Revision (CLEAN version)

Multi-center Evaluation of a PCR-based Digital Microfluidics and Electrochemical Detection System for the Rapid Identification of 15 Fungal Pathogens Directly from Positive Blood Cultures

(Running title: rapid detection of fungi in positive blood culture)

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Abstract

Background: Routine identification of fungal pathogens from positive blood cultures by culture-based methods can be time consuming, delaying treatment with appropriate antifungal agents. The GenMark Dx ePlex® Investigational Use Only Blood Culture Identification Fungal Pathogen Panel (BCID-FP) rapidly detects 15 fungal targets simultaneously in blood culture samples positive for fungi by Gram stain. We aimed to determine the performance of the BCID-FP in a multi-center clinical study.

Materials and Methods: Blood culture samples collected at 10 US sites and tested with BCID-FP at 4 sites were compared to the standard-of-care microbiological and biochemical techniques, PNA-FISH and MALDI-TOF MS. Discrepant results were analyzed by bi-directional PCR/sequencing of residual blood culture samples.

Results: A total of 866 clinical samples, 120 retrospectively- and 21 prospectively- collected, along with 725 contrived samples were evaluated. Sensitivity and specificity of the *Candida* species (*C. albicans, C. auris, C. dubliniensis, C. famata, C. glabrata, C. guilliermondii, C, kefyr, C. krusei, C. lusitaniae, C. parapsilosis, C. tropicalis*), ranged from 97.1-100% and 99.8-100%, respectively. For the other organism targets, sensitivity and specificity were as follows: 100% each for *Cryptococcus neoformans* and *C. gattii*, 98.6% and 100% for *Fusarium* spp. and 96.2% and 99.9% for *Rhodotorula* spp. respectively. In 4 of the 141 clinical samples, the BCID-FP Panel correctly identified an additional *Candida* species, undetected by standard-of-care methods.

Conclusion: The BCID-FP Panel offers a faster turnaround time for identification of fungal pathogens in positive blood cultures that may allow for earlier antifungal interventions and, includes *C. auris*, a highly multi-drug resistant fungus.

Introduction

Fungemia is a severe form of systemic and invasive fungal infection and delayed diagnosis of fungal bloodstream infections can result in significant increases in mortality. Candidemia, in particular, is one of the leading causes of bloodstream infections in hospital settings with a crude mortality rate of 40-75% (1). Previously, a multi-center study has shown that the mortality rate significantly increased for every hour delayed in diagnosis of candidemia (2). Rapid diagnosis of candidemia is even more crucial in immunocompromised patient populations because of a higher mortality rate in this patient group (3, 4). Conventional culture-based identification methods lack the speed needed to aid in choosing the appropriate antifungal drugs for timely management of patients suffering from these invasive fungal infections.

Three commercially available molecular tools have been applied to rapidly identify *Candida* spp. directly from positive blood culture bottles (without waiting for the growth of the organisms on the subsequent culture media): the *Candida* PNA FISH® assay (OpGen®) (5, 6), the BioFire® FilmArray® Blood Culture Identification Panel (BioMerieux®) (7), and PhenoTest® Blood Culture kit (Accelerate Diagnostics) (8). One major limitation of each method is the lack of broad coverage for fungal pathogen detection since the former two methods only target five *Candida* species: *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and the latter one only targets two *Candida* species: *C. albicans* and *C. glabrata*.

The ePlex® Investigational Use Only (IUO) Blood Culture Identification Fungal Pathogen (BCID-FP) Panel (GenMark Dx) is a fully automated one-step test to detect and identify 15 fungal pathogens directly from positive blood cultures. In this study, we conducted a multi-center evaluation to determine the clinical sensitivity and specificity of the ePlex IUO

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BCID-FP Panel for the rapid detection and identification of fungal pathogens directly from positive blood cultures.

MATERIALS AND METHODS

Study design and samples

Positive blood cultures from patients of all ages and genders were collected at 10 hospitals and medical centers from the following 9 cities located in the U.S.: Albuquerque, New Mexico; Baltimore, Maryland; Charleston, South Carolina; Danville, Pennsylvania; Detroit, Michigan (2 sites); Harvey, Illinois; Indianapolis, Indiana; New York City, New York; and San Diego, California.

Two sites prospectively collected samples in 2015 and 2016, and 4 sites collected samples from July-August 2018. In addition, samples with gram stain showing fungal organisms were retrospectively collected from 9 sites; they were stored in a freezer (≤-20°C) at the collection sites, and then shipped in frozen condition to the testing laboratory where they were stored in -70°C condition before testing. All prospectively and retrospectively-collected positive blood culture samples were tested by the standard-of-care testing (comparator method) performed at each site as per their standard laboratory procedures. The residual portion of these blood culture samples was de-identified and tested at 4 clinical sites with the GenMark Dx ePlex® IUO BCID-FP Panel. The study was approved by a central Institutional Review Board (IRB) and/or each site's IRB.

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The comparator method(s) included: traditional fungal culture, FDA-cleared Matrix assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (i.e., bioMérieux® Vitek® MS, Bruker® Biotyper®), microbiological and biochemical tests (i.e., Becton Dickinson® [BD] Phoenix™, bioMérieux® Vitek® 2, Beckman Coulter® MicroScan®), PNA-FISH testing. Discordant results between the BCID-FP Panel and the comparator method(s) were investigated by running molecular assays to determine the presence or absence of the organism directly in residual blood culture samples. The molecular assays employed polymerase chain reaction (PCR) amplification targeting genes associated with each fungal target followed by bi-directional sequencing (PCR/sequencing). The molecular assays were validated analytically with precision, limit-of-detection, inclusivity and exclusivity studies using spiked blood culture media and DNA or whole organisms. Descriptions of each gene target, primer sequences and PCR conditions are provided in Supplementary Table 1. As part of the comparator method, all prospective samples were tested with PCR/sequencing assays to determine the presence/absence of *Candida auris*, *Fusarium* (*F. dimerum*, *F. oxysporum*, *F. Ssacchari*, *F. solani*, *F. verticillioides*), and *Rhodotorula* (*R. glutinis*, *R. mucilaginosa*) because not all standard-of-care methods may have tested for these organisms initially on a consistent basis. Due to potential misidentification of *C. parapsilosis* with other cryptic species within the *C. parapsilosis* species complex, e.g. *C. orthopsilosis*, *C. metapsilosis*, by standard of care phenotypic methods (9, 10), samples with *Candida parapsilosis* identified by standard laboratory procedures were confirmed using the PCR/sequencing assay to determine the comparator method result.

Contrived samples were used to establish additional performance metrics for specific fungal targets due to very low prevalence from the prospectively and retrospectively collected

clinical samples. Each target had contrived samples prepared from at least 3 different strains. Contrived samples were prepared by aseptically injecting 3-10 mL of human whole blood (BioIVT, Westbury, NY) into a BD BacTec blood culture bottle (Plus Aerobic/F, Myco/F Lytic, or Peds Plus/F). The bottles were then inoculated with conidia or spores (in case of *Fusarium*) from a pure culture of a known organism grown on Sabouraud agar under 30°C between 36 – 72 hours. The fungal preparation was generated by diluting conidia or spores in saline to approximately 0.5 McFarland via OD_{600} reading (0.5 McFarland is equivalent to approximately 1.0 x10^6 CFU/mL for yeast cells at OD_{600nm}) (11, 12). The fungal preparation was used neat or diluted to either 1:10, 1:100, 1:1000, 1:10000, 1:20000, or 1:100000 and then 100µL (except for two samples used either 400µL or 1mL) was used to inoculate the bottle containing blood. The inoculum was adjusted based on successful growth and time to detection in preliminary samples. The time to detection varied from 11 hours to 5 days for 95% of the contrived samples; the remaining 5% varied from >5 days to 15 days. The contrived sample list is detailed in Supplementary Table 2.

GenMark Dx ePlex BCID-FP Panel Testing

The BCID-FP Panel runs on a single-use cartridge that automates all aspects of nucleic acid testing in combination with electrowetting and GenMark Dx's eSensor® technology based on the principles of competitive DNA hybridization and electrochemical detection (13). The BCID-FP Panel identifies the following 15 targeted fungal organisms from positive blood culture which contain fungal organism: *Candida albicans, C. auris, C. dubliniensis, C. famata, C. glabrata, C.*

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guilliermondii, C. kefyr, C. krusei, C. lusitaniae, C. parapsilosis, C. tropicalis, Cryptococcus gattii, C. neoformans, Fusarium spp., and *Rhodotorula* spp.

The test consists of a single-use cartridge to be used with the GenMark Dx ePlex instrument and software, in which all steps from sample extraction to detection of target DNA are performed from a positive blood culture. It combines two main technologies: digital microfluidics, or electrowetting, responsible for the movement and transfer of samples and reagents inside the cartridge, and the GenMark Dx eSensor® technology for electrochemical detection of target DNA. Nucleic acids are extracted and purified from blood culture samples (magnetic solid-phase extraction), and DNA is amplified to generate double-stranded PCR product. Amplification is followed by an exonuclease treatment to generate a single-stranded PCR product, which is mixed with a solution containing complementary signal probes labeled with ferrocene. If target DNA is present, hybridization between the single stranded PCR product and the signal probes occurs. The solution is then moved to the detection part of the cartridge, the eSensor microarray, consisting of target specific capture probes attached to gold electrodes. If present, the complex 'target DNA/signal probe' hybridizes with the capture probes, leading to the generation of a voltage signal detected by the ePlex instrument. Internal controls monitoring the performance of each step in the process and each amplification reaction are included on each cartridge.

Testing with the BCID-FP Panel was done following the manufacturer's instructions using the materials in the kit. Briefly, after inverting the blood culture bottle several times to mix, 50 µL was aspirated and loaded into the sample port of the BCID-FP Panel cartridge and the cap was depressed to close the port. Each cartridge was barcoded and scanned at the ePlex

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instrument and inserted into an available bay. Upon test completion, the ePlex instrument ejected the cartridge for disposal and a BCID-FP Panel report was generated (Supplementary Figure 1).

Statistical Methods

Sensitivity/positive percent agreement (PPA) and specificity/negative percent agreement (NPA) with comparator method results were determined for each targeted fungal organism detected by the BCID-FP Panel. Sensitivity/PPA was calculated as 100 x #TP/(#TP+#FN) and specificity/NPA was calculated as 100 x #TN/(#TN+#FP) where TP=true positive, FN=false negative, TN = true negative, and FP = false positive. The two-sided 95% score confidence interval (CI) was calculated for sensitivity/PPA and specificity/NPA.

RESULTS

Sample Disposition, Run/Sample Accountability, Demographic/Sample Information,

A total of 447 positive blood culture samples were collected prospectively at 6 sites in 2 phases. In phase I, 237 samples were collected at 2 sites and frozen for future testing (prospective frozen samples) from May 2015 through July 2016. In phase II, 210 samples were collected at 4 sites from July through August 2018, were never frozen and were tested fresh (prospective fresh samples). Of these 447 blood culture samples, 21 (10 from phase I samples; 11 from phase II samples) had a Gram stain result indicating fungal organisms, representing an overall prevalence of fungemia of 4.7%. Among the 21 cases, 18/21 included organisms targeted by the BCID-FP panel: 29% were caused by *C. glabrata*, followed by *C. albicans* (19%), 10% each by *C.*

tropicalis, *C. parapsilosis*, *C. krusei*, and 5% each by *C. dubliniensis* and *Rhodotorula* spp. (Supplementary Table 3).

A total of 120 positive blood culture samples with Gram stain results showing fungal organisms were retrospectively collected from 9 sites. In addition, 726 samples were contrived with targeted fungal organisms in BD BacTec™ bottles (Supplementary Table 2). Taken together, 867 samples were initially tested with the BCID-FP Panel, of which 839 yielded valid results for an initial validity rate of 96.8%. After repeat testing of the 28 initially invalid samples, 27 yielded valid results for a final validity rate of 99.9% (866/867). There was one contrived sample with an invalid result after repeat testing, and therefore it was excluded from the evaluation.

For prospective subjects, 67% were male and the mean age for this group was 48.1 years old; 71% of the prospective patients ranged in age from 18-64 years old. Among the retrospective subjects, 57% were male and the mean age for this group was 53.5 years old; 55% of these patients ranged in age from 18-64 years (Supplementary Table 4).

Ten different blood culture bottle types from 3 manufacturers (BD [Becton Dickinson], bioMérieux Inc, and Thermo Fisher Scientific®) were used. The majority of the blood culture bottles used in the prospectively collected samples were BacTec™ PLUS Aerobic/F, in the retrospectively collected samples were BacTec™ PLUS Aerobic/F and BacTec™ Standard/10 Aerobic/F, and in the contrived samples were BacTec™ Myco/F Lytic (Supplementary Table 5).

BCID-FP Panel Performance

Each of the 15 fungal targets on the BCID-FP Panel was tested by a range of 49 – 70 positive samples to determine sensitivity/PPA and a range of 796 – 817 negative samples to determine specificity/NPA (Table 1). For each fungal target, positive or negative samples (comparator results) consisted of prospectively- and retrospectively-collected clinical blood culture samples as well as contrived samples. Contrived samples were solely used to evaluate the sensitivity for the following fungal targets due to a lack of positive results from the prospective and retrospective sample collections: *Candida auris*, *C. famata*, *C. guilliermondii*, *C. kefyr*, *C. gattii*, and *Fusarium* spp.

Overall, test sensitivity/PPA and specificity/NPA were 100% for the following 6 fungal targets on the BCID-FP Panel: *C. auris*, *C. dubliniensis*, *C. famata*, *C. krusei*, *C. gattii*, and *C. neoformans*. The sensitivity/PPA for the remaining fungal targets ranged from 96.2% to 100%, and specificity/NPA ranged from 99.8% to 100%. A total of 9 false negative results were found in the samples containing the following fungal targets: 5 contrived samples each spiked with *C. albicans*, *C. guilliermondii*, *Fusarium* spp., and *Rhodotorula* spp. (n=2); 4 retrospectively collected clinical samples each positive for *C. albicans*, *C. glabrata*, *C. lusitaniae*, and *C. parapsilosis* (Table 2). A total of 9 false positive results were detected in the following samples: 5 were found to be positive in contrived samples without spiking the following fungal targets for *C. albicans*, *C. kefyr* (n=2), *C. lusitaniae*, and *Rhodotorula* spp.; 4 were from retrospectively collected clinical samples that were not identified by comparator methods but were detected by the BCID-FP Panel (2 *C. glabrata*, 1 *C. parapsilosis*, and 1 *C. tropicalis)* (Table 3). A discrepant analysis was performed by running PCR/sequencing for the above fungal targets in these 4 retrospectively collected samples. The target *Candida* spp. were detected by PCR/sequencing,

thus the 4 positive results by the BCID-FP Panel were deemed to be true positive. After discordant resolution for the 2 *C. glabrata*, 1 *C. parapsilosis*, and 1 *C. tropicalis*, the sensitivity increased to 98.4%, 98.4% and 100%, respectively for each target.

A total of 8 cases of mixed fungal infections were detected either by comparator methods or by the BCID-FP Panel among the 141 prospectively- and retrospectively-collected clinical samples (Table 3). Case numbers 1, 2, 3 and 8 were mixed infections detected by the comparator methods. Case number 8 contained only fungal pathogens that are not included on the BCID-FP Panel (i.e., *C. metapsilosis* and *Trichosporon asahii*) which resulted in no targets being detected on the BCID-FP Panel as expected. Case number 1 was a co-infection mixed with *C. albicans*, *C. glabrata*, and *C. dubliniensis*. The BCID-FP Panel was able to detect *C. albicans* and *C. dubliniensis*, but not *C. glabrata*. Subsequent PCR/sequencing was not able to confirm the presence of *C. glabrata* in that sample, rendering an inconclusive evaluation result. In case number 2, the BCID-FP Panel was able to detect both *C. albicans* and *C. parapsilosis*. In case number 3, the BCID-FP Panel was able to detect *C. albicans* but failed to detect *C. parapsilosis*. Case numbers 4-7 were positive for a single fungal target by the comparator methods. The BCID-FP Panel was able to detect not only the single target but also an additional fungal target in each of these 4 cases as described in the previous paragraph. These additional fungal targets were confirmed by PCR/sequencing results, indicating true co-infections detected by the BCID-FP Panel (Table 2).

DISCUSSION

One of the highest risk factors for mortality for patients with candidemia is time to diagnosis; therefore, rapid, accurate diagnosis is critical to improving patient care outcome (2, 14). The ePlex® BCID Fungal Pathogen Panel is currently the only rapid, commercial panel that detects the greatest number of fungal pathogens (up to 15 pathogens) directly in patients with positive blood cultures. The BCID-FP Panel has a straight forward easy-to-use workflow with hands-on time of less than 2 minutes to load each sample into the cartridge and a run time of approximately 100 minutes on the ePlex system, a scalable (3-24 bays) random-access instrument.

Our multicenter study showed that the ePlex BCID-FP Panel exhibited 100% sensitivity and specificity for 6 fungal targets (*C. auris*, *C. dubliniensis*, *C. famata*, *C. krusei*, *C. gattii*, and *C. neoformans*) and a range of sensitivity of 96.2% – 100% and specificity of 99.8% – 100% for the remaining fungal targets before resolution of discordant results. While the ePlex BCID-FP Panel missed the detection of fungal targets in 5 contrived samples and 4 retrospective clinical samples (Table 2), the panel did detect additional fungal targets in 4 cases that were missed by the standard-of-care testing, in turn delivering a faster set of complete results to the clinicians responsible for patient management, so that appropriate treatment can be initiated without delay. For example, the standard-of-care tests only detected *C. lusitaniae* in case numbers 6 and 7 of mixed fungal infections (Table 4). The ePlex BCID-FP Panel detected additional *C. glabrata* in both cases, which could have allowed the more appropriate choice of echinocandin over fluconazole as per current clinical practice guidelines for the management of candidiasis (15).

Importantly, it is the only FDA cleared rapid molecular panel that contains *C. auris*, which is an emerging multi-drug resistant fungal pathogen that has been reported to cause high

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mortality and nosocomial outbreaks in the hospital settings (16-18) and has recently been added to the CDC's Antimicrobial Resistance Urgent Threat list. Over 60% of patients infected by *C. auris* developed bloodstream infection with a mortality rate reaching up to 60% (19). Rapid detection of *C. auris* in blood cultures can not only result in early initiation of an appropriate antifungal regimen, (i.e., echinocandins due to its high resistance rate to azoles) (19, 20), but can also help prevent further spread of this nosocomial multi-drug resistant organism in health care facilities. A large, multi-institution outbreak of *C. auris* highlighted the clinical importance of its rapid identification as transmission occurs primarily among patients with extensive healthcare exposure and much like *Clostridioides difficile*, *C. auris* remains viable on inanimate objects for 7-14 days, longer in a non-culturable state, contributing to its nosocomial transmission (21-23). Although a positive *C. auris* result has clear epidemiological impact, a negative result for *C. auris* is also highly valuable as it would help hospital infection control to rule out this nosocomial pathogen due to the BCID-FP Panel's high specificity for this organism.

The ePlex BCID-FP Panel contains 2 non-*Candida* yeasts, *Cryptococcus* and *Rhodotorula*. Although bloodstream infection caused by these yeasts are less common than *Candida* spp. (24, 25), annually less than 10,000 cases compared to 25,000 cases in the U. S., rapid and accurate detection of these fungi are paramount because they contribute to a higher mortality rate and antifungal regimens are very different from candidemia (26, 27). For example, echinocandins are the most active category of antifungal agents against *Candida* spp. but they have no activity against *Cryptococcus* and *Rhodotorula* (27, 28).

Moreover, the ePlex BCID-FP Panel is the only commercial panel that also targets *Fusarium* spp., the most common filamentous fungus frequently isolated from patients' blood

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cultures (29). The broad coverage of the *Fusarium* target covers the most common and medically important *Fusarium* spp., including *F. solani, F. oxysporum*, *F. verticillioidis*, *F. dimerum*, and *F. sacchari*. Disseminated fusariosis occurs most commonly in immunocompromised patients particularly those with hematological malignant patients and stem cell transplant patients with prolonged and profound neutropenia and/or severe T-cell immunodeficiency (29). About 60–70% of those patients developed a *Fusarium* bloodstream infection and in this patient population the intrinsic resistance of *Fusarium* spp. to most antifungal agents results in high mortality rates (30). Rapid identification of *Fusarium* in these patients would aid in the initiation of proper antifungal treatment that is different from treatment of yeast infection, especially in persistently neutropenic patients with disseminated disease where the mortality rate approaches 100% (29, 31).

In summary, the ePlex BCID-FP Panel, which has recently been cleared by FDA, contains the largest breadth of fungal targets and proved to be an accurate, easy-to-use multiplex molecular tool suitable for clinical laboratories to detect common fungal pathogens causing bloodstream infections more rapidly than traditional and conventional microbiological methods.

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Table 1. Clinical Performance ePlex BCID-FP Panel With Comparator Methods

		Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	**% (95% CI)**	**TN/TN+FP**	**% (95% CI)**
Candida albicans					
	Clinical	53/54	98.1 (90.2-99.7)	87/87	100 (95.8-100)
	Contrived	13/14	92.9 (68.5-98.7)	710/711	99.9 (99.2-100)
	Combined	66/68	97.1 (89.9-99.2)	797/798	99.9 (99.3-100)
Candida auris					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	49/49	100 (92.7-100)	676/676	100 (99.4-100)
	Combined	49/49	100 (92.7-100)	817/817	100 (99.5-100)
Candida dubliniensis					
	Clinical	4/4	100 (51.0-100)	137/137	100 (97.3-100)
	Contrived	48/48	100 (92.6-100)	677/677	100 (99.4-100)
	Combined	52/52	100 (93.1-100)	814/814	100 (99.5-100)
Candida famata					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	51/51	100 (93.0-100)	674/674	100 (99.4-100)
	Combined	51/51	100 (93.0-100)	815/815	100 (99.5-100)
Candida glabrata					
	Clinical	43/44	97.7 (88.2-99.6)	95/97 [a]	97.9 (92.8-99.4)
		↓		↓	
		45/46 [a]		**95/95** [a]	
	Contrived	16/16	100 (80.6-100)	709/709	100 (99.5-100)
	Combined	59/60	98.3 (91.1-99.7)	804/806	99.8 (99.1-99.9)
Candida guilliermondii					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	49/50	98.0 (89.5-99.6)	675/675	100 (99.4-100)
	Combined	49/50	98.0 (89.5-99.6)	816/816	100 (99.5-100)
Candida kefyr					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	51/51	100 (93.0-100)	672/674	99.7 (98.9-99.9)
	Combined	51/51	100 (93.0-100)	813/815	99.8 (99.1-99.9)
Candida krusei					
	Clinical	4/4	100 (51.0-100)	137/137	100 (97.3-100)
	Contrived	46/46	100 (92.3-100)	679/679	100 (99.4-100)
	Combined	50/50	100 (92.9-100)	816/816	100 (99.5-100)
Candida lusitaniae					
	Clinical	3/4	75.0 (30.1-95.4)	137/137	100 (97.3-100)
	Contrived	45/45	100 (92.1-100)	679/680	99.9 (99.2-100)
	Combined	48/49	98.0 (89.3-99.6)	816/817	99.9 (99.3-100)
Candida parapsilosis					
	Clinical	18/19 [b]	94.7 (75.4-99.1)	121/122 [c]	99.2 (95.5-99.9)
		↓		↓	
		19/20 [c]		**121/121** [c]	
	Contrived	41/41	100 (91.4-100)	684/684	100 (99.4-100)

	Combined	59/60	98.3 (91.1-99.7)	805/806	99.9 (99.3-100)
Candida tropicalis					
	Clinical	5/5 ↓ **6/6** [d]	100 (56.6-100)	135/136 ↓ **135/135** [d]	99.3 (96.0-99.9)
	Contrived	45/45	100 (92.1-100)	680/680	100 (99.4-100)
	Combined	50/50	100 (92.9-100)	815/816	99.9 (99.3-100)
Cryptococcus gattii					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	50/50	100 (92.9-100)	675/675	100 (99.4-100)
	Combined	50/50	100 (92.9-100)	816/816	100 (99.5-100)
Cryptococcus neoformans					
	Clinical	5/5	100 (56.6-100)	136/136	100 (97.3-100)
	Contrived	52/52	100 (93.1-100)	673/673	100 (99.4-100)
	Combined	57/57	100 (93.7-100)	809/809	100 (99.5-100)
Fusarium					
	Clinical	0/0	---	141/141	100 (97.3-100)
	Contrived	69/70	98.6 (92.3-99.7)	655/655	100 (99.4-100)
	Combined	69/70	98.6 (92.3-99.7)	796/796	100 (99.5-100)
Rhodotorula					
	Clinical	2/2	100 (34.2-100)	139/139	100 (97.3-100)
	Contrived	48/50	96.0 (86.5-98.9)	674/675	99.9 (99.2-100)
	Combined	50/52	96.2 (87.0-98.9)	813/814	99.9 (99.3-100)

PPA: positive percent agreement; NPA: negative percent agreement; TP: true positive; FN: false negative; TN: true negative; FP: false positive

[a] *C. glabrata* was detected by ePlex BCID-FP panel in two samples that only grew *C. lusitaniae* (that was also detected by ePlex BCID-FP Panel). *C. glabrata* was further detected in the residual of these two samples by PCR/sequencing, thus confirming these two samples are true positive for *C. glabrata*. These two samples are also listed as Case 6 and 7 in Table 4.

[b] The false negative sample is also listed as Case 3 in Table 4.

[c] *C. parapsilosis* was detected by ePlex BCID-FP Panel in a sample that only grew *C. dubliniensis* (that was also detected by ePlex BCID-FP Panel). *C. parapsilosis* was further detected in the residual of that sample by PCR/sequencing, thus confirming this sample is true positive for *C. parapsilosis*. This sample as also listed as Case 4 in Table 4.

[d] *C. tropicalis* was detected by ePlex BCID-FP Panel in a sample that only grew *C. dubliniensis* (that was also detected by ePlex BCID-FP Panel). *C. tropicalis* was further detected in the residual of that sample by PCR/sequencing, thus confirming this sample is true positive for *C. tropicalis*. This sample is also listed as Case 5 in Table 4.

Table 2. Summary of discrepant results between the standard-of-care (SOC) testing or spiked organism and the ePlex BCID-FP Panel run

	SOC positive /BCID-FP negative	PCR /Sequencing	Interpretation	SOC negative /BCID-FP positive	PCR /Sequencing	Interpretation
C. albicans						
Clinical sample (Retrospective)	1	Positive for *C. albicans*	False negative			
Contrived sample	1[a]	ND	False negative	1[b]	ND	False positive
C. glabrata						
Clinical sample (Retrospective)	1[c]	Negative for *C. glabrata*	Indeterminate	2[d]	Positive for *C. glabrata*	True positive
C. guillermondii						
Contrived sample	1[e]	ND	False negative			
C. kefyr						
Contrived sample				2[f]	ND	False positive
C. lusitaniae						
Clinical sample (Retrospective)	1	Positive for *C. lusitaniae*	False negative			
Contrived sample				1[g]	ND	False positive
C. parapsilosis						
Clinical sample (Retrospective)	1[h]	Positive for *C. parapsilosis*	False negative	1[i]	Positive for *C. parapsilosis*	True positive
C. tropicalis						
Clinical sample (Retrospective)				1[j]	Positive for *C. tropicalis*	True positive
Fusarium						
Contrived sample	1[k]	ND	False negative			
Rhodotorula						
Contrived sample	2[l]	ND	False negative	1[m]	ND	False positive
Total	***9***			***9***		

ND: Not done.

[a] The sample was spiked with *C. albicans* ATCC10231. It was flagged positive on day 6 but was negative by BCID-FP Panel.

[b] The sample was spiked with *C. dubliniensis* ATCCMYA-578. *C. dubliniensis* was correctly detected by BCID-FP panel, but the sample was also positive for *C. albicans* and *C. kefyr* (same sample discussed in footnote *f*).

[c] The sample grew *C. albicans*, *C. glabrata*, and *C. dubliniensis*. BCID-FP Panel detected *C. albicans*, *C. dubliniensis*, but not *C. glabrata*. Subsequently, *C. glabrata* was not detected in the residual of that sample by PCR/sequencing. This sample is also listed as Case 1 in Table 4.

[d] These two samples are also listed as Case 6 and 7 in Table 4.

[e] The sample was spiked with *C. guilliermondii* ATCC90198. It was flagged positive on day 2 but was negative by BCID-FP Panel.
[f] One sample was spiked with *C. dubliniensis* ATCCMYA-578. *C. dubliniensis* was correctly detected by BCID-FP Panel, but the sample was also positive for *C. kefyr* and *C. albicans* (same sample discussed in footnote *b*). The other sample was spiked with *C. auris* CDC#0390. *C. auris* was correctly detected by BCID-FP, but the sample was also positive for *C. kefyr*.
[g] The sample was spiked with *C. neoformans* ATCC14116. *C. neoformans* was correctly detected by BCID-FP Panel, but the sample was also positive for *C. lusitaniae*.
[h] This sample is also listed as Case 3 in Table 4.
[i] This sample is also listed as Case 4 in Table 4.
[j] This sample is also listed as Case 5 in Table 4.
[k] The sample was spiked with *Fusarium dimerum* CBS110317. It was flagged positive on day 3 but was negative by BCID-FP Panel.
[l] Two contrived samples were each spiked with *Rhodotorula mucilaginosa* ATCC66034 and *R. mucilaginosa* ATCC9449 and flagged positive on day 3 and day 6, respectively, but were negative by BCID-FP Panel.
[m] The sample was spiked with *C. auris* CDC#0389. *C. auris* was correctly detected by BCID-FP Panel, but the sample was also positive for *Rhodotorula*.

Table 3. Detection of mixed fungal organisms by ePlex BCID-FP Panel in positive blood cultures (prospective/retrospective clinical samples)

Case	SOC Testing	BCID-FP	PCR/Sequencing	Interpretation
1	*C. albicans* *C. glabrata* *C. dubliniensis*	*C. albicans* - C. dubliniensis	Negative	Inconclusive
2	*C. albicans* *C. parapsilosis*	*C. albicans* *C. parapsilosis*		
3	*C. albicans* *C. parapsilosis*	*C. albicans* -	*C. parapsilosis*	BCID-FP false negative
4	*C. dubliniensis*	*C. dubliniensis* *C. parapsilosis*	*C. parapsilosis*	BCID-FP true positive
5	*C. dubliniensis*	*C. dubliniensis* *C. tropicalis*	*C. tropicalis*	BCID-FP true positive
6	*C. lusitaniae*	*C. lusitaniae* *C. glabrata*	*C. glabrata*	BCID-FP true positive
7	*C. lusitaniae*	*C. lusitaniae* *C. glabrata*	*C. glabrata*	BCID-FP true positive
8	*C. metapsilosis* *Trichosporon asahii*	Off-panel Off-panel		

SOC: Standard-of-care