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Multicenter Prospective Study of Biomarkers for Diagnosis of Invasive Candidiasis in Children and Adolescents

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Background. Diagnosis of invasive candidiasis (IC) relies on insensitive cultures; the relative utility of fungal biomarkers in children is unclear.

Methods. This multinational observational cohort study enrolled patients aged >120 days and <18 years with concern for IC from 1 January 2015 to 26 September 2019 at 25 centers. Blood collected at onset of symptoms was tested using T2Candida, Fungitell (1→3)-β-D-glucan, Platelia *Candida* Antigen (Ag) Plus, and Platelia *Candida* Antibody (Ab) Plus assays. Operating characteristics were determined for each biomarker, and assays meeting a defined threshold considered in combination. Sterile site cultures were the reference standard.

Results. Five hundred participants were enrolled at 22 centers in 3 countries, and IC was diagnosed in 13 (2.6%). Thirteen additional blood specimens were collected and successfully spiked with *Candida* species, to achieve a 5.0% event rate. Valid T2Candida, Fungitell, Platelia *Candida* Ag Plus, and Platelia *Candida* Ab Plus assay results were available for 438, 467, 473, and 473 specimens, respectively. Operating characteristics for T2Candida were most optimal for detecting IC due to any *Candida* species, with results as follows: sensitivity, 80.0% (95% confidence interval, 59.3%–93.2%), specificity 97.1% (95.0%–98.5%), positive predictive value, 62.5% (43.7%–78.9%), and negative predictive value, 98.8% (97.2%–99.6%). Only T2Candida and Platelia *Candida* Ag Plus assays met the threshold for combination testing. Positive result for either yielded the following results: sensitivity, 86.4% (95% confidence interval, 65.1%–97.1%); specificity, 94.7% (92.0%–96.7%); positive predictive value, 47.5% (31.5%–63.9%); and negative predictive value, 99.2% (97.7%–99.8%).

Conclusions. T2Candida alone or in combination with Platelia *Candida* Ag Plus may be beneficial for rapid detection of *Candida* species in children with concern for IC.

Clinical Trials Registration. NCT02220790.

Keywords. Pediatrics; invasive candidiasis; biomarkers.

Invasive candidiasis (IC) is a common cause of bloodstream infections in hospitalized patients [1] and associated with increased hospital lengths of stay, charges, and all-cause mortality rates [2, 3]. These infections can be particularly severe in children with cancer [4, 5] and after organ or hematopoietic cell transplantation [6–8].

Diagnosing IC in pediatric patients is challenging. Cultures are insensitive [9] and often take >24 hours for a preliminary positive result, delaying initial therapy [10] and leading to increased mortality rate [11, 12]. A single-center prediction rule to identify candidemia in pediatric intensive care unit patients found that multiple factors had a predictive probability of 46% [13]. However, an independent multicenter study failed to validate the model [14].

Fungal biomarkers are potential novel tools to identify *Candida* spp. in children with increased concern for IC. T2Candida, Fungitell, Platelia *Candida* Antigen (Ag) Plus, and Platelia *Candida* Antibody (Ab) Plus assays are approved for diagnosis of candidemia or IC in adults [15–23]. Furthermore,

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T2Candida and Fungitell are endorsed by an international consensus guideline for the diagnosis of IC in adults [24]. However, there are limited data on these biomarkers to diagnose IC in children [24–27].

The International Pediatric Fungal Network performed a prospective, multinational observational cohort study, BIOmarkers in Pediatric Invasive Candidiasis (BIOPIC), to define the operating characteristics of T2Candida, Fungitell (1→3)-β-D-glucan, Platelia *Candida* Ag Plus, and Platelia *Candida* Ab Plus assays individually and in combination among at-risk children and adolescents with signs concerning for IC.

METHODS

Study Design

BIOPIC is a prospective observational cohort study conducted by the International Pediatric Fungal Network [28]. Twenty-five sites (23 in the United States and 2 international) opened for enrollment (Supplementary Table 1). Each site obtained institutional review board approval locally.

Study Cohort

Eligible participants were aged >120 days and <18 years with clinical characteristics concerning for IC (Table 1). Participants were ineligible if they (1) had possible, probable, or proven invasive fungal disease according to European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria within the past 30 days; (2) had been previously included in the study; (3) weighed <4 kg, disallowing sufficient phlebotomy; or (4) were receiving empirical or targeted antifungal therapy.

Blood Sample Collection and Processing

A single blood sample (≤10.5 mL) was obtained within 24 hours of blood culture collection and aliquoted into ethylenediaminetetraacetic acid (EDTA) and serum separator tubes in a specified order (Supplementary Table 2). Sites shipped

frozen specimens to a central repository; after enrollment completion, specimens were shipped to 2 testing laboratories for performance of the 4 different biomarker assays (Appendix B in the Supplementary Materials). Laboratory personnel completing these assays were blinded to the clinical outcomes of the enrolled participants.

Preparation of Spiked Specimens

The estimated 5% event rate (pretest probability) of proven or probable IC was not achieved. An institutional review board amendment was approved to prospectively enroll 15 additional pediatric patients without concern for IC at 1 site. These patients' blood specimens were spiked using a similar technique, detailed in Appendix A (Supplementary Materials), that led to Food and Drug Administration approval for the T2Candida assay [23]. Distribution of species spiked was determined by epidemiology in a large pediatric cohort of IC [3]. All biomarker assay testing for these specimens was done directly on the spiked blood specimen.

Fungal Biomarker Assays

T2Candida Assay

The T2Candida assay (T2Biosystems) was performed using frozen whole-blood samples thawed at room temperature. Samples with volumes ≥3 mL were loaded directly to the sample inlet snorkel. Samples with volumes of 2–3 mL were pipetted by hand into the sample inlet; the opening of each sample inlet was pierced with a sterile 1-mL pipette tip, and a new sterile 1-mL pipette tip was used to load each sample. Samples <2.0 mL were excluded from testing. After a run time of approximately 4–8 hours, depending on number of samples, results of either “detected” or “not detected” were displayed. The lower limit of detection is 1 colony-forming unit/mL.

Fungitell Assay

Fungitell assay (Associates of Cape Cod) was performed according to manufacturer using frozen serum. Each specimen was tested in duplicate. Specimens required a percentage coefficient of variation <20% to be resulted; excessive percentage coefficient of variation values caused specimens to be retested or resulted as invalid. Following manufacturer guidance, (1→3)-β-D-glucan values <60 pg/mL were negative, values of 60–79 pg/mL were indeterminate, and values ≥80 pg/mL were positive. Figure 1 shows the testing algorithm for interpretation based on manufacturer guidance and prior publications [29, 30].

Platelia *Candida* Ag Plus Assay

Platelia *Candida* Ag Plus (Bio-Rad) was performed using frozen serum, according to the manufacturer's instructions. Specimens with concentrations <62.5 pg/mL were negative; concentrations from 62.5 to <125 pg/mL were indeterminate; and concentrations ≥125 pg/mL were positive.

Table 1. Criteria for Increased Clinical Concern for Invasive Candidiasis

At least one of the following underlying diagnoses/clinical conditions:
Intestinal failure (eg, short-gut syndrome)
Solid tumor or hematologic malignancy
Aplastic anemia
Solid organ transplant recipient
Hematopoietic stem cell transplant recipient
Any patient currently in or imminently transferred to a non-neonatal ICU
All of the following current clinical management:
Presence of at least one central catheter (arterial or venous)
Blood culture drawn for clinical concern of infection
Initiation or change in systemic nonantifungal antimicrobial therapy at time of blood culture attainment

Abbreviation: ICU, intensive care unit.

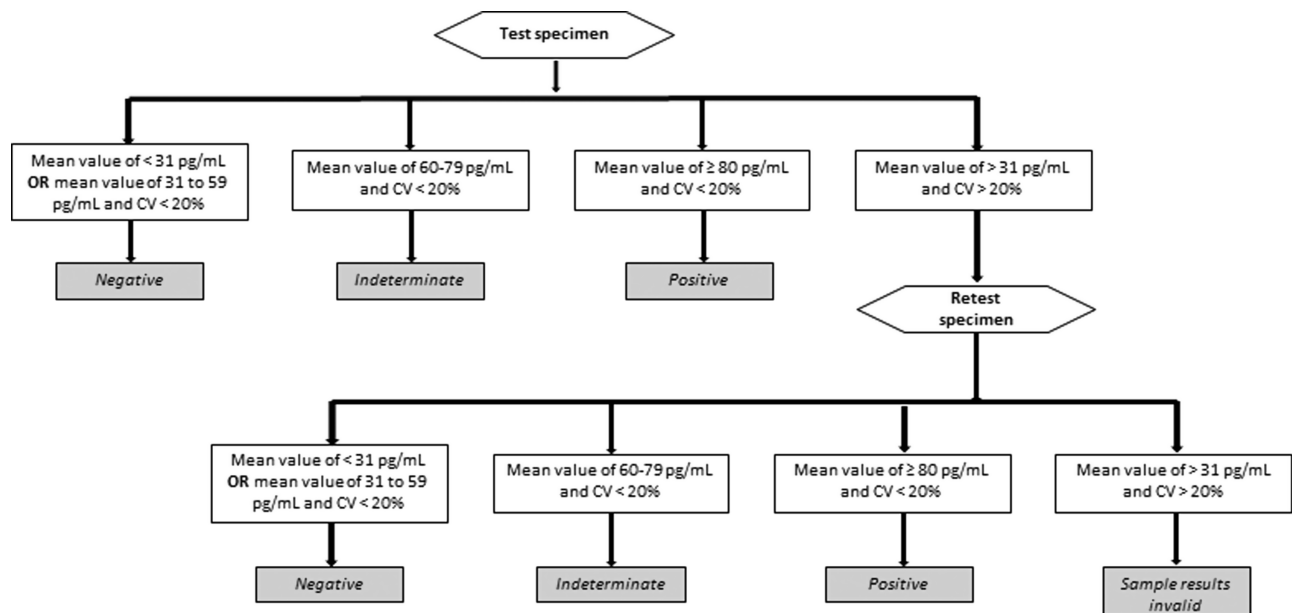


Figure 1. Testing algorithm for the Fungitell (1→3)-β-D-glucan assay. Abbreviation: CV, coefficient of variation.

Table 2. Demographic and Clinical Characteristics of Participants Contributing a Blood Specimen

	Any Specimen (N = 486)	EDTA Testing ^a (n = 474)	SST Testing ^b (n = 460)
Age	Median, (IQR)	Median, (IQR)	Median, (IQR)
	6.0, (2.5–11.8)	6.1, (2.6–11.8)	6.2, (2.8–11.7)
Race	n, (%)	n, (%)	n, (%)
Asian	18, 3.7	18, 3.8	18, 3.9
Black	55, 11.4	55, 11.6	51, 11.1
Other	24, 4.9	24, 5.1	22, 4.8
Unknown/not reported	25, 5.1	24, 5.1	21, 4.6
White	364, 74.9	353, 74.5	348, 75.7
Ethnicity			
Hispanic/Latino	65, 13.4	64, 13.5	59, 12.8
Not Hispanic/Latino	413, 85.0	402, 84.8	393, 85.4
Unknown/not reported	8, 1.6	8, 1.7	8, 1.7
Gender			
Female	239, 49.2	231, 48.7	224, 48.7
Male	247, 50.8	243, 51.3	236, 51.3
Underlying Condition^c			
Cardiac disorders	31, 6.4	28, 5.9	28, 6.1
Genetic/metabolic/congenital disorders	42, 8.6	40, 8.4	38, 8.3
Hematologic malignancy	158, 32.5	156, 32.9	154, 33.5
Intestinal failure ^d	93, 19.1	92, 19.4	86, 18.7
Neurologic disorders	36, 7.4	34, 7.2	32, 7.0
Respiratory disorders	42, 8.6	39, 8.2	37, 8.0
Solid organ transplant	31, 6.4	30, 6.3	29, 6.3
Solid tumor malignancy	114, 23.5	113, 23.8	112, 24.3
Stem cell transplant	46, 9.5	42, 9.2	42, 9.1
Surgery/Trauma in the last 2 weeks	45, 9.3	42, 8.9	43, 9.3
Other ^e	23, 4.7	23, 4.9	20, 4.3
Previously healthy	1, 0.2	1, 0.2	1, 0.2

Abbreviations: EDTA, ethylenediaminetetraacetic acid; IQR, interquartile range; SST, serum separator tube.

^a Used by T2Candida assay.

^b Used by Fungitell, Platelia Candida Antigen Plus and Platelia Candida Antibody Plus assays.

^c Sum of categories may be greater than the overall number of subjects because some subjects may have multiple underlying conditions.

^d Intestinal failure includes conditions such as short gut, bowel dysmotility syndrome, etc.

^e Other includes: autoimmune disease, cutaneous disorders, endocrine disorders, inherited immunodeficiency, invasive infection, lymphatic disorders, prematurity, and renal disorders.

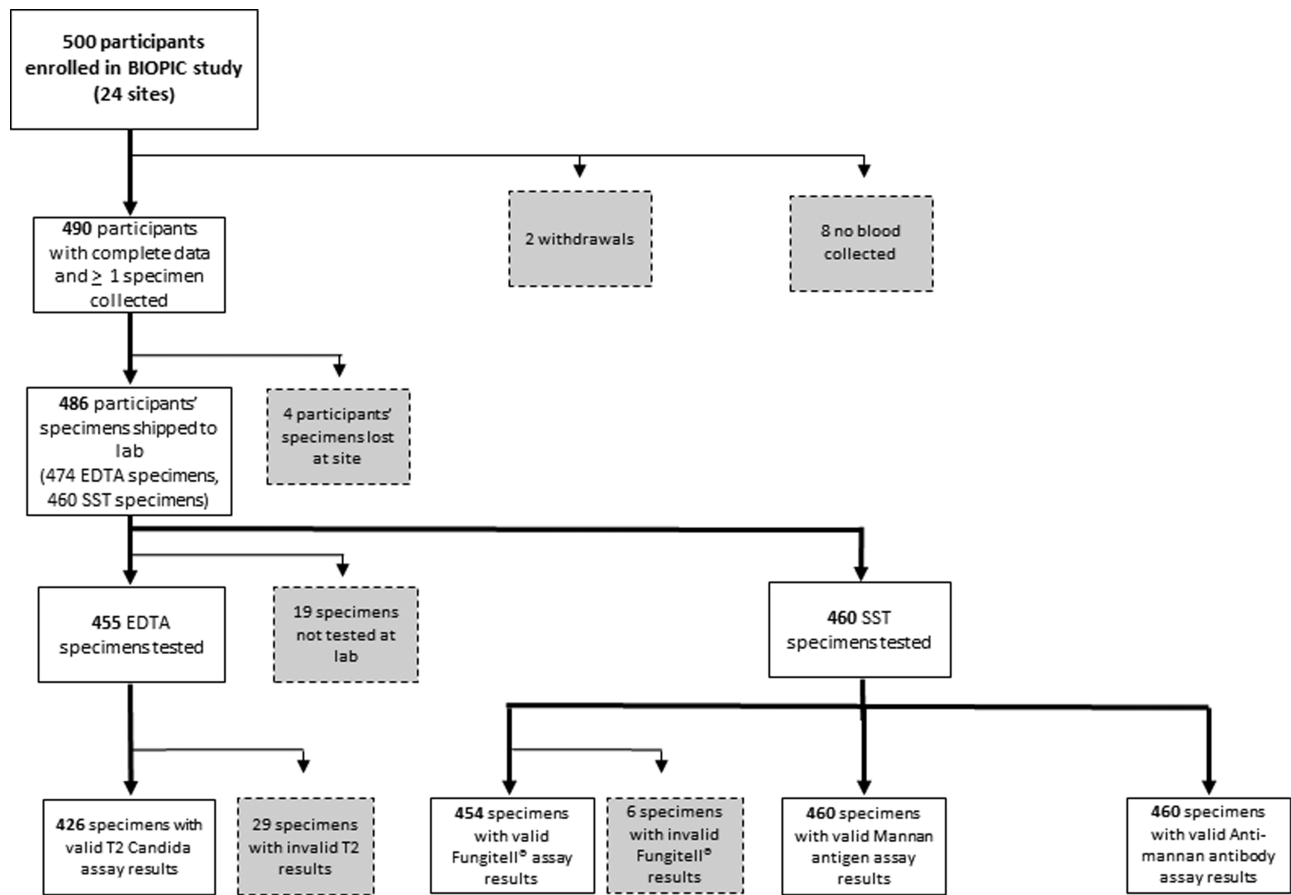


Figure 2. Application of exclusion criteria to determine evaluable specimens for analysis. Gray boxes represent participants excluded from analysis. Results of 29 T2Candida assays were reported as invalid, included 5 specimens with instrument errors during assay performance and 24 whose results were invalidated owing to failure of the internal positive control. Abbreviations: BIOPIC, Biomarkers in Pediatric Invasive Candidiasis; EDTA, ethylenediaminetetraacetic acid; SST, serum separation tube.

Platelia Candida Ab Plus Assay

Platelia Candida Ab Plus (Bio-Rad) was performed using frozen serum, according to manufacturer instructions, to detect antibodies to *Candida* mannan antigen. Specimens with concentrations <5 AU/mL were negative, concentrations from 5 to <10 AU/mL were indeterminate, and concentrations ≥10 AU/mL were positive.

Outcome

The primary end point of proven or probable IC was defined by the 2008 European Organization for Research and Treatment of Cancer/Mycoses Study Group criteria for invasive fungal disease [31]. For a patient to meet the primary end point, diagnosis of proven or probable IC must have been made on or between day 0 (day of enrollment) through day 14.

Data Collection

Data were prospectively collected using Research Electronic Data Capture (REDCap) [32]. The coordinating center was Duke University, and the analysis center was Children's Hospital of Philadelphia. Patient data were entered by sites

and reviewed, with quarterly automated data checks and queries to sites. Baseline data elements collected included patient demographics, underlying disease, and clinical and immunologic risk factors. In addition, exposures to a range of products that may lead to a false result for ≥1 assay were collected.

Statistical Analysis

Sample Size and Power

Based on a study sample size of 500 and a pretest probability of 5%, power calculations estimated an assay with sensitivity and specificity of 90% would provide a positive predictive value (PPV) of 32% (95% confidence interval [CI], 26%–39%) and a negative predictive value (NPV) of 99% (98%–100%). These predictive probabilities were deemed a priori to be clinically useful, as a positive test result would substantially increase concern for IC (ie, increase from a 5% pretest probability of disease to a 32% posttest probability) and a negative test would substantially decrease a clinician's concern for IC (ie, decrease from a 5% pretest probability of disease to a 1% posttest probability).

Table 3. Listing of *Candida* Species Identified From Enrolled Patients and Used in Spiked Specimens

	Species	Day of first detection ^a	Site of First Detection ^a	Spiking Concentration ^b (CFU/ mL)
Specimens				
Clinical infections				
	<i>C. albicans</i>	0	Blood	...
	<i>C. albicans</i>	4	Peritoneal	...
	<i>C. guilliermondii</i>	7	Blood	...
	<i>C. parapsilosis</i>	0	Blood	...
	<i>C. parapsilosis</i>	0	Blood	...
	<i>C. parapsilosis</i>	0	Blood	...
	<i>C. parapsilosis</i>	0	Blood	...
	<i>C. parapsilosis</i>	0	Blood	...
	<i>C. parapsilosis</i>	11	Blood	...
	<i>C. tropicalis</i>	0	Blood	...
	<i>C. tropicalis</i>	0	Blood	...
	<i>C. tropicalis</i>	0	Blood	...
	<i>C. tropicalis</i>	0	Blood	...
Spiked Specimens				
	<i>C. albicans</i>	8.7
	<i>C. albicans</i>	8.7
	<i>C. albicans</i>	8.7
	<i>C. albicans</i>	13.2
	<i>C. glabrata</i>	7.5
	<i>C. krusei</i>	10.0
	<i>C. lusitanae</i>	11.1
	<i>C. parapsilosis</i>	6.5
	<i>C. parapsilosis</i>	8.6
	<i>C. parapsilosis</i>	10.0
	<i>C. parapsilosis</i>	15.0
	<i>C. tropicalis</i>	7.4
	<i>C. tropicalis</i>	7.4

Abbreviation: CFU, colony-forming unit.

^aDay and site of first detection applies only to natural infections.

^bSpiking concentration applies only to spiked specimens.

Primary Analysis

For each assay, sensitivity, specificity, PPV, and NPV with 95% CIs were calculated using manufacturer-recommended positivity thresholds; calculated sensitivity and specificity then informed likelihood ratios and posttest probability for the presence or absence of IC for a range of hypothetical IC prevalence rates (1%, 2%, 5%, and 10%). Each assay sensitivity, specificity, PPV, and NPV were recalculated with 95% CIs, excluding from the analysis spiked specimens ([Appendix B \[Supplementary Materials\]](#)).

Sensitivity Analysis

To consider diagnostic ability in a more contemporaneous time frame, sensitivity analyses considered shorter outcome windows (0–7 and 0–2 days). Because the T2Candida assay is designed to target 5 *Candida* spp. (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*), we repeated analyses for this assay limiting outcome to these 5 species. A final sensitivity analysis excluded specimens exposed within relevant exposure windows to products reported as possible sources of false-positive Fungitell (1→3)-β-D-glucan, Platelia *Candida*

Ag Plus, or Platelia *Candida* Ab Plus results and false-negative T2Candida results ([Appendix B \[Supplementary Materials\]](#)).

Assay Results Considered in Combination

We hypothesized that multiple biomarker assays considered in combination could potentially optimize operating characteristics. We decided ad hoc that only quantitative assays with an area under the receiver operating characteristic (ROC) curve (AUC) >0.65 would be considered in subsequent combination algorithms. Potential combination testing considered assays performed in parallel or series. Further details are in the statistical analysis plan ([Appendix B \[Supplementary Materials\]](#)). Instead of using the manufacturer-recommended positivity threshold when evaluating the combinations incorporating quantitative assays, the positivity threshold was determined using an optimal operating slope (OOS) approach to minimize expected cost of mistakes from diagnosis decisions based on the test result [33–35]. The OOS was defined as $(1 - p)C_{FP}/pC_{FN}$, where p was the pretest probability; C_{FP} , the cost of false-positive mistakes; and C_{FN} , the cost of false-negative mistakes. The value at which the tangency on the ROC curve equals the OOS was

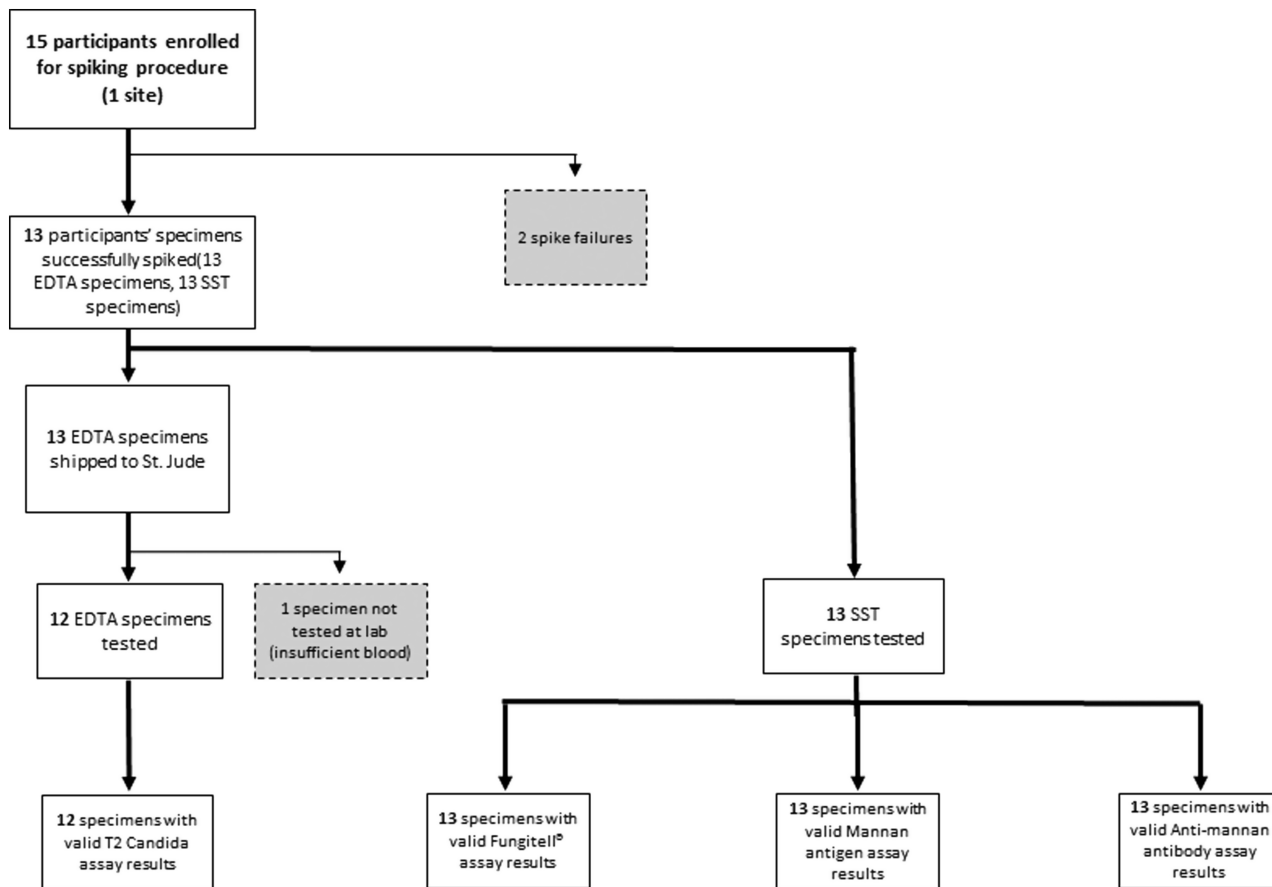


Figure 3. Processing of specimens for subjects enrolled under specimen spiking protocol. Gray boxes represent specimens excluded from analysis. Abbreviations: EDTA, ethylenediaminetetraacetic acid; SST, serum separation tube.

considered the optimal threshold. If none of the candidate points' tangencies on the ROC equaled the OOS, then we used the value whose tangency most closely approximated the OOS.

RESULTS

Between 1 January 2015 and 26 September 2019, a total of 500 patients with concern for IC were enrolled at 22 centers (Table 2). The cohort's median age (interquartile range) was 6.0 (2.5–11.8) years, and patients were predominantly white (74.9%) and not Hispanic or Latino (84.9%). Accounting for withdrawals and inadequate or lost specimens, 486 participants provided ≥ 1 specimen for testing (Figure 2). Only 1 specimen was collected >24 hours after the blood culture was obtained. The 474 EDTA specimens yielded 426 valid T2Candida results (89.9%), and the 460 serum separator tube specimens yielded valid Fungitell, Platelia *Candida* Ag Plus, and Platelia *Candida* Ab Plus results for 454 (98.7%), 460 (100%), and 460 (100%) of the specimens, respectively. Thirteen IC events were identified in the 14-day follow-up period. All but 1 of the clinical infections were candidemia events (Table 3).

Separately, 15 patients without concern for IC were enrolled at 1 center and had a blood specimen collected, 13 of which

were successfully spiked with *Candida* spp. (Table 3). All successfully spiked specimens had a valid result for the Fungitell, Platelia *Candida* Ag Plus, and Platelia *Candida* Ab Plus assays; 12 spiked specimens had a valid result for the T2Candida assay (Figure 3).

Primary Analysis

Table 4 presents the sensitivity, specificity, PPV, and NPV individually for all 4 biomarkers across the outcome window of 0–14 days after specimen collection. The T2Candida assay had the best operating characteristics (sensitivity, 79.2%; specificity, 97.1%; PPV, 61.3%; NPV, 98.8%) for the 14-day outcome window, followed by the Platelia *Candida* Ag Plus (sensitivity, 39.1%; specificity, 96.7%; PPV, 37.5%; NPV 96.9%), Platelia *Candida* Ab Plus (sensitivity, 21.7%; specificity, 94.9%; PPV, 17.9%; NPV, 96.0%), and Fungitell (sensitivity, 9.1%; specificity, 85.4%; PPV, 3.0%; NPV, 95.0%). Supplementary Table 3 compares the operating characteristics for each biomarker and each outcome window when including and excluding spiked specimens. Positive and negative likelihood ratios for each assay are presented in Supplementary Table 4.

Table 4. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for Each Biomarker by Different Outcome Assessment Windows

Biomarker	Sensitivity		Specificity		Positive Predictive Value		Negative Predictive Value	
	n, (%)	95% CI	n, (%)	95% CI	n, (%)	95% CI	n, (%)	95% CI
T2Candida^a								
Any <i>Candida</i> spp.								
0–14 d	19/24, (79.2)	57.9–92.9	402/414, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	402/407, (98.8)	97.2–99.6
0–7 d	19/23, (82.6)	61.2–95.1	403/415, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	403/407, (99.0)	97.5–99.7
0–2 d	19/21, (90.5)	69.6–98.8	405/417, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	405/407, (99.5)	98.2–99.9
Targeted <i>Candida</i> spp. ^b								
0–14 d	19/22, (86.4)	65.1–97.1	404/416, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	404/407, (99.3)	97.9–99.9
0–7 d	19/21, (90.5)	69.6–98.8	405/417, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	405/407, (99.5)	98.2–99.9
0–2 d	19/20, (95.0)	75.1–99.9	406/418, (97.1)	95.0–98.5	19/31, (61.3)	42.2–78.2	406/407, (99.8)	98.6–100.0
Fungitell^c								
Any <i>Candida</i> spp.								
0–14 d	2/22, (9.1)	1.1–29.2	380/445, (85.4)	81.8–88.5	2/67, (3.0)	0.4–10.4	380/400, (95.0)	92.4–96.9
0–7 d	2/21, (9.1)	1.2–30.4	381/446, (85.4)	81.8–88.6	2/67, (3.0)	0.4–10.4	381/400, (95.3)	92.7–97.1
0–2 d	2/19, (10.5)	1.3–33.1	383/448, (85.5)	81.9–88.6	2/67, (3.0)	0.4–10.4	383/400, (95.8)	93.3–97.5
Platelia <i>Candida</i> Antigen Plus^d								
Any <i>Candida</i> spp.								
0–14 d	9/23, (39.1)	19.7–61.5	435/450, (96.7)	94.6–98.1	9/24, (37.5)	15.4–30.8	435/449, (96.9)	94.8–98.3
0–7 d	9/22, (40.9)	20.7–63.7	436/451, (96.7)	94.6–98.1	9/24, (37.5)	15.4–30.8	436/449, (97.1)	95.1–98.5
0–2 d	8/20, (40.0)	19.1–64.0	437/453, (96.5)	94.3–98.0	8/24, (33.3)	15.6–55.3	437/449, (97.3)	95.4–98.6
Platelia <i>Candida</i> Antibody Plus^e								
Any <i>Candida</i> spp.								
0–14 d	5/23, (21.7)	7.5–43.7	427/450, (94.9)	92.4–96.7	5/28, (17.9)	6.1–36.9	427/445, (96.0)	93.7–97.6
0–7 d	5/22, (22.7)	7.8–45.4	428/451, (94.9)	92.5–96.7	5/28, (17.9)	6.1–36.9	428/445, (96.2)	94.0–97.8
0–2 d	4/20, (20.0)	5.7–43.7	429/453, (94.7)	92.2–96.6	4/28, (14.3)	4.0–32.7	429/445, (96.4)	94.2–97.9

Abbreviations: CI, confidence interval.

^a467 total specimens (455 specimens, 12 spikes) tested at lab and 438 specimens with valid results (93.8%); 24 specimens with valid result ever had event detected (5.5%).^bOne enrolled subject's specimen and one spiked specimen had a *Candida* species which is not detectable by the T2Candida assay and was not considered an event for this analysis. Effective event rate was 5.0%.^c473 specimens (460 specimens, 13 spikes) received at lab, 467 specimens with valid result (98.7%), 22 specimens with valid result ever had event detected (4.7%); manufacturer's recommended positivity cutoff: ≥ 80 pg/mL.^d473 specimens (460 specimens, 13 spikes) received at lab, all had valid result; 23 specimens ever had event detected (4.9%); manufacturer's recommended positivity cutoff: ≥ 125 pg/mL.^e473 specimens (460 specimens, 13 spikes) received at lab, all had valid result; 23 specimens ever had event detected (4.9%); manufacturer's recommended positivity cutoff: ≥ 10 AU/mL.

Using sensitivity and specificity metrics for each biomarker, the positive and negative posttest probabilities were calculated for disease prevalence rates of 1%, 2%, 5%, and 10% (Table 5). The probabilities of IC after a positive assay result across the range of prevalence rates were most optimal for the T2Candida assay, ranging from 21.6% in a 1% prevalence scenario to 75.2% in a 10% prevalence scenario. Of the remaining assays, Platelia *Candida* Ag had the highest posttest probability positive results, ranging from 10.6% in a 1% prevalence scenario to 56.6% in a 10% prevalence scenario.

Sensitivity Analysis

When limiting events to those *Candida* spp. targeted by T2Candida, the sensitivity, specificity, PPV, and NPV of T2Candida were 86.4%, 97.1%, 61.3%, and 99.3%, respectively (Table 4). Compared with the 0–14-day outcome window, windows of 0–7 and 0–2 days improved T2Candida sensitivity. Sensitivity was highest (90.5%) when restricting the outcome window to 0–2 days, while retaining similar specificity. Adjustment of the outcome window

did not demonstrably affect the other 3 biomarkers (Table 4). The *Candida* spp. identified by traditional cultures for each patient with a false-negative biomarker result are displayed by biomarker in Supplementary Table 5.

Removing specimens that tested positive but in the presence of a condition associated with false-positive results decreased sensitivity and increased specificity and PPV for Fungitell and decreased the sensitivity and increased PPV for Platelia *Candida* Ag Plus. Removing specimens that tested negative but in the presence of a condition associated with false-negative results slightly improved T2Candida sensitivity (Table 6).

Assay Results Considered in Combination

The AUCs from the Fungitell (0.5265) and Platelia *Candida* Ab (0.5959) assays did not reach the ad hoc threshold of 0.65 necessary to be included in combination testing algorithms. The AUC for the Platelia *Candida* Ag (0.6535) exceeded the threshold, and this assay was thus considered in combination with T2Candida.

Table 5. Calculated Post-Test Probabilities for Each Biomarker by Fixed Prevalence Rates and by Different Outcome Assessment Windows

	Posttest Probability With 0–14-d Window, %		Posttest Probability With 0–7-d Window, %		Posttest Probability With 0–2-d Window, %	
	Candidiasis (%)	No Candidiasis (%)	Candidiasis (%)	No Candidiasis (%)	Candidiasis (%)	No Candidiasis (%)
T2Candida Any <i>Candida</i> spp.	(79.2% Sens, 97.1% Spec)		(82.6% Sens, 97.1% Spec)		(90.5% Sens, 97.12% Spec)	
Prevalence (%)						
1	21.6	99.8	22.4	99.8	21.4	99.9
2	35.8	99.6	36.8	99.6	39.1	99.8
5	59.0	98.9	60.1	99.1	62.3	99.5
10	75.2	97.7	76.0	98.0	77.7	98.9
T2 Candida Targeted <i>Candida</i> spp.	(86.4% Sens, 97.1% Spec)		(90.5% Sens, 97.1% Spec)		(95.0% Sens, 97.1% Spec)	
Prevalence (%)						
1	23.2	99.9	24.1	99.9	25.1	99.9
2	37.9	99.7	39.1	99.8	40.3	99.9
5	61.2	99.3	62.3	99.5	63.5	99.7
10	76.9	98.5	77.7	98.9	78.6	99.4
Fungitell	(91% Sens, 85.4% Spec)		(9.1% Sens, 85.4% Spec)		(10.5% Sens, 85.5% Spec)	
Prevalence (%)						
1	0.6	98.9	0.7	98.9	0.7	99.0
2	1.3	97.9	1.3	97.9	1.5	97.9
5	3.2	94.5	3.3	94.7	3.7	94.8
10	6.5	89.4	6.8	89.5	7.5	89.6
Platelia <i>Candida</i> Antigen Plus	(39.1% Sens, 96.7% Spec)		(40.9% Sens, 96.7% Spec)		(40.0% Sens, 96.5% Spec)	
Prevalence (%)						
1	10.6	99.4	11.1	99.4	10.3	99.4
2	19.3	98.7	20.1	98.8	18.8	98.7
5	38.2	96.8	39.3	96.9	37.3	96.8
10	56.6	93.5	57.7	93.6	55.7	93.5
Platelia <i>Candida</i> Antibody Plus	(21.7% Sens, 94.9% Spec)		(22.7% Sens, 94.9% Spec)		(20.0% Sens, 94.7% Spec)	
Prevalence (%)						
1	4.1	99.2	4.3	99.2	3.7	99.2
2	8.0	98.3	8.3	98.4	7.2	98.3
5	18.3	95.8	19.0	95.9	16.6	95.7
10	32.1	91.6	33.1	91.7	30.0	91.4

Abbreviations: Sens, sensitivity; Spec, specificity

The prevalence in the subset of 418 specimens, including 12 spiked specimens, with both a valid T2Candida and Platelia *Candida* Ag Plus assay result available, was 5.3%; to approximate this, a prevalence of 5% was used to calculate the Platelia *Candida* Ag Plus OOS. The Platelia *Candida* Ag Plus cutoff point of 168.96 pg/mL performed best in scenarios where the cost of a false-negative was up to approximately 8 times the cost of a false-positive; this cutoff point was used as the positivity threshold for combination testing. The sensitivity was 86.4%, the specificity 94.7%, the PPV 47.5%, and the NPV 99.2% when either the T2Candida assay or Platelia *Candida* Ag Plus assay result was positive. Requiring both assay results to be positive resulted in a sensitivity of 31.8%, a specificity of 99.8%, a PPV of 87.5%, and an NPV of 96.3% (Table 7).

DISCUSSION

This is the largest prospective study of fungal biomarkers for the diagnosis of IC in any age group. In this cohort of hospitalized children at increased risk for IC, the T2Candida assay had the highest sensitivity (79.2%) and specificity (97.1%), followed by the Platelia *Candida* Ag Plus assay (sensitivity, 39.1%; specificity, 96.7%). The T2Candida assay sensitivity improved when the outcome window was narrowed to 0–2 days (90.5% [95% CI, 69.6%–98.8%]), but narrowing the outcome window did not demonstrably affect operating characteristics of the Platelia *Candida* Ag Plus assay. While the specificities of Platelia *Candida* Ab Plus and the Fungitell assays were adequate, neither exceeded 25% sensitivity for any outcome window considered.

Based on these results, the T2Candida assay is the only assay of the 4 investigated with sufficient sensitivity and specificity

Table 6. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for Each Biomarker By Different Outcome Assessment Windows, Excluding Specimens Exposed to Conditions Increasing Likelihood of False Positive or False Negative Results

T2Candida ^a	Sensitivity		Specificity		Positive Predictive Value		Negative Predictive Value	
	n, (%)	95% CI	n, (%)	95% CI	n, (%)	95% CI	n, (%)	95% CI
Any <i>Candida</i> spp.								
0–14 d	19/23, (82.6)	61.2–95.1	311/323, (96.3)	93.6–98.1	19/31, (61.3)	42.2–78.2	311/315, (98.7)	96.8–99.7
0–7 d	19/22, (86.4)	65.1–97.1	312/324, (96.3)	93.6–98.1	19/31, (61.3)	42.2–78.2	312/315, (99.1)	97.2–99.8
0–2 d	19/21, (90.5)	69.6–98.8	313/325, (93.6)	93.6–98.1	19/31, (61.3)	42.2–78.2	313/315, (99.4)	97.7–100.0
Targeted <i>Candida</i> spp.^b								
0–14 d	19/22, (86.4)	65.1–97.1	312/324, (96.3)	93.6–98.1	19/31, (61.3)	42.2–78.2	312/315, (99.1)	97.2–99.8
0–7 d	19/21, (90.5)	69.6–98.8	313/325, (96.3)	93.6–98.1	19/31, (61.3)	42.2–78.2	313/315, (99.4)	97.7–99.9
0–2 d	19/20, (95.0)	75.1–99.9	314/326, (96.3)	93.7–98.1	19/31, (61.3)	42.2–78.2	314/315, (99.7)	98.2–100.0
Fungitell^c								
Any <i>Candida</i> spp.								
0–14 d	1/21, (4.8)	0.1–23.8	380/387, (98.2)	96.3–99.3	1/8, (12.5)	0.3–52.7	380/400, (95.0)	92.4–96.9
0–7 d	1/20, (5.0)	0.1–24.9	381/388, (98.2)	96.3–99.3	1/8, (12.5)	0.3–52.7	381/400, (95.3)	92.7–97.1
0–2 d	1/18, (5.6)	0.1–27.3	383/390, (98.2)	96.3–99.3	1/8, (12.5)	0.3–52.7	383/400, (95.8)	93.3–97.5
Platelia <i>Candida</i> Antigen Plus^d								
Any <i>Candida</i> spp.								
0–14 d	6/20, (30.0)	11.9–54.3	435/441, (98.6)	97.1–99.5	6/12, (50.0)	21.1–78.9	435/449, (96.9)	94.8–98.3
0–7 d	6/19, (31.6)	12.6–56.6	436/442, (98.6)	97.1–99.5	6/12, (50.0)	21.1–78.9	436/449, (97.1)	95.1–98.5
0–2 d	6/18, (33.3)	13.3–59.0	437/443, (98.7)	97.1–99.5	6/12, (50.0)	21.1–78.9	437/449, (97.3)	95.4–98.6
Platelia <i>Candida</i> Antibody Plus^e								
Any <i>Candida</i> spp.								
0–14 d	5/23, (21.7)	7.46–43.7	427/450, (94.9)	92.4–96.7	5/28, (17.9)	6.1–36.9	427/445, (96.0)	94.0–97.6
0–7 d	5/22, (22.7)	7.82–45.4	428/451, (94.9)	92.5–96.7	5/28, (17.9)	6.1–36.9	428/445, (96.2)	94.0–97.8
0–2 d	4/20, (20.0)	5.73–43.7	429/453, (94.7)	92.2–96.6	4/28, (14.3)	4.0–32.7	429/445, (96.4)	94.2–97.9

See Table 2 for list of conditions and exposures considered to cause false positives for each assay.

Abbreviation: CI, confidence interval.

^a467 total specimens (455 specimens, 12 spikes) tested at lab and 438 specimens with valid results (93.8%); 92 negative specimens (92 specimens, 0 spikes) excluded as potential false negatives, 23/346 retained specimens had event detected (6.7%).

^bOne retained spiked specimen had a *Candida* species cultured which is not detectable by the T2Candida assay and was not considered an event for this analysis. Effective event rate was 6.4%.

^c473 specimens (460 specimens, 13 spikes) received at lab, 467 specimens with valid result; 59 positive specimens (58 specimens, 1 spike) excluded as potential false positives, 21/408 retained specimens had event detected (5.2%); manufacturer's recommended positivity cutoff: ≥ 80 pg/mL.

^d473 specimens (460 specimens, 13 spikes) received at lab; 12 positive specimens (11 specimens, 1 spike) excluded as potential false positives, 20/461 retained specimens had event detected (4.3%); manufacturer's recommended positivity cutoff: ≥ 125 pg/mL.

^e473 specimens (460 specimens, 13 spikes) received at lab; no potential false positives identified; 23 specimens ever had event detected (4.9%); manufacturer's recommended positivity cutoff: ≥ 10 AU/mL.

to be considered individually as a tool for diagnosis of IC in at-risk children and adolescents. Notably, when IC prevalence (ie, the pretest probability) was 5% or 10%, a positive test result increased posttest probability to almost 60%, and a negative test result decreased posttest probability to just above 2%.

These operating characteristics are similar to those reported in a cohort of adult patients for a similar clinical indication [23]. In contrast, the operating characteristics of the Fungitell assay in our cohort were inconsistent with prior adult studies [36, 37]. Sensitivity among adult patients is reportedly much higher,

Table 7. Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value for the T2Candida and Platelia *Candida* Antigen Plus Results Used in Combination

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Specimens Exceeding Cutoff
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	n
T2Candida only	77.3 (54.6, 92.2)	97.2 (95.1, 98.6)	60.7 (40.6, 78.5)	98.7 (97.0, 99.6)	28
Platelia <i>Candida</i> Antigen Plus only	40.9 (20.7, 63.7)	97.2 (95.1, 98.6)	45.0 (23.1, 68.5)	96.7 (94.5, 98.3)	20
At least one test positive	86.4 (65.1, 97.1)	94.7 (92.0, 96.7)	47.5 (31.5, 63.9)	99.2 (97.7, 99.8)	40
Both tests positive	31.8 (13.9, 54.9)	99.8 (98.6, 100.0)	87.5 (47.4, 99.7)	96.3 (94.0, 97.9)	8

418 specimen pairs (406 enrolled, 12 spikes) included in analysis, 22 specimens with event (5.3%).

resulting in guideline endorsement of the assay for diagnosis of IC in adults [24].

When evaluating posttest probabilities of the T2Candida assay, one must consider similar metrics for blood cultures, the current diagnostic standard. In a review of autopsy-informed investigations, the sensitivity of blood cultures was as low as 50% [9]. Assuming the same specificity as the T2Candida assay of 97.1% and disease prevalence of 5.0%, the associated positive and negative posttest probabilities would be 51% and 3%, respectively, worse than for the T2Candida assay. In addition, using sterile culture results as the reference standard for T2Candida assay may have limited the estimated operating characteristics of this assay. Limited clinical details for patients with a false-positive T2Candida assay result were available and provided in [Supplementary Table 6](#). Most patients had either gastrointestinal insufficiency, cancer, or both. It is possible that the assay was detecting *Candida* spp. transiently present in the bloodstream that were not detected by routine culture. The clinical relevance of this possibility cannot be determined from these data.

We assessed whether combining results of the Platelia *Candida* Ag with T2Candida could improve the diagnostic potential of either test alone. A testing approach of ≥ 1 positive result improved sensitivity to 86.4% and negative posttest probability to $< 1\%$ compared with either assay alone, but it reduced specificity (94.7%) and positive posttest probability (47.5%). Requiring that both test results be positive optimized specificity (99.8%) and positive posttest probability (87.5%) but compromised sensitivity (31.8%) and negative posttest probability (3.7%).

Ultimately, choosing a diagnostic approach that considers T2Candida assay alone or in combination with the Platelia *Candida* Ag Plus assay depends on the goals of care for a clinical situation. Posttest probabilities across differing prevalence rates of T2Candida assay performed alone may sufficiently inform initiation or cessation of antifungal therapy in many clinical circumstances. However, in clinical situations where greater certainty is desired before starting or stopping antifungal therapy, a 2-test approach may be more optimal. Additional considerations for testing approaches include assay availability, result turnaround time, and assay costs. Each of these factors will differ by institution; it is recommended that each center develop its own systematic diagnostic approach.

These results must be interpreted in the context of limitations. First, the prevalence rate for the assembled cohort was less than anticipated. The study inclusion criteria required patients to have an underlying medical condition and clinical presentation consistent with patients enrolled to a previous cohort of pediatric patients with IC [3]. Even with these inclusion criteria, it is possible that there was selection bias for less severely ill patients who were less likely to have IC. Future investigation of nonculture diagnostic tools should consider refined inclusion criteria that enrich for a higher pretest probability of IC. The lower rate required inclusion of spiked specimens, which

could have altered the calculated operating characteristics of each biomarker. While inclusion of spiked specimens did not significantly alter the calculated sensitivity for each biomarker ([Appendix B, section 1.7.1.5 \[Supplementary Materials\]](#)) it did result in more favorable estimates of sensitivity ([Supplementary Table 3](#)).

Second, specimens were tested after enrollment was completed; certain assays could have performed better in real time. Third, the reference standard was reliant on detection of *Candida* spp. with conventional, imperfect diagnostic studies, potentially increasing the possibility of mislabeling a positive assay result as false-positive. Fourth, while the T2Candida assay tests 2 mL of sample, the automated loading feature for the testing system requires a 3-mL sample. We hand-pipetted samples of < 3 mL into the system, obviating the automatic loading mechanism. Manual pipetting should not adversely affect test results. Moreover, this study's findings suggest manually loading specimens is possible and potentially beneficial for pediatric patients in whom access to blood volume is limited. Fifth, although the outcome was inclusive of any form of IC, only 1 event in this cohort met the definition of IC in the absence of candidemia. As such, the findings are limited to candidemia and may not be generalizable to all forms of IC. Sixth, the results reported only provide the operating characteristics of the biomarkers studied and do not assess the impact of results on clinical outcomes. Finally, there are other available nonculture diagnostic tools that we did not assess, such as *Candida*-specific multiplex polymerase chain reaction [38].

The estimated operating characteristics of T2Candida alone or in combination with the Platelia *Candida* Ag Plus assay in children and adolescents with clinical characteristics associated with increased risk for candidemia were reasonable. The decision to order T2Candida alone or in combination with the Platelia *Candida* Ag Plus assay is reliant on the clinician's assessed applicability of these data to the patient and clinical scenario under consideration (ie, starting or stopping antifungal therapy) in conjunction with awareness of the cost and turnaround time for test results.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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