

Supplementary Table 1. Description of each gene target used in the PCR/sequencing identification assay*

Pathogen Target	Molecular Target	Description	Primer Sequence (5'–3')	Size
<i>Candida albicans</i>	RPB1	DNA-directed RNA polymerase II subunit rpb1	F: M13–TCAGTGGCAAAAATAGAGTATCCTG	430
			R: M13–CATCTCTACGAATAGTTGGTTGTGG	
<i>Candida auris</i>	GABA	gamma-aminobutyric acid (GABA) A receptor	F: GGATACCGAGGAGATCATCCAG	377
			R: CATTTCCTTGACGTGGTCAGG	
<i>Candida dubliniensis</i>	HWP1	Hyphal wall protein 1	F: M13–CCACTCCAGAAGCTTCCACT	485
			R: M13–CCAAAAAAGAACAGACACGGAT	
<i>Candida famata</i>	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	F: M13–ATGATGACATCATTAGACATTCGGATT	384
			R: M13–TTTCCGGCAACTTGACTTCTG	
<i>Candida glabrata</i>	RPB1	DNA-directed RNA polymerase II subunit rpb1	F: M13–CAAGCAATTCAAATCAAGGATCCA	445
			R: M13–CCTTCAAGATATCTGCCAATTTGAAT	
<i>Candida guilliermondii</i>	FKS1	Beta-1,3-glucan synthase catalytic subunit 1	F: M13–GAATCAAGCACTGTGAATACTACCAG	488
			R: M13–CAACGAGATAAGATGGCGACAG	
<i>Candida kefyr</i>	EIF-2 α	Eukaryotic Translation Initiation Factor 2 Subunit Alpha	F: M13–GCAGTTATGGATGCTGCTCTATTA	477
			R: M13–TCTTACCTTGATCGTCCTTAGTAACT	
<i>Candida krusei</i>	CS1	Citrate synthase 1	F: M13–TTGCTGAATTGTTACCTGGCTG	441
			R: M13–CCTTCGCGAATTTGGATTCTGA	
<i>Candida lusitanae</i>	FKS1	Beta-1,3-glucan synthase catalytic subunit 1	F: M13–CTGTGTTTTCTCCATTGGACGT	504
			R: M13–GAAAATTGATCGTCTTCCCTGATGATC	
<i>Candida parapsilosis</i>	RPB1	DNA-directed RNA polymerase II subunit rpb1	F: M13–GGGTGATGATGTCATTAACAAG	418
			R: M13–CAACTTCTTTGCTTGGTCACGAG	
<i>Candida orthopsilosis</i>	RPB1	DNA-directed RNA polymerase II subunit rpb1	F: M13–TGGTGATGATGTGAMTAGTTAYAAAG	418
			R: M13–CAAYTTCTTAGCTTGGTCACGAG	
<i>Candida metapsilosis</i>	RPB1	DNA-directed RNA polymerase II subunit rpb1	F: M13–AGGTGAAGATRTRCTGGATACAAG	418
			R: M13–CAACTTCTTRGCTTGGTCACGMG	
<i>Candida tropicalis</i>	SADH	Alcohol dehydrogenase	F: M13–GGTTTATTGCCAAGATCAAGAAATGT	333
			R: M13–TTTCCAGTGGAGTCAATAAACGAC	
<i>Cryptococcus</i>	RNA Pol	RNA polymerase	F: M13–CCTCTGGYGAGAACTGGAGAC	387

			R: M13–GCCTTGGCAATCTTCTCAACTC	
<i>Fusarium</i>	CAP10	Capsular associated protein	F: M13–GATCTACCAGTGCGGTGGTAT	410
			R: M13–GAGGAGTCTCGAACTCCAGAG	
<i>Rhodotorula</i>	MF1	Anthocyanin methyltransferase	F: M13–TCTGCYATCCCATGGATTGG	314
			R: M13–TTGTCAGTGTCCYAGGTAG	

*Sanger sequencing assays were developed to detect and differentiate fungi present in bloodstream infections. The PCR primers were designed to detect clinically relevant strains, and they incorporate M13 universal sequencing tags. All PCR reactions utilized a primer annealing temperature of 58 °C and consisted of 40 cycles. With the exception of *Fusarium*, all assays used the Qiagen HotStarTaq Master Mix. Amplification of *Fusarium* was complicated by the presence of homopolymer tracts that cause polymerase slippage, resulting in poor quality sequencing results; therefore, this assay utilized the New England Biosciences Phusion High-Fidelity Polymerase, a proof-reading polymerase fused to a nonspecific dsDNA binding domain that enhances read-through of the mononucleotide repeat regions. Following PCR, amplicons were visualized by gel electrophoresis to verify successful amplification and then purified using Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (SAP). Each amplicon was sequenced with M13 primers using the BigDye Terminator v3.1 Cycle Sequencing Kit to yield 2x bi-directional coverage of at least 200 bp. Sequence reads were assembled using Sequencher software to generate a bi-directional consensus sequences. The resulting consensus sequences were then identified using the NCBI Basic Local Alignment Search Tool (BLAST).

Supplementary Table 2. Contrived sample list *

Fungal Organism	Strain	Number Tested	Dilution (1 to x) (min-max)	Spiked-in CFUs/mL (min-max) ^a	Time to Flag Positive ^b (DD:HH:MM) (min-max)
<i>Candida albicans</i>	ATCC10231	2	10 - 100	24.83 - 279.38	00:18:12 - 05:18:26 ^c
<i>Candida albicans</i>	ATCC14053	2	100	24.83 - 27.05	01:05:08 - 02:02:21
<i>Candida albicans</i>	ATCC24433	2	10 - 10000	0.26 - 239.47	00:19:12 - 02:23:09
<i>Candida albicans</i>	ATCC90028	5	1 - 100	19.45 - 2350.33	00:16:07 - 04:10:44
<i>Candida albicans</i>	ATCCMYA-4441	3	10 - 10000	0.25 - 243.90	00:19:12 - 01:15:08
<i>Candida auris</i>	ATCC10913	1	10	235.03	00:20:09
<i>Candida auris</i>	ATCC12372	1	10	230.60	00:18:19
<i>Candida auris</i>	ATCC12766	1	10	248.34	00:17:09
<i>Candida auris</i>	CBS10913	3	10	199.56 - 234.41	00:17:08 - 00:22:12
<i>Candida auris</i>	CBS12372	3	10	234.41 - 284.29	00:16:08 - 00:21:03
<i>Candida auris</i>	CBS12373	2	10	186.25 - 284.29	00:21:08 - 01:00:05
<i>Candida auris</i>	CBS12766	3	10	199.50 - 244.39	00:14:08 - 00:17:43
<i>Candida auris</i>	CBS12767	3	10	208.43 - 248.34	00:16:06 - 00:16:09
<i>Candida auris</i>	CBS12768	2	10	186.25 - 212.86	00:16:08 - 00:17:09
<i>Candida auris</i>	CDC#0385	5	10 - 100	26.61 - 309.23	00:12:30 - 01:12:11
<i>Candida auris</i>	CDC#0386	5	10 - 100	18.18 - 314.86	00:12:30 - 01:11:11
<i>Candida auris</i>	CDC#0387	5	10 - 100	26.61 - 243.90	00:12:30 - 00:22:10
<i>Candida auris</i>	CDC#0388	5	10 - 100	24.83 - 279.30	00:12:30 - 01:00:10
<i>Candida auris</i>	CDC#0389	5	10 - 100	23.06 - 294.26	00:12:30 - 00:22:10
<i>Candida auris</i>	CDC#0390	5	10 - 100	22.62 - 301.55	00:13:00 - 01:02:10
<i>Candida dubliniensis</i>	ATCCMYA-577	6	10 - 100	28.93 - 283.81	00:17:10 - 00:20:07
<i>Candida dubliniensis</i>	ATCCMYA-578	12	10	186.25 - 2434.78	00:14:03 - 00:20:14
	ATCCMYA-579	12	10 - 100000	0.02 - 257.21	00:17:30 - 04:09:43
	ATCCMYA-582	13	10 - 20000	0.11 - 257.21	00:17:04 - 04:08:03
	NCPF3949	5	10 - 100	23.06 - 243.90	00:18:03 - 01:14:06

Candida famata species complex

<i>Debaryomyces fabryi</i>	CBS789	21	10	195.12 - 310.42	01:22:15 - 14:18:40 ^d
<i>Candida famata</i>	CBS1961	3	10	243.90 - 274.31	01:04:14 - 01:11:07
<i>Debaryomyces subglobosus</i>	CBS1796	27	10 - 100000	0.01 - 301.55	01:01:03 - 04:18:32
<i>Candida glabrata</i>	128-4058	1	100	21.73	01:16:04
<i>Candida glabrata</i>	128-4072	1	100	31.42	03:17:40
<i>Candida glabrata</i>	ATCC15126	2	100	20.84 - 21.29	00:22:08 - 00:23:02
<i>Candida glabrata</i>	ATCC15545	2	100 - 10000	0.23 - 19.96	01:01:11 - 01:18:09
<i>Candida glabrata</i>	ATCC2001	1	100	22.17	01:09:04
<i>Candida glabrata</i>	ATCC66032	4	1 - 100000	0.02 - 2838.14	00:11:05 - 03:13:32
<i>Candida glabrata</i>	ATCC90030	2	1 - 100	19.07 - 2616.41	00:11:06 - 00:23:11
<i>Candida glabrata</i>	ATCCMYA-2950	3	1 - 100	15.52 - 3148.56	01:02:11 - 02:18:00
<i>Candida guilliermondii</i>	ATCC22017	13	10 - 10000	0.23 - 301.55	00:16:58 - 03:21:50
<i>Candida guilliermondii</i>	ATCC6260	12	10 - 10000	0.25 - 279.38	00:17:58 - 01:05:07
<i>Candida guilliermondii</i>	ATCC90197	10	10 - 100	23.95 - 288.25	00:16:58 - 00:23:10
<i>Candida guilliermondii</i>	ATCC90198	9	10 - 100	22.62 - 305.99	00:19:58 - 01:07:20
<i>Candida guilliermondii</i>	ATCC90199	6	10 - 100	23.95 - 270.51	00:18:07 - 01:00:05
<i>Candida kefyr</i>	ATCC204093	4	100 - 10000	0.29 - 30.16	00:15:03 - 01:12:06
<i>Candida kefyr</i>	ATCC2512	10	10 - 100	17.74 - 283.81	00:15:04 - 01:10:09
<i>Candida kefyr</i>	ATCC4135	13	10 - 100	18.18 - 266.08	00:14:04 - 00:23:09
<i>Candida kefyr</i>	ATCC66028	12	10 - 100	19.96 - 274.94	00:13:04 - 00:22:18
<i>Candida kefyr</i>	ATCC8553	12	1 - 100	19.51 - 2971.18	00:14:06 - 05:22:11 ^e
<i>Candida krusei</i>	ATCC14243	8	10 - 100	17.29 - 261.64	00:16:04 - 01:03:08
<i>Candida krusei</i>	ATCC22985	9	10 - 10000	0.22 - 270.51	00:14:04 - 03:01:16
<i>Candida krusei</i>	ATCC28870	9	10 - 10000	0.43 - 279.38	00:13:04 - 02:00:49
<i>Candida krusei</i>	ATCC32196	10	10 - 10000	0.35 - 270.51	00:14:03 - 02:14:30
<i>Candida krusei</i>	ATCC34135	10	10 - 10000	0.35 - 301.55	00:13:09 - 03:02:30
<i>Candida lusitanae</i>	ATCC26287	6 ^f	10 - 100	25.28 - 310.42	00:14:10 - 01:01:08
<i>Candida lusitanae</i>	ATCC34449	10	10 - 100	24.83 - 310.42	00:14:00 - 01:03:19
<i>Candida lusitanae</i>	ATCC42720	9	10 - 100	18.63 - 288.25	00:14:00 - 01:00:03
<i>Candida lusitanae</i>	ATCC66035	11	10 - 100	23.06 - 328.16	00:14:07 - 01:23:08

<i>Candida lusitanae</i>	ATCCMYA-766	10	10 - 100	21.29 - 288.25	00:16:00 - 01:19:08
<i>Candida parapsilosis</i>	ATCC22019	11	10 - 100	19.96 - 266.08	00:19:09 - 03:01:16
<i>Candida parapsilosis</i>	ATCC28474	5	10 - 100	18.18 - 208.82	02:13:30 - 03:07:17
<i>Candida parapsilosis</i>	ATCC28475	10	10 - 100	19.07 - 230.60	00:18:19 - 02:18:17
<i>Candida parapsilosis</i>	ATCC58895	7	10 - 100	26.16 - 243.90	00:17:08 - 02:06:11
<i>Candida parapsilosis</i>	ATCC90018	8	10 - 100	18.63 - 270.51	00:19:09 - 01:06:15
<i>Candida tropicalis</i>	ATCC1369	9	10 - 10000	0.25 - 270.51	00:12:39 - 02:02:10
<i>Candida tropicalis</i>	ATCC13803	12	10 - 10000	0.24 - 248.34	00:13:07 - 01:11:05
<i>Candida tropicalis</i>	ATCC201380	9	10 - 10000	0.24 - 270.51	00:14:06 - 02:01:10
<i>Candida tropicalis</i>	ATCC201381	7	10 - 10000	0.35 - 203.99	00:15:08 - 01:22:05
<i>Candida tropicalis</i>	ATCC750	8	10 - 10000	0.25 - 257.21	00:15:07 - 02:00:07
<i>Cryptococcus gattii</i>	ATCC14248	11	10 - 100	17.74 - 239.47	01:06:00 - 01:23:09
	ATCC76108	12	10 - 100	17.74 - 266.08	01:04:07 - 02:19:11
	ATCCMYA-4138	10	10 - 100	17.74 - 297.12	01:00:07 - 07:23:21 ^g
	ATCCMYA-4560	8	10 - 100	18.63 - 270.51	01:05:00 - 09:02:24 ^g
<i>Cryptococcus gattii</i>	ATCCMYA-4877	9	10 - 100	17.29 - 288.25	01:05:07 - 06:00:18 ^g
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	ATCC14116	9	1 - 100	17.29 - 2217.29	01:09:06 - 01:22:12
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	ATCC208821(H99)	8	10 - 100	18.63 - 292.68	01:07:00 - 03:00:17
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	ATCC90112	7	10 - 100	19.51 - 257.21	01:07:00 - 02:02:12
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	NCPF8195	7	10 - 100	19.51 - 235.03	01:13:03 - 02:01:11
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	NCPF8299	3	10 - 100	25.72 - 279.38	01:06:07 - 01:17:12
<i>Cryptococcus neoformans</i> var. <i>grubii</i>	NCPF8357	3	10 - 100	23.95 - 270.51	01:06:08 - 01:19:12
<i>Cryptococcus neoformans</i> var. <i>neoformans</i>	ATCC34873	3	10	190.69 - 261.64	01:14:17 - 01:20:12
<i>Cryptococcus neoformans</i> var. <i>neoformans</i>	ATCC36556	3	10	230.60 - 301.55	02:08:09 - 02:12:17
<i>Cryptococcus neoformans</i> var. <i>neoformans</i>	ATCCMYA-565	9	10 - 100	17.29 - 283.81	01:06:00 - 02:12:14
<i>Fusarium dimerum</i>	CBS108944	3	100000	2.26	01:13:09 - 01:19:05
<i>Fusarium dimerum</i>	CBS110317	9	10000 - 100000	2.35 - 3.44	01:21:09 - 08:12:17 ^h
<i>Fusarium dimerum</i>	CBS116520	3	100000	3.86	01:11:07 - 01:17:05
<i>Fusarium oxysporum</i>	CBS116611	1	1000	82.93	11:18:11

<i>Fusarium sacchari</i>	ATCC24379	10	10 - 10000	1.86 - 1862.53	02:04:17 - 04:18:15
	CBS119828	11	10 - 10000	13.46 - 13458.98	01:07:59 - 03:15:00
<i>Fusarium solani</i>	ATCC36031	11	10 - 10000	3.44 - 3436.81	01:22:11 - 03:03:30
<i>Fusarium verticillioides</i>	CBS100312	11	10 - 10000	6.27 - 6274.94	01:19:08 - 02:11:00
	ATCC38159	11	10 - 10000	4.57 - 4567.63	02:14:12 - 03:14:00
<i>Rhodotorula glutinis</i> ⁱ	ATCC32765	3	NA	N/A	NA
<i>Rhodotorula glutinis</i>	ATCC32766	3	NA	N/A	NA
<i>Rhodotorula glutinis</i>	ATCC96365	4	NA	N/A	NA
<i>Rhodotorula mucilaginosa</i>	ATCC66034	21	1 - 1000	2.04 - 2439.02	01:08:09 - 02:18:06
<i>Rhodotorula mucilaginosa</i>	ATCC9449	19	10	190.69 - 274.94	01:01:05 - 11:11:30 ^j

* A total of 678 contrived samples are listed; 47 negative contrived samples that are not included in this supplementary table as they did not contain organisms targeted by the BCID-FP Panel.

^a Volume spiked was 100uL except 1 replicate of *C. dubliniensis* ATCCMYA-578 (1 mL) and 1 replicate of *F. verticillioides* ATCC38159 (400 uL).

^b The majority of contrived samples were inoculated into the BacTec Myco/F lytic bottles (640). There were 39 samples inoculated in BacTec Plus Aerobic/F bottles, and 46 in BacTec Peds Pus/F bottles.

^c One replicate of the strain with 1:100 dilution took greater than 5 days to ring positive

^d Eleven replicates of the strain took greater than 5 days to ring positive.

^e One replicate of the strain took greater than 5 days to ring positive.

^f One of these replicates was a final, invalid result after ePlex BCID-FP testing, so this result was not included in the performance table (Table 2). There were 46 *C. lusitaniae* contrived samples tested but only 45 had valid results to be included.

^g One replicate of the strain took greater than 5 days to ring positive

^h Six replicates of the strain took greater than 5 days to ring positive.

ⁱ Contrived using quantified glycerol stocks (not grown in blood culture system).

^j Twelve replicates of the strain took greater than 5 days to ring positive.

Supplementary Table 3. Expected Value (Positivity Rate [%]) for the BCID-FP Panel by Age Group (Prospective Samples)

Target	All Ages (N=21)*	Age <1 (N=1)	Age 1-17 (N=2)	Age 18-44 (N=4*)	Age 45-64 (N=11*)	Age 65-84 (N=2)	Age 85+ (N=1*)
<i>Candida albicans</i>	4 (19.0)	1 (100)	0	0	2 (18.2)	1 (50.0)	0
<i>Candida auris</i>	0	0	0	0	0	0	0
<i>Candida dubliniensis</i>	1 (4.8)	0	0	0	1 (9.1)	0	0
<i>Candida famata</i>	0	0	0	0	0	0	0
<i>Candida glabrata</i>	6 (28.6)	0	1 (50.0)	1 (25.0)	3 (27.3)	1 (50.0)	0
<i>Candida guilliermondii</i>	0	0	0	0	0	0	0
<i>Candida kefyr</i>	0	0	0	0	0	0	0
<i>Candida krusei</i>	2 (9.5)	0	0	0	2 (18.2)	0	0
<i>Candida lusitanae</i>	0	0	0	0	0	0	0
<i>Candida parapsilosis</i>	2 (9.5)	0	1 (50.0)	0	1 (9.1)	0	0
<i>Candida tropicalis</i>	2 (9.5)	0	0	1 (25.0)	1 (9.1)	0	0
<i>Cryptococcus gattii</i>	0	0	0	0	0	0	0
<i>Cryptococcus neoformans</i>	0	0	0	0	0	0	0
<i>Fusarium</i>	0	0	0	0	0	0	0
<i>Rhodotorula</i>	1 (4.8)	0	0	1 (25.0)	0	0	0

* 3 samples only had off-panel organisms identified by standard-of-care methods (1 sample: *C. metapsilosis* and *Trichosporon asahii*; 1 sample: *Saccharomyces cerevisiae*; 1 sample: *Cryptococcus laurentii*); therefore, the ePlex BCID-FP Panel results were 'Not Detected' for all targets.

Supplementary Table 4. Demographics of subjects with positive blood culture for fungal organisms

	Prospectively-Collected (N=21)	Retrospectively-Collected (N=120)	All Clinical Samples (N=141)
Sex			
Male	14 (66.7)	68 (56.7)	82 (58.2)
Female	7 (33.3)	52 (43.3)	59 (41.8)
Age (years)			
Mean	48.1	53.5	52.7
Median	55.0	58.0	57.0
SD	22.4	22.5	22.5
Minimum	0.8	0.0	0.0
Maximum	90.0	89.0	90.0
<1 yr	1 (4.8)	2 (1.7)	3 (2.1)
1-17 yrs	2 (9.5)	8 (6.7)	10 (7.1)
18-44 yrs	4 (19.0)	27 (22.5)	31 (22.0)
45-64 yrs	11 (52.4)	39 (32.5)	50 (35.5)
65-84 yrs	2 (9.5)	39 (32.5)	41 (29.1)
85+ yrs	1 (4.8)	5 (4.2)	6 (4.3)

Supplementary Table 5. Blood Culture Bottle Type

	Contrived (N=725 ^a) n (%)	Prospective Fresh (N=11) n (%)	Prospective Frozen (N=10) n (%)	Retrospective (N=120) n (%)
BacT/Alert - SA (Standard Aerobic)	0	0	2 (20.0)	5 (4.2)
BacT/Alert - SN (Standard Anaerobic)	0	0	0	4 (3.3)
BacTec - Lytic/10 Anaerobic/F	0	0	1 (10.0)	19 (15.8)
BacTec - PLUS Aerobic/F	39 (5.4)	9 (81.8)	5 (50.0)	39 (32.5)
BacTec - PLUS Anaerobic/F	0	0	1 (10.0)	0
BacTec - Peds Plus /F	46 (6.3)	1 (9.1)	0	8 (6.7)
BacTec - Standard/10 Aerobic/F	0	0	0	27 (22.5)
BacTec - Standard Anaerobic/F	0	0	1 (10.0)	1 (0.8)
BacTec - Myco/F Lytic	640 (88.3)	0	0	0
VersaTREK - Redox 1 EZ Draw	0	1 (9.1)	0	17 (14.2)

^a Only including the evaluable samples. One contrived sample was excluded due to a final, invalid result.