Supplemental Information

METHODS

Details of Study Population

This study used banked, frozen serum sample remnants from the following sources: (1) pediatric patients prospectively recruited between 2008 and 2010 from the PEDs of 2 tertiary (Hôpitaux Universitaires de Genève, Geneva, and Centre Hospitalier Universitaire Vaudois, Lausanne) and 1 secondary hospital (Hôpital du Valais, Sion) in Switzerland, where the target populations were patients with clinical and radiological pneumonia (fever >38°C measured at ED and cough, tachypnea, or respiratory distress and abnormal lung infiltrates on chest radiograph) for infectious patients and patients with minor elective surgery for healthy (noninfectious) patients[26]; (2) pediatric patients prospectively recruited between 2010 and 2013 from the PED of a tertiary hospital (Hôpitaux Universitaires de Genève, Geneva), where the target population for infectious patients was patients with a fever without source, defined as the presence of a body temperature of 38°C or more with no identified source of infection after a careful review of the medical history and a thorough physical examination[27]; and (3) Pediatric patients presenting to the PED and pediatric wards of 2 secondary hospitals in Israel (Bnai Zion Medical Center, Haifa, and Hillel Yaffe Medical Center, Hadera), from whom blood was drawn and sent to the microbiology laboratories between 2011 and 2013 for serological analysis.

Although vaccination status was not recorded in the medical case records, it is noteworthy that pneumococcal, *Haemophilus influenzae* type b and meningitis C vaccines are routine for children from Switzerland, according to national guidelines,[48] whereas only pneumococcal and *H influenzae* type b vaccines are routine in Israel, [49] with high immunization rates in both countries.

It is noteworthy that the population in this study was constrained by availability of patient samples. For example, because banked serum samples were sourced from studies of children with pneumonia or fever without source and from serological test remnants, the study population does not include children with septic arthritis and osteomyelitis, important pediatric clinical infectious syndromes that could be captured in a prospective clinical study.

Reference Standard: Panel Expert Adjudication and Predetermined Criteria

The panel was made up of 3 experts from a pool of 11 pediatricians, each of whom had at least 10 years of clinical experience; panel composition from the pool was random, although at least 1 expert had the subspecialty of infectious disease. For every patient, the panel member independently assigned 1 of the following diagnoses: (1) bacterial infection (this diagnosis included cases of mixed bacterial and viral coinfection), (2) viral infection, (3) noninfectious, or (4) indeterminate. The diagnosis was based on a review of all available patient data gathered during routine care and accrued over the course of the patient's illness (including results obtained after discharge, eg, culture results), as recorded in an anonymized patient's case report, including but not limited to medical history; complete blood count; chemistry panel; radiological tests (report and image when available); blood, urine, or cerebrospinal fluid culture results; polymerase chain reaction analysis of nasal swab (when available; polymerase chain reaction analysis methods are detailed below); and the clinical syndrome recorded by attending clinician at discharge (which was not modified in light of data accrued after discharge).

For a subset of clinical syndromes, in order for an expert to assign a bacterial diagnosis fulfillment of predetermined criteria was required. Diagnoses with predetermined criteria included bacteremia (blood culture with positive results), bacterial meningitis (cerebrospinal fluid culture with positive results), lower/upper UTI (urine culture with >50 000 colony forming units and leukocytes and/or nitrite-positive urine), acute tonsillitis (throat culture positive for group A/C/G strep) and peritonsillar abscess (proven by surgical exploration or computerized tomography).

Two participants who were mistakenly assigned a bacterial reference standard outcome by the expert panel without meeting the predetermined criteria were reassigned as indeterminate.

Sample Size

The sample size was calculated on the basis of rejecting the null

hypothesis that sensitivity over the entire population, P, is lower than P_0 = 75% (H_0: $P \leq P_0$, H_1: $P > P_0$), with a significance level of 5% ($\alpha = .05$) and a power of 90% (= 1 − β) for a difference of 10% ($P_1 - P_0 \geq 0.1$). P_1 is the value of sensitivity under the alternative hypothesis. Assuming normal distribution for sensitivity, P, and a 1:3 ratio between patients with bacterial and viral infections, rejection of the null hypothesis was calculated to require 54 bacterial and 162 viral patients.

Polymerase Chain Reaction Analysis Methods

For Swiss patients (all from the pneumonia study and a small number from the fever without source study), reverse transcription polymerase chain reaction testing was performed for 13 viruses, including influenza A and B, respiratory syncytial virus A and B, rhinovirus, parainfluenza 1–3, enterovirus, human metapneumovirus, coronavirus OC43, E229, and NL63. Testing was also performed for 2 bacteria, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*.[50,51] For a small number of patients from Israel, viral detection was conducted by using the Anyplex II RV16 Detection Assay (Seegene Technologies, Inc, Walnut Creek, CA).

Other Sample Procedures

Serum samples were aliquoted and stored at −80°C. Frozen serum sample remnants were thawed and the index test was performed in batches. CRP measurements were conducted by using COBAS c501. PCT measurements were conducted by using Elecsys BRAHMS PCT on COBAS e601 or LIAISON BRAHMS PCT on a DiaSorin device or on KRYPTOR (Thermo Fisher, Waltham, MA).

Predefined Cutoffs of Routine Laboratory Parameters and Biomarkers

Predefined cutoffs were as follows: white blood cell count: 15 000/μL[32]; absolute neutrophil count: 10 000/μL[52]; percentage neutrophils: 75%[53]; CRP: 20, 40, 80 mg/L[32,33–35]; and PCT: 0.5, 1.0, 2.0 ng/mL.[35–39] In addition, the diagnostic performance of 2 predefined combinations of CRP and PCT were evaluated: (1) CRP <20 ug/mL and PCT <0.5 ng/mL to rule out bacterial infection and (2) CRP >80 ug/mL and PCT >2 ng/mL to rule in bacterial infection. Since PCT levels were not measured on all patients (and a limited number of patients did not have white blood cell count and absolute neutrophil count results), analyses that included these markers were performed only on the patients for whom they were measured; if multiple laboratory tests were performed for a given patient, then data from the tests performed closest to the date of the index test blood draw were used for comparison of diagnostic performance.

Analysis of Diagnostic Performance

Primary analysis of diagnostic accuracy was based on sensitivity (true-positive/positive) and specificity (true-negative/negative), with positive referring to bacterial infection. Specifically, sensitivity was defined as the number of cases in which the outcome of both the reference standard and the index test is bacterial divided by the number of cases in which the reference standard outcome is bacterial. Specificity was defined as the number of cases in which the outcome of both the reference standard and index test is viral divided by the number of cases in which the reference standard outcome is viral. Additional measures of accuracy included the positive likelihood ratio (sensitivity/[1 − specificity]), the negative likelihood ratio ([1 − sensitivity]/specificity), the negative predictive value (true-negative/[true-negative + false-negative]), the positive predictive value (true-positive/[true-positive + false-positive]), and the diagnostic odds ratio (positive likelihood ratio/negative likelihood ratio). Positives referred to the bacterial reference standard outcome and negatives referred to the viral reference standard outcome.

The P values were calculated as follows: for the mean and SD, t test; for the median and interquartile range, Wilcoxon rank test; for the number (n) and proportion (%), Fisher's exact test; for sensitivity and specificity, Fisher's exact test; and for more than 2 sensitivities or specificities, the χ^2 test. $P < .05$ was deemed statistically significant. P values smaller than .001 are reported as $P < .001$.

RESULTS

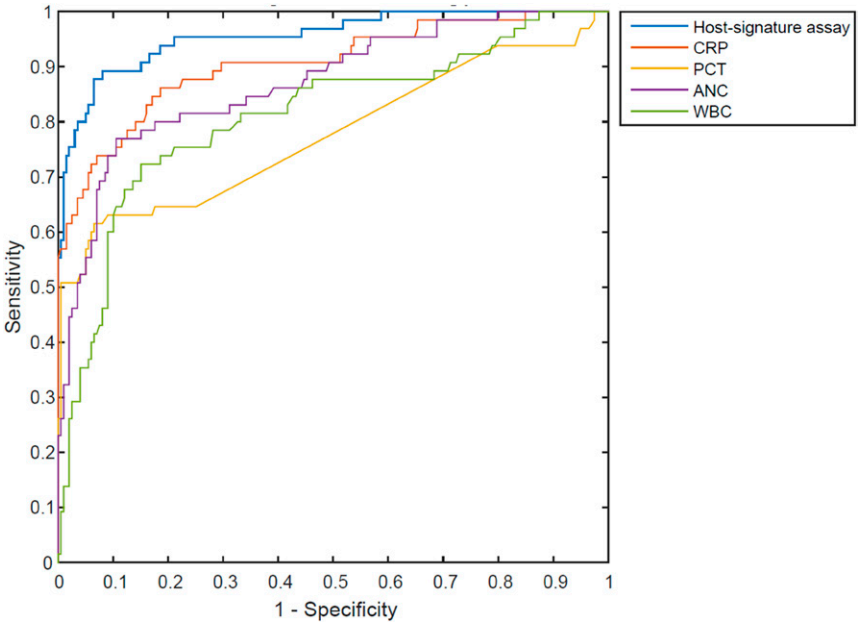
Performance of the Host-Signature Assay (for Patients With Suspicion of Acute Infection)

Additional data are presented to support the primary analysis as referenced in the text: Supplemental Figs 6–8, Supplemental Tables 6–8.

Exploratory Analysis of Host-Signature Assay's Performance for Patients Without and With Suspicion of Acute Infection

An exploratory analysis was conducted to evaluate the performance of the host-signature assay on a cohort that includes both patients without suspicion of acute infection (ie, healthy and/or noninfectious) and patients with suspicion of infectious disease

(Supplemental Fig 9). This is exploratory as the host-signature assay is currently intended for use on patients with suspicion of acute infection. In this exploratory analysis, the index test generates 3 possible outcomes: (1) nonbacterial etiology (including viral, healthy, and/or noninfectious): ImmunoXpert score <35; (2) equivocal: 35 ≤ ImmunoXpert score ≤65; and (3) bacterial etiology (including mixed bacterial and viral coinfection): ImmunoXpert score >65. Inclusion of healthy/noninfectious patients in the performance analysis did not impact the sensitivity of 93.8% (87.8%–99.8%) and increased the specificity to 91.8% (88.5%–95.2%). The details of other accuracy measures are given in Supplemental Table 9.

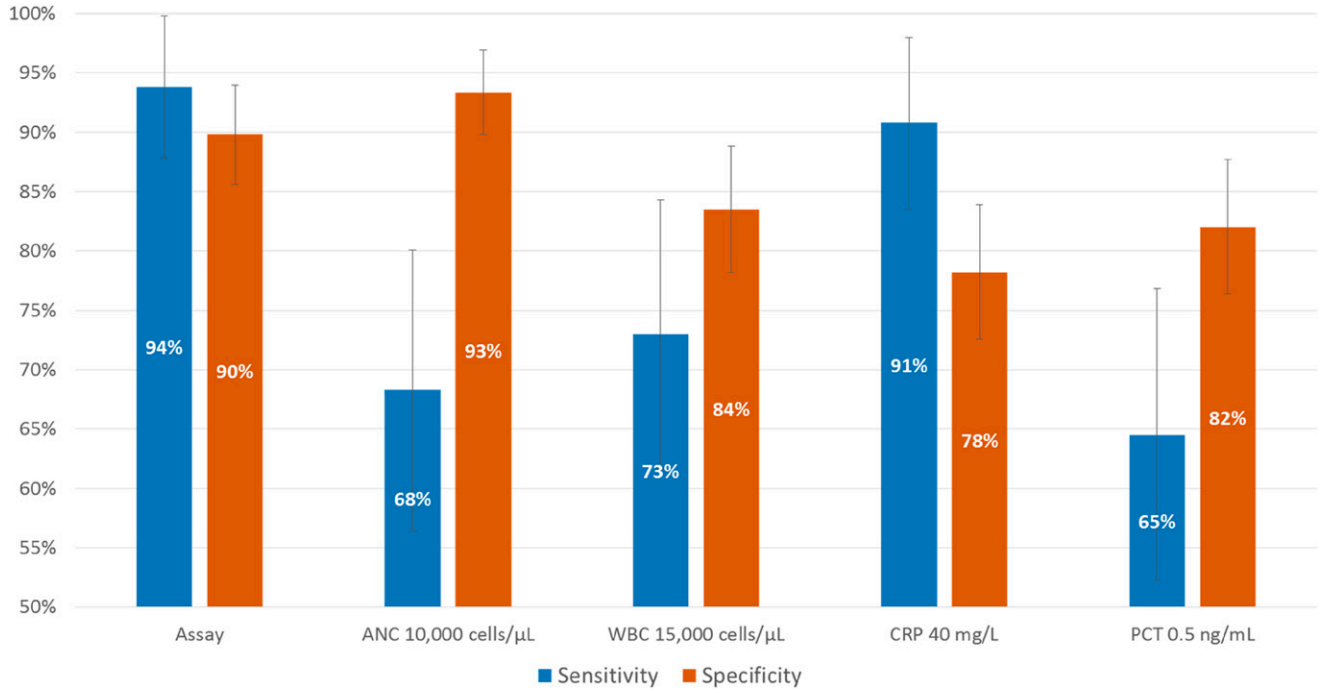


SUPPLEMENTAL FIGURE 6

Comparison of areas under the receiver-operating curve for the host-signature assay, CRP, PCT, absolute neutrophil count, and white blood cell count (for patients with suspicion of acute infection). The area under receiver operating curve of the host-signature assay (0.96 [0.92–0.99]) was significantly higher compared to that of CRP (0.91 [0.86–0.96], $P < .001$), PCT (0.78 [0.71–0.85], $P < .001$), absolute neutrophil count (0.87 [0.81–0.93], $P < .001$), and white blood cell count (0.81 [0.74–0.88], $P < .001$). Analysis was performed on patients with a bacterial or viral reference standard outcome (according to expert panel majority) for which the assay and the 4 laboratory markers were measured ($n = 264$).

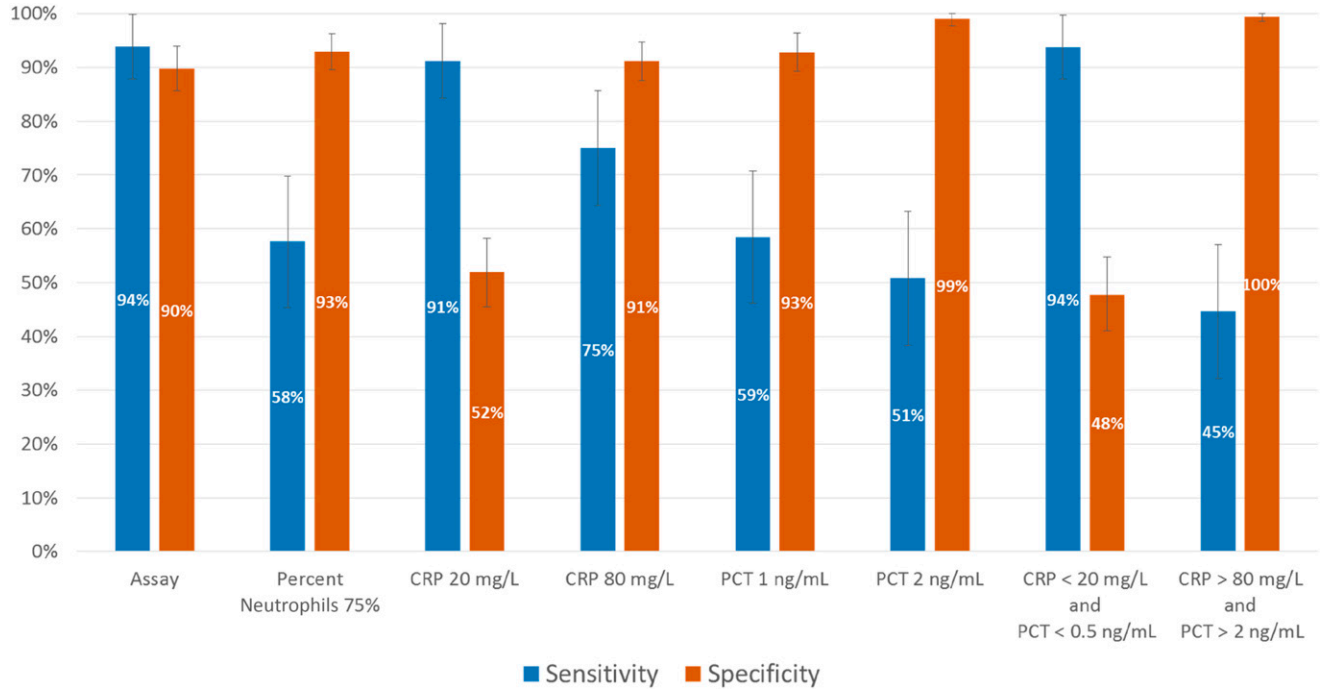
DISCUSSION

The influence of endemic pathogens (eg, *Mycobacterium tuberculosis*, *Plasmodium*, and HIV), on the host signature should be carefully examined. Furthermore, it is desirable to evaluate the applicability of the host-protein assay to febrile neonates and infants <3 months old. Of note, van Houten et al[25] reported correct bacterial versus viral classification for 22 out of 22 infants aged between 1 and 2 months, suggesting that the host-signature assay may be applicable also to infants.



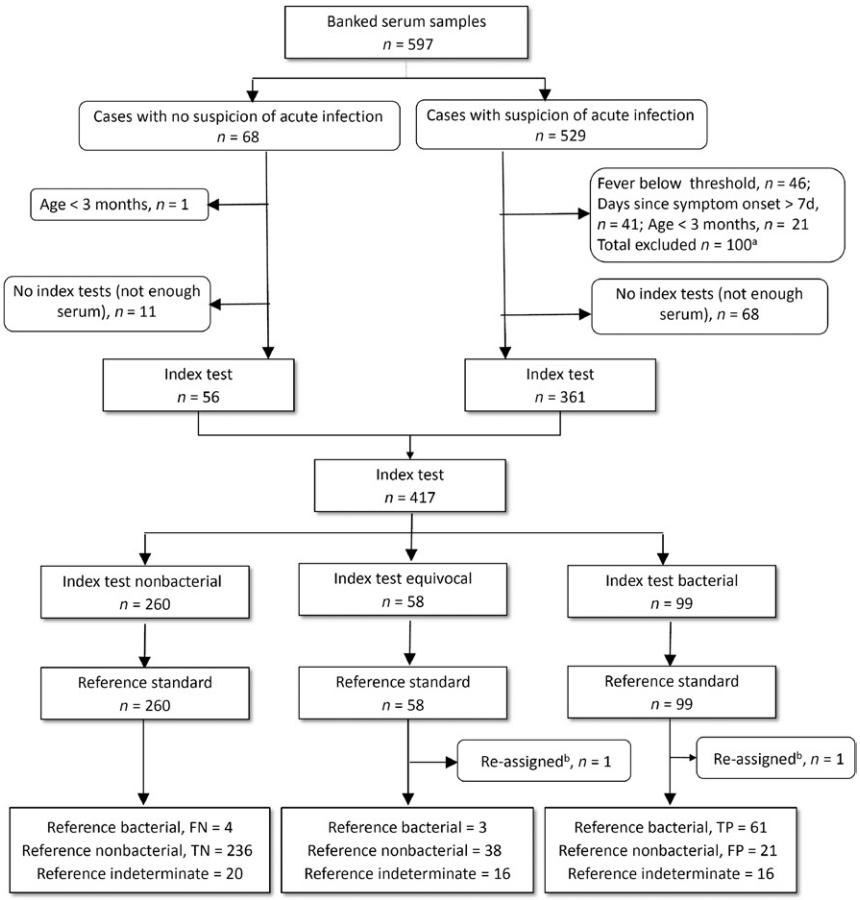
SUPPLEMENTAL FIGURE 7

Diagnostic performance of host-signature assay compared with routine laboratory parameters and other biomarkers after removing equivocal patients (as defined by host-signature assay for patients with suspicion of acute infection). Diagnostic performance was evaluated by comparing the reference standard outcome (according to expert panel majority) with the outcome classified by the host-signature assay, or other parameter or biomarker. Predefined cutoffs were applied, and, in addition, equivocal patients, as defined by host-signature assay outcomes, were removed before every performance calculation (11.7% had an equivocal result). Accordingly, the diagnostic performance was calculated on the basis of the following: 271 patients (65 bacterial and 206 viral) for the host-signature assay and CRP; 257 patients (63 bacterial and 194 viral) for absolute neutrophil count and white blood cell count; 245 (62 bacterial and 183 viral) for PCT.



SUPPLEMENTAL FIGURE 8

Diagnostic performance of host-signature assay compared with percent neutrophils, with additional predefined cutoffs of CRP, PCT, and their combinations (for patients with suspicion of acute infection). Diagnostic performance was evaluated by comparing the reference standard outcome (according to expert panel majority) with the outcome classified by the host-signature assay, or other parameter or biomarker. Predefined cutoffs were applied. The assay was run on 307 patients (68 bacterial and 239 viral; 11.7% had an equivocal result); absolute neutrophil count, white blood cell count, and percent neutrophil testing was run on 292 patients (66 bacterial and 226 viral), PCT testing was run on 274 patients (65 bacterial and 209 viral); and CRP testing was run on 307 patients (68 bacterial and 239 viral).



SUPPLEMENTAL FIGURE 9

Exploratory analysis of patients without and with suspicion of acute infection. Flow diagram is structured according to Standards for Reporting of Diagnostic Accuracy (STARD).[31] The study cohort (*n* = 417) includes the subjects on which the index test was performed (this includes cases with an indeterminate reference standard). An equivocal outcome is a nonmissing, nonerroneous result that does not provide diagnostic information. [a] Participant may satisfy >1 exclusion criteria. [b] Reassigned as reference indeterminate because expert panel did not employ predetermined criteria (see Supplemental Information, Methods). FN, false-negative; FP, false-positive; TN, true-negative; TP, true-positive.

SUPPLEMENTAL TABLE 6 Baseline Characteristics of Cases With Suspicion of Acute Infection That Fulfilled Inclusion Criteria Compared With Cases That Did Not Fulfill Inclusion Criteria

	Study Population, *n* = 361	Did Not Fulfill Inclusion Criteria, *n* = 100	*P*
Age, y (mean, SD)	4.1 (4.2)	5.2 (5.4)	.08
Sex, female, *n* (%)	170 (47)	43 (43)	.43
Maximal temperature, °C (mean, SD)	39.3 (0.8)	38.2 (1.4)	<.001
Receipt of antibiotics, *n* (%)	188 (52)	42 (42)	.06
Time from symptom onset, d (median, IQR)	4 (3)	5 (3)	<.001
Hospital admission, *n* (%)	139 (47)	58 (65)	.003
Hospitalization duration, d (median, IQR)	0 (3)	2 (4)	.001
Clinical syndrome, *n* (%)			
CNS infection	3 (1)	2 (2)	.3
FWS	125 (35)	32 (32)	.64
GE	11 (3)	5 (5)	.4
LRTI	56 (16)	9 (9)	.11
URTI	114 (32)	22 (22)	.06
UTI	17 (5)	6 (6)	.61
Other	35 (10)	25 (25)	<.001

Baseline characteristics of the study population, for whom the index test was conducted (left column) and patients who did not fulfill inclusion criteria (right column). The clinical syndrome was the diagnosis recorded by the attending clinician at discharge (and was not modified in light of data accrued after discharge). CNS infections included meningitis. FWS required a urine analysis with negative results. LRTIs included pneumonia and bronchiolitis. URTIs included acute tonsillitis, pharyngitis, sinusitis, acute otitis media, aphthous stomatitis, herpengina, retropharyngeal abscess, and scarlet fever. UTIs included cystitis and pyelonephritis. The "other" category included febrile convulsions, hepatitis, lymphadenitis, myositis, parotitis, sialoadenitis, and lymphadenopathy. CNS, central nervous system and encephalitis; FWS, fever without source; GE, gastroenteritis; IQR, interquartile range; LRTI, lower respiratory infection; URTI, upper respiratory infection; UTI, urinary tract infection.

SUPPLEMENTAL TABLE 7 Extrapolated Host-Signature Assay Positive Predictive Value and Negative Predictive Value in Different Clinical Settings

Setting	Bacterial Infection Prevalence (%)	PPV (%)	NPV (%)
Study population	22.1	72.4	98.1
Outpatient children	20[a]	69.7	98.3
Inpatient children	50[a]	90.2	93.6

PPV and NPV depend on the underlying frequency of bacterial versus viral etiologies in the target population. The table compares the PPV and NPV in the study population with that anticipated in the outpatient and inpatient population by adjusting for different bacterial prevalence. NPV, negative predictive value; PPV, positive predictive value.

[a] Estimates of bacterial infection prevalence are based on data reported in the bacterial etiology chapter, part 7, of *Harrison's Internal Medicine*, 17th Edition.[54]

SUPPLEMENTAL TABLE 8 Host-Signature Assay Performance for Cases With Suspicion of Acute Infection Per Patient Age

Patient Age (y)	TP (*n*)	FN (*n*)	TN (*n*)	FP (*n*)	Equivocal (*n*)	Total (*n*)
<1	11	0	53	1	4	69
≥ 1 and < 2	7	1	47	5	10	70
≥ 2 and < 3	9	1	25	5	5	45
≥ 3 and < 4	8	0	8	3	1	20
≥ 4 and < 5	4	0	12	1	4	21
≥ 5 and < 6	2	0	10	0	2	14
≥ 6 and < 7	2	0	3	2	0	7
≥ 7 and < 8	1	1	2	2	4	10
≥ 8 and < 9	2	1	3	1	0	7
≥ 9 and < 10	2	0	0	0	2	4
≥ 10 and < 11	1	0	3	0	1	5
≥ 11 and < 12	5	0	1	0	1	7
≥ 12 and < 13	0	0	4	0	1	5
≥ 13 and < 14	0	0	0	0	0	0
≥ 14 and < 15	2	0	5	1	0	8
≥ 15 and < 16	3	0	5	0	0	8
≥ 16 and < 17	1	0	3	0	0	4
≥ 17 and ≤ 18	1	0	1	0	1	3

Diagnostic performance was evaluated by comparing the reference standard outcome (according to expert panel majority) with the host-signature assay outcome ($n = 307$). The terms positive and negative correspond to bacterial and viral infections respectively. FN, false-negative; FP, false-positive; TP, true-positive; TN, true-negative.

SUPPLEMENTAL TABLE 9 Exploratory Analysis of the Host-Signature Assay's Diagnostic Performance in Patients Without and With Suspicion of Acute Infection

Accuracy Measure	Assay Performance (95% CI)
Sensitivity	93.8% (87.8%–99.8%)
Specificity	91.8% (88.5%–95.2%)
PPV	74.4% (65.7%–81.5%)
NPV	98.3% (95.8%–99.4%)
LR+	11.48 (7.59–17.38)
LR–	0.07 (0.03–0.17)
DOR	171.4 (56.7–517.8)
Equivocal	11.3%

Diagnostic performance was evaluated by comparing the reference standard outcome with the host-signature assay outcome; positive refers to a bacterial outcome. As predefined in the statistical analysis plan, the reference standard outcomes were assigned according to expert panel majority (n = 363). DOR, diagnostic odds ratio; LR+, positive likelihood ratio; LR–, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

SUPPLEMENTAL REFERENCES

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