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MAJOR ARTICLE

A Rapid Host Response Blood Test for Bacterial/Viral Infection Discrimination Using a Portable Molecular Diagnostic Platform

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Background. Difficulty discriminating bacterial versus viral etiologies of infection drives unwarranted antibacterial prescriptions and, therefore, antibacterial resistance.

Methods. Utilizing a rapid portable test that measures peripheral blood host gene expression to discriminate bacterial and viral etiologies of infection (the HR-B/V assay on Biomeme's polymerase chain reaction–based Franklin platform), we tested 3 cohorts of subjects with suspected infection: the HR-B/V training cohort, the HR-B/V technical correlation cohort, and a coronavirus disease 2019 cohort.

Results. The Biomeme HR-B/V test showed very good performance at discriminating bacterial and viral infections, with a bacterial model accuracy of 84.5% (95% confidence interval [CI], 80.8%–87.5%), positive percent agreement (PPA) of 88.5% (95% CI, 81.3%–93.2%), negative percent agreement (NPA) of 83.1% (95% CI, 78.7%–86.7%), positive predictive value of 64.1% (95% CI, 56.3%–71.2%), and negative predictive value of 95.5% (95% CI, 92.4%–97.3%). The test showed excellent agreement with a previously developed BioFire HR-B/V test, with 100% (95% CI, 85.7%–100.0%) PPA and 94.9% (95% CI, 86.1%–98.3%) NPA for bacterial infection, and 100% (95% CI, 93.9%–100.0%) PPA and 100% (95% CI, 85.7%–100.0%) NPA for viral infection. Among subjects with acute severe acute respiratory syndrome coronavirus 2 infection of ≤7 days, accuracy was 93.3% (95% CI, 78.7%–98.2%) for 30 outpatients and 75.9% (95% CI, 57.9%–87.8%) for 29 inpatients.

Conclusions. The Biomeme HR-B/V test is a rapid, portable test with high performance at identifying patients unlikely to have bacterial infection, offering a promising antibiotic stewardship strategy that could be deployed as a portable, laboratory-based test.

Keywords. antibacterial resistance; antibiotic stewardship; bacterial infection; host response diagnostic; infectious diseases; viral infection.

The current standard of care for discriminating bacterial versus viral infection often relies on clinical features and limited pathogen-based diagnostic testing. However, there is substantial overlap in the clinical presentation of bacterial and viral infections for syndromes such as acute respiratory infection, and pathogen-based diagnostic tests that are both sensitive and specific are lacking. The challenges associated with discriminating bacterial and viral etiologies of infection result in inappropriate antibacterial prescription in 20%–40% of cases [1–6], which leads to unnecessary drug-related adverse effects, contributes to increased healthcare costs, and is one of the primary drivers of antibacterial resistance. Tests that reliably discriminate bacterial and viral etiologies could decrease diagnostic uncertainty, decrease inappropriate antibacterial use, and improve patient outcomes.

Given the limitations associated with pathogen-based diagnostic tests, an alternative strategy involves measuring the host immunological response. This strategy is predicated on bacterial and viral infections inducing unique immunological responses [7]. Host immunological response may be assessed by quantifying changes in the peripheral blood of either protein biomarkers or gene expression associated with the immune response.

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Procalcitonin (PCT), the most widely used host protein biomarker for acute respiratory infection, tends to be elevated in bacterial infections, and rises less commonly in response to viral infections. This biomarker is used to support clinical decisions, including initiation (for PCT >0.25 ng/mL) or withholding (for PCT ≤0.25 ng/mL) of antibacterial therapy in patients with acute exacerbations of chronic bronchitis or community-acquired pneumonia. PCT levels can also increase when there is inflammation in the absence of a bacterial infection (eg, autoimmune disease, severe trauma, surgery, cardiac shock) [8, 9]. Therefore, PCT is limited by poor accuracy and lack of specificity and has exhibited mixed results for bacterial/viral discrimination and guiding antibacterial use [10–14]. Other protein biomarkers have also been described for bacterial/viral discrimination including myxovirus resistance protein A, erythrocyte sedimentation rate, C-reactive protein, tumor necrosis factor–related apoptosis-inducing ligand, interferon-γ–induced protein 10, and combinations thereof [15–17].

Another approach involves measuring peripheral blood host gene expression, which can now be done using clinically available platforms [18, 19]. We previously discovered a host gene expression signature discriminating bacterial and viral illness as well as noninfectious causes of illness, containing 71 genes for bacterial infection, 33 genes for viral infection, and 26 genes for noninfectious causes of illness [20]. This signature was further developed into a research use–only host response test for viral infection diagnosis using 10 genes, and later as a 45-gene test to discriminate host response for bacterial versus viral infection (HR-B/V) on the BioFire FilmArray System, showing excellent performance characteristics [18, 21]. In this study, we describe the performance of a novel host gene expression test (Biomeme HR-B/V) for bacterial/viral discrimination using a reduced set of targets compared to previous classifiers utilized on the BioFire FilmArray platform [20]. The Biomeme HR-B/V assay was tested on a portable, molecular diagnostic testing platform known as the Franklin. The Franklin three9 real-time polymerase chain reaction (PCR) thermocycler (Biomeme Inc, Philadelphia, Pennsylvania) is lightweight, portable, and battery-powered and is capable of multiplex detection of up to 27 targets from 1 sample (Figure 1). Nucleic acid is extracted, isolated, and purified from blood samples using the M1 Sample Prep Cartridge Kit, which requires no laboratory equipment, refrigeration, electricity, incubation, alcohol precipitation, or phenol-chloroform extraction. A smartphone connects to the Franklin instrument via Bluetooth or a USB cable to load the test protocol, input sample IDs, and display results of the test. The results are reported in approximately 1 hour, which has the ability to impact unnecessary antibacterial prescriptions in a range of care settings.

In this study, we assess the performance of the Biomeme HR-B/V test and its concordance with the BioFire HR-B/V test using a multisite, diverse, prospectively enrolled cohort including subjects with coronavirus disease 2019 (COVID-19).

Figure 1. Biomeme M1/Franklin HR-B/V workflow The system consists of 4 components: an M1 RNA 2.0 sample prep cartridge, 3 Go-Strips panels (A, B, C), the Franklin three9 real-time polymerase chain reaction (PCR) thermocycler, and a smartphone. The M1 sample prep cartridge allows rapid nucleic acid extraction without the need for any equipment. Each Go-Strip consists of 3 connected PCR tubes containing lyophilized master mix, multiplexed primers, and probes for 3 targets. Each handheld unit is equipped with 9 sample wells, enabling simultaneous quantitative detection of up to 27 targets per PCR run across 3 different color channels including green (FAM/SYBR), amber (Jun/TexRedX), and red (ATTO647N/Cy5). The thermocycler is just under 3 lb (1.36 kg); battery-operated for maximum portability, enabling a full day's work on a single charge; and provides results in less than an hour. The smartphone uses an intuitive app, the Biomeme Go, which pairs wirelessly with the Franklin thermocycler to easily run, monitor, analyze, and share the PCR data.

MATERIALS AND METHODS

Study Design

Studies were approved by each site's institutional review board. All subjects or legally authorized representatives provided written informed consent.

Subjects were analyzed as part of 3 cohorts: the HR-B/V training cohort, the HR-B/V technical correlation cohort, and a COVID-19 cohort. We chose to include a COVID-19 cohort separately to show that the Biomeme HR-B/V assay performed similarly in patients with this novel viral infection when compared to other known viral infections. Our team has previously shown that COVID-19 results in an alteration of the host immune system that is both similar to other viral infections (interferon driven) and different (early B-cell activation and antibody production) [22]. Samples from subjects in the bacterial/viral training and correlation cohorts were collected and banked as part of previously published studies (Community Acquired Pneumonia and Sepsis Outcome Diagnostics [CAPSOD], Community Acquired

Pneumonia and Sepsis Study [CAPSS], Austere Environments Consortium for Enhanced Sepsis Outcomes [ACESO], Clinico-Molecular Predictors of Presymptomatic Infectious Disease, Rapid Diagnostics in Categorizing Acute Lung Infections [RADICAL], RADICAL-2, and Molecular Epidemiology of Sepsis and Suspected Infection [MESSI]) [18, 23–27]. In brief, patients were enrolled by convenience sampling from 2008 through 2019 in the emergency departments of Duke University Medical Center (Durham, North Carolina), Henry Ford Hospital (Detroit, Michigan), UNC Health Care (Chapel Hill, North Carolina), Brigham and Women's Hospital (Boston, Massachusetts), University of Utah Medical Center (Salt Lake City, Utah), Newton-Wellesley Hospital (Newton, Massachusetts), University of California Hospital at Davis (Sacramento, California), the University of Texas Health Science Center at Houston (Houston, Texas), Hasbro Children's Hospital (Providence, Rhode Island), and Children's Hospital of Pittsburgh (Pittsburgh, Pennsylvania). Enrollment criteria varied across the studies, which were prospective observational studies to identify patients with suspected infectious syndromes and to bank samples for future research use. These cohorts include participants with either a suspected infection and 2 or more systemic inflammatory response syndrome (SIRS) criteria (CAPSOD, CAPSS, ACESO, Clinico-Molecular Predictors of Presymptomatic Infectious Disease studies), participants with an acute respiratory illness (RADICAL, RADICAL-2), or patients with exposure to or suspected or confirmed infection (MESSI). Patients were eligible for CAPSOD, CAPSS, ACESO, and Clinico-Molecular Predictors of Presymptomatic Infectious Disease studies if they had known or suspected infection of <28 days' duration and exhibited 2 or more SIRS criteria. CAPSOD and CAPSS enrolled patients aged ≥6 years while ACESO and Clinico-Molecular Predictors of Presymptomatic Infectious Disease enrolled patients aged ≥18 years. RADICAL and RADICAL-2 enrolled patients aged ≥2 years with acute respiratory illness of <28 days' duration. Acute respiratory illness was defined as having at least 2 qualifying symptoms or 1 qualifying symptom and at least 1 qualifying vital sign abnormality. Qualifying symptoms included headache, rhinorrhea, nasal congestion, sneezing, sore throat, itchy/watery eyes, conjunctivitis, cough, shortness of breath, sputum production, chest pain, and wheezing. Qualifying vital sign abnormalities included heart rate ≥90 beats per minute (or ≥110 beats per minute for children aged 2–6 years), respiratory rate ≥20 breaths per minute, and temperature ≥38.0°C or ≤36.0°C. Patients were eligible for MESSI if they were ≥2 years of age and had suspected infection, confirmed infection, exposure to someone with symptoms of suspected infection, or recent/planned vaccination. These overarching studies enrolled the following numbers of patients: 1274 in CAPSOD, 1320 in CAPSS, 944 in ACESO, 99 in Clinico-Molecular Predictors of Presymptomatic Infectious Disease studies, 796 in MESSI, 940 in RADICAL, and 783 in RADICAL-2.

At enrollment, a nasal or nasopharyngeal sample, urine, and blood in PAXgene Blood RNA tubes (Qiagen) were collected from all subjects and stored at −80°C. Etiologic testing included multiplex PCR testing for respiratory pathogens (ResPlex V2.0, Qiagen; Respiratory Viral Panel, Luminex Corporation; Respiratory Pathogen Panel, Luminex Corporation; or Respiratory 2.1 Panel, BioFire Diagnostics) and urine antigen testing for *Streptococcus pneumoniae* (BinaxNOW, Alere Inc).

Subjects were further selected from the pool of previously enrolled subjects based on the availability of a PAXgene Blood RNA sample and confirmatory microbiology to indicate the presence of a bacterial or viral infection. A subset of participants was selected to train the Biomeme HR-B/V model based on a clinically adjudicated bacterial or viral infection (adjudication described below). These individuals were adjudicated and later selected for model training without considering results from host gene expression testing, including the BioFire HR-B/V test. The BioFire HR-B/V test measures 42 host messenger RNA (mRNA) transcripts (plus 3 normalizing control genes) and differentiates viral, bacterial, and noninfectious causes of illness [18, 28]. Since the Biomeme HR-B/V test measures a subset of the BioFire-based panel (22 vs 42 genes), an additional cohort of subjects was identified for the purpose of correlating the Biomeme HR-B/V test to the BioFire HR-B/V test. This additional cohort (correlation cohort) of subjects had a clear viral or bacterial result on BioFire HR-B/V testing, which was concordant with clinical adjudication. We had previously developed a 1-model (bacterial vs other) and a 2-model (bacterial vs other; viral vs other) system for the BioFire HR-B/V. A large, multisite cohort showed compelling performance for the single-model system; thus, samples for the correlation cohort were chosen from this study [28].

The COVID-19 cohort included inpatients and outpatients enrolled from March 2020 to May 2021 at Duke University and the surrounding central North Carolina communities based on clinically suspected or confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [22].

Case Definitions and Reference Standard

Clinical adjudication served as the comparator method to determine the etiology of illness. Adjudicators were clinicians with experience in the diagnosis of infectious diseases (eg, pediatric or adult infectious diseases, internal medicine, or pulmonary/critical care medicine physicians), and used a combination of clinical history such as exposures, symptoms, physical examination findings, treatments received, and clinical outcomes; results from laboratory testing performed during routine clinical care, such as white blood cell count and C-reactive protein level; results from etiological testing for research purposes (with the exception of results from the HR-B/V assays); and radiographic test results during routine

clinical care to conduct adjudications. Two adjudicators were randomly assigned to independently determine the likelihood of bacterial and/or viral infection, noninfectious syndrome, or indeterminate diagnosis. Adjudicator discordance was resolved by a consensus panel of at least 3 experts, with a simple majority determining final diagnosis [18, 28].

Biomeme HR-B/V Testing

The Biomeme HR-B/V tested performed on the Franklin molecular diagnostic platform can detect up to 27 targets per sample through real-time reverse-transcription quantitative PCR (RT-qPCR) including 22 discriminating targets (BATF, CFAP45, CTBP1, DEFA3, DSC2, EXOG, FOLR3, GCAT, HLA-DRB1, IFI27, LAMP1, LAPTM4B, MCTP1, OAS3, PLAC8, RPS21, SIGLEC1, SIRPB1, SLC29A1, STAP1, TNFAIP2, USP18) along with 2 normalization controls (DECR1 and PPIB) and an RNA process control (RNA extraction and RT-PCR control utilizing MS2 bacteriophage).

Whole blood was collected in PAXgene Blood RNA tubes at the time of enrollment and stored at −80°C. Preservatives incompatible with the chemistry used in the Biomeme sample preparation system were removed by centrifugation, and pellets were washed with RNase-free water. The resuspended sample was centrifuged again and the final pellet was resuspended in 6M Biomeme Lysis Buffer (Biomeme, Philadelphia, Pennsylvania) followed by the addition of an equivalent volume of 100% ethanol. Then RNA was extracted and purified using Biomeme's M1 RNA 2.0 Sample Prep Cartridge (Biomeme). The processed product of 300 µL PAXgene Blood RNA sample along with 20 µL of MS2 bacteriophage external process control was added to the first well of the reagent cartridge containing lysis buffer (Biomeme). Sample was pumped through the Biomeme M1 sample prep column, which contains silica membranes, a barbed tip, and Luer lock for attachment to a 1-mL syringe. The column's barbed tip pierces the foil sealed cartridge chambers, which contain lysis buffer, protein, salt wash, and drying buffers. For the final air-drying step, we transferred the column to a clean 20-mL syringe and dried it onto a clean low-lint wipe with 5–10 pumps, and eluted the RNA with 800 µL 0.1 M ethylenediaminetetraacetic acid TE buffer. Purified RNA samples were added to lyophilized HR-B/V assay reagents, then run on the Franklin three9 thermocycler (Biomeme) (Figure 1). Primers/probes were multiplexed for triplex reactions. Each sample required approximately 7 minutes of hands-on time, 20 minutes in a centrifuge, and 55 minutes in the Franklin three9 thermocycler. One laboratory technician was able to keep 4 Franklin three9 thermocyclers in continuous operation throughout a workday without difficulty.

Statistical Analysis

Days post–symptom onset (DPSO) was calculated from the first day a participant reported 1 or more symptoms. Symptom severity in the COVID-19 cohort was calculated as a cumulative score from a patient-reported survey tracking over 39 symptoms, each scaled from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe). The maximum score a participant could report was therefore 156.

Raw RT-PCR values were exported from the Biomeme Franklin mobile RT-qPCR thermocyclers via XML worksheets. As a quality control measure, samples with reference targets (DECR1 and PPIB) with cycle threshold (Ct) >31 were removed from downstream analysis. Missing or nondetected values were imputed to the maximum observed value per target plus 1 Ct, that is, max (observed Ct) + 1. RT-PCR values were normalized with the delta Ct method [29], which is the target Ct value minus the mean of the reference targets (DECR1 and PPIB). Principal component analysis (PCA) plots were generated for dimensionality reduction of the training, correlation, and COVID-19 cohorts.

Separate models for bacterial versus nonbacterial and viral versus nonviral infections were built using linear sparse logistic regression. Specifically, elastic net regularization favoring ridge regression ($\alpha = .01$) was implemented in the glmnet R package [30]. The optimal regularization parameter (λ) was obtained via leave-one-out cross-validation (LOOCV) and used to build the final model on the entire discovery set for predictions on the correlation set. We considered the area under the receiver operating characteristic curve (AUC), summaries of the confusion matrix (accuracy, positive percent agreement [PPA], negative percent agreement [NPA], positive predictive value [PPV], and negative predictive value [NPV]), scatter and box plots of predicted probabilities for bacterial and viral infection, and calibration plots (for predictions grouped by deciles) as performance metrics. The threshold for both bacterial and viral classifiers was estimated via average weighted accuracy with parameters relative importance $r = 0.25$ and prevalence band $(a, b) = (0.10, 0.30)$ for bacterial and $r = 2$ and $(a, b) = (0.50, 0.80)$ for viral [31]. Confidence intervals (CIs) were generated from confusion matrices using epiR, Wilson method [32]. Calibration plots were generated by selecting a random subset (70%) from the training and correlation cohorts and sampling with replacement over 1000 iterations. Linear values are averaged over all iterations. All statistical analyses were completed using R Statistical Software version 4.2.0 [33].

RESULTS

Subject Characteristics

Among 444 subjects in the training cohort, the median age was 44.0 years (interquartile range [IQR], 27.0–57.0 years); 239 (54%) were female and 203 (46%) were male (Table 1). Subjects were racially and ethnically diverse (self-reported), as displayed in Table 1. Among subjects, 320 (72%) subjects had a respiratory site of infection and 304 (69%) subjects were sampled during the acute phase of illness (≤7 DPSO). Based on clinical adjudications, 267 (60%) subjects had viral

Table 1. Subject Demographics and Clinical Characteristics by Cohort

Characteristic	Training (n = 444)	Correlation (n = 82)	COVID-19 Inpatient (n = 60)	COVID-19 Outpatient (n = 51)
Demographic characteristics				
Age, y, median (IQR)	44.0 (27.0–57.0)	19.5 (13.1–31.7)	55.8 (48.0–67.9)	44.5 (30.9–54.5)
Sex				
Female	239 (54)	46 (56)	35 (58)	25 (49)
Male	203 (46)	36 (44)	25 (42)	26 (51)
Missing	2 (1)	0	0	0
Race				
American Indian/Alaska Native	3 (1)	1 (1)	0	3 (6)
Asian	7 (2)	1 (1)	0	4 (8)
Black/African American	205 (46)	40 (49)	28 (47)	14 (28)
Native Hawaiian/Pacific Islander	2 (1)	0	0	0
Other	9 (2)	12 (15)	0	0
>1 race	0	0	5 (8)	3 (6)
Unknown	5 (1)	0	3 (5)	0
White	211 (48)	28 (34)	24 (40)	27 (53)
Missing	2 (1)	0	0	0
Ethnicity				
Hispanic/Latino	32 (7)	21 (26)	11 (18)	7 (14)
Not Hispanic/Latino	391 (88)	59 (72)	48 (80)	43 (84)
Unknown	19 (4)	2 (2)	1 (2)	1 (2)
Missing	2 (1)	0	0	0
Clinically adjudicated type of illness				
Bacterial infection	113 (26)	23 (28)	0	0
COVID-19	0	0	60 (100)	51 (100)
SIRS	64 (14)	0	0	0
Viral infection	267 (60)	59 (72)	0	0
Characteristics of illness				
Infection at respiratory site	320 (72)	74 (90)	60 (100)	51 (100)
Days post–symptom onset				
≤7	304 (69)	82 (100)	29 (48)	30 (59)
>7	82 (18)	0	25 (42)	9 (18)
No DPSO data	58 (13)	0	6 (10)	12 (24)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: COVID-19, coronavirus disease 2019; DPSO, days post–symptom onset; IQR, interquartile range; SIRS, systemic inflammatory response syndrome.

infection, 113 (26%) subjects had bacterial infection, and 64 (14%) subjects had noninfectious SIRS.

Among 82 subjects in the technical correlation cohort, the median age was 19.5 years (IQR 13.1–31.7 years); 46 (56%) were female and 36 were male (44%) (Table 1). The majority of subjects had a respiratory infection (n = 74 [90%]) and all were sampled at ≤7 DPSO (n = 82 [100%]). A total of 59 (72%) subjects had viral infection and 23 (28%) subjects had bacterial infection.

Of a total of 111 subjects who were part of the COVID-19 cohort, 60 (54%) were inpatients and 51 (46%) were outpatients (Table 1). The median age was 55.8 years (IQR, 48.0–67.9 years) for the inpatients and 44.5 years (IQR, 30.9–54.5 years) for the outpatients. Subjects were again racially and ethnically diverse. Twenty-nine inpatients (48%) had ≤7 DPSO and 25 (42%) had >7 DPSO. Thirty outpatients (59%) had ≤7 DPSO and 9 (18%) had >7 DPSO. Median symptom count (assessed within 7 days of enrollment, with 94% assessed on the day of enrollment) was higher in the inpatient population (6.00 [IQR, 3.00–9.50]) than in the outpatient population (4.00 [IQR, 0.00–7.00]). As expected, median symptom severity score was higher (12.00 [IQR, 0.50–20.00]) in the inpatient population than in the outpatient population (4.00 [IQR, 0.00–14.00]).

Training Cohort

Biomeme HR-B/V Test Performance Compared to Clinical Adjudication. The Biomeme HR-B/V test was trained on a cohort of 444 subjects. The Biomeme HR-B/V test was used to measure transcript abundance, which was used to build a logistic regression model trained on subjects with an adjudicated clinical diagnosis of bacterial or viral infection and then validated using LOOCV.

For the diagnosis of bacterial infection, the AUC was 0.91 (95% CI, .88–.94) (Supplementary Figure 1*A*), with accuracy of 84.5% (95% CI, 80.8%–87.5%), PPA of 88.5% (95% CI, 81.3%–93.2%), NPA of 83.1% (95% CI, 78.7%–86.7%), PPV of 64.1% (95% CI, 56.3%–71.2%), and NPV of 95.5% (95% CI, 92.4%–97.3%) given a bacterial infection prevalence of

Table 2. Performance Metrics of the Biomeme HR-B/V Bacterial and Viral Models and of Procalcitonin in the Training Cohort

Model	Testing Modality	Overall Accuracy, %	AUC	PPV, %	NPV, %	PPA, %	NPA, %
Biomeme HR-B/V (n = 444)	Bacterial model	84.5 (80.8–87.5)	0.91 (.88–.94)	64.1 (56.3–71.2)	95.5 (92.4–97.3)	88.5 (81.3–93.2)	83.1 (78.7–86.7)
	Viral model	86.7 (83.2–89.6)	0.92 (.89–.94)	87.4 (83.0–90.8)	85.5 (79.4–90.1)	91.0 (87.0–93.9)	80.2 (73.7–85.4)
PCT (n = 430[a])	PCT	77.2[b] (73.0–80.9)	0.75[b] (.69–.80)	56.4 (47.0–65.3)	84.4[b] (80.0–87.9)	55.4[b] (46.1–64.2)	84.9 (80.6–88.4)

95% confidence intervals are displayed in parentheses.

Abbreviations: AUC, area under the curve; HR-B/V, host response bacterial versus viral; NPA, negative percent agreement; NPV, negative predictive value; PCT, procalcitonin; PPA, positive percent agreement; PPV, positive predictive value.

[a]Fourteen missing PCT values in the training cohort.

[b]Significant differences ($P < .05$) observed between Biomeme HR-BV bacterial model and PCT.

26% (Table 2). For the diagnosis of viral infection, the AUC was 0.92 (95% CI, .89–.94) (Supplementary Figure 1*B*) with accuracy of 86.7% (95% CI, 83.2%–89.6%), PPA of 91.0% (95% CI, 87.0%–93.9%), NPA of 80.2% (95% CI, 73.7%–85.4%), PPV of 87.4% (95% CI, 83.0%–90.8%), and NPV of 85.5% (95% CI, 79.4%–90.1%) given a prevalence of 60% of viral infection. No meaningful differences were observed for the probabilities in either bacterial or viral samples in the ≤7 or >7 DPSO groups. The scatter plot of viral and bacterial probabilities is shown in Figure 2*A*.

The aggregate expression of genes assigned to each pathogen type is summarized in a PCA plot (Supplementary Figure 2). The viral samples appear more distinct, whereas there is overlap between bacterial and SIRS samples. The outcome versus predicted probability for bacterial and viral models in the training cohort is displayed in the calibration plots (Supplementary Figure 3).

Biomeme HR-B/V Test Performance Compared to Procalcitonin. Next, we aimed to compare Biomeme HR-B/V and PCT performance in the training cohort. PCT levels >0.25 ng/mL were used to identify bacterial infection, as per standard cutoffs [34]. Procalcitonin data were available for 430 of the 444 subjects in the training cohort (Table 2).

For PCT, the AUC for identifying bacterial infection was 0.75 (95% CI, .69–.80), with an accuracy of 77.2% (95% CI, 73.0%–80.9%), PPA of 55.4% (95% CI, 46.1%–64.2%), NPA of 84.9% (95% CI, 80.6%–88.4%), PPV of 56.4% (95% CI, 47.0%–65.3%), and NPV of 84.4% (95% CI, 80.0%–87.9%). Across these metrics, the Biomeme HR-B/V test consistently displayed better performance. Using a test of 2 proportions, HR-BV performed significantly better ($P < .05$) than PCT in accuracy, PPA, and NPV metrics. Additionally, using the DeLong test to compare AUC values for the 430 subjects for whom both tests were run, the HR-B/V test performed significantly better than PCT ($P = 2.636 \times 10^{-6}$).

Technical Correlation Cohort

Biomeme HR-B/V Test Performance Compared to BioFire HR-B/V Test Performance. The purpose of the correlation cohort was to determine how closely the Biomeme HR-B/V test would perform using 22 host response genes when compared to the BioFire HR-B/V test using 42 genes. The correlation cohort consisted of 82 subjects: 59 (72%) with viral infection and 23 (28%) with bacterial infection. The Biomeme HR-B/V test identified all 23 cases of bacterial infection correctly (PPA, 100% [95% CI, 85.7%–100%]) (Tables 3 and 4 and Figure 2*B*). Three viral cases were predicted to have a bacterial–viral coinfection. For 59 subjects clinically predicted to be viral by the BioFire test, the Biomeme HR-B/V test identified 59 as viral (PPA, 100% [95% CI, 93.9%–100%]) and correctly excluded all 23 nonviral cases as nonviral (NPA, 100% [95% CI, 85.7%–100%]). Two-dimensional data representation using PCA showed viral samples as appearing more distinct and having only partial overlay with bacterial and SIRS samples, while SIRS samples overlapped with bacterial samples in the training cohort (Supplementary Figure 2). Outcome versus predicted probability for bacterial and viral models in the correlation cohort is displayed in the calibration plots (Supplementary Figure 3).

COVID-19 Cohort

We next assessed how the Biomeme HR B/V test performed at identifying patients with SARS-CoV-2 infection, since SARS-CoV-2 infections were not included in the signature's discovery, model training, or correlation cohorts (which were all enrolled prior to 2020). PCA data visualization showed good overlap between COVID-19 samples and viral samples in the training cohort (Figure 3*A*). When stratifying by duration of symptoms, outpatients mostly presented for care during the acute phase, while inpatients were also seen at later stages of infection (Figure 3*B*).

The Biomeme HR-B/V test correctly identified 86 of 111 subjects with COVID-19 (77.5%) as having viral infection (Table 5). Performance was better among outpatients and for subjects with shorter durations of symptoms. For outpatients, viral accuracy was 93.3% (95% CI, 78.7%– 98.2%) for 30 subjects with ≤7 DPSO and 88.9% (95% CI, 56.5%–98.0%) for 9 subjects with >7 DPSO. For inpatients, viral accuracy was 75.9% (95% CI, 57.9%–87.8%) for 29 subjects with ≤7 DPSO and 56.0% (95% CI, 37.1%–73.3%) for 25 subjects with >7 DPSO.

DISCUSSION

The overlap in the clinical presentation of bacterial and viral infections, together with the limitations associated with

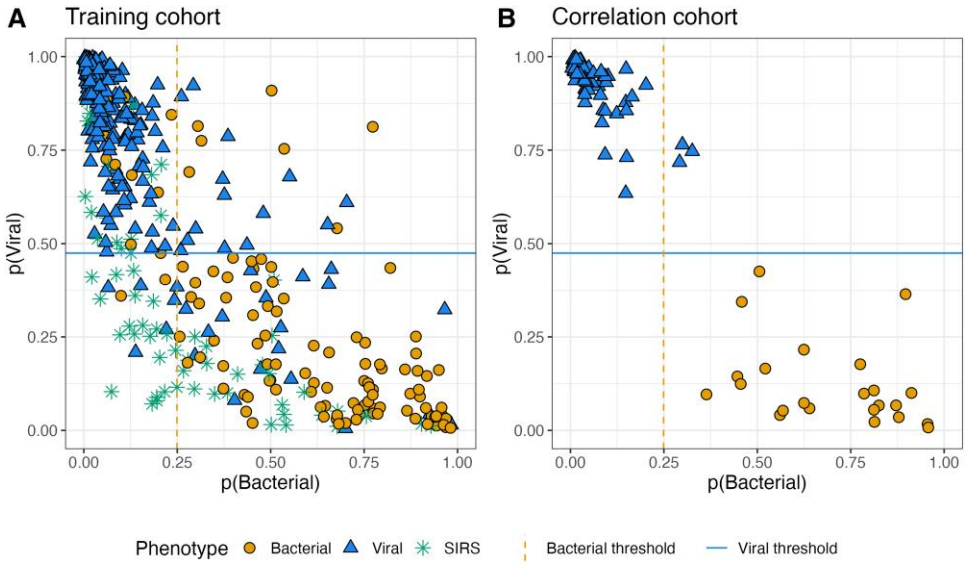

Figure 2. Predicted probabilities for the training cohort (*A*) and correlation cohort (*B*) for the bacterial model (x-axis) and viral model (y-axis). In the correlation cohort, all bacterial samples were correctly predicted to have a bacterial infection. All viral samples were also correctly predicted to have a viral infection, although 4 viral samples were predicted to have a coinfection, falling above both viral and bacterial thresholds. Abbreviation: SIRS, systemic inflammatory response syndrome.

Table 3. Performance Metrics Versus BioFire HR-B/V Results for Subjects in the Biomeme HR-B/V Correlation Cohort (Bacterial and Viral Models)

Assay	Testing Modality	Overall Accuracy, %	AUC	PPA, %	NPA, %
Biomeme HR-B/V (n = 82)	Bacterial model	96.3 (89.8–98.7)	1 (1–1)	100.0 (85.7–100.0)	94.9 (86.1–98.3)
	Viral model	100.0 (95.5–100.0)	1 (1–1)	100.0 (93.9–100.0)	100.0 (85.7–100.0)
BioFire HR-B/V (n = 82)	Bacterial model	100 (95.5–100.0)	1 (1–1)	100.0 (85.7–100.0)	100.0 (93.9–100.0)

95% confidence intervals are displayed in parentheses.

Abbreviations: AUC, area under the curve; HR-B/V, host response bacterial versus viral; NPA, negative percent agreement; PPA, positive percent agreement.

Table 4. Confusion Matrices for Correlation Cohort Subjects Tested Using Both the Biomeme and BioFire HR-B/V Assays

Assay	Truth → Test ↓	Bacterial	Viral	Sum
HR-BV bacterial model	B	23 (100%[a])	3	26
	Non-B	0	56 (95%[b])	56
	Sum	23	59	82
BioFire	B	23 (100%[a])	0	24
	V (non-B)	0	59 (100%[b])	58
	Sum	23	59	82

Only the bacterial model for the Biomeme HR-B/V test is displayed since the BioFire test did not generate a viral infection probability. Positive percent agreement (PPA) and negative percent agreement (NPA) percentages are displayed on the diagonals of the table.

Abbreviations: B, bacterial; HR-B/V, host response bacterial versus viral; V, viral.

[a]Test PPA.

[b]Test NPA.

pathogen-based diagnostic tests, drives inappropriate antibacterial use and exacerbates the problem of antimicrobial resistance. The newly developed Biomeme HR-B/V test showed excellent performance characteristics at differentiating bacterial versus viral infection, similar to the prior BioFire HR-B/V test, and may be useful at mitigating inappropriate antibacterial use.

Measuring the host immune response to different categories of pathogens provides an attractive and rapid diagnostic solution. The Biomeme HR-B/V test performed well compared to the previously developed classifier on the BioFire technology, despite using considerably fewer targets (22 vs 42) [20]. The Biomeme HR-B/V test also showed superior performance when compared to PCT, both with respect to the identification

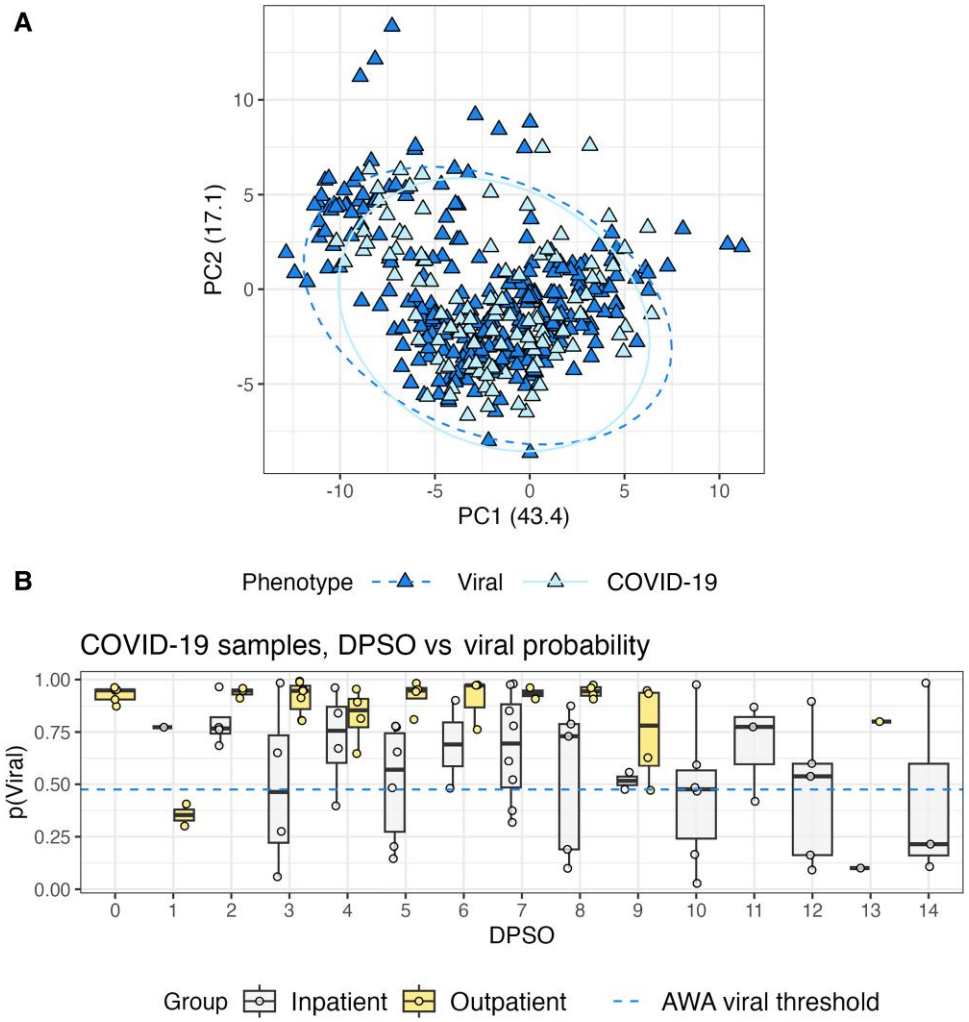

Figure 3. *A,* Principal component analysis of the viral training and coronavirus disease 2019 (COVID-19) samples. Principal component (PC) 1 and PC2, on the x- and y-axis, respectively, explain 60.5% of the variance. COVID-19 samples overlay closely with the viral samples in the training cohort. *B,* Viral probability (p(Viral)) vs days post–symptom onset (DPSO) in the COVID-19 cohort. A viral threshold is established using average weighted accuracy (AWA) metrics. Eighteen DPSO values were not available in the COVID-19 cohort and are not displayed here.

Table 5. Performance Characteristics of the Biomeme HR-B/V Test in the Stratified Coronavirus Disease 2019 Cohort

Model	DPSO[a]	Inpatient (n = 60)	95% CI	Outpatient (n = 51)	95% CI
Bacterial model	DPSO ≤7 (n = 59)	93.1% (n = 29)	78.0–98.1	96.7% (n = 30)	83.3–99.4
	DPSO >7 (n = 34)	68.0% (n = 25)	48.4–82.8	100.0% (n = 9)	70.1–100.0
Viral model	DPSO ≤7 (n = 59)	75.9% (n = 29)	57.9–87.8	93.3% (n = 30)	78.7–98.2
	DPSO >7 (n = 34)	56.0% (n = 25)	37.1–73.3	88.9% (n = 9)	56.5–98.0

Abbreviations: CI, confidence interval; DPSO, days post–symptom onset; HR-B/V, host response bacterial versus viral.

[a]No DPSO data, n = 18.

of bacterial infection and the ability to discriminate viral infection from SIRS. This improved performance is likely due to the Biomeme HR-B/V test offering a more complex analysis of the patient's underlying host immune state by reporting independent probabilities of bacterial and viral infection. This is also favorable compared to some other host response approaches. For example, there are 2 commercially available tests measuring host response proteins for bacterial versus viral infection: the MeMed BV test and the Lumos FebriDx test. Both tests only report the likelihood of a bacterial infection, without providing independent information about viral infections, coinfections, or noninfectious illness (ie, bacterial vs nonbacterial models).

The use of 2 models (ie, bacterial vs nonbacterial and viral vs nonviral) provides the ability to diagnose bacterial–viral coinfection (both positive) and to identify patients with noninfectious etiologies (both negative) [20]. This approach has also been utilized by Inflammatix, a company that is likewise developing an mRNA-based test to measure the host response to infection [35].

The Biomeme HR-B/V assay performed well in the COVID-19 cohort, with better performance among outpatients than among inpatients. Inpatients likely had a more severe immune response that may share host response features similar to those patients with bacterial infection or SIRS [36]. This pattern has also been shown in patients with other severe viral infections, such as influenza [37, 38]. In addition, inpatients with COVID-19 may have been more likely to receive treatment with immunomodulatory agents and were also more likely to be enrolled later in illness, both of which may affect assay performance.

A focus on the host rather than the pathogen is also useful to distinguish infection from colonization, as some organisms (human rhinovirus/enterovirus, *Streptococcus pneumoniae*, *Streptococcus pyogenes*) are carried in a high proportion by the general population. Moreover, host response tests are suited for emerging diseases, when pathogen-specific diagnostics are not yet available. Indeed, the HR-B/V test showed excellent performance at identifying patients with SARS-CoV-2 infection as being viral in etiology, especially early in the disease course when symptoms are largely in response to active viral replication. Although host-based immunodiagnostics are not able to identify the pathogen, and therefore direct specific treatment, they could complement current pathogen-based diagnostics. Simply identifying viral infection could reduce inappropriate antibacterial use, which is driving the global crisis of antibacterial resistance, itself associated with almost 5 million deaths annually [39]. Moreover, host response signatures derived from nasal or nasopharyngeal tissues could support development of a combined host and pathogen test that simultaneously measures the host and pathogen aspects of the infection.

Strengths of this study include the use of a racially and ethnically diverse cohort and validation of our results relative to another host gene expression test using a different platform. In addition, we used expert clinical adjudication as the comparator method for our training cohort given the absence of a single test or composite of tests to identify etiology. Given the limitations in clinical adjudication, it is possible the comparator method was incorrect, which would tend to lower estimates of a test's performance. Additionally, we did not include patients with immunocompromised status or who had coinfections; thus, performance in these cohorts cannot be extrapolated. Further, we excluded patients who had an "indeterminate" primary diagnosis based on clinical adjudication, as we were comparing performance of the Biomeme HR-B/V test to performance on the BioFire HR-B/V, and thus including indeterminate cases would not have provided useful information. The performance of the Biomeme HR-B/V assay needs to be determined in future unselected clinical cohorts. Finally, approximately 30% of patients in the training cohort had samples collected >7 DPSO, which may have affected model performance as the host response changes over time. However, models trained only on the most obvious cases may struggle to identify patients who may be at the fringes in terms of illness duration, while a model that can see the full spectrum of data may generate a more robust algorithm that is better suited to classify patients in less controlled settings.

In conclusion, the Biomeme HR-B/V test is a rapid, portable, laboratory-based test with excellent performance at identifying patients with bacterial or viral infection, offering a promising antibiotic stewardship strategy to combat the public health threat of antimicrobial resistance. Our findings need to be confirmed in future large, independent cohorts and in prospective studies.

Supplementary Data

Supplementary materials are available at *Open Forum 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

Author contributions. All authors were involved in the conduct of the study and analysis, as well as the decision to publish.

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Patient consent. Written informed consent was obtained from all patients. Approval was obtained from the institutional review boards of each of the enrolling sites: Duke University Medical Center (Durham, North Carolina), Henry Ford Hospital (Detroit, Michigan), UNC Health Care (Chapel Hill, North Carolina), Brigham and Women's Hospital (Boston, Massachusetts), University of Utah Medical Center (Salt Lake City, Utah), Newton-Wellesley Hospital (Newton, Massachusetts), University of California Hospital at Davis (Sacramento, California), the University of Texas Health Science Center at Houston (Houston, Texas), Hasbro Children's Hospital (Providence, Rhode Island), and Children's Hospital of Pittsburgh (Pittsburgh, Pennsylvania).

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Potential conflicts of interest. C. W. W. and E. L. T. owned equity in Biomeme during the conduct of the study, and received personal fees from Biomeme. T. W. B. consulted and owned equity in Biomeme during the conduct of the study. E. L. T., R. H., T. W. B., G. S. G., C. W. W., and M. T. M. are listed as inventors of a patent (WO 2017/004390 A1) for bacterial versus viral discrimination (licensed to Biomeme). P. G. M., J. S., C. C., S. N., A. C., M. P., and J. v. W. owned equity in Biomeme during the conduct of the study and received personal fees from Biomeme during the conduct of the study. C. W. W. serves as Biomeme's Chief Medical Officer. E. L. T. is presently an employee of Danaher Corporation and owns equity. All other authors report no potential conflicts of interest.

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