using fluorescent DNA probes. J Clin Microbiol **1999**; 37:165–70.
33. Metwally L, Hogg G, Coyle PV, et al. Rapid differentiation between fluconazole-sensitive and -resistant species of *Candida* directly from positive blood-culture bottles by real-time PCR. J Med Microbiol **2007**; 56:964–70.
34. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Clin Infect Dis **2002**; 34:7–14.