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Rapid Detection and Differentiation of Clinically Relevant *Candida* Species Simultaneously from Blood Culture by Use of a Novel Signal Amplification Approach

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ABSTRACT Fungal bloodstream infections are a significant problem in the United States, with an attributable mortality rate of up to 40%. An early diagnosis to direct appropriate therapy has been shown to be critical to reduce mortality rates. Conventional phenotypic methods for fungal detection take several days, which is often too late to impact outcomes. Herein, we describe a cost-effective multiplex assay platform for the rapid detection and differentiation of major clinically relevant *Candida* species directly from blood culture. This approach utilizes a novel biotin-labeled polymer-mediated signal amplification process combined with targeting rRNA to exploit phylogenetic differences for sensitive and unambiguous species identification; this assay detects seven pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae*, and *C. guilliermondii*) simultaneously with very high specificity to the species level in less than 80 min with the limits of detection at 1×10^3 to 10×10^3 CFU/ml or as few as 50 CFU per assay. The performance of the described assay was verified with 67 clinical samples (including mixed multiple-species infections as well), with an overall 100% agreement with matrix-assisted laser desorption ionization (MALDI) mass spectrometry-based reference results. By providing a species identity rapidly, the clinician is aided with information that may direct appropriate therapy sooner and more accurately than current approaches, including PCR-based tests.

KEYWORDS AMPED, blood culture, *Candida*, *Candida* detection, molecular diagnostics, signal amplification

Candidemia is one of the major nosocomial bloodstream infections in the United States and worldwide. The main causative agent for this infection are the fungal *Candida* species, and the top seven species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae*, and *C. guilliermondii*) account for more than 95% of the candidemia cases globally (1–4). This infection is opportunistic and may be life threatening for immunocompromised and critically ill patients, such as those with cancers, ongoing chemotherapy, and broad-spectrum antibiotic treatment, as well as those undergoing organ transplant or other major surgeries, particularly, those with the use of central venous and arterial catheters (5–8). In addition to the most common species *C. albicans*, it has also been reported that *C. parapsilosis* and *C. tropicalis* are more frequently associated with neonatal and pediatric patients with low birth weights, parental malnutrition, and hematological malignancies (9–11).

It is well established that the rapid initiation of appropriate therapy is critical for the treatment of fungal bloodstream infections (3, 4, 12). It has also been shown that individual *Candida* species exhibit unique antimicrobial susceptibility profiles, and so

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species information is an attractive approach to more rapidly determine the appropriate treatment for patients suspected of having a *Candida* bloodstream infection. Over the last decades, the prevalence of different species in specific regions and antifungal susceptibility have been closely studied and monitored by the ARTEMIS global antifungal surveillance program team to provide general guidance for local clinicians (3, 4). The focus has been on *in vitro* susceptibility testing for two major antifungal drugs, namely, fluconazole and voriconazole, as the differential cost is significant for these two drugs (13). More recently, the Infectious Disease Society of America (IDSA) has updated the practice guidelines for the treatment of *Candida* bloodstream infection (14). Although echinocandins have been recommended as the frontline therapy, the follow-up treatment is more dependent on the specific species and the associated antifungal susceptibility. Therefore, a low-cost and rapid detection of *Candida* at the species level is valuable for the efficacious treatment of candidemia.

The current gold standards for *Candida* species isolation and identification are still conventional culture-based methods, which take up to several days to get results. In addition, the conventional biochemical, immunological, and serological assays have often been shown to have poor sensitivity and specificity (15, 16, 17). Recently, mass spectrometry (such as matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) testing has improved the accuracy on colonies isolated from positive blood cultures and has shown some promise, but there are some inconsistencies in pretreatment procedures and systems (18, 19, 20, 21). PCR and other nucleic acid amplification methods have been developed for detecting *Candida* species directly from a blood draw (22), and although they have significantly shortened the time to an answer, they are still controversial with some limitations. These methods still cannot provide accurate species-specific information (23, 24, 25). Some other molecular-based assays are either not rapid enough, associated with poor specificity, too costly for reagents and instrumentation, or the process cannot be easily automated (23, 24, 25). However, in general, nucleic acid-based molecular methods could provide more accurate and useful species-specific information if the detection probes are properly designed compared with that from morphology-based culture methods.

Here, we report the development of a rapid and cost-efficient molecular assay based on combining a novel signal amplification process (26) with our chip-array technology (27, 28) to target rRNA for detecting and differentiating seven clinically relevant *Candida* species simultaneously with high specificity. The amplification process is mediated by a high-molecular-weight polysaccharide polymer conjugated with multiple biotin molecules. Using a multivalent bridge, streptavidin, this polymer is conjugated to a biotin-labeled DNA detection probe hybridized to rRNA sequences in *Candida* to allow for amplification of the single probe biotin into ~80 biotin signaling events (Fig. 1). More importantly, the overall high sensitivity of this process eliminates the need for target amplification approaches such as PCR, reducing the test expense and complexity. Furthermore, this process is easily automated on our Portrait device (originally developed for an isothermal amplification process) (29) to provide a rapid and accurate approach for clinicians to identify *Candida* infection with valuable species information.

MATERIALS AND METHODS

Chemicals, reagents, and blood culture medium. All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA) unless otherwise indicated below. 3,3′,5,5′-Tetramethylbenzidine (TMB)-enhanced horseradish peroxidase (HRP) membrane substrate was obtained from SurModics (Eden Prairie, MN, USA). Unspun human whole blood (EDTA, sodium) was purchased from Biological Specialty Corporation (Colmar, PA, USA). BD Bactec instrumented blood culture system and blood culture bottles were from BD Diagnostics (Sparks, MD, USA).

AMP polymer preparation. AMP polymer (originally termed PED polymer) (26) was prepared in-house using the protocol described below. One milligram per milliliter of amino dextran (500 kDa; Molecular Probes) was reacted with 500 μM sulfo-*N*-hydroxysuccinamide (NHS)-biotin (Pierce) in 0.1 M borate buffer (pH 8.5) at room temperature for 3 h with constant mixing. The biotinylated dextran was purified and desalted using a PD-10 column (Fisher GE Healthcare) equilibrated in 0.1 M borate buffer (pH

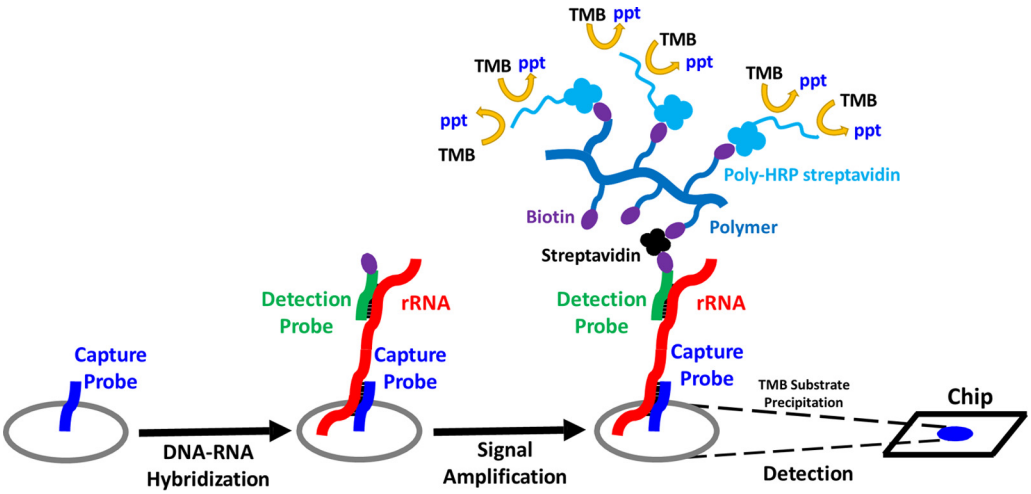

FIG 1 Diagram showing the principle of the AMPED method. TMB, 3,3′,5,5′-tetramethylbenzidine; ppt, precipitation.

8.5) according to the manufacturer's instructions. The polymer solution was then diluted to 5 ml in 0.1 M borate buffer (pH 8.5) and reacted with 100 μM sulfo-NHS-acetate at room temperature for 3 h with constant mixing. The reaction mixture was passed over another PD-10 column equilibrated with 1× phosphate-buffered saline ([PBS] pH 7.0). The biotinylated and acetylated AMP polymer was eluted with 2.5 ml of 1× PBS (pH 7.0) and stored at 4°C until further use.

AMPED *Candida* identification assay probe design. DNA probes were designed against the 28S rRNA of the target *Candida* species. The 28S rRNA sequences of relevant species obtained from GenBank were aligned and analyzed using the CLC sequence viewer (CLC Bio, Aarhus, Denmark). A desired candidate region with maximal mismatches between different target species was chosen for capture probe design to distinguish different target *Candida* species from other closely related species. Detection probes were then designed against the immediate upstream region of the corresponding capture probes. BLAST analyses were performed for all capture probe sequences to determine any potential cross-reactivity. Capture probes were designed using MeltCalc (30, 31), which uses nearest neighbor calculations to optimize the discrimination of all the mismatches. Criteria were set for a melting temperature (T_m) of 65 to 70°C under our assay condition of 825 mM monovalent cation. Higher T_m probes (up to 74°C) were also required for some capture probes, depending on the secondary structure of that region. Each probe was screened to maximize the sensitivity and specificity against other closely related species. All the capture and detection probes used in this study are listed in Table 1. These oligonucleotide probes were ordered from Integrated DNA Technologies (Coralville, IA, USA).

Strains and blood culture process. All the strains used in this study were purchased or were gifts from the American Type Culture Collection (Manassas, VA, USA), Microbiologics, Inc. (St. Cloud, MN, USA), or ARUP Laboratories (Salt Lake City, UT, USA). All samples were subcultured on yeast extract-peptone-dextrose (YEPD) agar plates. To prepare spiked blood cultures, 5 to 7 ml of human blood was injected into BD Bactec bottles. The bottles were then seeded with 3 to 5 isolated colonies of the strain of interest from subcultures suspended in 100 μl of 1× PBS and placed in a BD Bactec 9240 instrumented blood culture system until microbial activity was detected. Blood cultures were serially diluted and plated out to determine the culture titers and then aliquoted and stored at −80°C before use.

Chip production. Crystalline silicon wafers were coated with the polymer amine-functional T-structure polydimethylsiloxane ([TSPS] United Chemical Technologies, Bristol, PA, USA) and cured at 150°C for 24 h. The TSPS-coated wafers were further prepared by soaking in a solution of poly-(Lys-Phe) (50 mg/liter) in 1× PBS (pH 6) containing NaCl (2 mol/liter) overnight at room temperature. The poly-(Lys-Phe)-coated wafers were then washed and incubated with 10 μM succimidyl-4-formyl benzoate ([SFB] Sigma) for 2 h at room temperature, and then washed thoroughly with water, dried with a stream of nitrogen, and stored at room temperature before use. Capture probes contain a reactive hydrazide group on the 5′ end designed to interact with and attach to the aldehyde-functionalized surface of the silicon wafers with a 12-carbon atom spacer to separate the surface from the capture probe sequence, and they were spotted (75 nl) on the SFB-coated silicon wafers in spotting buffer (0.1 M phosphate buffer [pH 7.8], 10% glycerol) using a nanoliter dispenser (BioDot, Irvine, CA, USA). The probe concentration spotted was 300 nM for all the species, except for 100 nM for the *C. krusei* probe and 50 nM for the *C. lusitaniae* probe. To orient the chips for subsequent processing, a fiducial marker (carboxylated polystyrene microspheres) was also printed. After incubating for 2 h, the wafers were washed with 0.1% sodium dodecyl sulfate (SDS), dried, and scribed into 6.5-mm^2 chips using DTX Scribe and Break (Dynatek International, Santa Rosa, CA, USA).

Assay process. For manual assays, chips were attached to the bottoms of the wells of a 96-well plate using double-sided tape, were covered with a microplate sealer, and were preheated on the heater block

TABLE 1 List of detection and capture probe sets

Target species	Probe			Accession no.	Location (nt)[a]
	Type	Name	Sequence		
C. albicans	Capture	Calb853-CP7	/5Ilink12//iSp18/CGCAGCGGCCGCTCCAGAGAGAGCAGCATGC	MF767829.1	835–865
	Paired detection	Calb-DP12	AAAATACCAAGTCTGATCTCAAGCCCTTCCCTTT/3BioEG/	MF767829.1	800–834
C. glabrata	Capture	Cgla284rCP3	/5Ilink12//iSp18/ACTCTTCGAGCACCCTTTACAA	AB499020.1	185–206
	Paired detection	Cand234DP1	mAmCmGmGmGmATTCTCACCCTC/3BioEG/	AB499020.1	158–173
C. tropicalis	Capture	Ctro264rCP2	/5Ilink12//iSp18/TTACATAGGCCTGGATCAT	KY928442.1	198–216
	Paired detection	Cand234DP1	mAmCmGmGmGmATTCTCACCCTC/3BioEG/	KY928442.1	173–189
C. parapsilosis	Capture	Cpar1260-CP5	/5Ilink12//iSp18/CGCTAGTCCACTCCTAAAGGAGGTCCTAC	GQ254874.1	457–485
	Paired detection	Cpar1260-DP1	CTACGTTCACTTTCATTACGCGTACGGGTTTTACA/3BioTEG/	GQ254874.1	422–456
C. krusei	Capture	Ckru262rCP1	/5Ilink12//iSp18/CACTGCTTCCGCCGGCATCCC	LC015645.1	173–193
	Paired detection	Cand234DP1	mAmCmGmGmGmATTCTCACCCTC/3BioEG/	LC015645.1	156–172
C. guilliermondii	Capture	Cgui2600-CP2	/5Ilink12//iSp18/ACCGCCGGTTCTGCTGGGTATGGTAAAG	NG_042640.1	1896–1923
	Paired detection	Cgui2600-DP1	GCCCAAGACACCCGATCCTTAGAGCCAATCCTTA/3BioEG/	NG_042640.1	1862–1895
C. lusitaniae	Capture	Clus3900-CP1	/5Ilink12//iSp18/ACCGCCGCCAAACGCCGCCTTG	JQ689030.1	3030–3051
	Paired detection	Clus3900-DP1	TATGGTCCACATCGTATTTGTATCCAACTG/3BioTEG/	JQ689030.1	3000–3029
S. pombe/SPC	Capture	Spombe2600-CP1	/5Ilink12//iSp18/AGTCCAGCAACCGTTCAGGTTCCAAG	Z19136.1	2019–2044
	Paired detection	Spombe2600-DP1	GCCCAACGTACCCAACCCTTAGAGCCAATCCTTA/3BioEG/	Z19136.1	1985–2018

[a]nt, nucleotide.

in a 60°C oven for 5 to 10 min. Sixty microliters of fungal lysis buffer (30% *N*-lauroyl sarcosine with 20 nM each detection probe) was mixed well with 60 μl of blood culture sample in PCR tubes and lysed at 95°C for 30 min. Then, 100 μl from each of the lysed samples was transferred into the wells of the preheated plate with chips, which was sealed and incubated on the heated block for 20 min at 60°C. After this incubation, the polymer-enhanced detection was performed at room temperature. Briefly, chips were washed 3 times with wash buffer B (0.1× SSC, 0.05%Tween 20), and 100 μl of streptavidin (10 μg/ml in 1× hybridization buffer) was added to each well and incubated for 4 min. Chips were further washed 3 times with buffer B, and 100 μl of AMP-polymer (1:1,000 dilution in 1× hybridization buffer) was added and incubated for 4 min. Again, the chip solution was removed and 100 μl of poly-HRP streptavidin (1:1,000 dilution in Guardian buffer) was added and incubated for 4 min. The chips were washed as before, and 100 μl of BioFX-enhanced small particle TMB was added and incubated for 10 min. Finally, chips were washed 2 times with distilled water and dried 2 times with ethanol. An image was taken using a charge-coupled-device (CCD) camera for each chip.

Clinical specimen testing. A total of 67 frozen specimens from blood culture-positive bottles seeded directly with whole blood of patients suspected with candidemia were collected over 6 months in the Midwest region of the United States. These specimens were characterized in a reference clinical laboratory using MALDI-TOF MS and were identified at the species level. There were a total of 60 *Candida* specimens, representing the 5 most prevalent species, with 22 of *C. albicans*, 25 of *C. glabrata*, 7 of *C. parapsilosis*, 3 of *C. tropicalis*, and 3 of *C. krusei*. There were no *C. lusitaniae*, *C. guilliermondii*, or other nontarget *Candida* species detected in the collection. Two other specimens in this collection were mixed-species infections with *C. albicans* plus *C. dubliniensis* or *C. glabrata*. Furthermore, there were also five specimens identified as bacterial infections rather than fungal infections in this collection. These clinical specimens were tested using the AMPED method according to the assay process described.

RESULTS

Novel signal amplification approach targeting rRNA. Previously, we described the polymer-based enzymatic detection (AMPED) approach and its application for the detection of single-copy genomic DNA targets in *Staphylococcus* species, in which the signal is amplified to provide a 10- to 100-fold improvement in the assay limit of detection, allowing for direct-from-blood culture detection (26). Here, we have applied the AMPED approach to target multicopy rRNA for the detection of fungal *Candida* species in positive blood culture bottles from patients suspected with candidemia as an attempt to further improve the assay limit of detection. Because phylogenetic differences in *Candida* species are significant, unambiguous detection to the species level is possible (32). The AMPED process is briefly depicted in Fig. 1.

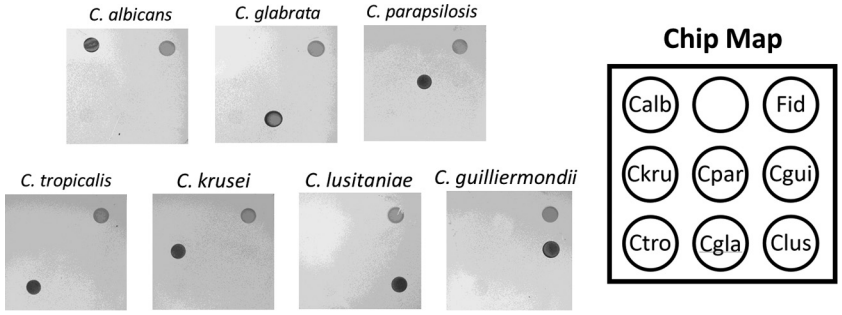

FIG 2 Reactivity panel of AMPED *Candida* identification assay target species. (Left) Chip images for each target species. (Right) Chip map. Calb, *C. albicans*; Ckru, *C. krusei*; Cpar, *C. parapsilosis*; Cgui, *C. guilliermondii*; Ctro, *C. tropicalis*; Cgla, *C. glabrata*; Clus, C. *lusitaniae*; Fid, fiducial (colored carboxylated polystyrene microspheres spotted for easy orientation identification).

AMPED *Candida* identification assay analytical performance. To validate the sensitivity and specificity of the final probe set for the identification of *Candida* species, negative blood culture bottles were spiked with cultured cells, incubated until they became positive as indicated by an alarm on the Bactec blood culture instrument, and then tested in the described assay (see Materials and Methods). For each of the species present in the array, an unambiguous signal was observed with no cross-reaction with any of the other target species (Fig. 2).

Additionally, we determined the reactivity of this AMPED *Candida* identification assay with other closely related *Candida* species. All other *Candida* species tested showed no cross-reactivity, except *C. orthopsilosis* and *C. metapsilosis*, which reacted with the *C. parapsilosis* probe (Table 2, top). However, these results may be expected, as both are members of *C. parapsilosis* complex strains and they have identical sequence in the probe region. Also, there was no cross-reactivity observed for other non-*Candida* fungal and bacterial species tested (Table 2, middle and bottom), including the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe*, which will be used as a sample preparation control strain in our automated assay system.

The limit of detection (LOD) was determined for this AMPED *Candida* identification assay by titrating known quantities of CFU into negative blood cultures for each target. A visual LOD ranging from 1.0×10^3 to 5.0×10^4 CFU/ml was determined for all the target species (Table 3, column 4). Chip images for the *C. krusei* LOD determination are shown in Fig. 3 below. Actually, the LOD of *C. krusei* is lower than 1.0×10^3 CFU/ml, but the signal is faint below these levels (Fig. 3). These limits of detection are far below the measured blood culture titers of alarmed *Candida* positive bottles with *C. albicans* alarm positive at the lowest level (2.7×10^5 CFU)/ml and with all other species alarm positive at 3.1×10^6 CFU/ml or higher (Table 3). The titers measured here confirm the titers previously reported from the Bactec blood culture instrument (33).

Clinical specimen verification. We verified 67 clinical specimens from frozen blood culture-positive bottles from patients suspected with candidemia on our AMPED *Candida* identification assay platform. We correctly detected 62 of the 67 total specimens as positive (including 2 mixed infections) and the other 5 as negative (non-*Candida* infections) in perfect (100%) concordance with the reference method (MALDI-TOF MS) (Table 4).

DISCUSSION

We have herein described a novel multiplex *Candida* identification test that combines a sensitive modified silicon chip with an array of nucleic acid probes and a signal amplification method, AMPED, for the detection of multicopy rRNA sequences, allowing for the direct detection of *Candida* species from positive blood cultures. An advantage of nucleic acid detection approaches that do not require

TABLE 2 List of strains in exclusivity test panel

Species	Source and strain	Input (CFU/ml)	Reactivity
Non-target *Candida*			
C. auris	CDC 0391-3090	~1.0E+7	None
C. catenulata	ATCC 10565	~1.0E+6	None
C. dubliniensis	ATCC MYA-646	~1.0E+6	None
C. duobushaemulonii	CDC 0391	~1.0E+7	None
C. haemulonii	ATCC 22991	4.60E+6	None
C. kefyr	Microbiologic 2512	2.75E+5	None
C. metapsilosis	ATCC 14054	8.56E+6	*C. parapsilosis* probe
C. norvegensis	ATCC 96301	1.61E+6	None
C. orthopsilosis	ATCC 20503	1.35E+7	*C. parapsilosis* probe
C. rugosa	ATCC 20263	3.30E+6	None
C. utilis	ATCC 9905	1.81E+7	None
C. viswanathii	ATCC 28269	1.87E+7	None
Non-*Candida* fungi			
Aspergillus flavus	ATCC 9643	~1.0E+6	None
Aspergillus fumigatus	ATCC 204305	~1.0E+6	None
Aspergillus niger	ATCC 6275	~1.0E+6	None
Aspergillus terreus	ATCC 1012	~1.0E+6	None
Fusarium proliferatum	ATCC 201904	~1.0E+6	None
Rhizopus oryzae	ATCC 56536	~1.0E+6	None
Saccharomyces cerevisiae	ATCC MYA-796	~1.0E+6	None
Schizosaccharomyces pombe	ATCC 10667	~1.0E+6	None
Bacteria			
Acinetobacter baumannii	ATCC 19606	6.15E+7	None
Bacillus subtilis	ATCC 23857	~1.0E+6	None
Bacteroides fragilis	ATCC 23745	5.00E+6	None
Enterococcus faecalis	ATCC 29212	7.80E+7	None
Enterobacter cloacae	ATCC 13047	1.86E+9	None
Escherichia coli	ATCC 43888	~1.0E+6	None
Klebsiella pneumoniae	ATCC 13883	8.95E+7	None
Klebsiella oxytoca	ATCC 49134	1.71E+9	None
Morganella morganii	ATCC 25829	4.30E+7	None
Serratia marcescens	ATCC 13880	5.00E+6	None
Staphylococcus aureus	ATCC 700699	3.70E+7	None
Staphylococcus epidermidis	ATCC 700576	4.70E+7	None

target amplification, such as by PCR, is that the level of multiplexing is only limited by the probe density on the chip. Primer interactions that harm the sensitivity and limit the number of primer sets that may be used in PCR-based multiplex designs are also eliminated. The AMPED approach uses inexpensive, stable, and easy-to-produce biotinylated polymers. This polymer is highly water soluble, chemically simple, and uncharged, limiting nonspecific interactions. Additionally, we have demonstrated that the approach is efficient, with the signal amplification proportional to the number of biotin molecules present on the polymer (26).

The AMPED *Candida* identification test displayed excellent analytical specificity for species-level detection of seven important *Candida* species. No cross talk was observed between the target species or with other key pathogenic *Candida* species, with the exception of *C. orthopsilosis* and *C. metapsilosis* which cross-react with the *C. parapsilosis*

TABLE 3 Limit of detection of each target *Candida* species

Species	Strain	Titer at alarm (CFU/ml)	Limit of detection (CFU/ml)
C. albicans	ARUP1	2.7×10^5	1.0×10^3
C. glabrata	ATCC 2001	4.2×10^6	5.0×10^4
C. tropicalis	ATCC 750	3.0×10^7	5.0×10^3
C. parapsilosis	ATCC 22019	9.4×10^6	1.0×10^4
C. krusei	ATCC 24210	1.1×10^7	1.0×10^3
C. guilliermondii	ARUP2	4.8×10^6	1.0×10^4
C. lusitaniae	ATCC 60247	3.1×10^6	1.0×10^4

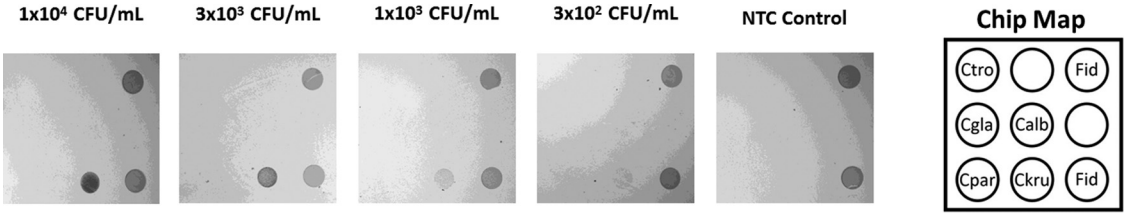

FIG 3 Limit detection of *C. krusei*. (Left) Chip images showing the detection of *C. krusei* with different cell inputs. (Right) Chip map. Calb, *C. albicans*; Ckru, *C. krusei*; Cpar, *C. parapsilosis*; Ctro, *C. tropicalis*; Cgla, *C. glabrata*; Fid, fiducial (colored carboxylated polystyrene microspheres spotted for easy orientation identification).

probes; however, these species belong to the same complex of strains. The limits of detection of 10^3 to 10^4 CFU/ml for this assay are 30- to 5,000-fold below those reported at alarm positivity for *Candida* species, including *C. albicans*, which has been observed elsewhere and measured here to alarm positive at $\sim 3 \times 10^4$ CFU/ml, or 30- to 100-fold lower than the other *Candida* species (Table 3) (33). These limits of detection permit excellent clinical sensitivity in detecting alarm-positive blood cultures, as evidenced herein with 100% sensitivity compared with MALDI reference data in a retrospective study of 62 *Candida*-positive blood cultures. To achieve these limits of detection using the described AMPED method, it is critical to target multicopy genes such as rRNA. A previous work focused on the detection of single-copy genomic sequences specific to *Staphylococcus* using the AMPED approach found lower limits of detection (LLODs) of 2×10^6 to 6×10^6 copies/ml using the same chip formulation (26). This suggests that rRNA is contributing to an up to 6,000-fold improvement in detection sensitivity. Variability in the LODs across the probe set is likely related to differences in secondary structures for the targeted regions of the *Candida* species, as it has been observed that sensitivity can vary by up to 200-fold in targeting structured RNA (34). We applied an approach used in a previous work aimed at targeting the GC-rich, and therefore highly structured, *Mycobacterium tuberculosis* genome in which the probe sets were longer, with higher T_ms, than those for targeting less-structured nucleic acid targets under the same assay conditions (35). Combined with a coaxial stack of the capture and detect probes, the secondary structures could be more effectively unfolded or invaded to enable highly sensitive detection (34).

TABLE 4 Comparison of clinical specimen and reference identifications by MALDI-TOF MS and the AMPED *Candida* assay

Species	No. positively identified	
	MALDI-TOF MS	AMPED assay
Reference *Candida* species (MALDI-TOF MS)		
C. albicans	22	22
C. glabrata	25	25
C. parapsilosis	7	7
C. tropicalis	3	3
C. krusei	3	3
C. lusitaniae	0	0
C. guilliermondii	0	0
Mixed-infection specimens		
C. albicans and *C. dubliniensis*[a]	1	1
C. albicans and *C. glabrata*[b]	1	1
Non-*Candida* infection specimens		
Histoplasma capsulatum	1	0
Malassezia pachydermatis	2	0
Rhodotorula species	1	0
Gram-positive rods resembling *Bacillus*[c]	1	0

[a]*C. dubliniensis* was not detected in this study as it is not a target species.
[b]Both *C. albicans* and *C. glabrata* were detected.
[c]No yeast growth.

The AMPED identification assay was designed to provide species-specific information to aid in making appropriate treatment decisions as early as possible from positive blood cultures. The rapid initiation of appropriate therapy has significant positive outcomes in patients with fungal bloodstream infections. The determination of drug susceptibility profiles using phenotypic methods is time consuming; however, the determination of the *Candida* species identity has been shown to be a good indicator of what treatments may be efficacious. On the basis of the local epidemiology information from national or regional surveys, some basic guidelines for the treatment of candidemia have been provided to local clinicians for selecting empirical antifungal therapy with species-level information by some government agencies (14, 36, 37). For example, *C. tropicalis*, like *C. albicans*, is susceptible to most antifungal agents available on the market but with a relatively high-level sensitivity to fluconazole; thus, the most cost-effective drug, fluconazole, is the best choice (38, 39). *C. glabrata* and *C. krusei* display increased resistance to fluconazole; therefore, it has been recommended that treatment should be with echinocandins (14, 36, 37). *C. parapsilosis* has been the key species for catheter-related bloodstream infections. The removal of catheters and the use of echinocandins as the frontline therapy are recommended for *C. parapsilosis* infection patients. *C. lusitaniae* and *C. guilliermondii* are infrequently observed but display resistance to amphotericin B or fluconazole, respectively, making echinocandins the treatment of choice. More recently, outbreaks of *Candida auris* across different continents have been observed with potentially even broader drug resistance (40, 41). We are currently adding the ability to detect *Candida auris* in the AMPED format to improve the diagnostic potential of this test.

Currently, there are several other systems on the market for fungal and *Candida* identification with different advantages and limitations. AdvanDx offers a peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH)-based test for the identification of a few key *Candida* species from positive blood culture bottles (42). This is a rapid method, but the process is not automated and it provides limited species-specific information. BioFire's BCID test is a large blood culture identification panel (including both fungal and bacterial species) with good sensitivity and specificity (43). However, the system is too costly for small clinical hospitals. T2 Biosystems' *Candida* panel enables a more rapid detection of candidemia directly from whole blood samples but with limited target species numbers and species-specific information (22, 44). Although MALDI-TOF MS has some improved accuracy, it cannot detect mixed infections with the current libraries available on the market, and also, the sample preparation procedure is inconsistent (18, 19, 20, 21). Despite some limitations (such as the need for a blood culture step), the AMPED method does have its own advantages. In addition to the low reagent cost, it provides more accurate species-specific information and also detects more species, including mixed-species infections, with the possibility to further expand the multiplex in a straightforward manner.

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