



Original Investigation | Infectious Diseases

Diagnostic Accuracy of a Bacterial and Viral Biomarker Point-of-Care Test in the Outpatient Setting

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Abstract

IMPORTANCE Acute respiratory infections (ARIs) account for most outpatient visits. Discriminating bacterial vs viral etiology is a diagnostic challenge with therapeutic implications.

OBJECTIVE To investigate whether FebriDx, a rapid, point-of-care immunoassay, can differentiate bacterial- from viral-associated host immune response in ARI through measurement of myxovirus resistance protein A (MxA) and C-reactive protein (CRP) from finger-stick blood.

DESIGN, SETTING, AND PARTICIPANTS This diagnostic study enrolled adults and children who were symptomatic for ARI and individuals in a control group who were asymptomatic between October 2019 and April 2021. Included participants were a convenience sample of patients in outpatient settings (ie, emergency department, urgent care, and primary care) who were symptomatic, aged 1 year or older, and had suspected ARI and fever within 72 hours. Individuals with immunocompromised state and recent vaccine, antibiotics, stroke, surgery, major burn, or myocardial infarction were excluded. Of 1685 individuals assessed for eligibility, 259 individuals declined participation, 718 individuals were excluded, and 708 individuals were enrolled (520 patients with ARI, 170 patients without ARI, and 18 individuals who dropped out).

EXPOSURES Bacterial and viral immunoassay testing was performed using finger-stick blood. Results were read at 10 minutes, and treating clinicians and adjudicators were blinded to results.

MAIN OUTCOMES AND MEASURES Bacterial- or viral-associated systemic host response to an ARI as determined by a predefined comparator algorithm with adjudication classified infection etiology.

RESULTS Among 520 participants with ARI (230 male patients [44.2%] and 290 female patients [55.8%]; mean [SD] age, 35.3 [17.7] years), 24 participants with missing laboratory information were classified as unknown (4.6%). Among 496 participants with a final diagnosis, 73 individuals (14.7%) were classified as having a bacterial-associated response, 296 individuals (59.7%) as having a viral-associated response, and 127 individuals (25.6%) as negative by the reference standard. The bacterial and viral test correctly classified 68 of 73 bacterial infections, demonstrating a sensitivity of 93.2% (95% CI, 84.9%-97.0%), specificity of 374 of 423 participants (88.4% [95% CI, 85.0%-91.1%]), positive predictive value (PPV) of 68 of 117 participants (58.1% [95% CI, 49.1%-66.7%]), and negative predictive value (NPV) of 374 of 379 participants (98.7% [95% CI, 96.9%-99.4%]). The test correctly classified 208 of 296 viral infections, for a sensitivity of 70.3% (95% CI, 64.8%-75.2%), a specificity of 176 of 200 participants (88.0% [95% CI, 82.8%-91.8%]), a PPV of 208 of 232 participants (89.7% [95% CI, 85.1%-92.9%]), and an NPV of 176 of 264 participants (66.7% [95% CI, 60.8%-72.1%]).

(continued)

Key Points

Question What is the diagnostic performance of a point-of-care immunoassay measuring C-reactive protein and myxovirus resistance protein A to differentiate bacterial- from viral-associated host immune response in outpatients with symptoms of suspected acute respiratory infection (ARI)?

Findings In this diagnostic study of 520 participants with suspected ARI, the bacterial and viral test had a sensitivity of 93.2%, negative predictive value of 98.7%, specificity of 88.4%, and positive predictive value of 58.1% to detect a bacterial-associated host immune response.

Meaning These findings suggest that this immunoassay may differentiate bacterial from viral infection in patients with ARI.

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

CONCLUSIONS AND RELEVANCE In this study, a rapid diagnostic test demonstrated diagnostic performance that may inform clinicians when assessing for bacterial or viral etiology of ARI symptoms.

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Introduction

Acute respiratory infections (ARIs), including acute bronchitis, pharyngitis, pneumonia, sinusitis, and the common cold, are the most common causes of infectious illnesses¹⁻³ and account for more than 150 million annual US outpatient visits.⁴⁻⁸ Overlap in presentation of bacterial and viral ARI, as well as noninfectious respiratory illnesses, makes it difficult to differentiate etiology and distinguish new from resolving infection with persistent symptoms.⁹ Clinicians tend to liberally prescribe antibiotics to avoid missing a bacterial infection that could progress to a serious infection or sepsis. The judicious use of antibiotics for an individual patient is balanced against overprescribing antibiotics and fostering antibiotic resistance. Many visits are associated with antibiotic prescription, and approximately 50% are classified as unnecessary or inappropriate.^{2,6,10-15} Diagnostic uncertainty is a key contributor to antibiotic overuse. Given this challenge, a diagnostic test with sufficient accuracy may be associated with improved decision-making and antibiotic use practices.

The FebriDx bacterial and viral test (Lumos Diagnostics) is a point-of-care immunoassay designed to detect and differentiate bacterial- from viral-associated host immune response in patients with a suspected ARI with the goal of aiding therapeutic decisions during the patient visit. This qualitative, 10-minute test simultaneously measures myxovirus resistance protein A (MxA) and C-reactive protein (CRP) biomarkers in finger-stick whole blood.¹⁶ MxA is induced by type I interferon as part of the innate immune response to a wide range of viral infections and forms circulating immune complexes with viral nuclear proteins.^{17,18} CRP is a nonspecific, acute-phase protein predominantly produced in response to inflammatory cytokines that are upregulated by acute inflammation and infection.^{16,19-21} Increased MxA levels, with or without increased CRP levels, are indicative of a viral infection, while increased CRP levels alone are indicative of a bacterial infection.^{9,22} Independently, MxA or CRP provide incomplete information for clinical decision-making. However, in combination, results may aid in clinical and therapeutic decisions.

To investigate whether this bacterial and viral test may assist clinicians in therapeutic decision-making, we conducted a prospective study evaluating the test's ability to detect and differentiate bacterial from viral infection. The primary aim was to investigate the performance characteristics of the bacterial and viral test for the detection and differentiation of bacterial- or viral-associated host immune response in ARI.

Methods

Institutional review board approval and written informed consent were obtained for this diagnostic study. Results are presented in accordance with the Standards for Reporting of Diagnostic Accuracy (STARD) 2015 essential items for reporting diagnostic accuracy studies.

Study Design

This was a prospective, blinded, multicenter, observational study of a bacterial and viral test device's performance in identifying bacterial vs viral etiology of infection. Participants were enrolled October 2019 to April 2021 at 20 outpatient sites throughout the United States. These consisted of 9 emergency departments, 6 urgent care clinics, and 5 primary care clinics.

Study Population

Asymptomatic Control Cohort

A control group of individuals who were asymptomatic was enrolled to evaluate the bacterial and viral test in individuals without ARIs. The study required a minimum enrollment of 50 participants in each age group (ages 1-21 years, 22-64 years, and ≥ 65 years). Inclusion criteria: aged 1 year or older and without infectious signs or symptoms. Exclusion criteria: fever (≥ 100.5 °F [38.1 °C]) within 14 days, cough, chills, dyspnea, purulent sputum, fatigue, pleuritic pain, nasal congestion, rhinorrhea, sore throat, hoarseness, earache, and autoimmune or rheumatologic disease.

ARI Cohort

Patients who presented to outpatient settings with new-onset respiratory symptoms and a recent fever were included. Inclusion criteria: aged 1 year or older, fever of 100.5 °F or greater at the time of or within 3 days of enrollment, clinical suspicion for ARI, and new onset of at least 1 of 6 symptoms (rhinorrhea, nasal congestion, sore throat, cough, hoarseness, or shortness of breath) within 7 days before enrollment. Exclusion criteria: incomplete or invalid testing for comparator method, unwilling to participate in 7-day follow-up, interferon therapy, immunosuppression, significant trauma or burns, major surgery, myocardial infarction or stroke within 30 days, antibiotics, antiviral medications, live immunization, or history of earache consistent with otitis media within 14 days, and chronic fever more than 7 days.

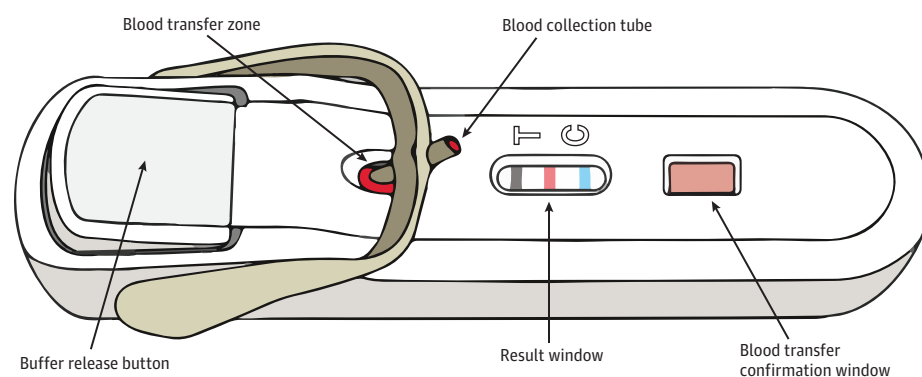
Immunoassay Measurements

The bacterial and viral test was a rapid, single-use, point-of-care lateral-flow immunoassay designed to detect host immune response to infection and differentiate bacterial and viral ARI through detection of MxA and CRP directly from finger-stick whole blood (**Figure 1**).^{16,23} The test provided qualitative results for increased CRP (≥ 2 mg/dL [≥ 20 mg/L]) and MxA (≥ 40 ng/mL) levels. Operators visually interpreted results (bacterial, viral, or negative) and were blinded to comparator testing. Treating clinicians were blinded to bacterial and viral test results. The finger-stick sample was collected concurrently with comparator samples. Results were read at 10 minutes.

Primary Outcome

The primary outcome was a bacterial- or viral-associated systemic host response to an ARI. A predefined comparator algorithm with expert adjudication was used to classify whether bacterial or viral infection was the etiology of the host response (eFigure 1 in the [Supplement](#)). Bacterial and viral test results were compared with adjudicated comparator algorithm results, and performance characteristics were calculated for bacterial and viral infection.

Figure 1. Bacterial and Viral Test



This drawing displays a used test with a viral positive result. The test is visually interpreted using lines that indicate each biomarker. A black line indicates increased C-reactive protein levels and is interpreted as bacterial if a red line is not present. A red line indicates increased MxA and is interpreted as viral with or without a black line. Presence of only a blue control line indicates the absence of increased C-reactive protein or myxovirus resistance protein A levels and is interpreted as negative. A blue control line indicates that the test result is valid.

Comparator Algorithm

Specimens were sent to central laboratories for testing. Laboratory personnel were blinded to bacterial and viral test results. Participants underwent pathogen detection via multiplex polymerase chain reaction (PCR) testing using combined nasopharyngeal and oropharyngeal swab for BioFire FilmArray Respiratory Panel (BioMerieux); real-time quantitative PCR testing for herpes simplex virus 1 and 2, Epstein-Barr virus, cytomegalovirus, *Neisseria gonorrhoeae*, and SARS-CoV-2 (Eurofins Viracor); target-enriched multiplex PCR testing for human bocavirus and *Fusobacterium necrophorum* (Eurofins Viracor); oropharyngeal swab for bacterial culture; and venous whole blood testing for complete blood count with differential (Siemens Advia), serum procalcitonin (BRAHMS PCT BioMerieux), and serum Epstein-Barr virus IgM antibody (DiaSorin Liaison) level testing. The comparator algorithm tested for 28 pathogens via PCR and culture (eTable 1 in the [Supplement](#)).

Final diagnosis was determined by comparator algorithm (eFigure 1 in the [Supplement](#)) in conjunction with adjudication by 2 independent reviewers who were blinded to results of the bacterial and viral test and could accept or override the comparator algorithm based on participant clinical data. If reviewers disagreed, 1 additional adjudicator (M.S.P) served as arbitrator.

Follow-up

All participants with ARIs were followed up by text message or telephone on study day 7. If the first contact attempt failed, participants were contacted daily for 21 days before being considered lost to follow-up. If hospital admission (defined as a hospital overnight stay >24 hours) was required by study day 7, the study team attempted to obtain the hospitalization record to determine the reason for admission. It was determined a priori that participants with a final diagnosis classified by the comparator algorithm as negative or viral at the initial visit who subsequently required hospitalization for pneumonia with objective evidence of a bacterial infection would be reclassified to a bacterial diagnosis. Objective evidence suggesting a bacterial infection was defined as at least 1 of the following: lobar pneumonia on chest imaging, identification of a bacterial pathogen with a newly increased serum procalcitonin level greater than 0.75 ng/mL, or death while hospitalized due to a suspected community acquired bacterial infection.

Statistical Analysis

The study was powered based on the confidence bounds of the sensitivity and specificity for bacterial-associated and viral-associated systemic host response, with an overall power of at least 80% and 1-sided type I error rate of 0.025 testing that the performance exceeded the a priori performance goal of 60% for each of 4 assessments. A sample size of 100 participants per hypothesis test met these criteria. The performance goal of 60% was based on the expected performance of other diagnostic tests plus a noninferiority margin.^{24,25}

Additional participants were enrolled beyond these calculations for each group to include a range of pathogens and participant ages. Race, ethnicity, and sex were collected to assess potential difference in performance based on demographics. Race and ethnicity were self-reported by participants. Categories for race were American Indian or Alaskan Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White or other; categories for ethnicity were Hispanic and non-Hispanic. Other was an option for participants who did not categorize themselves with the list provided. We attempted to provide an exhaustive list of possible races from which participants could make a selection but also provided other as an option because it was possible a portion of the cohort would not identify with races provided. We anticipated that a small number of participants would not identify with races listed. Races other than Black and White were combined because of small population numbers among American Indian, Asian, and Pacific Islander participants. Diagnostic accuracy was calculated by comparing the bacterial and viral test result (bacterial, viral, or negative) with the final diagnosis (bacterial, viral, or negative) as determined by the adjudicated comparator algorithm. Primary analysis of diagnostic performance was calculated separately for bacterial- and viral-associated host immune response. A sensitivity analysis was

conducted to assess diagnostic performance in microbiologically confirmed infections (results were pathogen detected and determined to be associated with the host immune response). Analyses were conducted using SAS statistical software version 9.4 (SAS Institute). CIs were calculated using the Wilson score. Additionally, posttest probabilities were calculated using bayesian theorem in which pretest probability (p) was converted to pretest odds ($p/[1 - p]$). Pretest odds were multiplied by the immunoassay likelihood ratio [LR] to determine posttest odds (O), which were converted to the posttest probability ($O/[O + 1]$). We report 2-sided 95% CIs for diagnostic performance assessments.

Results

Of 1685 individuals assessed for eligibility, 259 individuals declined participation, 718 individuals were excluded, and 708 individuals were enrolled, among whom 18 individuals exited prior to cohort identification. There were 520 individuals in the ARI group (230 male patients [44.2%] and 290 female patients [55.8%]; mean [SD] age, 35.3 [17.7] years; 110 Black individuals [21.2%], 356 White individuals [68.5%], and 54 individuals with other race, including American Indian or Alaskan Native, Asian, Native Hawaiian or other Pacific Islander, and other not specified [10.4%]; 97 Hispanic individuals [18.7%]) and 170 individuals in the asymptomatic group (**Table 1; Figure 2**). In the ARI group, there were 108 individuals aged 1 to 21 years (20.8%), 373 individuals aged 22 to 64 years (71.7%), and 39 individuals aged 65 years or older (7.5%). The most common symptoms included

Table 1. Baseline Characteristics

Characteristic	Patients, No. (%)					
	Total (N = 520)	Bacterial classification (n = 73)	Viral classification (n = 296)	Negative classification (n = 127)	Unknown classification (n = 24) ^a	Asymptomatic cohort (N = 170)
Sex						
Female patients	290 (55.8)	44 (60.3)	153 (51.7)	93 (73.2)	8 (33.3)	94 (55.3)
Male patients	230 (44.2)	29 (39.7)	143 (48.3)	34 (26.8)	16 (66.7)	76 (44.7)
Age, mean (SD), y	35.3 (17.7)	37.9 (17.2)	34.4 (17.7)	37 (18)	37 (12)	44 (24)
Age group, y						
1-21	108 (20.8)	13 (17.8)	69 (23.3)	23 (18.1)	3 (12.5)	53 (31.2)
22-64	373 (71.7)	52 (71.2)	207 (69.9)	93 (73.2)	21 (87.5)	54 (31.8)
≥65	39 (8)	8 (11)	20 (7)	11 (9)	0	63 (37)
Race						
African American or Black	110 (21.2)	8 (11.0)	78 (26.4)	22 (17.3)	2 (8.3)	29 (17.1)
White	356 (68.5)	58 (79.5)	180 (60.8)	96 (75.6)	22 (91.7)	135 (79.4)
Other ^b	54 (10.4)	7 (9.6)	38 (12.8)	9 (7.1)	0	6 (3.5)
Ethnicity						
Hispanic	97 (18.7)	12 (16.4)	58 (19.6)	27 (21.3)	0	21 (12.4)
Not Hispanic	419 (80.6)	60 (82.2)	235 (79.4)	99 (78.0)	24 (100)	149 (87.6)
Declined to answer	4 (0.8)	1 (1.4)	3 (1.0)	1 (0.8)	0	0
Fever						
At time of visit	166 (31.9)	33 (45.2)	117 (39.5)	14 (11.0)	2 (8.3)	0
Reported within 72 h of visit	354 (68.1)	40 (54.8)	179 (60.5)	113 (89.0)	22 (91.7)	0
Symptom						
Cough	425 (81.7)	46 (63.0)	265 (89.5)	97 (76.4)	18 (75.0)	0
Sore throat	278 (53.5)	49 (67.1)	139 (46.9)	77 (60.6)	13 (54.2)	0
Sinus symptom	329 (63.3)	27 (37.0)	168 (56.7)	43 (33.9)	23 (95.8)	0
Shaking or chills	270 (51.9)	44 (60.3)	154 (52.0)	52 (40.9)	18 (75.0)	0
Shortness of breath	119 (22.9)	23 (31.5)	73 (24.7)	21 (16.5)	0	0

^a Final diagnosis could not be determined for 24 participants due to insufficient laboratory test results.

^b Other included American Indian, Asian, and Pacific Islander.

cough, sore throat, sinus symptoms, and shortness of breath (Table 1). The bacterial and viral test needed to be repeated in 13 of 690 enrollments (1.9%) due to device-related issues.

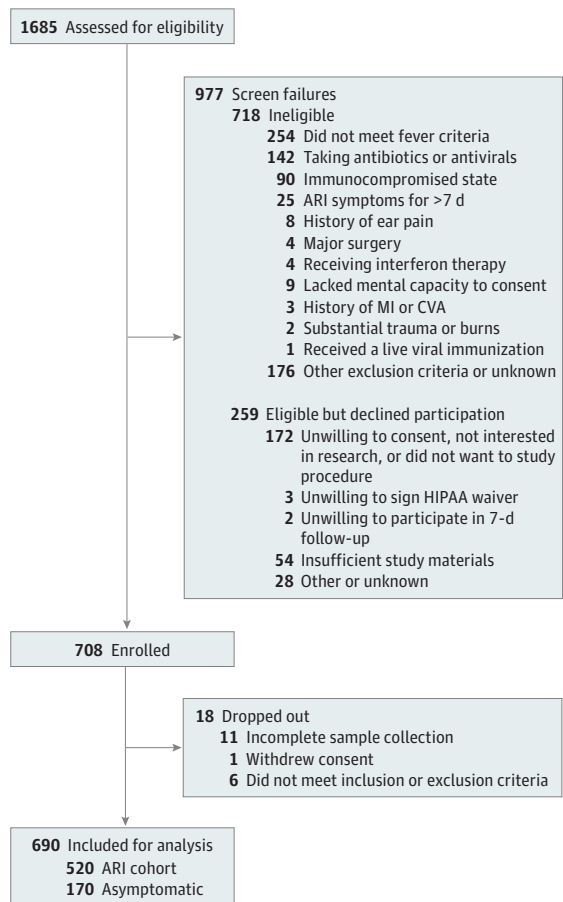
Diagnostic Accuracy of Immunoassay

Final diagnosis could not be determined for 24 participants due to insufficient laboratory test results (classified as unknown and not included in the analysis). Among 496 study participants with a clinically adjudicated final diagnosis with an immunoassay test result, the comparator algorithm classified the diagnosis as bacterial for 73 participants (14.7%), viral for 296 participants (59.7%), and negative for infection among 127 participants (25.6%), and no participants had coinfection. The most common pathogens identified were influenza A (117 participants [23.6%]), influenza B (63 participants [12.7%]), SARS-CoV-2 (45 participants [9.1%]), and group A *Streptococcus* (37 [7.4%]) (eTable 2 in the Supplement).

Bacterial-Associated Host Response Immune Identification

A total of 73 participants were classified as having a bacterial-associated host response. The bacterial and viral test correctly classified 68 of 73 participants with a bacterial infection, demonstrating a sensitivity of 93.2% (95% CI, 84.9%-97.0%), a specificity of 374 of 423 participants (88.4% [95% CI, 85.0%-91.1%]), a PPV of 68 of 117 participants (58.1% [95% CI, 49.1%-66.7%]), and an NPV of 374 of 379 participants (98.7% [95% CI, 96.9%-99.4%]), with a positive LR of 8 (95% CI, 6-11) and negative LR of 0.08 (95% CI, 0.03-0.18) (Table 2). Diagnostic performance for bacterial-associated systemic host response exceeded the prespecified study success criteria. Pretest and posttest probabilities

Figure 2. Screening and Enrollment Flow Diagram



ARI indicates acute respiratory infection; CVA, cerebrovascular accident; HIPAA, Health Insurance Portability and Accountability Act of 1996; MI, myocardial infarction.

were calculated using positive and negative LR_s (bayesian theorem). Pairing the immunoassay with clinician pretest probability improved posttest probabilities to rule in and rule out bacterial infection. Calculations can be found in eTable 4 and eFigure 2 in the [Supplement](#).

Viral-Associated Host Response Immune Identification

A total of 296 participants were classified as having a viral-associated host response. The test correctly classified 208 of 296 viral infections, for a sensitivity of 70.3% (95% CI, 64.8%-75.2%), a specificity of 176 of 200 participants (88.0% [95% CI, 82.8%-91.8%]), a PPV of 208 of 232 participants (89.7% [95% CI, 85.1%-92.9%]), and an NPV of 176 of 264 participants (66.7% [95% CI, 60.8%-72.1%]), with a positive LR of 6 (95% CI, 4-9) and negative LR of 0.3 (95% CI, 0.3-0.4) (Table 2). Diagnostic performance for detection of viral-associated systemic host response exceeded the prespecified study success criteria. Pretest and posttest probabilities were calculated using positive and negative LR_s (bayesian theorem). Pairing the immunoassay with clinician pretest probability improved posttest probabilities to rule in and rule out viral infection. Calculations can be found in eTable 4 and eFigure 2 in the [Supplement](#).

Coinfection and Codetection

No participants had coinfection, defined as the identification of bacterial and viral pathogens plus a host response, by the comparator algorithm. Codetection, the identification of bacterial and viral pathogens, with or without a host response, occurred in 143 participants with final diagnoses (28.8%).

Sensitivity Analysis

A sensitivity analysis was conducted to further assess the diagnostic performance of the bacterial and viral test to detect microbiologically confirmed infections. Performance was similar when stratified by microbiologically confirmed infection. Moreover, the sensitivity for the bacterial and viral test to detect microbiologically confirmed bacterial infections was 33 of 33 infections (100% [95% CI, 89.6%-100%]) (eTable 3 in the [Supplement](#)).

Association With Antibiotic Prescribing Patterns

Because the test is meant to serve as an adjunct to clinical assessment to help guide treatment strategies, we collected treatment information. Assuming that antibiotics should be prescribed for patients with bacterial infection and withheld from those without bacterial infection, prescribing according to the bacterial and viral test would have appropriately prompted the addition of antibiotics for 25 participants who did not receive them, improving the antibiotic treatment rate for individuals classified as having a bacterial infection from 45 participants (61.6%) to 68 participants (93.2%) (Table 3). The test would have appropriately withheld antibiotics in 67 participants who did

Table 2. Bacterial and Viral Test Performance Characteristics

Characteristic	Associated host immune response			
	Bacterial		Viral	
	No./No. ^a	Value, % (95% CI)	No./No.	Value, % (95% CI)
Prevalence	73/496	14.7 (11.7-17.0)	296/496	59.7 (53.0-61.0)
Sensitivity	68/73	93.2 (84.9-97.0)	208/296	70.3 (64.8-75.2)
Specificity	374/423	88.4 (85.0-91.1)	176/200	88.0 (82.8-91.8)
Predictive value				
Positive	68/117	58.1 (49.1-66.7)	208/232	89.7 (85.1-92.9)
Negative	374/379	98.7 (96.9-99.4)	176/264	66.7 (60.8-72.1)
Likelihood ratio				
Positive	NA	8.0 (6.1-10.5)	NA	5.9 (4.0-8.6)
Negative	NA	0.08 (0.03-0.2)	NA	0.3 (0.3-0.4)

^a Final diagnosis could not be determined for 24 participants due to insufficient laboratory test results, so there were 496 participants with a clinically adjudicated final diagnosis with a bacterial and viral test result.

not have a bacterial infection and were unnecessarily prescribed antibiotics. However, 38 participants without a bacterial infection but a test indicative of a host response to a bacterial infection may have received antibiotics when not needed (Table 3). On balance, bacterial and viral test use may be associated with a decrease in overprescription from 78 of 423 participants with viral infection or negative test results (18.4%) to 49 participants (11.6%) and a decrease in underprescription due to missed bacterial infection from 28 of 73 participants with bacterial infection test results (38.4%) to 5 participants (6.8%) (Table 3). The net outcome would be improved coverage and reduced overprescribing. The bacterial and viral test had a correct change at an approximately 2.4:1 ratio (25 participants changed to positive in bacterial infection + 67 participants changed to no antibiotics in viral infection/38 participants changed to antibiotics in viral infection), associated with an improved rate of prescription for bacterial infection and a reduced overprescription rate (Table 3).

Follow-up

Patients were contacted on study day 7. There were 5 patients admitted to the hospital within 7 days, among whom records were available for 4 patients. There were 2 patients who were admitted for a bacterial infection. They had bacterial and viral tests that were positive for bacterial-associated systemic host response at the index visit. Of these patients, 1 was appropriately treated with antibiotics and the other was not. There were 2 patients classified as having a viral-associated systemic host response by the bacterial and viral test at the index visit and admitted for worsening symptoms that were not attributed to a bacterial infection. There were no deaths within 7 days of study visit or during hospital admission, and 9 patients were considered lost to follow-up.

Asymptomatic Control Group

There were 170 participants enrolled in the asymptomatic control cohort, among whom there were 53 individuals aged 1 to 21 years (31.2%), 54 individuals aged 22 to 64 years (31.8%), and 63 individuals aged 65 years or older (37.1%), with a mean (SD) age of 44 (24) years. We excluded 9 participants from the analysis due to insufficient comparator sample or lack of test results to determine final diagnosis. There were 3 participants who were asymptomatic but had a final bacterial or viral diagnosis determined by the comparator method. Of the remaining 158 patients with confirmed negatives, the bacterial and viral test results were negative in 156 individuals (98.7% [95% CI, 95.5%-99.7%]). Bacterial or viral pathogens without associated host immune response were detected in 75 participants (47.5%), indicating colonization.

Discussion

We conducted a prospective, blinded, multicenter diagnostic study that assessed the diagnostic accuracy of a bacterial and viral test, a rapid immunoassay that uses CRP and MxA biomarkers to detect bacterial- or viral-associated host immune response in patients with ARI.^{20,26} Compared with an adjudicated comparator algorithm, the bacterial and viral test demonstrated sensitivity (93.2%) and NPV (98.7%) to detect and rule out bacterial-associated host immune response and specificity (88.0%) and PPV (89.7%) to detect viral-associated host immune response. The test detected all

Table 3. Antibiotic Prescription

Bacterial outcome	Patients prescribed antibiotics by clinician, No.					
	With diagnosis of bacterial infection			With diagnosis of viral infection or negative classification		
	Without assay outcome	With assay outcome	Total	Without assay outcome	With assay outcome	Total
Positive	25	43	68	38	11	49
Negative	3	2	5	307	67	374
Total	28	45	73	345	78	423

microbiologically confirmed bacterial infections with a sensitivity and NPV of 100%, suggesting an ability to help guide antibiotic therapy in patients with ARIs. The test's PPV (58.1%) to detect bacterial infection may be associated with overprescription of antibiotics. This underscores the importance of using the test as an aid to clinical judgement rather than as a stand-alone test. Of note, when we evaluated outcomes associated with the test based on real-world actions among patients studied, a 2.4:1 decrease in antibiotic prescribing patterns was found when using the test in the context of clinical judgement.

CRP is an established marker associated with inflammation that is a sensitive yet unspecific measure of an acute-phase response.²⁷ Bacterial infection stimulates increased CRP levels; however, viral infections, including influenza, adenovirus, and SARS-CoV-2, are associated with increased CRP levels,^{17,19,20} and therefore, CRP is unable to differentiate viral or bacterial etiology independently.^{28,29} Studies have found increased MxA levels associated with viruses (eg, influenza, rotavirus, adenovirus, respiratory syncytial virus, cytomegalovirus, coronavirus, hepatitis C virus, and SARS-CoV-2),^{23,30-35} suggesting that MxA may be an adept marker associated with acute viral infection.³⁶ Our data, along with results from previously published studies, suggest that simultaneous detection of CRP and MxA may distinguish viral and bacterial ARI with sensitivity and specificity ranging from 80% to 95%³⁷ and 76% to 94%,²² respectively.

From a practical perspective, clinicians often prescribe antibiotics to ensure that they address a potential bacterial infection at the risk of contributing to antibiotic resistance. Due to ARI's nonspecific clinical presentation, it is challenging to diagnose the underlying cause of infection or whether persistent symptoms represent a newly developing infection or resolving infection or are associated with another autoimmune, immunological, or environmental source rather than infectious stimuli. When clinicians face diagnostic uncertainty (ie, a pretest probability of 40%), using a bayesian approach, one may calculate that the bacterial and viral test was associated with an improvement to an 84% posttest probability for detecting a bacterial infection when the test was positive and 5% when the test was negative. It is important to underscore that a test that was negative for bacterial infection was correct in 374 of 379 patients (NPV, 98.7%), which may provide clinicians with reassurance to withhold antibiotics when supported by the clinical assessment. Of note, 25.6% of patients in the cohort were classified as negative for infection by comparator algorithm, which suggests that many patients present in convalescence. We submit that patients have fidelity in testing; thus, when treating clinicians deduce that a patient does not have a bacterial or viral infection and the bacterial and viral test confirms the suspicion, the tangible display of the results may help clinicians to communicate their clinical management strategy.

Limitations

This study has several limitations, including a minority representation of patients older than 65 years (7.5%). A large number of screen failures, with the most common reasons being not having a fever within 72 hours of enrollment, taking an antimicrobial medication in the previous 7 days, or being unwilling to undergo study procedures. The enrolled cohort represents the proposed use of the test (new-onset ARI) in a low-risk population presenting to an outpatient setting. Studies in clinical settings that include patients who were excluded in our study should be conducted given that test performance in these groups is currently undefined. All-comer enrollment was not used owing to lack of study staff availability during the COVID-19 pandemic, when many research assistants were not able to approach patients with respiratory symptoms suspected of COVID-19; therefore, patients may have been missed during screening. Our study did not include patients who were immunocompromised, and perhaps most importantly, there is a lack of a criterion standard to differentiate viral and bacterial respiratory infections. Coinfection is relatively rare in the outpatient setting,³⁸⁻⁴¹ and our study algorithm did not identify patients with coinfection; therefore, it is possible that these patients were underrepresented or misclassified. Pathogen-specific tests are limited by an inability to differentiate colonization and active infection given that they may remain

positive after clinical resolution of the acute infection. Our study used an algorithm with expert adjudication to mitigate errors and maximize validity; however, misclassification may have occurred.

Conclusions

This diagnostic study found that a bacterial and viral test that detects MxA and CRP biomarkers in finger-stick blood met prespecified success criteria for differentiating bacterial- from viral-associated host immune response to ARI. The ability to detect a bacterial infection that may benefit from antibiotic treatment, while providing confidence that a bacterial infection is not present and thus antibiotic therapy is not needed, is essential to optimizing clinical management and addressing global antimicrobial resistance. Improved treatment decisions in ARI may be associated with decreases in preventable disease progression, adverse events, antimicrobial resistance, and costs associated with antimicrobial misuse in millions of patients at low risk who present in the outpatient setting with suspected ARI.

ARTICLE INFORMATION

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Author Contributions: Dr Shapiro had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Acquisition, analysis, or interpretation of data: Shapiro, Filbin, Hou, Kurz, Han, Aufderheide, Ward, Pulia, Birkhahn, Diaz, Hughes, Harsch, Bell, Suarez-Cuervo.

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SUPPLEMENT.

eTable 1. Comparator Algorithm, Tests for Pathogen Detection and Host Response

eTable 2. Bacterial and Viral Pathogens Detected

eTable 3. Performance Characteristics, Microbiologically Confirmed Infection

eTable 4. Pretest and Posttest Probability

eFigure 1. Comparator Algorithm

eFigure 2. Pretest and Posttest Probability