510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K182690

B. Purpose for Submission:

To obtain clearance for the ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel

C. Measurand:

Candida albicans, Candida auris, Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Cryptococcus gattii, Cryptococcus neoformans, Fusarium, and *Rhodotorula.*

D. Type of Test:

A multiplexed nucleic acid-based test intended for use with the GenMark's ePlex instrument for the qualitative *in vitro* detection and identification of multiple, pathogenic fungal organisms. The BCID-FP assay is performed directly on positive blood culture samples that demonstrate the presence of organisms as determined by Gram stain.

E. Applicant:

GenMark Diagnostics, Incorporated

F. Proprietary and Established Names:

ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3365 - Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures

2. Classification:

   Class II

3. Product codes:

   PEO

4. Panel:

   83 (Microbiology)

H. Intended Use:

1. Intended use(s):

   The GenMark ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on GenMark's ePlex Instrument for simultaneous detection and identification of multiple potentially pathogenic fungal organisms in positive blood culture. The ePlex BCID-FP Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and which contain fungal organism.

   The following fungal organisms are identified using the ePlex BCID-FP Panel: *Candida albicans, Candida auris, Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Cryptococcus gattii, Cryptococcus neoformans, Fusarium* and *Rhodotorula*.

   The detection and identification of specific fungal nucleic acids from individuals exhibiting signs and/or symptoms of bloodstream infection aids in the diagnosis of bloodstream infection when used in conjunction with other clinical information. The results from the ePlex BCID-FP Panel are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

   Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by the ePlex BCID-FP Panel may not be the definite cause of disease. Additional laboratory testing (e.g. sub-culturing of positive blood cultures for identification of organisms not detected by ePlex BCID-FP Panel, susceptibility testing and differentiation of mixed growth) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

   Limitations:

   - For prescription use only.
   - This test is a qualitative test and does not provide a quantitative value.
   - This product should not be used with blood culture media that contains charcoal.
   - False results were observed for some targets in a single lot of BACT/Alert PF Plus and BACT/Alert FA Plus bottle types (see the Sample Matrix Equivalency (Bottle Evaluation) section of the package insert for additional details).
   - There is a risk of false negative values due to the presence of sequence variants in the fungal targets of the test.
   - In mixed cultures, the ePlex BCID-FP Panel may not identify all organisms in the specimen, depending upon the concentration of each target present.
   - The results of the ePlex BCID-FP Panel should not be used as the sole basis for diagnosis, treatment or other patient management decisions.
   - The genus level assays included as a part of the BCID-FP Panel (*Fusarium, Rhodotorula*) are designed to detect a broad range of species but will not necessarily detect all species within a genus or group.

4. Special instrument requirements:

   For use with the GenMark ePlex instrument

I. Device Description:

The ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel is based on the principles of competitive nucleic acid hybridization using a sandwich assay format, wherein a single-stranded target binds concurrently to a sequence-specific solution-phase signal probe and a solid-phase electrode-bound capture probe. The test employs nucleic acid extraction, target amplification via polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) and hybridization of target DNA. In the process, the double-stranded PCR amplicons are digested with exonuclease to generate single-stranded DNA suitable for hybridization.

Nucleic acid extraction from biological samples occurs within the cartridge via cell lysis, nucleic acid capture onto magnetic beads, and release for amplification. The nucleic acid extraction is processed through microfluidic liquid handling. Once the nucleic acid targets are captured and inhibitors are washed away, the magnetic particles are delivered to the electrowetting environment on the printed circuit board (PCB) and the targets are eluted from the particles and amplified.

During hybridization, the single-stranded target DNA binds to a complementary, single-stranded capture probe immobilized on the working gold electrode surface. Single-stranded signal probes (labeled with electrochemically active ferrocenes) bind to specific target sequence / region adjacent to the capture probe. Simultaneous hybridization of target to signal probes and capture probe is detected by alternating current voltammetry (ACV). Each working electrode on the array contains specific capture probes, and sequential analysis of each electrode allows detection of multiple analyte targets.

Table 1: Materials provided in each kit:

Product	Item number	Components (quantity)	Storage
ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel	EA005012	ePlex BCID-FP Panel Cartridge (12)	2–8 ˚C

Materials required but not provided:

- GenMark ePlex Instrument and Software
- Pipettes capable of delivering 50µL
- Printer (optional) - See ePlex Operator Manual for compatibility guidelines
- Pipette tips, aerosol resistant, RNase/DNase-free
- Disposable, powder free gloves
- 10% bleach for appropriate surfaces
- 70% ethanol or isopropyl alcohol (or equivalent) for appropriate surfaces
- 1.5mL RNase/DNase-free microcentrifuge tube or equivalent (optional)

Interpretation of Results

Results interpretation of the ePlex BCID-FP Panel is performed by the ePlex instrument, and the interpretation of results on the ePlex BCID-FP Panel Detection Report for each targeted analyte is summarized in Table 2 below.

Table 2: Interpretation of Results on the ePlex BCID-FP Panel Detection Report

Target Result	Explanation	Action
Detected	The test was completed successfully and the target has generated signal above its defined threshold and the Internal Control was reported as PASS.	All results are displayed on the ePlex BCID-FP Panel Detection Report. Test is valid, report results.
Not Detected	The test was completed successfully and the target did not generate signal above its defined threshold and the Internal Control was reported as PASS.	All results are displayed on the ePlex BCID-FP Panel Detection Report. Test is valid, report results.

Invalid	The test has not successfully completed and results for this test are not valid. This may be due to an instrument or software error.	No results are displayed on the ePlex BCID-FP Panel Detection Report. Test is not valid, repeat test.

ePlex BCID-FP Panel Test Reports

Several different reports are available on the ePlex System. Results are provided in a printable format and may be viewed electronically or exported for additional analysis. Reports can be customized with account specific information such as the address, logo and institutional specific footers on each report.

Detection Report

The ePlex BCID-FP Panel Detection Report includes the results for each individual sample run on the ePlex System. The Summary section indicates the overall test result and lists all detected targets in that sample. The Results section includes a list of all targets on the panel with an individual result for each target. Results are reported as Detected, Not Detected, or Invalid (displayed as a red x); results for the Internal Control are reported as PASS, FAIL, or INVALID.

External Control Report

The ePlex BCID-FP Panel External Control Report is generated for an external control that has been pre-defined in the ePlex BCID-FP Panel Software. For more information on defining external controls on the ePlex System, refer to the ePlex Operator Manual.

The Summary section indicates the overall result (PASS or FAIL status) and lists all detected targets for that external control. The Results section includes a list of all panel targets with the result, expected result and PASS/FAIL status for each. Results are reported as Detected, Not Detected, or Invalid (displayed as a red x). A target is reported as PASS if the actual result matches the expected result (as defined for that control); a target is reported as FAIL if the actual result does not match the expected result. If the actual result for each target matches the expected result (all targets reported as PASS), the overall result for the external control is reported as PASS in the Summary section. If the actual result for any target does not match the expected result, the overall result for the external control is reported as FAIL in the Summary section.

Summary Report

The Summary Report allows the operator to use searchable criteria to create customized reports, using specified targets, dates, range of dates, sample, external control, test bay, or operator. For more information on creating Summary Reports, refer to the ePlex Operator Manual.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BioFire Diagnostics; FilmArray Blood Culture Identification (BCID) Panel

2. Predicate 510(k) number(s):

   k130914

3. Comparison with predicate:

Similarities		
Item	**Device: ePlex BCID-FP Panel (K182690)**	**Predicate: FilmArray BCID Panel (K130914)**
Indication for Use	The GenMark ePlex Blood Culture Identification Fungal Pathogen (BCID-FP) Panel is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on GenMark's ePlex Instrument for simultaneous detection and identification of multiple potentially pathogenic fungal organisms in positive blood culture. The ePlex BCID-FP Panel is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system and which contain fungal organism. The following fungal organisms are identified using the ePlex BCID-FP Panel: *Candida albicans, Candida auris, Candida dubliniensis, Candida famata, Candida glabrata, Candida guilliermondii, Candida kefyr, Candida krusei, Candida lusitaniae, Candida parapsilosis, Candida tropicalis, Cryptococcus gattii, Cryptococcus neoformans, Fusarium* and *Rhodotorula*. The detection and identification of specific fungal nucleic acids from individuals exhibiting signs and/or symptoms of bloodstream infection aids in the diagnosis of bloodstream infection when used in conjunction with other clinical information. The results from the ePlex BCID-FP Panel are intended to be interpreted in conjunction with Gram stain results and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.	The FilmArray Blood Culture Identification (BCID) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray Instrument. The FilmArray BCID Panel is capable of simultaneous detection and identification of multiple bacterial and yeast nucleic acids and select genetic determinants of antimicrobial resistance. The BCID assay is performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain. The following gram-positive bacteria, gram-negative bacteria, and yeast are identified using the FilmArray BCID Panel: *Enterococci, Listeria monocytogenes,* commonly encountered *Staphylococci* (including specific differentiation of *Staphylococcus aureus),* commonly encountered *Streptococci* (with specific differentiation of *Streptococcus agalactiae, Streptococcus pneumoniae,* and *Streptococcus pyogenes*), *Acinetobacter baumannii,* commonly encountered *Enterobacteriaceae* (including specific differentiation of the *Enterobacter cloacae* complex, *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus,* and *Serratia marcescens*), *Haemophilus influenzae, Neisseria meningitidis* (encapsulated), *Pseudomonas aeruginosa, Candida albicans,*

Similarities		
	Negative results in the setting of a suspected bloodstream infection may be due to infection with pathogens that are not detected by this test. Positive results do not rule out co-infection with other organisms; the organism(s) detected by the ePlex BCID-FP Panel may not be the definite cause of disease. Additional laboratory testing (e.g. sub-culturing of positive blood cultures for identification of organisms not detected by ePlex BCID-FP Panel, susceptibility testing and differentiation of mixed growth) and clinical presentation must be taken into consideration in the final diagnosis of bloodstream infection.	*Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis*. The FilmArray BCID Panel also contains assays for the detection of genetic determinants of resistance to methicillin (*mecA*), vancomycin (*vanA* and *vanB*), and carbapenems (bla_{KPC}) to aid in the identification of potentially antimicrobial resistant organisms in positive blood culture samples. The antimicrobial resistance gene detected may or may not be associated with the agent responsible for disease. Negative results for these select antimicrobial resistance gene assays do not indicate susceptibility, as multiple mechanisms of resistance to methicillin, vancomycin, and carbapenems exist. FilmArray BCID is indicated as an aid in the diagnosis of specific agents of bacteremia and fungemia and results should be used in conjunction with other clinical and laboratory findings. Positive FilmArray results do not rule out co-infection with organisms not included in the FilmArray BCID Panel. FilmArray BCID is not intended to monitor treatment for bacteremia or fungemia. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing and epidemiological typing, to identify organisms in the blood culture that are not detected by the FilmArray BCID Panel, and for species determination of some *Staphylococci*, *Enterococci*, *Streptococci*, and *Enterobacteriaceae* that are not specifically identified by the FilmArray BCID Panel assays
Analyte	DNA	DNA
Sample Processing	Automated by instrument	Automated by instrument

Differences		
Item	**Device: ePlex BCID-FP Panel (k182690)**	**Predicate: FilmArray BCID Panel (k130914)**
Specimen Type	Gram-Fungal Blood Culture	Gram-Positive & Gram-Negative Blood

Differences		
		Culture
Test Principles	Reagents on cartridge include: sample lysis and nucleic acid extraction, PCR amplification and hybridization-based electrochemical detection reagents.	The FilmArray BCID pouch contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex PCR with DNA melt analysis.
Instrumentation and Hardware	GenMark ePlex Instrument & Single Use Cartridge	FilmArray Instrument and assay pouch
Software Interface Result Reporting	GenMark ePlex System Software GenMark ePlex BCID-FP Panel Software	The FilmArray Software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism and antimicrobial resistance gene on the panel.

K. Standard/Guidance Document Referenced

- Class II Special Controls Guideline: Multiplex Nucleic Acid Assay for Identification of Microorganisms and Resistance Markers from Positive Blood Cultures (May 2015)
- CLSI MM17-A, Vol. 28, No. 9, Verification and Validation of Multiplex Nucleic Acid Assays
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition (June 2012)
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition (November 2005)
- CLSP EP25-A Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline (May 2013)

L. Test Principle:

The ePlex Blood Culture Identification Gram-Positive (BCID-FP) Panel is based on the principles of competitive nucleic acid hybridization using a sandwich assay format, wherein a single-stranded target binds concurrently to a sequence-specific solution-phase signal probe and a solid-phase electrode-bound capture probe. The test employs nucleic acid extraction, target amplification via polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) and hybridization of target DNA. In the process, the double-stranded PCR amplicons are digested with exonuclease to generate single-stranded DNA suitable for hybridization.

Nucleic acid extraction from biological samples occurs within the cartridge via cell lysis, nucleic acid capture onto magnetic beads, and release for amplification. The nucleic acid extraction is processed through microfluidic liquid handling. Once the

nucleic acid targets are captured and inhibitors are washed away, the magnetic particles are delivered to the electrowetting environment on the printed circuit board (PCB) and the targets are eluted from the particles and amplified.

During hybridization, the single-stranded target DNA binds to a complementary, single-stranded capture probe immobilized on the working gold electrode surface. Single-stranded signal probes (labeled with electrochemically active ferrocenes) bind to specific target sequence/region adjacent to the capture probe. Simultaneous hybridization of target to signal probes and capture probe is detected by alternating current voltammetry (ACV). Each working electrode on the array contains specific capture probes, and sequential analysis of each electrode allows detection of multiple analyte targets.

M. Performance Characteristics:

1. Analytical performance:

a. *Analytical Sensitivity:*

The limit of detection (LoD), or analytical sensitivity, was identified and verified for each assay on the BCID-FP Panel using quantified reference strains in simulated blood culture sample matrix, which is defined as the matrix from a negative blood culture bottle mixed with whole blood and EDTA in the same ratio as the manufacturer recommends for blood culture. At least 20 replicates per target were tested for each condition. The limit of detection was defined as the lowest concentration of each target that is detected in ≥95% of tested replicates. The confirmed LoD for each ePlex BCID-FP Panel organism is shown in Table 3 below.

Table 3: BCID-FP Panel LoD Results Summary

Target	Organism	Strain	LOD Concentration (CFU/mL)
Candida albicans	*Candida albicans*	ATCC 14053	1×10^6
	Candida albicans	ATCC 24433	1×10^5
Candida auris	*Candida auris*	CBS 10913	1×10^5
	Candida auris	CBS 12766	1×10^5
Candida dubliniensis	*Candida dubliniensis*	ATCC MYA-577	1×10^4
	Candida dubliniensis	NCPF 3949	1×10^5
Candida famata	*Candida famata*	CBS 767	1×10^3
	Candida famata	CBS 766	1×10^4
Candida glabrata	*Candida glabrata*	ATCC 2001	1×10^6
	Candida glabrata	ATCC 15545	1×10^6
Candida guilliermondii	*Candida guilliermondii*	ATCC 22017	1×10^5
	Candida guilliermondii	ATCC 6260	1×10^5

Target	Organism	Strain	LOD Concentration (CFU/mL)
Candida kefyr	*Candida kefyr*	ATCC 4135	1 x 10^3
	Candida kefyr	ATCC 8553	1 x 10^4
Candida krusei	*Candida krusei*	ATCC 22985	1 x 10^5
	Candida krusei	ATCC 28870	1 x 10^6
Candida lusitaniae	*Candida lusitaniae*	ATCC 34449	1 x 10^6
	Candida lusitaniae	ATCC 66035	1 x 10^5
Candida parapsilosis	*Candida parapsilosis*	ATCC 28474	1 x 10^4
	Candida parapsilosis	ATCC 28475	1 x 10^5
Candida tropicalis	*Candida tropicalis*	ATCC 13803	1 x 10^5
	Candida tropicalis	ATCC 1369	1 x 10^6
Cryptococcus gattii	*Cryptococcus gattii*	ATCC MYA-4877	1 x 10^3
	Cryptococcus gattii	ATCC MYA-4138	1 x 10^3
Cryptococcus neoformans	*Cryptococcus neoformans*	ATCC 208821	1 x 10^5
	Cryptococcus neoformans	ATCC MYA-565	1 x 10^5
Fusarium	*Fusarium oxysporum*	CBS 116611	1 x 10^6 spores/mL
	Fusarium solani	ATCC 36301	1 x 10^6 spores/mL
Rhodotorula	*Rhodotorula mucilaginosa*	ATCC 4058	1 x 10^5
	Rhodotorula glutinis	ATCC 32765	1 x 10^5

b. *Reproducibility:*

A multisite reproducibility study of the ePlex BCID-FP Panel was conducted at three testing sites (two external sites and one internal site) across major potential sources of variability, such as site-to-site, lot-to-lot, day-to-day, and operator-to-operator. One ePlex instrument per study site with four towers was employed in this reproducibility study. Two operators performed testing at each site on six days (five nonconsecutive days) with three unique lots of ePlex BCID-FP Panel cartridges. A reproducibility study panel consisting of four positive panel members including 5 on-panel organisms at 2 concentrations reflecting bottle positivity plus 8 hours and bottle positivity, and 1 negative panel member, was tested in triplicate. Concentrations in the positive mixes reflected those observed at time of bottle positivity plus 8 hours (BP+8) and time of bottle positivity (BP) and one mix containing an off-panel organism grown to bottle positivity, which is expected to yield a negative result. Bottle concentrations used in this study are summarized in Table 4.

Table 4: Bottle Positivity Concentrations

Organism	Bottle Positivity Concentration	Bottle Positivity +8 Hours Concentration
Candida albicans	1 x 10^6 CFU/mL	1 x 10^7 CFU/mL
Candida kefyr	1 x 10^6 CFU/mL	1 x 10^7 CFU/mL
Cryptococcus neoformans	1 x 10^7 CFU/mL	1 x 10^8 CFU/mL
Fusarium sacchari	6.5 x10^6 spores/mL	6.1 x10^6 spores/mL
Rhodotorula mucilaginosa	1 x 10^6 CFU/mL	1 x 10^7 CFU/mL

Summary results for the ePlex BCID-FP Panel reproducibility study are provided in Table 5 below.

Table 5: Summary of Reproducibility Results

BCID-FP Test Result	Organism Test Concentration	Site	Agreement with Expected Results		
			Agreed/N	%	95% CI
Candida albicans	Bottle Positive + 8 Hours (1x10^7 CFU/mL)	1	34/35	97.1	(85.5-99.5)
		2	35/36	97.2	(85.8-99.5)
		3	36/36	100	(90.4-100)
		All	**105/107**	**98.1**	**(93.4-99.5)**
	Bottle Positive (1x10^6 CFU/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	(96.6-100)
	Negative	1	108/108	100	(96.6-100)
		2	108/108	100	(96.6-100)
		3	108/108	100	(96.6-100)
		All	**324/324**	**100**	**(98.8-100)**

BCID-FP Test Result	Organism Test Concentration	Site	Agreement with Expected Results		
Candida kefyr	Bottle Positive + 8 Hours ($1x10^7$ CFU/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	**(96.6-100)**
	Bottle Positive ($1x10^6$ CFU/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	**(96.6-100)**
	Negative	1	107/107	100	(96.5-100)
		2	108/108	100	(96.6-100)
		3	108/108	100	(96.6-100)
		All	**323/323**	**100**	**(98.8-100)**
Cryptococcus neoformans	Bottle Positive + 8 Hours ($1x10^8$ CFU/mL)	1	35/35	100	(90.1-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**107/107**	**100**	**(96.5-100)**
	Bottle Positive ($1x10^7$ CFU/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	**(96.6-100)**
	Negative	1	108/108	100	(96.6-100)
		2	108/108	100	(96.6-100)
		3	108/108	100	(96.6-100)
		All	**324/324**	**100**	**(98.8-100)**
Fusarium	Bottle Positive + 8 Hours ($6.12x10^6$ spores/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	**(96.6-100)**
	Bottle Positive ($6.54x10^6$ spores/mL)	1	36/36	100	(90.4-100)
		2	36/36	100	(90.4-100)
		3	36/36	100	(90.4-100)
		All	**108/108**	**100**	**(96.6-100)**
	Negative	1	107/107	100	(96.5-100)
		2	108/108	100	(96.6-100)
		3	108/108	100	(96.6-100)
		All	**323/323**	**100**	**(98.8-100)**

BCID-FP Test Result	Organism Test Concentration	Site	Agreement with Expected Results		
Rhodotorula	Bottle Positive + 8 Hours ($1x10^7$ CFU/mL)	1	36/36	100	(90.4-100)
		2	34/36	94.4	(81.9-98.5)
		3	35/36	97.2	(85.8-99.5)
		All	**105/108**	**97.2**	**(92.1-99.1)**
	Bottle Positive ($1x10^6$ CFU/mL)	1	35/36	97.2	(85.8-99.5)
		2	36/36	100	(90.4-100)
		3	35/36	97.2	(85.8-99.5)
		All	**106/108**	**98.1**	**(93.5-99.5)**
	Negative	1	107/107	100	(96.5-100)
		2	108/108	100	(96.6-100)
		3	108/108	100	(96.6-100)
		All	**323/323**	**100**	**(98.8-100)**

The initial invalid rate was 4.7%. Initially invalid samples were re-tested resulting in a final invalid rate of 0.2%.

c. Linearity/assay reportable range:

Not applicable

d. Traceability, Stability, Expected values (controls, calibrators, or methods):

Assay Controls

Internal Controls:
Each ePlex BCID- FP Panel cartridge includes internal controls that monitor performance of each step of the testing process, including extraction, amplification and detection of targets.

Each amplification reaction on the cartridge has an internal control and in each reaction either the internal control or a target must generate signal above the defined threshold for a valid test result. Internal control results are interpreted by the ePlex Software and displayed on the ePlex BCID-FP Panel Reports as Internal Control with a result of PASS, FAIL, or INVALID. Table 6 includes details on the interpretation of Internal Control results.

Table 6: Internal Control Results

Internal Control Result	Explanation	Action
PASS	Signal above threshold has been detected from each amplification reaction. The test was completed and internal controls were successful, indicating valid results were generated.	All results are displayed on the ePlex BCID-FP Panel Detection Report. Test is valid, report results.
FAIL	Signal above threshold has not been detected from at least one amplification reaction. The test was completed but internal controls were not detected, indicating that results may not be valid.	No results are displayed on the ePlex BCID-FP Panel Detection Report. Test is not valid, repeat the test using a new cartridge.
INVALID	An error has occurred during processing that prevents analysis of signal data. The test has not successfully completed and results for this test are not valid. This may be due to an instrument or software error.	No results are displayed on the ePlex BCID-FP Panel Detection Report. Test is not valid, repeat the test using a new cartridge.

Recommended External Controls: External controls are not provided with the ePlex Identification Fungal Pathogen (BCID-FP) Panel, but are recommended in the package insert. Positive and negative external controls should be tested with each new lot of reagents or monthly, whichever occurs first. Blood culture medium can be used as the negative control. Previously characterized positive samples or blood culture medium spiked with well characterized organisms can be used as the external positive control. External controls should be run in accordance with laboratory protocols and accrediting organizations, as applicable.

Specimen Stability

A specimen stability study was performed to confirm the stability of specimens stored under four different temperature conditions over multiple time points which vary based on storage temperature. Additionally, specimen stability was compared at bottle ring and 12 hours after bottle ring.

Organism mixes whose members were at a concentration approximating bottle positivity were stored at ≤-70°C, ≤-20°C, 2°-

8°C, and ambient temperature over various time points described in Table 7 below. These two mixes include representative targets from each of the four multiplex primer pools on the BCID- FP Panel and include diverse fungal targets. A description of the test mix used for this study is summarized in Table 8.

Table 7: Storage Conditions and Testing Time Points

Storage Condition	Time Points							
Ambient	T0	1 day	3 days	5 days	7 days	14 days	30 days	--
Refrigerated (2-8˚C)	--	1 day	3 days	5 days	7 days	14 days	30 days	--
Frozen (≤-20˚C)	1 week	2 weeks	1 mo.	3 mo.	6 mo.	12 mo.	18 mo.	24 mo.
Frozen (≤-70˚C)	1 week	2 weeks	1 mo.	3 mo.	6 mo.	12 mo.	18 mo.	24 mo.
Bottle Incubator	Bottle positivity	12 hours post positivity	--	--	--	--	--	--

Table 8: Representative Organism Test Mixes

Mix	Primer Pool	Organism	Testing Concentration (CFU/mL)
1	1	*Candida kefyr*	$1x10^6$
	3	*Rhodotorula mucilaginosa*	$1x10^6$
	2	*Fusarium sacchari*	$6x10^6$ spores/mL
2	2	*Cryptococcus neoformans*	$1x10^7$
	4	*Candida albicans*	$1x10^6$

Twenty replicates were tested at the beginning of the study when organism mixes were freshly made (time point T0). Ten replicates were tested for each additional temperature and time point. If any of the targets were not detected in 1 of 10 replicates, an additional 10 replicates were tested to determine whether 95% detection rate could be achieved.

Ambient Storage Condition

The positivity rate and mean nA signals for all 5 targets for the Ambient Storage temperature were calculated (Table 9). All analytes were ≥95% positive at all time points at ambient temperature storage, with the exception of *Rhodotorula*. Detection of *Rhodotorula* was 85% at day 7, but passed the criteria of ≥95% detection at the subsequent two time points (14 and 30 days), which passes overall acceptance criteria for the ambient storage condition.

Table 9: Summary of Ambient Storage Sample Stability

Time Point	Metric	Target (nA Cutoff)				
		C. albicans **(10 nA)**	***C. kefyr*** **(25 nA)**	***C. neoformans*** **(25 nA)**	***Fusarium*** **(25 nA)**	***Rhodotorula*** **(25 nA)**
T0 n=20	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	899	791	825	671	263
1 day n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	869.5	787.2	1037.6	714.1	247.1
3 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	818.4	867.8	921.6	585.4	265.9
5 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	599	388.9	552.3	631.5	282.2
7 days n=20 Mix1 n=10 Mix 2	% positive	100%	95%	100%	95%	85%
	Mean Signal (nA)	706.6	314.6	680	358.1	144.4
14 days n=20 Mix1 n=10 Mix 2	% positive	100%	100%	100%	100%	95%
	Mean Signal (nA)	677.8	489.8	643.7	649.1	281.6
30 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	631.9	657.2	617	587	252.7

Refrigerated (2-8°C) Storage Condition

The positivity rate and mean nA signals for all 5 targets for the 2°C - 8°C temperature condition were calculated (Table 10). All analytes were 100% positive for all time points up to 30 days of the 2°C - 8°C condition of the study.

Table 10: Summary of 2-8°C Storage Sample Stability

Time Point	Metric	Target (nA Cutoff)				
		C. albicans (10 nA)	*C. kefyr* (25 nA)	*C. neoformans* (25 nA)	*Fusarium* (25 nA)	*Rhodotorula* (25 nA)
T0 n=20	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	899	791	825	671	263
1 day n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	849	821.7	970.9	626.9	283.9
3 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	804.2	793.5	849.7	522.1	286.5
5 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	708.6	349.6	656.6	648.9	249
7 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	568.7	586.6	641.5	648.6	254.0
14 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	647.6	516.5	761	656.7	280.5
30 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	710.2	525.8	648.2	600.3	282.8

Frozen (≤-20˚C) Storage Condition

The positivity rate and mean nA signals for all 5 targets for the -20 °C temperature storage condition were calculated (Table 11). All analytes were 100% positive at all time points up to 3 months at -20 °C temperature storage, with the exception of *Rhodotorula*. Detection of *Rhodotorula* was 85% at 30 days, but passed the criteria of ≥95% detection at the subsequent time point (3 months), which passes overall acceptance criteria for the -20 °C storage condition.

Table 11: Summary ≤-20˚C Storage Sample Stability

Time Point	Metric	Target (nA Cutoff)				
		C. albicans (10 nA)	*C. kefyr* (25 nA)	*C. neoformans* (25 nA)	*Fusarium* (25 nA)	*Rhodotorula* (25 nA)
T0 n=20	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	899	791	825	671	263
7 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	686.6	513.6	705.6	642.4	296.6
14 days n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	620.3	486.5	637.8	737.8	349.5
30 days n=20 Mix 1 n =10 Mix 2	% positive	100%	100%	100%	100%	85%
	Mean Signal (nA)	802	823.6	930.1	781.1	221.3
3 months n=10	% positive	100%	100%	100%	100%	100%
	Mean Signal (nA)	756.5	847.8	896.2	730.8	308.5

Frozen (≤-70˚C) Storage Condition

The positivity rate and mean nA signals for all 5 targets for ≤-70°C temperature storage conditions are shown in Table 12. All analytes were ≥95% positive when stored for up to 3 months at ≤-70°C.

Table 12: Summary ≤ -70˚C Storage Sample Stability

Time Point	Metric	Target (nA Cutoff)				
		C. albicans (10 nA)	*C. kefyr* (25 nA)	*C. neoformans* (25 nA)	*Fusarium* (25 nA)	*Rhodotorula* (25 nA)
T0	% positive	100%	100%	100%	100%	100%
n=20	Mean Signal (nA)	899	791	825	671	263
7 days	% positive	100%	100%	100%	100%	100%
n=10	Mean Signal (nA)	627.3	561.6	528.4	704.3	302
14 days	% positive	100%	100%	100%	100%	100%
n=10	Mean Signal (nA)	653.4	474.3	554.5	665	275.9
30 days	% positive	100%	100%	100%	100%	95%
n=20 Mix 1 n=10 Mix 2	Mean Signal (nA)	778.1	787.1	872.9	625.1	243.5
3 months	% positive	100%	100%	100%	100%	100%

Time Point	Metric	Target (nA Cutoff)				
n =10	Mean Signal (nA)	808.2	851.2	812.5	716.4	277.4

Bottle Ring and 12 Hours Post Bottle Ring

The positivity rate and mean nA signals for the two representative organisms grown to bottle positivity and incubated 12 hours post positivity were calculated and included below. The positivity rate for *C. albicans* and *C. neoformans* at bottle ring and 12 hours post bottle ring was 100% for both organisms. Results are summarized in Table 13.

Table 13: Bottle Ring and 12 hours Post Bottle Ring

Target	Metric	Bottle Ring (n=20)	12hrs Post Bottle Ring *C. albicans* (n=10) *C. neoformans* (n=20)^
C. albicans	% positive	100%	100%
	Mean Signal (nA)	866.3	707.2
	Standard Deviation	236.6	156.8
	Mean minus 2 SD	393.1	393.6
C. neoformans	% positive	100%	100%
	Mean Signal (nA)	660.2	804.2
	Standard Deviation	173.0	193.1
	Mean minus 2 SD	314.2	417.9

^ n=20 for *C. neoformans* BP+12 sample, *Fusarium* false positive was detected in 1 out of 20 replicates.

Results demonstrated that specimens can be stored under the following temperature conditions without impacting the performance of the BCID-FP Panel:

- Up to 1 month at ambient temperature and refrigerated (2°C-8°C) temperature conditions
- Up to 3 months at frozen conditions (-20˚C and ≤-70˚C).
- Specimens can also be tested when incubated up to 12 hours after bottle ring in a continuously monitoring blood culture device.

The specimen stability claims are summarized in the table below.

Storage Condition	Specimen Stability
Ambient	7 days
Refrigerated (2°C-8°C)	1 month
Frozen (≤-20˚C)	3 month
Frozen (≤-70˚C)	3 month
Post Bottle Ring	12 hours

Freeze-Thaw Study

A study was performed to assess the tolerance of the ePlex BCID-FP to correctly identify specimens containing fungal organisms that have gone through one or two freeze/thaw cycles prior to testing. One hundred and twelve positive clinical samples that were tested fresh during the BCID-FP Panel Clinical Performance Study were selected to best represent a variety of organisms in clinical samples. The samples were frozen at ≤-70°C and then thawed once or twice prior to re-testing with the BCID-FP Panel.

The positive agreement with the fresh condition at each freeze-thaw cycle was calculated and was above 95% for each condition. Table 14 and 15 below summarizes the concordance at each freeze-thaw cycle with a 95% confidence level and the mean nA signal.

Table 14: Concordance of Results with Fresh Condition

Metric	Fresh	1x Freeze Thaw	2x Freeze Thaw
Total Valid Samples	112	103	108
False Negative Samples	0	0	1
Concordant Samples	112	103	107
Positive Agreement	**100%**	**100%**	**99.1%**
CI Low	NA	96.5%	93.9%
CI High	NA	100%	99.8%

Table 15: Mean Signal Results

Target	Total Replicates	Mean Signal (nA)		
		Fresh	1xFT	2xFT
Candida albicans	10	834.2	811.4	738.2
Candida auris	5	490.3	375.5	362.8
Candida dubliniensis	5	901.8	983.2	912.2
Candida famata	5	367.6	503.3	512.3
Candida glabrata	12	694.7	731.4	685.2
Candida guilliermondii	2	154.0	186.1	73.9
Candida kefyr	8	811.6	736.5	733.5
Candida krusei	7	713.6	656.4	614.6
Candida lusitaniae	5	716.6	648.8	763.8
Candida parapsilosis	10	778.6	824.1	746.7
Candida tropicalis	10	673.0	693.8	702.2
Cryptococcus gattii	9	698.2	862.6	749.7
Cryptococcus neoformans	9	953.4	906.6	871.1
Fusarium	11	536.1	700.1	533.1
Rhodotorula	4	175.9	267.2	263.5

Results demonstrate that the BCID-FP Panel can detect the same organisms in samples that were tested fresh and in samples that have been frozen and thawed up to 2 times.

In-Cartridge Sample Stability Study

An analytical study was carried out to demonstrate performance under the following conditions:

1) Condition 1: The hands-on-time for pipetting the sample into the sample reservoir and starting the consumable run on the ePlex instrument is ≤ 2 minutes.
2) Condition 2 (Open Pouch): the cartridge is stable for 2 hours after the pouch has been opened and the unloaded cartridge is exposed to the environment.
3) Condition 3 (In-Consumable Sample Stability): the cartridge can be stored at room temperature for at least 2 hours after the sample has been loaded prior to running the cartridge in an ePlex bay.

A test mix of four organisms was used in the study. The selected organisms represent all four of the multiplex primer pools on the BCID-FP Panel. Each organism was tested at a concentration approximating bottle positivity. A description of the test mix used for this study is summarized in Table 16 below.

Table 16: Representative Organisms Contained in Test Mix

Organism	Concentration (CFU/mL)	Primer Pool	ePlex BCID-FP Panel Expected Result
Candida albicans	1×10^6	4	*Candida albicans*
Candida kefyr	1×10^6	1	*Candida kefyr*
Rhodotorula mucilaginosa	1×10^6	3	*Rhodotorula*
Cryptococcus neoformans	1×10^7	2	*Cryptococcus neoformans*

The results of the study which assessed the interim storage conditions are summarized in Table 17. All targets were detected in 100% of test replicates in each condition tested (Open Pouch Stability Condition and In-Consumable Sample Stability Condition)

Table 17: Detection Rates for All Conditions Tested

Detection Rate (n=20)			
Target	**Control Condition**	**Open Pouch Stability**	**In-Consumable Sample Stability Condition**

Candida albicans	100%	100%	100%
Candida kefyr	100%	100%	100%
Rhodotorula mucilaginosa	100%	100%	100%
Cryptococcus neoformans	100%	100%	100%

Hands-on time study results demonstrated that each consumable met the ≤ 2 minute hands-on time requirement, with an overall average time of 27 seconds. Sample loading time averaged 9 seconds, and consumable loading time on the ePlex Instrument averaged 18 seconds.

Cartridge Shelf-Life Stability Study

An analytical study was carried out to generate supporting stability data to establish the shelf-life of the ePlex BCID-FP Panel cartridge when stored under refrigerated conditions (2ºC - 8ºC). The study design uses an approach consistent with CLSI guideline EP25-A.

A minimum of three lots of ePlex BCID-FP Panel cartridges were stored under refrigerated conditions and were tested throughout the duration of the real-time stability study. A positive test sample was comprised of a mix containing qualified organisms, each at concentrations approximating bottle positivity (Table 18). This sample mix represents all four multiplex primer pools on the BCID-FP Panel.

Table 18: Representative Organisms Contained in Test Mix

Organism in Test Mix	Concentration in Mix (CFU/mL)	Primer Pool	ePlex BCID-FP Panel Expected Result
Candida albicans	1×10^6	4	*Candida albicans*
Candida kefyr	1×10^6	1	*Candida kefyr*
Rhodotorula mucilaginosa	1×10^6	3	*Rhodotorula*
Cryptococcus neoformans	1×10^7	2	*Cryptococcus neoformans*

On each day of time point testing, positive test samples were made to a final concentration. Positive test samples were created in negative blood matrix. A negative control consisting of negative blood matrix was also included with each time point. The following were tested at each time point; 4 replicates of negative blood matrix as negative control and 50 replicates of positive test samples.

Real-time study data support that the ePlex BCID-FP Panel cartridges continue to be stable for up to ten weeks when stored under refrigerated conditions (2ºC - 8ºC). The stability study is ongoing and the product will be labeled with an expiration date that is supported by the real-time stability

data from this study.

e. Growth and Detection Study

A study was performed to establish the range of expected organism concentrations present in incubated blood cultures at bottle positivity (i.e., bottle "ring") and eight hours after bottle positivity/bottle "ring" Several representative fungal organisms were spiked into blood culture bottles along with the manufacturer's recommended volume of human whole blood and grown to positivity in a commercially-available continuously monitoring blood culture system. Bottles were removed from the incubator within two hours of being identified as positive as well as eight hours after bottle positivity. At least two independent positive blood culture replicates were quantified for each organism on culture plates. Organisms tested and approximate bottle positivity concentrations are summarized in Table 19. Concentrations shown below represent approximate levels that may be observed in a clinical setting. All estimated bottle positivity concentrations are equivalent or greater than the established Limit of Detection (LOD) for each of the assays of the ePlex BCID-FP Panel.

Table 19: Bottle Positivity Concentrations

Organism	Strain ID	Mean Bottle Positivity Concentration	Mean Bottle Positivity +8 hours Concentration
Candida albicans	ATCC 90082	1.6 x 10^6 CFU/mL	1.4 x 10^6 CFU/mL
Cryptococcus neoformans var. grubii	ATCC 14116	1.3 x 10^7 CFU/mL	6.5 x 10^7 CFU/mL
Fusarium solani	ATCC 36031	9.6 x 10^6 spores/mL	7.7 x 10^6 spores/mL
Rhodotorula mucilaginosa	ATCC 66034	1.6 x 10^6 CFU/mL	4.2 x 10^6 CFU/mL

f. *Analytical Reactivity (Inclusivity):*

The analytical reactivity of the ePlex BCID-FP Panel was evaluated with a collection of 51 fungal organisms that represent the diversity of the ePlex BCID-FP Panel analytes. Isolates were selected to represent relevant species or serotypes and selection with specific inclusion of more commonly encountered species and known human pathogens.

Each isolate was initially tested in blood culture matrix at a concentration

consistent with the levels of organism enumerated from blood cultures at the time of bottle positivity (1 x 10^6 CFU/mL for *Candida* and *Rhodotorula*, 1 x 10^7 CFU/mL for *Cryptococcus*, and 1 x 10^8 spores/mL for *Fusarium*) and were tested in triplicate. If an isolate was not detected initially, the sample was retested at higher concentrations. If detected at the higher concentration(s), the species/isolate is indicated as detected with reduced sensitivity and the concentration of organism that was detected is indicated. If not detected at the highest concentration the isolate is listed as not detected by the ePlex BCID-FP Panel. Results are provided in Table 20 below:

Table 20: Analytical Reactivity (Inclusivity) Results for the BCID-FP Panel

Target	Organism	Strain
Candida albicans	*Candida albicans*	ATCC 10231
	Candida albicans	ATCC 90028
	Candida albicans	ATCC MYA-4441
Candida auris	*Candida auris*	CDC#385
	Candida auris	CDC#386
	Candida auris	CDC#387
	Candida auris	CDC#388
	Candida auris	CDC#389
	Candida auris	CDC#390
	Candida auris	CBS 12766
Candida dubliniensis	*Candida dubliniensis*	ATCC MYA-578
	Candida dubliniensis	ATCC MYA-579
	Candida dubliniensis	ATCC MYA-582
Candida famata	*Candida famata*	ATCC 20850
	Candida famata	CBA 1961
	Candida famata	CBS 789
Candida glabrata	*Candida glabrata*	ATCC 15126
	Candida glabrata	ATCC 66032
	Candida glabrata	ATCC MYA-2950
Candida guilliermondii	*Candida guilliermondii*	ATCC 90197
	Candida guilliermondii	ATCC 90198
	Candida guilliermondii	ATCC 90199
Candida kefyr	*Candida kefyr*	ATCC 204093
	Candida kefyr	ATCC 2512
	Candida kefyr	ATCC 66028
Candida krusei	*Candida krusei*	ATCC 14243
	Candida krusei	ATCC 32196
	Candida krusei	ATCC 34135
Candida lusitaniae	*Candida lusitaniae*	ATCC 42720
	Candida lusitaniae	ATCC MYA-766
	Candida lusitaniae	Z010
Candida parapsilosis	*Candida parapsilosis*	ATCC 22019

Target	Organism	Strain
	Candida parapsilosis	ATCC 58895
	Candida parapsilosis	ATCC 90018
Candida tropicalis	*Candida tropicalis*	ATCC 201380
	Candida tropicalis	ATCC 201381
	Candida tropicalis	ATCC 750
Cryptococcus gattii	*Cryptococcus gattii*	ATCC 14248
	Cryptococcus gattii	ATCC 4560
	Cryptococcus gattii	ATCC 76108
Cryptococcus neoformans	*Cryptococcus neoformans*	ATCC 14116
	Cryptococcus neoformans	ATCC 90112
	Cryptococcus neoformans	NCPF 8299
Fusarium	*Bisifusarium dimerum*	CBS 110317
	Fusarium moniliforme	ATCC 38159
	Fusarium proliferatum	CBS 131570
	Fusarium sacchari	CBS 119828
	Fusarium verticillioides	CBS 100312
Rhodotorula	*Rhodotorula glutinis*	ATCC 96365
	Rhodotorula mucilaginosa	ATCC 66034
	Rhodotorula mucilaginosa	ATCC 9449

In addition to species-specific assays, the ePlex BCID-FP Panel contains two broader genus-level assays: *Fusarium* and *Rhodotorula*. Table 21 and Table 22 highlight the predicted (*in silico*) reactivity (inclusivity) for these assay targets.

Table 21: Predicted (in silico) Reactivity (Inclusivity) Results for *Fusarium*

Detection Predicted for ≥95% of target sequences		
Fusarium acaciae-mearnsii	*Fusarium cortaderiae*	*Fusarium napiforme*
Fusarium acuminatum	*Fusarium culmorum*	*Fusarium nisikadoi*
Fusarium acutatum	*Fusarium denticulatum*	*Fusarium nygamai*
Fusarium aethiopicum	*Bisifusarium dimerum**	*Fusarium oxysporum**
Fusarium ananatum	*Fusarium dlaminii*	*Fusarium palustre*
Fusarium andiyazi	*Fusarium equiseti*	*Fusarium phyllophilum*
Fusarium anthophilum	*Fusarium falciforme*	*Fusarium poae*
Fusarium armeniacum	*Fusarium foetens*	*Fusarium polyphialidicum*
Fusarium asiaticum	*Fusarium fujikuroi*	*Fusarium proliferatum**
Fusarium austroamericanum	*Fusarium gaditjirri*	*Fusarium pseudoanthophilum*
Fusarium avenaceum	*Fusarium globosum*	*Fusarium pseudocircinatum*
Fusarium aywerte	*Fusarium guttiforme*	*Fusarium pseudograminearum*
Fusarium bactridioides	*Fusarium hostae*	*Fusarium pseudonygamai*

Fusarium begoniae	*Fusarium incarnatum*	*Fusarium ramigenum*
Fusarium beomiforme	*Fusarium inflexum*	*Fusarium sacchari**
Fusarium boothii	*Fusarium konzum*	*Fusarium secorum*
Fusarium brachygibbosum	*Fusarium lacertarum*	*Fusarium sinensis*
Fusarium brasilicum	*Fusarium lactis*	*Fusarium solani**
Fusarium brevicatenulatum	*Fusarium langsethiae*	*Fusarium sporotrichioides*
Fusarium bulbicola	*Fusarium lichenicola (Cylindrocarpon lichenicola)*	*Fusarium sterilihyphosum*
Fusarium bullatum	*Fusarium louisianense*	*Fusarium subglutinans*
Fusarium camptoceras	*Fusarium lunulosporum*	*Fusarium temperatum*
Fusarium cerealis	*Fusarium mangiferae*	*Fusarium thapsinum*
Fusarium circinatum	*Fusarium meridionale*	*Fusarium udum*
Fusarium commune	*Fusarium mesoamericanum*	*Fusarium verticillioides**
Fusarium concentricum	*Fusarium mexicanum*	
Fusarium concolor	*Fusarium musae*	
Detection Predicted for 85%-94% of target sequences		
Fusarium torulosum	*Fusarium xylarioides*	
Detection Predicted for <85% of target sequences		
Fusarium chlamydosporum (66.7%)	*Fusarium graminearum (59.4%)*	*Fusarium longipes (25.0%)*
Fusarium coeruleum (50.0%)	*Fusarium lateritium (50.0%)*	*Fusarium nelsonii (16.7%)*
Detection Not Predicted		
Fusarium kyushuense	*Fusarium sambucinum*	*Fusarium venenatum*
Fusarium miscanthi	*Fusarium stilboides*	
Fusarium redolens	*Fusarium succisae*	

Detection Predicted for ≥95% of target sequences		
Rhodotorula araucariae	*Rhodotorula graminis*	*Rhodotorula taiwanensis*
*Rhodotorula glutinis**	*Rhodotorula mucilaginosa**	
Detection Predicted for 85%-94% of target sequences		
None Identified		
Detection Predicted for <85% of target sequences		
None Identified		
Detection Not Predicted		
Rhodotorula acheniorum	*Rhodotorula fragariae*	*Rhodotorula marina*
Rhodotorula acuta	*Rhodotorula fujisanensis*	*Rhodotorula minuta*
Rhodotorula armeniaca	*Rhodotorula hinnulea*	*Rhodotorula muscorum*
Rhodotorula aurantiaca	*Rhodotorula hordea*	*Rhodotorula nothofagi*
Rhodotorula auriculariae	*Rhodotorula hylophila*	*Rhodotorula philyla*
Rhodotorula bacarum	*Rhodotorula ingeniosa*	*Rhodotorula phylloplana*
Rhodotorula bogoriensis	*Rhodotorula javanica*	*Rhodotorula pilati*
Rhodotorula buffonii	*Rhodotorula lactosa*	*Rhodotorula pustula*
Rhodotorula ferulica	*Rhodotorula lignophila*	*Rhodotorula sonckii*

** The performance of the ePlex BCID-FP Panel has not been established for all of the organisms listed in the tables above. See the Analytical Reactivity (Inclusivity) and Limit of Detection (Analytical Sensitivity) sections for data on organisms for which performance characteristics have been established. Some species were not assessed in silico due to lack of sequence data, though they may appear in the analytical sensitivity or specificity studies.*

** The performance of the ePlex BCID-FP Panel has not been established for all of the organisms listed in the tables above. See the Analytical Reactivity (Inclusivity) and Limit of Detection (Analytical Sensitivity) sections for data on organisms for which performance characteristics have been established. Some species were not assessed in silico due to lack of sequence data, though they may appear in the analytical sensitivity or specificity studies.*

Table 22: Predicted (*in silico*) Reactivity (Inclusivity) Results for *Rhodotorula*

g. Analytical specificity (Exclusivity):

Cross-reactivity of on-panel and off-panel analytes was evaluated with the BCID-FP Panel. On-panel organisms were tested in triplicate at concentrations approximating bottle positivity are noted in Table 23. Off-panel organisms were tested at concentrations of $\geq 1x10^9$ CFU/mL for bacteria and $\geq 1x10^7$ CFU/mL or spores/mL for fungi unless otherwise noted in Table 24. If the target concentration could not be reached, the organism was diluted 2-fold from stock for use.

Table 23: On-Panel Organisms Assessed for Cross-reactivity with the ePlex BCID-FP Panel (Exclusivity)

Organism	Strain	Organism	Strain
Candida albicans	ATCC 10231	*Candida krusei*	ATCC 32196
Candida albicans	ATCC 90028	*Candida krusei*	ATCC 34135
Candida albicans	ATCC MYA-4441	*Candida lusitaniae*	ATCC 42720
Candida auris	CBS 12766	*Candida lusitaniae*	ATCC MYA-766
Candida auris	CDC#385	*Candida lusitaniae*	Z010
Candida auris	CDC#386	*Candida parapsilosis*	ATCC 22019

Organism	Strain	Organism	Strain
Candida auris	CDC#387	*Candida parapsilosis*	ATCC 58895
Candida auris	CDC#388	*Candida parapsilosis*	ATCC 90018
Candida auris	CDC#389	*Candida tropicalis*	ATCC 201380
Candida auris	CDC#390	*Candida tropicalis*	ATCC 201381
Candida dubliniensis	ATCC MYA-578	*Candida tropicalis*	ATCC 750
Candida dubliniensis	ATCC MYA-579	*Cryptococcus gattii*	ATCC 14248
Candida dubliniensis	ATCC MYA-582	*Cryptococcus gattii*	ATCC 4560
Candida famata	ATCC 20850	*Cryptococcus gattii*	ATCC 76108
Candida famata	CBA 1961	*Cryptococcus neoformans*	ATCC 14116
Candida famata	CBS 789	*Cryptococcus neoformans*	ATCC 90112
Candida glabrata	ATCC 15126	*Cryptococcus neoformans*	NCPF 8299
Candida glabrata	ATCC 66032	*Bisifusarium dimerum*	CBS 110317
Candida glabrata	ATCC MYA-2950	*Fusarium lichenicola (Cylindrocarpon lichenicola)*	ATCC 204306
Candida guilliermondii	ATCC 90197	*Fusarium moniliforme*	ATCC 38159
Candida guilliermondii	ATCC 90198	*Fusarium proliferatum*	CBS 131570
Candida guilliermondii	ATCC 90199	*Fusarium sacchari*	CBS 119828
Candida kefyr	ATCC 204093	*Fusarium verticillioides*	CBS 100312
Candida kefyr	ATCC 2512	*Rhodotorula glutinis*	ATCC 96365
Candida kefyr	ATCC 66028	*Rhodotorula mucilaginosa*	ATCC 66034
Candida krusei	ATCC 14243	*Rhodotorula mucilaginosa*	ATCC 9449

Table 24: Cross-reactivity with Organisms Not Targeted by the ePlex BCID-FP Panel (Exclusivity)

Organism	Strain	Organism	Strain
Acinetobacter Iwoffii	ATCC 15309	*Kodamaea ohmeri*	CDC#0396
Acremonium kiliense	ATCC 4301	*Lactobacillus rhamnosus*	ATCC 53103
Aspergillus fumigatus	ATCC 204305[A]	*Malassezia furfur*	ATCC 12078
Bacteroides fragilis	ATCC 25285	*Malassezia furfur*	ATCC 14521
Bordetella pertussis	ATCC 9340	*Malassezia furfur*	CBS 7710
Candida bracarensis	CBS 10154	*Malassezia globosa*	ATCC MYA-4612
Candida carpophila	CBS 5256	*Malassezia restricta*	ATCC MYA-4611
Candida	CDC#394	*Malassezia sympodialis*	ATCC 44031

Organism	Strain	Organism	Strain
duobushaemulonii			
Candida haemulonii	CDC#393	*Meyerozyma caribbica (Candida fermentati)*	ATCC 20296
Candida inconspicua	ATCC 16783	*Micrococcus luteus*	ATCC 19212
Candida lambica	ATCC 24750	*Morganella morganii*	ATCC 25830
Candida lipolytica	ATCC 20177	*Mucor velutinosus*	ATCC MYA-4766
Candida metapsilosis	ATCC 96144	*Penicillium marneffei*	ATCC 200050
Candida nivariensis	CBS 9984	*Proteus mirabilis*	ATCC 35659
Candida norvegensis	ATCC 22977	*Rhodotorula minuta*	ATCC 36236
Candida orthopsilosis	ATCC 96139	*Saccharomyces cerevisiae*	ATCC 18824
Candida pelliculosa	ATCC 10262	*Salmonella enterica (Typhi)*	ATCC 19430
Candida rugosa	CBS 96275	*Scedosporium prolificans*	ATCC 200543
Candida sake	ATCC 22021	*Schizosaccharomyces pombe*	LPY 02387
Candida utilis	ATCC 9256	*Serratia marcescens*	ATCC 43861
Citrobacter freundii	ATCC 6879	*Sporidiobolus salmonicolor*	ATCC 24217
Clostridium perfringens	ATCC 13124	*Sporothrix schenckii*	ATCC 18616
Corynebacterium striatum	ATCC 7094	*Staphylococcus hominis*	ATCC 27844
Enterobacter aerogenes	ATCC 29751	*Staphylococcus intermedius*	ATCC 29663
Enterobacter cloacae	ATCC 23373	*Staphylococcus saprophyticus*	ATCC 15305
Enterococcus faecium	ATCC 31282	*Streptococcus agalactiae*	ATCC 12401
Exophiala jeanselmei	ATCC 12734	*Streptococcus anginosus*	ATCC 9895
Filobasidium elegans	CBS 7637	*Streptococcus pyogenes*	ATCC 12384
Filobasidium globisporum	CBS 7642	*Trichosporon asahii*	ATCC 201110
Klebsiella oxytoca	ATCC 43165	*Trichosporon asteroides*	ATCC 90043
Kluyveromyces lactis	ATCC 10689	*Trichosporon dermatis*	ATCC 204094

[A.] Tested at 1 x 10^6 spores/mL

No cross-reactivity was observed between any of the off-panel bacteria and fungi with the ePlex BCID-FP Panel targets.

h. Assay cut-off:

Analytical studies were conducted to establish the signal boundaries for all targets and controls of the ePlex BCID-FP Panel.

A mixture of clinical samples, contrived bottle positives, and organisms spiked at or above the analytically determined limit of detection were used as samples for the study. Positive data points from samples at or greater than the determined LoD test

concentration in the Limit of Detection study were used to supplement the Cutoff study data. Negative data points for all targets were obtained from the Limit of Blank study. Expected negative data points from replicates that were positive for other targets in the LoD and Cutoff studies were also combined to increase statistical power of the ROC analysis.

For each target, the signals for positive and negative tests were analyzed. An appropriate boundary was established wherein specificity and sensitivity were maximized. The analysis was verified by ROC analysis. The final boundary set points are listed in Table 25 below.

Table 25: Summary of BCID-FP Panel Boundary Set

Target Name	Boundary (nA)
Candida albicans	10
Candida auris	10
Candida dubliniensis	25
Candida famata	10
Candida glabrata	15
Candida guilliermondii	25
Candida kefyr	25
Candida krusei	25
Candida lusitaniae	20
Candida parapsilosis	25
Candida tropicalis	25
Cryptococcus gattii	25
Cryptococcus neoformans var. *grubii*	25
Cryptococcus neoformans var. *neoformans*	25
Fusarium	25
Rhodotorula	25
Schizosaccharomyces pombe (IC1-4)	50

i. Interference:

A study was performed to investigate the effect of 18 potentially interfering substances on the BCID-FP Panel. Two organism mixes consisting of 5 on-panel organisms and negative blood matrix were used to assess potentially interfering substances for interference. Potentially interfering test substances were spiked at levels predicted to be above the concentration of the substance likely to be found in a blood culture specimen. The positivity of the organisms for each potentially interfering substance at the initial testing concentration is summarized in Table 26 and 27.

Table 26: Interfering Substance and Bottle Equivalency Concentrations

Organism	Concentration
Candida albicans	1 x 10^6 CFU/mL

Candida kefyr	1 x 10^6 CFU/mL
Cryptococcus neoformans	1 x 10^7 CFU/mL
Fusarium sacchari	6.5 x 10^6 spores/mL
Rhodotorula mucilaginosa	1 x 10^6 CFU/mL

Table 27: List of Potentially Interfering Substances

Endogenous Substances	**Testing Concentration**
Bilirubin	60 µg/mL
Hemoglobin	0.6 g/L
Human genomic DNA	6 x 10^5 copies/mL
Triglycerides	1000 mg/dL
γ-globulin	0.85 g/dL
Exogenous Substances	**Testing Concentration**
Amoxicillin/Clavulanate	3.5 µg/mL
Amphotericin B	2 µg/mL^
Caspofungin	5 µg/mL^^
Ceftriaxone	0.23 mg/mL
Ciprofloxacin	3 mg/L
Fluconazole	25 mg/L
Flucytosine	90 µg/mL
Gentamicin sulfate	3 µg/mL
Heparin	0.9 U/mL
Imipenem	83 µg/mL
Sodium Polyanetholsulfonate	0.25% w/v
Tetracycline	5 mg/L
Vancomycin	30 mg/L

No false positives were detected in negative blood matrix runs without or with interfering substances.

j. Mixed Culture Study (Microbial interference):

Competitive inhibition was evaluated for the ePlex BCID-FP Panel by pairing twelve clinically relevant organisms (including 9 off-panel organisms) in thirteen simulated dual infection sample mixes. *Candida albicans* was tested at low titer (concentration expected at bottle positivity) while in the presence of other organisms at higher titer (concentrations expected at 8 hours beyond bottle positivity, or one log higher than that expected at bottle positivity). *Candida glabrata* and *Candida parapsilosis* were also tested at the concentration expected at bottle positivity in the presence of *Candida albicans* at a higher titer concentration. No competitive inhibition was observed in any of the sample mixes evaluated at the concentrations listed in Table 28.

Table 28: Competitive Inhibition Organisms and Concentrations Tested

On-panel Organisms	High Concentration	Low Concentration
Candida albicans	1 x 10^7 CFU/mL	1 x 10^6 CFU/mL
Candida glabrata	1 x 10^7 CFU/mL	1 x 10^6 CFU/mL
Candida parapsilosis	1 x 10^7 CFU/mL	1 x 10^6 CFU/mL
Off-panel Organisms	**High Concentration**	
Acinetobacter baumannii	1 x 10^9 CFU/mL	
Cutibacterium acnes	1 x 10^9 CFU/mL	
Enterococcus faecalis	1 x 10^8 CFU/mL	
Escherichia coli	1 x 10^9 CFU/mL	
Klebsiella pneumoniae	1 x 10^9 CFU/mL	
Staphylococcus aureus	1 x 10^8 CFU/mL	
Staphylococcus epidermidis	1 x 10^8 CFU/mL	
Streptococcus pneumoniae	4 x 10^8 CFU/mL	
Pseudomonas aeruginosa	4 x 10^8 CFU/mL	

Study results demonstrated that high concentrations of the on-panel BCID-FP microorganisms spiked into blood culture samples produced positive results for the relevant assays on the BCID-FP Panel but did interfere with any expected results for other analytes. High concentrations of off-panel BCID-FP microorganisms also showed no interference with the detection of any ePlex BCID-FP organism with no unexpected false negative or false positive results observed.

k. Testing of Additional Blood Culture Bottle Types

Fifteen different blood culture bottle types from three different blood culture systems (BacT/Alert, BACTEC and VersaTREK) were evaluated analytically with the BCID-FP Panel. Blood culture bottles/media were tested with the recommended ratio of blood to media. Testing was performed using two organism mixes whose members were present at a concentration approximating blood culture bottle positivity in a clinical sample. The mixes contain representative organisms from four genera, including the most prevalent *Candida* organism. Each organism was present at a testing concentration approximating bottle positivity. The 15 bottle types listed in the table below were evaluated and study results demonstrated correct positive and negative BCID-FP Panel results with each bottle type.

Table 29: Sample Matrix Equivalency (Bottle Evaluation) Bottle Types

Manufacturer	Bottle Brand	Bottle Type	Study Outcome
BD	BACTEC	Plus Aerobic	No interference observed
BD	BACTEC	Plus Anaerobic	No interference observed
BD	BACTEC	Standard Aerobic	No interference observed
BD	BACTEC	Standard Anaerobic	No interference observed

BD	BACTEC	Peds Plus	No interference observed
BD	BACTEC	Lytic Anaerobic	No interference observed
BD	BACTEC	Myco	No interference observed
bioMérieux	BACT/ALERT	SA Standard Aerobic	No interference observed
bioMérieux	BACT/ALERT	SN Standard Anaerobic	No interference observed
bioMérieux	BACT/ALERT	FA Plus	A false negative result was observed for the *Candida albicans* target in one lot.
bioMérieux	BACT/ALERT	FN Plus	No interference observed
bioMérieux	BACT/ALERT	PF Plus	False negative results were observed for the *Rhodotorula* target in one lot.
bioMérieux	BACT/ALERT	MP Mycobacteria	No interference observed
Thermo Scientific	VersaTREK	REDO 1 EZ Draw Aerobic	No interference observed
Thermo Scientific	VersaTREK	REDOX 2 EZ Draw Anaerobic	No interference observed

Thirteen bottle types tested showed no interference for any of the targets tested. The BACT/Alert® PF Plus and BACT/Alert® FA Plus bottle types each showed lower sensitivity for one organism tested in one of the two bottle lots and may have reduced sensitivity for some targets. The following discordance was observed in the study:

- BACT/Alert PF Plus

  Both lots of BacT/ Alert PF Plus failed to detect *Rhodotorula* in one of five initial replicates. Upon retest, one of ten replicates resulted in a *Rhodotorula* false negative for lot 3048740. The second lot of the same bottle type (lot 3047800), detected all targets in all 10 repeat test replicates. Given the lower detection rate of *Rhodotorula* in one of two bottle lots tested, the sensitivity for some targets may potentially be reduced in BacT/ Alert PF Plus bottles.

- BacT/ Alert FA Plus

  The BacT/ Alert FA Plus lot 3048018 had one run with a *Candida parapsilosis* false positive in the initial five test replicates with mix 2. Upon retest, no false positives were observed but one *Candida albicans* false negative was observed. Given the lower detection rate of C*andida albicans* in one of two bottle lots tested, the sensitivity for some targets may potentially be reduced in BacT/ Alert FA Plus bottles.

The following statements are included in the package insert:

Section of the Package Insert	Statement
Interfering Substances and Sample Matrix Equivalency (Bottle Evaluation)	Thirteen bottle types tested showed no interference for any of the targets tested. The BACT/Alert PF Plus and BACT/Alert FA Plus bottle types each showed lower sensitivity for one organism tested in one of the two bottle lots and may have reduced sensitivity for some targets.
Limitations of the Procedure	Decreased sensitivity has been observed for some targets in BACT/Alert PF Plus and BACT/Alert FA Plus bottle types.

l. Carryover *Study*:

The carryover/cross-contamination rate of the ePlex BCID-FP Panel and ePlex instrument was evaluated using a checkerboard approach by running high positive and negative samples interspersed in all bays of a four-tower ePlex instrument (i.e., 24 bays total) over five separate runs on five separate days. The positive sample was a contrived blood culture sample containing *Fusarium sacchari*. This organism was contrived and grown to positivity in blood culture, then incubated for an additional 8 hours in a continuously monitoring blood culture instrument. The contrived *F. sacchari* sample was then spiked with *Candida albicans* at $1x10^7$ CFU/mL. The organism levels were higher than typically found in positive blood cultures in order to challenge the system for potential cross contamination. No false positives were detected in the negative runs indicating no carryover or cross-contamination was observed between bays or within bays with the ePlex BCID-FP Panel when testing samples consecutively or in adjacent bays with an ePlex instrument. Two false negatives were detected in positive samples, which were repeated and produced expected results.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies Section of this document.

b. Matrix comparison:

N/A

3. Clinical studies:

Prospective Clinical Study

The clinical performance of the ePlex BCID-FP Panel was established during multi-center clinical studies conducted at six distinct U.S. test sites, with 21

evaluable samples. Each test site was representative of the intended use setting (clinical laboratories) and testing was performed by trained clinical laboratory personnel. Demographic information for prospectively collected samples is described in Table 30.

To supplement the results of the prospective collection, 120 samples were collected retrospectively from a total of 9 sites, and 725 evaluable samples were contrived for organisms with low prevalence. Demographic information for retrospectively collected samples is described in Table 31.

Table 30: Demographic Data for Clinical Samples by Collection Site (Prospective Collection)

	All Sites (N=21) n (%)	**Site 1 (N=1) n (%)**	**Site 2 (N=8) n (%)**	**Site 3 (N=2) n (%)**	**Site 4 (N=4 n (%))**	**Site 5 (N=4) n (%)**	**Site 6 (N=2) n (%)**
Sex, n (%)							
Male	14 (66.7)	1 (100)	7 (87.5)	1 (50.0)	3 (75.0)	1 (25.0)	1 (50.0)
Female	7 (33.3)	0 (0.0)	1 (12.5)	1 (50.0)	1 (25.0)	3 (75.0)	1 (50.0)
Age (years)							
<1 yr	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
1-17 yrs	2 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (50.0)	0 (0.0)
18-44 yrs	4 (19.0)	0 (0.0)	2 (25.0)	0 (0.0)	1 (25.0)	1 (25.0)	0 (0.0)
45-64 yrs	11 (52.4)	1 (100)	4 (50.0)	1 (50.0)	2 (50.0)	1 (25.0)	2 (100)
65-84 yrs	2 (9.5)	0 (0.0)	1 (12.5)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
85+ yrs	1 (4.8)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 31: Demographic Summary for Retrospective Arm of ePlex BCID-FP Clinical Evaluation

	All Sites (N=120) n (%)	**Site 1 (N=13) n (%)**	**Site 2 (N=14) n (%)**	**Site 3 (N=17) n (%)**	**Site 4 (N=4) n (%)**	**Site 5 (N=3) n (%)**	**Site 6 (N=13) n (%)**	**Site 7 (N=16) n (%)**	**Site 8 (N=5) n (%)**	**Site 9 (N=35) n (%)**
Sex, n (%)										
Male	68 (56.7)	10 (76.9)	8 (57.1)	8 (47.1)	1 (25.0)	2 (66.7)	8 (61.5)	9 (56.3)	3 (60.0)	19 (54.3)

	All Sites (N=120) n (%)	Site 1 (N=13) n (%)	Site 2 (N=14) n (%)	Site 3 (N=17) n (%)	Site 4 (N=4) n (%)	Site 5 (N=3) n (%)	Site 6 (N=13) n (%)	Site 7 (N=16) n (%)	Site 8 (N=5) n (%)	Site 9 (N=35) n (%)
Female	52 (43.3)	3 (23.1)	6 (42.9)	9 (52.9)	3 (75.0)	1 (33.3)	5 (38.5)	7 (43.8)	2 (40.0)	16 (45.7)
Age (years)										
<1 yr	2 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.7)
1-17 yrs	8 (6.7)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (38.5)	0 (0.0)	0 (0.0)	2 (5.7)
18-44 yrs	27 (22.5)	4 (30.8)	2 (14.3)	2 (11.8)	1 (25.0)	0 (0.0)	3 (23.1)	3 (18.8)	1 (20.0)	11 (31.4)
45-64 yrs	39 (32.5)	2 (15.4)	6 (42.9)	6 (35.3)	1 (25.0)	2 (66.7)	2 (15.4)	7 (43.8)	1 (20.0)	12 (34.3)
65-84 yrs	39 (32.5)	6 (46.2)	6 (42.9)	8 (47.1)	2 (50.0)	0 (0.0)	2 (15.4)	5 (31.3)	2 (40.0)	8 (22.9)
85+ yrs	5 (4.2)	0 (0.0)	0 (0.0)	1 (5.9)	0 (0.0)	1 (33.3)	1 (7.7)	1 (6.3)	1 (20.0)	0 (0.0)

The performance of the ePlex BCID-FP Panel was compared to standard laboratory procedures, including traditional and automated culture, MALDI-TOF IVD, and microbiological and biochemical techniques. In addition, all prospective samples were tested with analytically validated PCR assays followed by bi-directional sequencing to determine the presence or absence of *Candida auris*, *Fusarium*, and *Rhodotorula*. Identification for samples with *Candida parapsilosis* identified by standard laboratory procedures was confirmed using analytically validated PCR assays followed by bi-directional sequencing.

The comparator method(s) results were used to determine the Detected / Not Detected status for each target organism on the ePlex BCID-FP Panel. The comparator methods for each target are summarized in Table 32.

Table 32: Reference/Comparator Methods used to Assess BCID-FP Performance

BCID-FP Panel Target	Comparator Method(s)
Candida albicans, *Candida dubliniensis*, *Candida famata*, *Candida glabrata*, *Candida guilliermondii*, *Candida kefyr*, *Candida krusei*, *Candida lusitaniae*, *Candida tropicalis*, *Cryptococcus gattii*,	Standard laboratory procedures for organism identification

BCID-FP Panel Target	Comparator Method(s)
Cryptococcus neoformans	
Candida parapsilosis	Standard laboratory procedures for organism identification. PCR/sequencing to confirm *C. parapsilosis* or identify *C. metapsilosis*, *C. orthopsilosis*
Candida auris, *Fusarium*, *Rhodotorula*	Standard laboratory procedures for organism identification. PCR/sequencing in prospective samples with yeast and/or fungal Gram stain results

Clinical sensitivity or positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of TP and false negative (FN) results, while specificity or negative percent agreement (NPA) was calculated by dividing the number of true negative (TN) results by the sum of TN and false positive (FP) results. A TP result was defined as a sample where the detected ePlex BCID-FP Panel result matched the detected comparator method result, while a TN result was one where a negative ePlex BCID-FP Panel result matched a negative comparator method result. The two-sided 95% confidence interval was also calculated.

A total of 866 positive blood culture samples with a Gram stain result indicating fungal organism consisting of 11 fresh prospective, 10 frozen prospective, 120 retrospective, and 725 contrived samples were evaluated for the ePlex BCID-FP Panel targets. Contrived samples were prepared by spiking an isolate into a blood culture bottle and growing until flagged positive by a continuously monitoring blood culture system. Samples were removed from the system within 8 hours of positivity and stored frozen until the time of testing. PPA and NPA results are summarized by target in Tables 33-48 below and the strains used to contrive samples are summarized in Table 49.

Table 33: Clinical Performance for *Candida albicans*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida albicans	Prospective (Fresh)	2/2	100 (34.2-100)	9/9	100 (70.1-100)
	Prospective (Frozen)	2/2	100 (34.2-100)	8/8	100 (67.6-100)
	Prospective (All)	**4/4**	**100 (51.0-100)**	**17/17**	**100 (81.6-100)**
	Retrospective	49/50	98.0 (89.5-99.6)	70/70	100 (94.8-100)
	Prospective/Retrospective	**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)
	Overall	**66/68**	**97.1 (89.9-99.2)**	**797/798**	**99.9 (99.3-100)**

CI= Confidence Interval

Table 34: Clinical Performance for *Candida auris*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida auris	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	49/49	100 (92.7-100)	676/676	100 (99.4-100)
	Overall	**49/49**	**100 (92.7-100)**	**817/817**	**100 (99.5-100)**

Table 35: Clinical Performance for *Candida dubliniensis*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida dubliniensis	Prospective (Fresh)	1/1	100 (20.7-100)	10/10	100 (72.2-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**1/1**	**100 (20.7-100)**	**20/20**	**100 (83.9-100)**
	Retrospective	3/3	100 (43.9-100)	117/117	100 (96.8-100)
	Prospective/Retrospective	**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)
	Overall	**52/52**	**100 (93.1-100)**	**814/814**	**100 (99.5-100)**

Table 36: Clinical Performance for *Candida famata*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida famata	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	51/51	100 (93.0-100)	674/674	100 (99.4-100)
	Overall	**51/51**	**100 (93.0-100)**	**815/815**	**100 (99.5-100)**

Table 37: Clinical Performance for *Candida glabrata*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida glabrata	Prospective (Fresh)	4/4	100 (51.0-100)	7/7	100 (64.6-100)
	Prospective (Frozen)	2/2	100 (34.2-100)	8/8	100 (67.6-100)
	Prospective (All)	**6/6**	**100 (61.0-100)**	**15/15**	**100 (79.6-100)**
	Retrospective	37/38	97.4 (86.5-99.5)	80/82	97.6 (91.5-99.3)

	Prospective/Retrospective	**43/44**	**97.7 (88.2-99.6)**	**95/97[A]**	**97.9 (92.8-99.4)**
	Contrived	16/16	100 (80.6-100)	709/709	100 (99.5-100)
	Overall	**59/60**	**98.3 (91.1-99.7)**	**804/806**	**99.8 (99.1-99.9)**

[A.] *C. glabrata* was detected by PCR/sequencing in 2/2 false positive clinical samples.

Table 38: Clinical Performance for *Candida guilliermondii*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	**% (95% CI)**	**TN/TN+FP**	**% (95% CI)**
Candida guilliermondii	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	49/50	98.0 (89.5-99.6)	675/675	100 (99.4-100)
	Overall	**49/50**	**98.0 (89.5-99.6)**	**816/816**	**100 (99.5-100)**

Table 39: Clinical Performance for *Candida kefyr*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	**% (95% CI)**	**TN/TN+FP**	**% (95% CI)**
Candida kefyr	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	51/51	100 (93.0-100)	672/674	99.7 (98.9-99.9)
	Overall	**51/51**	**100 (93.0-100)**	**813/815**	**99.8 (99.1-99.9)**

Table 40: Clinical Performance for *Candida krusei*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	**% (95% CI)**	**TN/TN+FP**	**% (95% CI)**
Candida krusei	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	2/2	100 (34.2-100)	8/8	100 (67.6-100)
	Prospective (All)	**2/2**	**100 (34.2-100)**	**19/19**	**100 (83.2-100)**
	Retrospective	2/2	100 (34.2-100)	118/118	100 (96.8-100)
	Prospective/Retrospective	**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)
	Overall	**50/50**	**100 (92.9-100)**	**816/816**	**100 (99.5-100)**

Table 41: Clinical Performance for *Candida lusitaniae*

Target	Sample Type	Sensitivity/PPA	Specificity/NPA

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida lusitaniae	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	3/4	75.0 (30.1-95.4)	116/116	100 (96.8-100)
	Prospective/Retrospective	**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)
	Overall	**48/49**	**98.0 (89.3-99.6)**	**816/817**	**99.9 (99.3-100)**

Table 42: Clinical Performance for *Candida parapsilosis*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida parapsilosis	Prospective (Fresh)	2/2	100 (34.2-100)	9/9	100 (70.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**2/2**	**100 (34.2-100)**	**19/19**	**100 (83.2-100)**
	Retrospective	16/17	94.1 (73.0-99.0)	102/103	99.0 (94.7-99.8)
	Prospective/Retrospective	**18/19**	**94.7 (75.4-99.1)**	**121/122[A]**	**99.2 (95.5-99.9)**
	Contrived	41/41	100 (91.4-100)	684/684	100 (99.4-100)
	Overall	**59/60**	**98.3 (91.1-99.7)**	**805/806**	**99.9 (99.3-100)**

[A.] *C. parapsilosis* was detected by PCR/sequencing in 1/1 false positive clinical sample.

Table 43: Clinical Performance for *Candida tropicalis*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Candida tropicalis	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	2/2	100 (34.2-100)	8/8	100 (67.6-100)
	Prospective (All)	**2/2**	**100 (34.2-100)**	**19/19**	**100 (83.2-100)**
	Retrospective	3/3	100 (43.9-100)	116/117	99.1 (95.3-99.8)
	Prospective/Retrospective	**5/5**	**100 (56.6-100)**	**135/136[A]**	**99.3 (96.0-99.9)**
	Contrived	45/45	100 (92.1-100)	680/680	100 (99.4-100)
	Overall	**50/50**	**100 (92.9-100)**	**815/816**	**99.9 (99.3-100)**

[A.] *C. tropicalis* was detected by PCR/sequencing in 2/2 false positive clinical samples.

Table 44: Clinical Performance for *Cryptococcus gattii*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Cryptococcus gattii	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**

	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	50/50	100 (92.9-100)	675/675	100 (99.4-100)
	Overall	**50/50**	**100 (92.9-100)**	**816/816**	**100 (99.5-100)**

Table 45: Clinical Performance for *Cryptococcus neoformans*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Cryptococcus neoformans	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	5/5	100 (56.6-100)	115/115	100 (96.8-100)
	Prospective/Retrospective	**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)
	Overall	**57/57**	**100 (93.7-100)**	**809/809**	**100 (99.5-100)**

Table 46: Clinical Performance for *Fusarium*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Fusarium	Prospective (Fresh)	0/0	---	11/11	100 (74.1-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**0/0**	**---**	**21/21**	**100 (84.5-100)**
	Retrospective	0/0	---	120/120	100 (96.9-100)
	Prospective/Retrospective	**0/0**	**---**	**141/141**	**100 (97.3-100)**
	Contrived	69/70	98.6 (92.3-99.7)	655/655	100 (99.4-100)
	Overall	**69/70**	**98.6 (92.3-99.7)**	**796/796**	**100 (99.5-100)**

Table 47: Clinical Performance for *Rhodotorula*

Target	Sample Type	Sensitivity/PPA		Specificity/NPA	
		TP/TP+FN	% (95% CI)	TN/TN+FP	% (95% CI)
Rhodotorula	Prospective (Fresh)	1/1	100 (20.7-100)	10/10	100 (72.2-100)
	Prospective (Frozen)	0/0	---	10/10	100 (72.2-100)
	Prospective (All)	**1/1**	**100 (20.7-100)**	**20/20**	**100 (83.9-100)**
	Retrospective	1/1	100 (20.7-100)	119/119	100 (96.9-100)
	Prospective/Retrospective	**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)
	Overall	**50/52**	**96.2 (87.0-98.9)**	**813/814**	**99.9 (99.3-100)**

The ePlex BCID-FP Panel reports genus level results for *Fusarium* and *Rhodotorula* targets. Sensitivity/PPA of these genus and group level targets for species from all clinical and contrived samples tested are summarized in Table 48 below.

Table 48: Species Detected by Genus Assays

Target Species Detected	All Samples	
	Sensitivity/PPA	
	TP/TP+FN	% (95% CI)
Fusarium	**69/70**	**98.6 (92.3-99.7)**
Bisifusarium dimerum	14/15	93.3 (70.2-98.8)
Fusarium moniliforme	11/11	100 (74.1-100)
Fusarium oxysporum	1/1	100 (20.7-100)
Fusarium sacchari	21/21	100 (84.5-100)
Fusarium solani	11/11	100 (74.1-100)
Fusarium verticillioides	11/11	100 (74.1-100)
Rhodotorula	**50/52**	**96.2 (87.0-98.9)**
*Rhodotorula**	2/2	100 (34.2-100)
Rhodotorula glutinis	6/6	100 (61.0-100)
Rhodotorula glutinis (Fresenius) Harrison	4/4	100 (51.0-100)
Rhodotorula mucilaginosa	38/40	95.0 (83.5-98.6)

*Results are from 2 clinical samples (1 prospective, 1 retrospective). All other *Fusarium* and *Rhodotorula* samples were contrived.

Table 49: Contrived Sample Summary

Target	Organism	Strain	Independent Contrived Samples Tested
Candida albicans	*Candida albicans*	ATCC 10231	2
		ATCC 14053	2
		ATCC 24433	2
		ATCC 90028	5
		ATCC MYA-4441	3
	Candida albicans total		14
Candida auris	*Candida auris*	ATCC 10913	1
		ATCC 12372	1
		ATCC 12766	1
		CBS 10913	3
		CBS 12372	3
		CBS 12373	2
		CBS 12766	3
		CBS 12767	3
		CBS 12768	2
		CDC#0385	5
		CDC#0386	5
		CDC#0387	5
		CDC#0388	5
		CDC#0389	5
		CDC#0390	5

Target	Organism	Strain	Independent Contrived Samples Tested
	Candida auris total		49
Candida dubliniensis	*Candida dubliniensis*	ATCC MYA-577	6
		ATCC MYA-578	12
		ATCC MYA-579	12
		ATCC MYA-582	13
		NCPF3949	5
	Candida dubliniensis total		48
Candida famata	*Debaryomyces fabryi*	CBS 789	21
	Debaryomyces hansenii	CBS 1961	3
	Debaryomyces subglobosus	CBS 1796	27
	Candida famata total		51
Candida glabrata	*Candida glabrata*	128-4058	1
		128-4072	1
		ATCC 15126	2
		ATCC 15545	2
		ATCC 2001	1
		ATCC 66032	4
		ATCC 90030	2
		ATCC MYA-2950	3
	Candida glabrata total		16
Candida guilliermondii	*Candida guilliermondii*	ATCC 22017	13
		ATCC 6260	12
	Meyerozyma guilliermondii	ATCC 90197	10
		ATCC 90198	9
		ATCC 90199	6
	Candida guilliermondii total		50
Candida kefyr	*Candida kefyr*	ATCC 204093	4
		ATCC 2512	10
		ATCC 4135	13
		ATCC 66028	12
		ATCC 8553	12
	Candida kefyr total		51
Candida krusei	*Candida krusei*	ATCC 14243	8
		ATCC 22985	9
		ATCC 28870	9
		ATCC 32196	10
		ATCC 34135	10
	Candida krusei total		46
Candida lusitaniae	*Candida lusitaniae*	ATCC 26287	5

Target	Organism	Strain	Independent Contrived Samples Tested
		ATCC 34449	10
		ATCC 42720	9
		ATCC 66035	11
		ATCC MYA-766	10
	Candida lusitaniae total		45
Candida parapsilosis	*Candida parapsilosis*	ATCC 22019	11
		ATCC 28474	5
		ATCC 28475	10
		ATCC 58895	7
		ATCC 90018	8
	Candida parapsilosis total		41
Candida tropicalis	*Candida tropicalis*	ATCC 1369	9
		ATCC 13803	12
		ATCC 201380	9
		ATCC 201381	7
		ATCC 750	8
	Candida tropicalis total		45
Cryptococcus gattii	*Cryptococcus gattii*	ATCC 14248	11
		ATCC 76108	12
		ATCC MYA-4138	10
		ATCC MYA-4560	8
		ATCC MYA-4877	9
	Cryptococcus gattii total		50
Cryptococcus neoformans	*Cryptococcus neoformans* var. *grubii*	ATCC 14116	9
		ATCC 208821(H99)	8
		ATCC 90112	7
		NCPF8195	7
		NCPF8299	3
		NCPF8357	3
	Cryptococcus neoformans var. *neoformans* (*Filobasidiella bacillispora* Kwon-Chung, teleomorph (serotype D))	ATCC 34873	3
	Cryptococcus neoformans var. *neoformans* (serotype D)	ATCC 36556	3
		ATCC MYA-565	9
	Cryptococcus neoformans total		52
Fusarium	*Bisifusarium*	CBS 108944	3
		CBS 110317	9

Target	Organism	Strain	Independent Contrived Samples Tested
	dimerum	CBS 116520	3
	Fusarium moniliforme	ATCC 38159	11
	Fusarium oxysporum	CBS 116611	1
	Fusarium sacchari	ATCC 24379	10
		CBS 119828	11
	Fusarium solani	ATCC 36031	11
	Fusarium verticillioides	CBS 100312	11
	Fusarium total		70
Rhodotorula	*Rhodotorula glutinis*	ATCC 32765	3
		ATCC 32766	3
	Rhodotorula glutinis (Fresenius) Harrison	ATCC 96365	4
	Rhodotorula mucilaginosa	ATCC 66034	21
		ATCC 9449	19
	Rhodotorula total		50

Co-detections in Clinical Samples

The ePlex BCID-FP Panel did not identify any fungal co-detections in prospective samples, and 6 fungal co-detections were identified in the retrospective samples. Of the 120 retrospective samples, 114 (95.0%) had single detections and 6 (5.0%) had double detections. In the 6 co-detections, 4 included 1 organism that comparator method(s) did not detect. See Table 50 below for a summary of co-detections in retrospective samples.

Table 50: Co-Detections Identified by the ePlex BCID-FP Panel (Retrospective Samples)

Distinct Organism Combinations Detected by the ePlex Panel in Retrospective Samples		Number of Samples (Number Discrepant)	Discrepant Organism(s)[A,B]
Target 1	**Target 2**		
Candida albicans	*Candida dubliniensis*	1 (0)	
Candida albicans	*Candida parapsilosis*	1 (0)	
Candida dubliniensis	*Candida parapsilosis*	1 (1)	*C. parapsilosis* (1)
Candida dubliniensis	*Candida tropicalis*	1 (1)	*C. tropicalis* (1)
Candida glabrata	*Candida lusitaniae*	2 (2)	*C. glabrata* (2)

[A] A discrepant organism is defined as one that was detected by the BCID-FP Panel but not by the comparator method(s).
[B] 4/4 organisms were investigated using PCR/sequencing and the discrepant organism was detected in 4/4 cases; 1/1

sample, C. parapsilosis was detected, 1/1 sample, C. tropicalis was detected and 2/2 samples, C. glabrata was detected.

Summaries of additional distinct fungal co-detections detected by the comparator method in prospective and retrospective samples are provided in Tables 51 and 52. These tables include additional distinct fungal co-detections not included in the co-detections identified by the BCID-FP Panel; they have 1 or more organism not detected by the BCID-FP Panel or an off-panel fungal organism.

Table 51: Co-Detections Identified by the Comparator Method (Prospective Samples)

Distinct Organism Combinations Detected by the ePlex Panel in Prospective Samples		Number of Samples (Number Discrepant)	Discrepant Organism(s)[A]
Target 1	**Target 2**		
*Candida metapsilosis**	*Trichosporon asahii**	1 (0)	

[A] A discrepant organism is defined as one that was detected by the comparator method(s) but not by the BCID-FP Panel (excludes organisms not targeted by the BCID-FP Panel).
* Off-panel organism not targeted by the BCID-FP Panel.

Table 52:Co-Detections Identified by the Comparator Method (Retrospective Samples)

Distinct Organism Combinations Detected by the ePlex Panel in Retrospective Samples			Number of Samples (Number Discrepant)	Discrepant Organism(s)[A]
Target 1	**Target 2**	**Target 3**		
Candida albicans	*Candida dubliniensis*	*Candida glabrata*	1 (0)	*C. glabrata* (1)
Candida albicans	*Candida parapsilosis*		1 (0)	*C. parapsilosis* (1)

[A] A discrepant organism is defined as one that was detected by the comparator method(s) but not by the BCID-FP Panel (excludes organisms not targeted by the BCID-FP Panel).

A total of 867 samples (including prospective, retrospective, and contrived samples) were initially tested in the clinical evaluations. Of these, 7/867 (0.8%) did not complete the run and the sample was retested. After repeat testing, all 867 samples completed testing and 839/867 (96.8%, 95% CI: 95.4%-97.8%) generated valid results and 28/867 (3.2%, 95% CI: 2.2%-4.6%) generated invalid results on the first completed attempt.

Upon repeat testing of the 28 samples with initially invalid results, all completed the run and 27/28 (96.4%) generated valid results. Overall, after final testing, 1/867 (0.1%, 95% CI: 0.0%-0.7%) had final, invalid results, resulting in a final validity rate of 866/867 (99.9%, 95% CI: 99.3%-100%).

External Control testing in the Clinical Study:

External controls used in the clinical study consisted of 2 positive controls and 1 negative control. The positive controls together represent all the targets on the ePlex BCID-FP Panel. On each day of testing with the ePlex BCID-FP Panel, each site tested one positive and one negative control. Sites rotated through the 2 positive controls throughout the study.

Table 53: External Controls Utilized in the Clinical Evaluations

Mix ID	Plasmids in Mix	Concentration in Mix (Plasmid Copies/mL)
Mix 1	*Candida albicans*	2×10^6
	Candida glabrata	2×10^6
	Candida kefyr	1×10^6
	Candida krusei	2×10^6
	Cryptococcus neoformans	1×10^5
	Candida famata	5×10^7
	Candida dublinensis	1×10^7
	Rhodotorula	1×10^8
Mix 2	*Candida auris*	1×10^7
	Candida guillermondii	2×10^5
	Candida lusitaniae	2×10^5
	Candida parapsilosis	2×10^6
	Candida tropicalis	2×10^6
	Cryptococcus gattii	3×10^5
	Fusarium	2×10^6

Over 47 days of investigational testing across 4 sites (median number of days/site was 12), there were 93 external control samples tested with valid results: 23 Positive Control A, 24 Positive Control B, and 46 Negative Control. Of these, 92/93 had the expected results. The remaining 1 external control sample had the expected results except as follows: 1 Positive Control B sample had 1 *Candida glabrata* false positive result.

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

A prospective, multicenter clinical study was conducted to evaluate the clinical performance of the ePlex BCID-FP Panel in positive blood culture samples. A total of 447 positive blood culture samples were collected at 6 clinical sites in 2 phases from patients of all ages and genders. Samples were collected and frozen for future testing from May 2015 through July 2016. Samples were collected from July through August 2018 and tested fresh (never frozen). Of these 447 samples, 21 had a Gram stain result indicating fungal organism. The expected values of individual analytes based on the ePlex BCID-FP Panel results in the 21 prospective samples are summarized by age group and by site in Tables 54 and 55 below.

Table 54: Expected Value by Age Group (Prospective Samples)

Target	All Ages (N=445) n (%)	Age <1 (N=16) n (%)	Age 1-17 (N=23) n (%)	Age 18-44 (N=70) n (%)	Age 45-64 (N=158) n (%)	Age 65-84 (N=145) n (%)	Age 85+ (N=33) n (%)
Candida albicans	4 (19.0)	1 (100)	0 (0.0)	0 (0.0)	2 (18.2)	1 (50.0)	0 (0.0)
Candida auris	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida dubliniensis	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)
Candida famata	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida glabrata	6 (28.6)	0 (0.0)	1 (50.0)	1 (25.0)	3 (27.3)	1 (50.0)	0 (0.0)
Candida guilliermondii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida kefyr	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida krusei	2 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (18.2)	0 (0.0)	0 (0.0)
Candida lusitaniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida parapsilosis	2 (9.5)	0 (0.0)	1 (50.0)	0 (0.0)	1 (9.1)	0 (0.0)	0 (0.0)
Candida tropicalis	2 (9.5)	0 (0.0)	0 (0.0)	1 (25.0)	1 (9.1)	0 (0.0)	0 (0.0)
Cryptococcus gattii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cryptococcus neoformans	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fusarium	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rhodotorula	1 (4.8)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 55: Expected Value by Collection Site (Prospective Samples)

Target	All Sites (N=445) n (%)	Site 1 (N=25) n (%)	Site 12 (N=162) n (%)	Site 3 (N=74) n (%)	Site 4 (N=65) n (%)	Site 5 (N=75) n (%)	Site 6 (N=44) n (%)
Candida albicans	4 (19.0)	1 (100)	0 (0.0)	2 (100)	1 (25.0)	0 (0.0)	0 (0.0)
Candida auris	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida dubliniensis	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)
Candida famata	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida glabrata	6 (28.6)	0 (0.0)	2 (25.0)	0 (0.0)	1 (25.0)	2 (50.0)	1 (50.0)
Candida guilliermondii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida kefyr	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Target	All Sites (N=445) n (%)	Site 1 (N=25) n (%)	Site 12 (N=162) n (%)	Site 3 (N=74) n (%)	Site 4 (N=65) n (%)	Site 5 (N=75) n (%)	Site 6 (N=44) n (%)
Candida krusei	2 (9.5)	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida lusitaniae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida parapsilosis	2 (9.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	1 (50.0)
Candida tropicalis	2 (9.5)	0 (0.0)	2 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cryptococcus gattii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cryptococcus neoformans	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fusarium	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rhodotorula	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)

N. Instrument Name:

GenMark ePlex Instrument

O. System Descriptions:

1. Modes of Operation:

   After samples have been loaded in the ePlex BCID-FP Panel cartridge, the remaining processing steps are executed under control of the ePlex instrument.

2. Software

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes _____X___ or No _____

3. Specimen Identification:

   The Sample ID can be entered manually or scanned via barcode.

4. Specimen Sampling and Handling:

   N/A

6. Quality Control:

   See Section M (1d) above

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

R. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

S. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.