JCM
Journals.ASM.org

Detection of Yeasts in Blood Cultures by the Luminex xTAG Fungal Assay

Joan-Miquel Balada-Llasat, Heidi LaRue, Kamal Kamboj, Lisa Rigali, Debra Smith, Keelie Thomas, and Preeti Pancholi

Department of Pathology, The Ohio State University Medical Center, Columbus, Ohio, USA

A multiplex-PCR Luminex xMAP bead probe fluid array using xTAG analyte-specific reagents (multiplex xTAG fungal ASR assay) was employed for detection of clinically significant *Candida* species, *Cryptococcus neoformans*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis* from blood cultures. We tested 132 blood cultures negative ($n = 10$) or positive ($n = 97$) for yeasts and/or bacteria ($n = 25$). The assay showed sensitivity and specificity of 100% and 99%, respectively. The xTAG fungal ASR assay is a rapid assay that allows simultaneous identification of multiple yeast species.

Blood *Candida* species infections account for significant morbidity and mortality (13, 17). Candidemia has risen to be the fourth most common cause of bloodstream infection (BSI) in the developed world (17). Most BSIs are caused by *Candida albicans*; however, about 45% are caused by *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* (19). While the majority of the yeasts remain susceptible to antifungals, it has to be taken into consideration that *C. krusei* is resistant to fluconazole and that there are increasing rates of resistance to triazole in *C. glabrata* and variable rates of resistance to fluconazole and voriconazole among *C. tropicalis* strains (13). Furthermore, *C. parapsilosis* has higher echinocandin MICs (13). Faster diagnosis guides the clinician on appropriate therapy, improving patient outcomes (9, 11).

In the current work, we studied the use of the multiplex-PCR Luminex xMAP bead probe fluid array using xTAG analyte-specific reagents (multiplex xTAG fungal ASR assay), which combines amplification and detection based on xMAP technology for the accurate identification of yeast-like pathogens directly from positive blood culture bottles.

A total of 132 blood culture samples from aerobic or anaerobic bottles randomly collected from the Bactec 9240 blood culture system (Becton, Dickinson Diagnostics, Sparks, MD) were tested with the multiplex xTAG fungal ASR assay (Table 1). Of these, 95 were positive for yeasts, 2 for yeasts and bacteria, and 25 for bacteria. Ten were negative for bacteria and/or yeasts. Of the positive cultures, 86 were obtained from patients and 18 were simulated aerobic blood bottles spiked with yeast as described previously (21) (Table 1). Only one positive blood culture per patient was tested in a 48-h period. Of the 97 positive yeast blood cultures (79 from patients and 18 spiked), 90 were positive for the presence of one yeast and 7 had two yeast species. A total of 10 yeast species were targeted based on the frequency of recovery at our institution and likely resistance to commonly used antifungal drugs. The yeast species and their corresponding gene targets were *C. albicans* (hyphal wall protein 1), *C. glabrata* (RNase P), *C. parapsilosis* (RNase P), *C. tropicalis* (RNase P), *C. krusei* (RNase P), *Candida lusitaniae* (RNase P), *Candida guilliermondii* (RNase P), *Cryptococcus neoformans* (elongation factor 1 α), *Histoplasma capsulatum* (M antigen), and *Blastomyces dermatitidis* (WI-1 adhesion gene). *Tremella fuciformis* (ATCC 58859) was used as the internal control for extraction and amplification. A blood culture sample (1.3 ml) was spiked with 100 μl of *T. fuciformis* resuspended in water (50,000 CFU) to achieve a median fluorescence intensity (MFI) of 6,000 ± 1,500. Afterwards, samples were hemolyzed with 75 μl of xTAG RW (Luminex Molecular Diagnostics, Toronto, Canada) for 5 min, followed by two washes with water. The samples were resuspended in 1.0 ml of NucliSENS easyMAG lysis buffer (bioMérieux, Durham, NC), transferred to a tube containing 1.4-mm ceramic beads, vortexed for 2 min, incubated at 95°C for 10 min, and filtered with a 0.22-μm filter. One milliliter of the filtered sample was transferred to an easyMAG sample cartridge (bioMérieux, Durham, NC) for DNA extraction using the NucliSENS easyMAG-specific B 2.0.1 profile, with a final elution volume of 40 μl. Five microliters of the extracted sample was amplified using 2.5 μl of nuclease-free water, 4.4 μl of 10× PCR buffer (Qiagen), 0.8 μl of 25 mM $MgCl_2$ (Qiagen), 1 μl of a 5 mM concentration of the deoxynucleoside triphosphates (dNTPs), 11 μl of a 50× concentration of the ASR primers (0.5 μl each primer per reaction mixture; Luminex Molecular Diagnostics, Toronto, Canada), and 0.3 μl of HotStarTaq polymerase (Qiagen). The cycling conditions were denaturation at 95°C for 15 min, followed by 35 cycles of amplification at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and 1 cycle of extension at 72°C for 5 min. For the bead hybridization/capture assay, 1 μl of the PCR product was added to 20 μl of bead mix (MagPlex-TAG; Luminex, Austin, TX). Then, 75 μl of the reporter solution (xTAG ASR–streptavidin–(R)-phycoerythrin G75 [Luminex], diluted 1/75 in 1× hybridization buffer [Luminex Molecular Diagnostics, Toronto, Canada]), was added, and the solution was mixed and incubated at 45°C for 45 min. Detection was performed using a Luminex 100 instrument with xPONENT 3.1 software (7). In each assay, the internal control and the specimen's bead count were read and the results were interpreted as positive if the MFI was >300, indeterminate if the MFI was 150 to 299, and negative if the MFI was <150 (3). *T. fuciformis* was detected in all specimens (mean MFI, 6,282 ± 1,319). For 87 of the blood cultures for which fungal primers were

Received 14 November 2011 **Returned for modification** 15 November 2011
Accepted 23 November 2011
Published ahead of print 14 December 2011
Address correspondence to Preeti Pancholi, Preeti.pancholi@osumc.edu.

doi:10.1128/JCM.06375-11

TABLE 1 Correlation of multiplex xTAG fungal ASR assay results with fungal culture results

Organism(s) detected	No. of positive blood culture samples ($n = 122$)		No. of positive xTAG assay samples[a]	Avg MFI ± SD
	From patients	Spiked		
C. albicans	28	1	29	12,056 ± 2,112
C. glabrata[b]	19	1	20	7,373 ± 1,667
C. parapsilosis[b]	9	2	11	7,428 ± 364
C. neoformans	3	2	5	8,069 ± 2,681
C. krusei	3	2	5	9,308 ± 1,703
C. tropicalis	4	1	5	8,063 ± 3,205
C. guilliermondii	1	3	4	9,930 ± 1,923
C. lusitaniae	0	2	2	10,815 ± 344
C. parapsilosis, *C. neoformans*	2	0	2	7,684 ± 153, 8,343 ± 1,662
C. albicans, *C. glabrata*	1	0	1	
C. glabrata, *C. parapsilosis*	1	0	1	
C. krusei, *C. guilliermondii*	0	1	1	
C. krusei, *C. parapsilosis*	0	1	1	
C. glabrata, *C. dubliniensis*	1	0	1 (*C. glabrata*)	
C. dubliniensis	3	0	ND	0
Aureobasidium pullulans	1	0	ND	0
Candida norvegensis	0	1	ND	0
Rhodotorula spp.	1	0	ND	0
Saccharomyces cerevisiae	2	0	ND	0
C. ciferrii	0	1	1 (*C. neoformans*)	
Staphylococcus aureus	6	0	ND	0
Coagulase-negative staphylococci	5	0	ND	0
Escherichia coli	2	0	ND	0
Enterococcus faecalis	2	0	ND	0
Streptococcus pneumoniae	2	0	ND	0
Klebsiella oxytoca	1	0	ND	0
Klebsiella pneumoniae	1	0	ND	0
Abiotrophia spp.	1	0	ND	0
B. fragilis	1	0	ND	0
Viridans group streptococci	1	0	ND	0
Chryseomonas spp.	1	0	ND	0
E. coli, *S. aureus*	1	0	ND	0
Enterobacter spp., *Streptococcus* spp.	1	0	ND	0
Total	104	18		

[a] ND, not determined.

[b] One culture was coinfected with coagulase-negative staphylococci.

included, the multiplex xTAG fungal ASR assay, compared to culture, correctly identified all blood cultures with high MFIs (Table 1). For culture, the blood culture medium was plated onto 5% sheep blood agar (BBL CHROMagar Candida; BD, Sparks, MD) and potato dextrose agar (PDA), and the plates were incubated at 35°C or 30°C for the PDA for 24 to 48 h. Phenotypic identification was performed by routine methods, including determination of colony morphology and color on CHROMagar, the germ tube test, determination of urease activity, and an API 20C AUX strip test (bioMérieux, Marcy l'Etoile, France). One blood culture that was spiked with *Candida ciferrii* was determined to be *C. neoformans* by the multiplex xTAG fungal ASR assay. While repeat testing by xTAG blood culture (and extracted nucleic acid) yielded the same result, the reference laboratory confirmed the culture identity of the isolate as *C. ciferrii* by sequencing.

For the targets included, the multiplex xTAG fungal ASR assay showed a sensitivity of 100%, a specificity of 99%, a positive predictive value of 99%, and a negative predictive value of 100% compared to culture. Recently, Babady et al. published similar observations with 22 positive yeast blood cultures using the multiplex xTAG fungal ASR assay (2). The assay is an attractive alternative to reference methods, as it is rapid and allows simultaneous identification of multiple fungal species. Other rapid and sensitive assays that significantly reduce the time of yeast identification and can impact the appropriate use of antifungal therapy and outcome (18), such as PCR (1, 16), fluorescence *in situ* hybridization (20), and mass spectrometry (10, 14, 15), have been developed.

The Luminex fungal assay has the potential to guide empirical antifungal drug treatment. The selection of an effective treatment relies on the final identification and susceptibilities of the yeast, which in some instances require up to 5 days to determine by traditional culture methods (6, 12, 17, 22). Reducing the turnaround time (TAT) can potentially improve therapeutic efficacy, reduce adverse effects, lower costs, and contain resistance development.

When selecting a new test, the performance of the assay, TAT, ease of use, cost, and regulatory status are important factors to consider. Previous studies have shown the utility of Luminex-

based multiplex applications for the detection of clinically relevant fungi in clinical specimens and for surveillance (2, 4, 5, 8). However, this is the first extensive report of the utility of a Luminex-based assay with 11 probes for the rapid identification of yeast-like organisms, including *C. neoformans*, from blood cultures. The multiplex xTAG fungal ASR assay offered excellent sensitivity and specificity and considerably better TATs than culture. The assay detected mixed *Candida* infections that were observed in 5.8% of the cases. Furthermore, specificity of fungal identification was maintained in the presence of bacterial growth (Table 1). In its current format, the assay takes 5 h to complete, as well as a meticulous technique to prevent cross-contamination in the open system. Further optimization of the DNA extraction protocol will enhance the usability of the method in clinical practice. Furthermore, the inclusion of fungal probes for detection of *Candida dubliniensis* would be beneficial since this species can be recovered from blood cultures and should be distinguished from *C. albicans*.

In conclusion, the multiplex xTAG fungal ASR assay provides a reliable methodology for rapid yeast identification directly from positive blood cultures and has the potential to guide empirical antifungal drug selection.

ACKNOWLEDGMENTS

The research project was approved by the OSUMC Institutional Review Board.

We are grateful to Luminex for providing reagents for the study.

We declare no competing interests.

REFERENCES

1. **Avni T, Leibovici L, Paul M.** 2011. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. J. Clin. Microbiol. **49**:665–670.
2. **Babady NE, Miranda E, Gilhuley KA.** 2011. Evaluation of Luminex xTAG fungal analyte-specific reagents for rapid identification of clinically relevant fungi. Clin. Microbiol. **49**:3777–3782.
3. **Balada-Llasat JM, LaRue H, Kelly C, Rigali L, Pancholi P.** 2011. Evaluation of commercial ResPlex II v2.0, MultiCode-PLx, and xTAG respiratory viral panels for the diagnosis of respiratory viral infections in adults. J. Clin. Virol. **50**:42–45.
4. **Bovers M, et al.** 2007. Identification of genotypically diverse *Cryptococcus neoformans* and *Cryptococcus gattii* isolates by Luminex xMAP technology. J. Clin. Microbiol. **45**:1874–1883.
5. **Deak E, et al.** 2010. Utility of a Luminex-based assay for multiplexed, rapid species identification of Candida isolates from an ongoing candidemia surveillance. Can. J. Microbiol. **56**:348–351.
6. **De Pauw B, et al.** 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin. Infect. Dis. **46**:1813–1821.
7. **Dunbar SA.** 2006. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clin. Chim. Acta **363**:71–82.
8. **Etienne KA, Kano R, Balajee SA.** 2009. Development and validation of a microsphere-based Luminex assay for rapid identification of clinically relevant aspergilli. J. Clin. Microbiol. **47**:1096–1100.
9. **Fernandez J, Erstad BL, Petty W, Nix DE.** 2009. Time to positive culture and identification for Candida blood stream infections. Diagn. Microbiol. Infect. Dis. **64**:402–407.
10. **Ferroni A, et al.** 2010. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48**:1542–1548.
11. **Forrest GN, et al.** 2006. Peptide nucleic acid fluorescence in situ hybridization-based identification of *Candida albicans* and its impact on mortality and antifungal therapy costs. J. Clin. Microbiol. **44**:3381–3383.
12. **Garey KW, et al.** 2006. Time to initiation of fluconazole therapy impacts mortality in patients with candidemia: a multi-institutional study. Clin. Infect. Dis. **43**:25–31.
13. **Iatta R, Caggiano G, Cuna T, Montagna MT.** 2011. Antifungal susceptibility testing of a 10-year collection of Candida spp. isolated from patients with candidemia. J. Chemother. **23**:92–96.
14. **Marinach C, et al.** 2009. MALDI-TOF MS-based drug susceptibility testing of pathogens: the example of *Candida albicans* and fluconazole. Proteomics **9**:4627–4631.
15. **Marklein G, et al.** 2009. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. J. Clin. Microbiol. **47**:2912–2917.
16. **McMullan R, et al.** 2008. A prospective clinical trial of a real-time polymerase chain reaction assay for the diagnosis of candidemia in nonneutropenic, critically ill adults. Clin. Infect. Dis. **46**:890–896.
17. **Pappas PG, et al.** 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **48**:503–535.
18. **Perlin DS.** 2009. Antifungal drug resistance: do molecular methods provide a way forward? Curr. Opin. Infect. Dis. **22**:568–573.
19. **Pfaller MA, et al.** 2001. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and in vitro susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. J. Clin. Microbiol. **39**:3254–3259.
20. **Reller ME, Mallonee AB, Kwiatkowski NP, Merz WG.** 2007. Use of peptide nucleic acid-fluorescence in situ hybridization for definitive, rapid identification of five common *Candida* species. J. Clin. Microbiol. **45**:3802–3803.
21. **Sheppard DC, Locas MC, Restieri C, Laverdiere M.** 2008. Utility of the germ tube test for direct identification of *Candida albicans* from positive blood culture bottles. J. Clin. Microbiol. **46**:3508–3509.
22. **Tortorano AM, et al.** 2004. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur. J. Clin. Microbiol. Infect. Dis. **23**:317–322.