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Comparison of two rapid host-response tests for distinguishing bacterial and viral infection in adults with acute respiratory infection

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SUMMARY

Objectives: Distinguishing bacterial from viral acute respiratory infection (ARI) is challenging, leading to inappropriate antimicrobial use and antimicrobial resistance. We evaluated the accuracy of two host-response tests to differentiate bacterial and viral infection.

Methods: This study used patient blood samples previously collected during a randomised controlled trial of adults hospitalised with ARI. The aetiology for each patient was clinically adjudicated. PAXgene blood RNA samples were tested using the TriVerity test (which measures 29 mRNAs) and serum samples were tested using the MeMed BV test (which measures 3 proteins). Diagnostic accuracy was calculated against adjudicated aetiology.

Results: 169 patients were tested. Median age was 60 (45–74) years and 152 (90%) received antibiotics. 60 (36%) were adjudicated as bacterial, 54 (32%) as viral, 26 (15%) as viral/bacterial co-infection, and 29 (17%) as non-infected. For bacterial (including bacterial/viral co-infection) versus non-bacterial infection, the TriVerity bacterial score had a Positive Percentage Agreement (PPA) of 81% (95%CI 70–89) and a Negative Percentage Agreement (NPA) of 66% (95%CI 55–79) and the MeMed BV score had a PPA of 96% (95%CI 90–99) and NPA of 34% (95%CI 23–47). The AUROC for the two tests was 0.77 (95%CI 0.70–0.84) and 0.81 (95%CI 0.74–0.87) respectively, p = 0.388.

Conclusions: Both tests demonstrated similar overall accuracy for distinguishing bacterial infection with the Triverity test missing some bacterial infections and MeMed BV misclassifying most viral infections as bacterial. Prospective impact studies evaluating antibiotic use, safety and cost effectiveness are now required.

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Introduction

Acute respiratory infections (ARIs) are the commonest reason for antibiotic use worldwide.[1–3] In the United Kingdom, the vast majority of adult patients presenting to hospital with ARI are prescribed antibiotics despite a large proportion being caused by viruses.[4,5] Unnecessary antibiotic use is the key driver of antimicrobial resistance which is now recognised as a global threat to human health.[6,7] Diagnostic uncertainty over the causative pathogen is a key driver of this excessive use of antibiotics, as viral and bacterial ARIs cannot be reliably distinguished clinically.[8,9] Rapid diagnostic testing for pathogens is an attractive solution, however, for bacteria this is hampered by difficulty obtaining appropriate lower respiratory tract samples and by the inability to differentiate colonising organisms from those causing disease. Although the use of multiplex molecular point-of-care tests (POCT) for respiratory viruses has been associated with a range of benefits in clinical trials, the impact on antibiotic use was minimal.[10,11] This is likely to be due to their inability to rule out concomitant bacterial infection.

Testing for host immune response biomarkers has the potential to differentiate viral and bacterial infection and therefore reduce inappropriate antibiotic use by ruling out bacterial infection. Two acute phase inflammatory proteins, C-reactive protein (CRP) and procalcitonin (PCT) are already used widely in clinical practice but cannot differentiate bacterial from viral infection with sufficient accuracy.[12–14]

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The MeMed BV test (MeMed, Tirat Carmel, Israel) uses measurement of three host immune proteins: TNF-related apoptosis-inducing ligand; interferon gamma-induced protein 10 and C-reactive protein (TRAIL, IP-10 and CRP) in blood, using a laboratory immunoassay platform, which are then computationally integrated into a single score denoting the likelihood of either a bacterial (including viral/bacterial co-infection) or a viral (and other non-bacterial aetiologies) infection.[15] Studies evaluating MeMed BV in a range of different settings and including adults and children have reported high levels of accuracy.[16,17]

An alternative approach to host immune protein detection is the use of host mRNA transcriptomics-based assays to differentiate bacterial from viral infection. The TriVerity Acute Infection and Sepsis Test (Inflammatix, Sunnyvale, CA, USA), uses an isothermal reverse-transcribed loop-mediated amplification (qRT-LAMP) assay to measure levels of 29 host mRNAs in blood and incorporates machine learning to calculate 3 separate scores predicting the likelihood of bacterial infection, viral infection and illness severity.[18] This 29 gene set classifier has shown good levels of accuracy at distinguishing bacterial and viral infection across a range of clinical infection syndromes, in studies performed using laboratory instruments.[19–21] The Myrna platform is a newly developed small footprint rapid analyser that analyses TriVerity test cartridges and is designed for use at the point-of-care.

This study aimed to evaluate and compare the diagnostic accuracy of the TriVerity test, performed on the Myrna POCT platform, and the MeMed BV test, performed in a laboratory setting, in differentiating bacterial and viral infections in adults hospitalised with acute respiratory illness.

Methods

Study design, setting and patients

This retrospective diagnostic accuracy study used stored blood samples and data from the FluPOC trial: a multicentre randomised controlled trial evaluating the impact of POCT for Influenza in adults hospitalised with ARI, performed in the UK from 2017–2019. The results of the study and the protocol have been published previously and are publicly available https://www.thelancet.com/journals/lanres/article/PIIS2213–2600(20)30469–0/fulltext.[22,23] In brief, patients were eligible for recruitment if they: were aged ≥18 years, presented to hospital with symptoms of ARI of 10 days duration or less, were admitted and could be recruited within 16 h of presentation. All patients either gave written informed consent, or where patients lacked capacity to consent, consultee assent was obtained. All patients had upper respiratory tract samples (and, where available, lower respiratory tract samples) tested for respiratory viruses and atypical bacteria using the FilmArray Respiratory panel 2 (bioMérieux, Marcy l'Etoile, France) as part of the study. Patients had blood samples taken within 24 h following presentation to hospital and stored. All patients recruited within the parent study were approached for blood samples. These samples were processed and frozen at −80°C within 2 h of collection and did not undergo any freeze-thraw cycles prior to this study. All patients with stored PAXgene blood RNA tubes (PreAnalytix, Switzerland, a QIAGEN/BD company) were included in this study. Procalcitonin was retrospectively tested on stored serum samples and used in clinical adjudication along with the FilmArray results and routinely performed laboratory and radiological data.

Ethics approval

This FluPOC study was approved by the South Central – Hampshire A Research Ethics Committee on 7 September 2017 (reference[17]/SC/0368- amended once on 23 November 2017 to include an additional recruitment site). This included approval for the testing and analysis carried out in this study.

Clinical adjudication

In this diagnostic accuracy study, the reference standard was guidance tool-assisted clinical adjudication of infection status by an expert panel of Infectious diseases physicians with expertise in ARI, who had no input into the care of recruited participants. Clinical adjudication is the most widely accepted reference standard in diagnostic accuracy studies in the absence of gold standard diagnostic methods.[24,25] However, the process for clinical adjudication is not standardised and therefore we designed an adjudication guidance tool to assist with this process (supplementary tables S1 and S2). Our guidance tool describes four categories of likelihood for the presence of bacterial and viral infection: confirmed, probable, unlikely, and rejected, as has been used in previous studies.[19,20] Diagnostic clinical data (microbiological, virological, laboratory, and radiological) were used to categorise patients into these four categories. Three physicians independently reviewed anonymised clinical data from cases and adjudicated each participant for the presence both bacterial and viral infection into the category of the highest likelihood for which they qualified, using the guidance tool. All adjudicators were blinded to the TriVerity and MeMed BV results. Final adjudication status for each participant was determined by the majority rule as detailed in the supplementary appendix (table S3).[26]

Two methods were used for converting the four original assessment categories into a binary (i.e., present or absent) classification for bacterial and viral infection: "consensus adjudication" (CA) and "forced adjudication" (FA) as described previously.[24] The more stringent CA method only considers confirmed (bacterial/viral) adjudications as "(bacterial/viral) infection present" cases and rejected adjudications as "(bacterial/viral) infection absent" cases. The remaining cases (unlikely or probable adjudication) are considered inconclusive and removed from downstream analyses. The FA method "forces" every case into a binary classification for the presence of bacterial infection and presence of viral infection, at the risk of introducing more uncertainty into the adjudicated infection status, due to ambiguous clinical presentation; all cases adjudicated as confirmed or probable become "(bacterial/viral) infection present" cases and those adjudicated as unlikely or rejected become "(bacterial/viral) infection absent". The study profile with the numbers of patients analysed by each adjudication method is detailed in Fig. 1. In this study we focus on reporting of results of FA, consistent with reporting guidelines for diagnostic accuracy studies,[27] but also provide the results for CA for completeness.

TriVerity Acute Infection and Sepsis Test

The TriVerity test generates three numerical scores indicating the likelihood of bacterial infection, viral infection and illness severity by interpreting the levels of 29mRNAs, quantified via qRT-LAMP.[23] Each score ranges between 1 and 50 and falls within one of the five categorical bands of likelihood: very low (1–10), low (11–20), moderate (21–30), high (31–40), and very high (41–50) as shown in the supplementary Fig. S1. PAXgene blood RNA samples were thawed and inserted into TriVerity cartridges and tested on the Myrna instrument (shown in supplementary appendix). Full details of the testing process and quality controls are provided in the supplementary appendix. As well as the results of the test, the time to result and the run failure rate was recorded. Accuracy for the illness severity score component of the TriVerity test was not analysed due to the very low event rate for the validated outcomes (Intensive Care Unit [ICU] admission and death) in this cohort.

MeMed BV test

The MeMed BV test uses measurement of three host immune proteins (TRAIL, IP-10, and CRP) which are computationally integrated into a

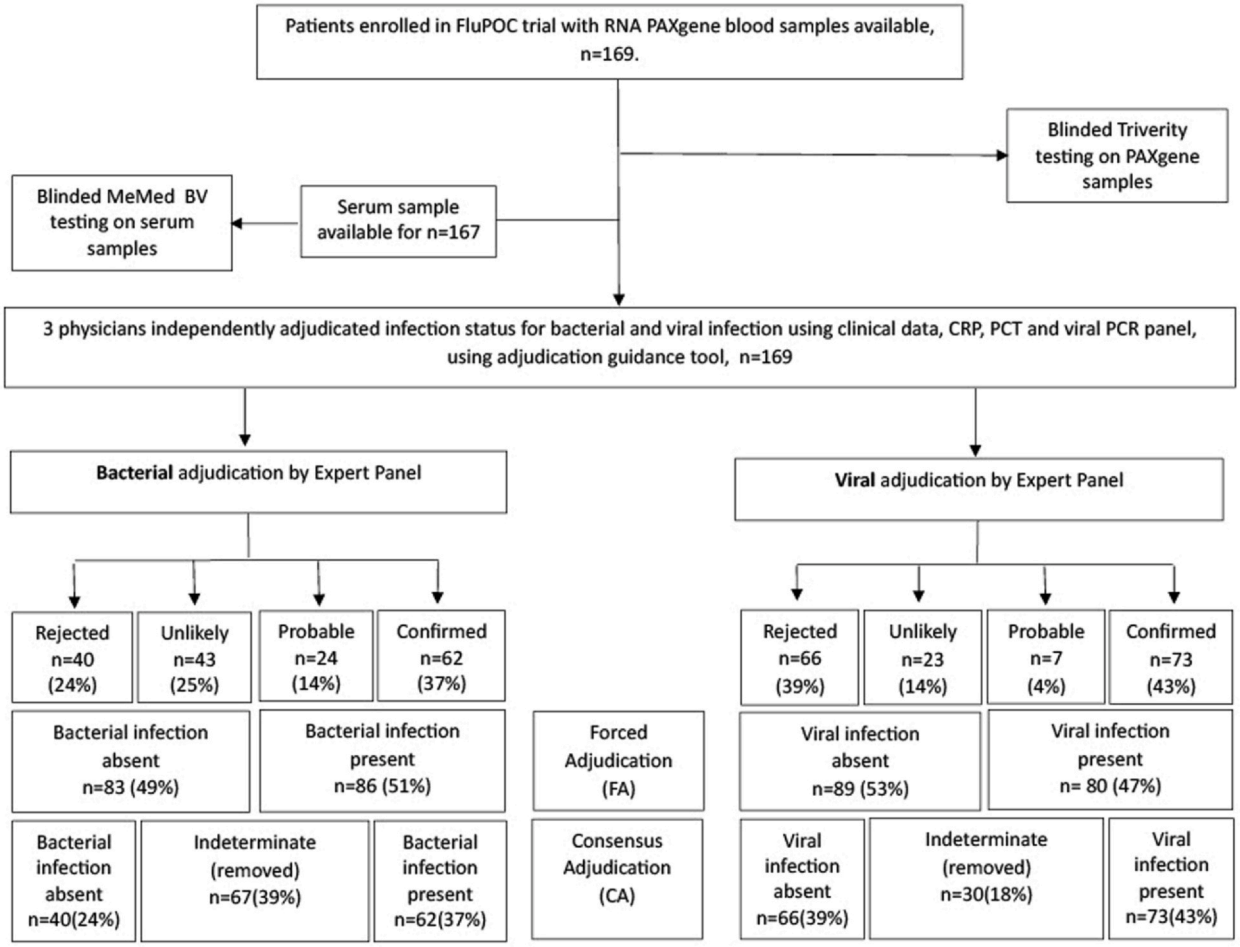

Fig. 1. Study profile.

single score from 0–100 denoting the likelihood of bacterial or 'viral and other non-bacterial aetiology'.[15] A score of 90–100 is classified as high likelihood of bacterial infection, 66–89 as moderate likelihood of bacterial infection, 35–65 as equivocal, 11–34 as moderate likelihood of viral infection, and ≤10 as high likelihood of viral infection. Overall, a score of > 65 is suggested to indicate bacterial infection and < 35 to indicate viral and 'other non-bacterial infection aetiology' (shown in supplementary Fig. S2). Stored serum samples frozen at −80 °C were shipped on dry ice to Labor Berlin-Charité Vivantes GmbH laboratory (Berlin, Germany) thawed and MeMed BV tested using the DiaSorin Liasion XL immunoassay platform.

Statistical analysis

Descriptive statistics were used to summarise baseline demographic and clinical variables. For continuous data, median and interquartile range were calculated and categorical or binary data variables were summarised as frequency and percentage of total. Measures of diagnostic accuracy with 95% confidence intervals were calculated for the TriVerity and MeMed test compared with clinical adjudication, overall and according to likelihood bands, for both bacterial (including co-infection with viruses) and viral infection. Accuracy was calculated using the two different methods of clinical adjudication (CA and FA) as previously described. The terms positive and negative percentage agreement (PPA and NPA) are used in preference to sensitivity and specificity to acknowledge the lack of a gold standard for comparison and the inherent uncertainty with using clinical adjudication. In addition, receiver operator characteristic (ROC) curves were generated for bacterial (including co-infection with viruses) versus non-bacterial infection and viral versus non-viral infection, for both CA and FA, and area under the curve (AUC) calculated with 95% confidence intervals. As MeMedBV does not distinguish between bacterial and bacterial/viral co-infection (categorising co-infections as bacterial) we also calculated MeMed BV performance for viral infection by categorising bacterial/viral co-infection as bacterial, i.e. bacterial (including viral/bacterial co-infection) versus viral infection. AUCs were compared using the Hanley & McNeil method.[28] All other analyses were performed using Prism version 9.4.1 (GraphPad Software, La Jolla, CA, USA) and Stata version 17.0 (StataCorp, College Station, Texas, USA). This report conforms to the STARD reporting guidelines for diagnostic accuracy studies.[27]

Results

Clinical adjudication

The results of clinical adjudication are detailed in the supplementary table S4. Using FA (i.e. all patients included) 60 (36%) of 169 patients were adjudicated as bacterial, 54 (32%) as viral, 26 (15%) as viral/bacterial co-infection and 29 (17%) as non-infected. Fig. 1 shows the study profile.

Baseline characteristics

Patient baseline characteristics and outcomes for all patients and by adjudicated aetiological category are shown in Table 1. For the entire cohort median (IQR) age was 60 (45 to 74) years and 69 (49%) were male. 146 (87%) had at least 1 co-morbidity, most commonly chronic respiratory disease. The median duration of illness prior to presentation was 4 (3–7) days and 54 (32%) had received antibiotics

Table 1
Baseline characteristics for all patients and according to infection status (by forced adjudication), n = 169.

	All patients n = 169	Bacterial alone n = 60	Viral alone n = 54	Co-infection[a] n = 26	Non-infected n = 29
Age, years	60 (45, 74)	61 (49, 76)	57 (40, 76)	63 (49, 76)	62 (49, 68)
Age > 65	69 (41%)	27 (45%)	20 (37%)	12 (46%)	10 (34%)
Male sex	82 (49%)	33 (55%)	23 (43%)	12 (46%)	14 (48%)
Current smoker	37/168 (22%)	15 (25%)	9/53 (17%)	4 (15%)	9 (31%)
Influenza vaccinated	112/168 (67%)	40 (67%)	34/53 (64%)	19 (73%)	19 (66%)
Ethnicity					
White British	156 (92%)	59 (98%)	48 (89%)	24 (92%)	25 (86%)
Other White	6 (3.5%)	1 (1.7%)	3 (5.6%)	1 (3.9%)	1 (3.5%)
Indian	4 (2.4%)	0 (0.0%)	2 (3.7%)	0 (0.0%)	2 (6.9%)
Black African	2 (1.2%)	0 (0.0%)	0 (0.0%)	1 (3.9%)	1 (3.5%)
Other	1 (0.6%)	0 (0.0%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
Comorbidity					
Any comorbidity	146 (86%)	49 (82%)	48 (89%)	21 (81%)	28 (97%)
Hypertension	45 (26%)	12 (20%)	14 (26%)	5 (19%)	14 (48%)
Cardiovascular disease	39 (23%)	14 (23%)	12 (22%)	3 (12%)	10 (34%)
Respiratory disease	124 (73%)	38 (63%)	44 (81%)	18 (69%)	24 (83%)
Liver disease	13 (7.7%)	4 (6.7%)	5 (9.3%)	3 (12%)	1 (3.5%)
Diabetes mellitus	7 (4.1%)	3 (5.0%)	3 (5.6%)	0 (0.0%)	1 (3.5%)
Cancer	33 (19%)	14 (23%)	4 (7%)	6 (23%)	9 (31%)
Immune suppression	12 (7.1%)	6 (10%)	2 (3.7%)	2 (7.7%)	2 (6.9%)
Charlson comorbidity score	4 (4, 9)	4 (2, 12)	4 (4, 8)	6 (3, 8)	4 (4, 12)
Clinical features					
Duration of symptoms, days	4 (3, 7)	5 (3, 7)	4 (2, 7)	6 (3, 7)	3 (2.5, 6)
Antibiotics prior to presentation	54 (32%)	17 (28%)	18 (33%)	10 (38%)	9 (31%)
Physiological parameters					
Pulse rate, bpm	102 (88, 118)[b]	105 (96, 120)[c]	98 (85, 115)[d]	105 (92, 127)[e]	100 (86, 110)
Systolic BP, mmHg	135 (123, 154)	130.5 (120, 148)	142 (123, 159)	133 (129, 146)	140 (128, 150)
Resp rate, bpm	24 (20, 28)[f]	23 (19, 26)	24 (22, 26)[d]	24 (20, 29)	24 (20, 28)
Temp, ∘C	37.0 (36.4, 37.7)[g]	37.2 (36.7, 38.0)[c]	36.8 (36.2, 37.3)[h]	37.9 (37.0, 38.6)	36.6 (36.3, 37.0)[i]
Supplementary Oxygen	36 (21%)	17 (28%)	8 (15%)	6 (23%)	5 (17%)
NEWS2 score at presentation	5 (3, 6)	5 (3, 6)	4 (3, 6)[j]	6 (2, 6)[e]	4 (3, 6)[i]
Laboratory and radiological					
CXR performed	167 (99%)	60 (100%)	54 (100%)	25 (96%)	28 (97%)
CT chest performed	16 (9.5%)	8 (13%)	2 (3.7%)	2 (7.7%)	4 (13.8%)
CRP, mg/L	47 (15, 115)[k]	108 (43, 175)	22 (13, 40)[l]	125 (82, 199)[m]	7 (4, 21)[n]
WCC, $x10^9$ per L	11.2 (8.7, 14.9)	14.1 (10.1, 18.4)	10.1 (8.0, 12.2)	13.3 (8.7, 16.6)	9.6 (8.2, 11.3)
PCT, ng/ml	0.12 (0.05, 0.36)[o]	0.35 (0.18, 4.13)[p]	0.06 (0.04, 0.12)[j]	0.28 (0.13, 5.04)	0.06 (0.03, 0.10)
Treatment in hospital					
Received antibiotics	152 (90%)	60 (100%)	47 (87%)	26 (100%)	19 (66%)
Received IV antibiotics	111 (66%)	54 (90%)	27 (50%)	18 (69%)	12 (41%)
Received influenza antivirals	61 (36%)	18 (30%)	22 (41%)	12 (46%)	9 (31%)
Diagnosis					
Pneumonia	49 (29%)	31 (52%)	5 (9.3%)	12 (46%)	1 (3.5%)
Asthma exacerbation	29 (17%)	2 (3.3%)	19 (35%)	1 (3.9%)	7 (24%)
COPD exacerbation	49 (29%)	15 (25%)	18 (33%)	4 (15%)	12 (41%)
NPLRTI	16 (9.5%)	5 (8.3%)	6 (11%)	3 (12%)	2 (6.9%)
ILI	8 (4.7%)	3 (5.0%)	3 (5.6%)	2 (7.7%)	0 (0.0%)
Other	18 (11%)	4 (6.7%)	3 (5.6%)	4 (15%)	7 (24%)
Outcomes					
Length of stay	2.9 (1.3, 5.4)	3.1 (1.6, 6.9)	1.9 (1.1, 4.2)	3.8 (1.9, 5.4)	3.1 (0.8, 5.4)
Prolonged LOS (≥7 days)	29 (17%)	15 (25%)	8 (15%)	5 (19%)	1 (3.5%)
Critical care unit admission	4 (2.4%)	3 (5.0%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
Death (in hospital)	2 (1.2%)	2 (3.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Re-admission	25 (15%)	7 (12%)	7 (13%)	2 (7.7%)	9 (31%)

All data are presented as n(%) or median (Inter-quartile range).
CRP; C reactive protein. WCC; white cell count. PCT; Procalcitonin. IV; intravenous. ILI; Influenza-like illness. NPLRTI; Non-pneumonic lower respiratory tract infection.
[a] Viral/bacterial co-infection.
[b] measured in 166.
[c] measured in 59.
[d] measured in 53.
[e] measured in 25.
[f] measured in 168.
[g] measured in 165.
[h] measured in 52.
[i] measured in 28.
[j] measured in 51.
[k] measured in 156.
[l] measured in 46.
[m] measured in 24.
[n] measured in 26.
[o] measured in 163.
[p] measured in 57.

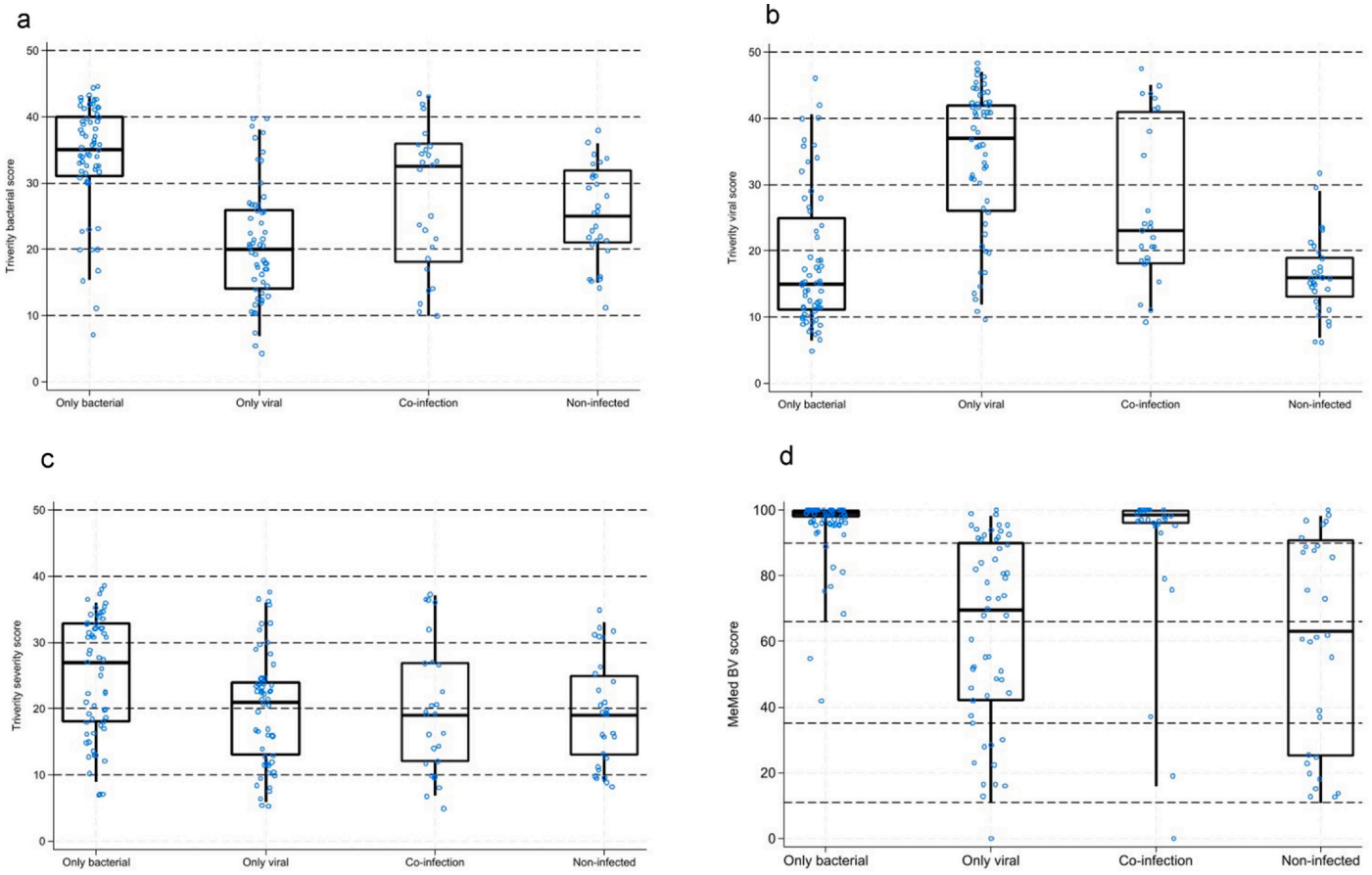

Fig. 2. a-d. Box plots showing median (IQR) scores for TriVerity bacterial (a), viral (b) and illness severity scores (c) and for MeMed BV scores (d), for forced adjudication (all patients).

in the preceding two weeks. 152 (90%) received antibiotics during admission, median length of hospital stay was 2.9 (1.3 to 5.4) days, 4 patients (2.4%) required critical care unit admission (ICU or High Dependency Unit) and 2 patients (1.2%) died. According to adjudicated aetiological category, the median CRP, WCC, and PCT level and NEWS2 score were higher in those adjudicated as bacterial infection or bacterial/viral co-infection compared with those adjudicated as viral infection or non-infected. Details of the pathogens detected in each group are shown in supplementary table S5. More patients adjudicated as bacterial infection (52%) or bacterial/viral coinfection (46%) had a diagnosis of pneumonia compared to those adjudicated as viral infection (9.3%) or non-infected (3.4%). For patients adjudicated as viral most had a diagnosis of either asthma exacerbation (35%) or COPD exacerbation (33%). Length of hospital stay was lower for those adjudicated as viral infection compared to the other groups.

Median scores

The median (IQR) values for the TriVerity bacterial, viral and severity scores (out of 50) and for the MeMed BV score (out of 100) are shown in Fig. 2a-d. Median (IQR) TriVerity bacterial score was higher for bacterial (31 [31–40]) and bacterial/viral coinfection (33[18–36]) compared to viral (20[14–26]) and non-infected patients (25[21–32]). Median (IQR) TriVerity viral score was higher in viral infection (37[26–42]) compared to bacterial/viral co-infection (23[18–41]), bacterial infection (15[11–25]), and non-infected (16[13–19]) patients. Median (IQR) TriVerity illness severity score was higher in bacterial infection (27[18–33]) compared to the other categories. Median (IQR) MeMed BV score was higher for bacterial (99 [98–100]) and bacterial-viral coinfection (99 [96–100]) compared to viral (70 [42–90]) and non-infective patients (63[34–91]); however, the median score for viral patients was above the cut-off of 65 used for interpretation as bacterial.

Overall accuracy and likelihood bands for bacterial (including co-infection with viruses) versus non-bacterial infection

Measures of diagnostic accuracy overall and according to TriVerity bacterial score likelihood band and MeMed BV score band for FA are shown in Tables 2a 2b (measures for CA are shown in the supplementary table S6a and S6b).

For the TriVerity bacterial score, scores in the very high band had a positive predictive value (PPV) of 100% (95%CI 81–100) and a likelihood ratio (LR) of 33.8 (95%CI 2.1 to 553) for bacterial infection. Scores in the high band had a PPV of 72% (95%CI 59–82) and a LR of 2.47 (95%CI 1,57–3.88). The negative predictive value (NPV) and LR for scores in the low band were 71% (95%CI 55–84) and 0.39 (0.22–0.73) and was 63% (95%CI 25–91) and 0.58 (0.14–2.35) in the very low band. Several patients adjudicated as bacterial with low or very low TriVerity bacterial scores (i.e. 'false negatives') had high or very high TriVerity viral scores and had *Mycoplasma pneumoniae* detected by PCR. Further clinical details for patients adjudicated as bacterial but with a TriVerity bacterial scores in the low and very low band are provided in the supplementary appendix. Overall positive percentage agreement (PPA) for the TriVerity bacterial score was 81% (95%CI 70–89) and negative percentage agreement (NPA) was 66% (95%CI 52–79). Overall accuracy for bacterial infection (with very high and high bands considered positive and low and very low bands considered negative) was 75% (95%CI 66–82).

Table 2a
Measures of diagnostic accuracy for bacterial infection for **TriVerity Bacterial score** compared with clinical adjudication according to likelihood band and overall, for forced adjudication (all patients), n = 169.

TriVerity score Band	Yes bacterial	No bacterial	PPA (95%CI)	NPA (95%CI)	LR (95%CI)	% of results in band	PPV (95%CI)	NPV (95%CI)
Very high	17	0	20 (12, 30)	100 (96, 100)	33.79 (2.07, 553)	10	100 (81, 100)	N/A
High	46	18	53 (42, 64)	78 (68, 87)	2.47 (1.57, 3.88)	38	72 (59, 82)	N/A
Moderate	8	30	9 (4, 18)	64 (53, 74)	0.26 (0.13, 0.53)	22	21 (10, 37)	79 (63, 90)
Low	12	30	86 (77, 93)	36 (26, 47)	0.39 (0.22, 0.73)	25	N/A	71 (55, 84)
Very Low	3	5	97 (90, 99)	6 (2, 14)	0.58 (0.14, 2.35)	5	N/A	63 (25, 91)
Total	86	83						
Overall result								
Yes (high or very high band)	63	18	81 (70, 89)	66 (52, 79)	2.38 (1.61, 5.42)	62	78 (67, 86)	70 (55, 82)
No (low or very low band)	15	35	-	-	-	38	-	-
						Overall accuracy = 75% (66, 82)		

PPA; Positive percentage agreement. NPA; Negative percentage agreement. LR; Likelihood ratio. PPV; Positive predictive value. NPV; Negative predictive value. CI; confidence interval. N/A; Non-applicable.

Table 2b
Measures of diagnostic accuracy for bacterial infection for **MeMed BV score** compared with clinical adjudication according to likelihood band and overall, for forced adjudication (all patients), n = 169.

MeMed score Band	Yes bacterial	No bacterial	PPA (95%CI)	NPA (95%CI)	LR (95%CI)	% of results in band	PPV (95%CI)	NPV (95%CI)
High bacterial	72	22	86 (76, 92)	73 (63, 83)	3.23 (2.24, 4.68)	56	77 (67, 85)	N/A
Mod bacterial	7	21	8 (3, 16)	75 (64, 84)	0.33 (0.15, 0.73)	17	25 (11, 45)	N/A
Equivocal	2	18	2 (0.3, 8)	78 (68, 87)	0.11 (0.03, 0.5)	12	10 (1, 32)	90 (68, 99)
Moderate viral	2	21	98 (92, 100)	25 (16, 36)	0.09 (0.02, 0.39)	14	N/A	91 (72, 99)
High viral	1	1	99 (94, 100)	1 (0.03, 7)	0.99 (0.06, 16)	1	N/A	50 (42, 58)
Total	84	83						
Overall result								
Yes (high or very high band)	79	43	96 (90, 99)	34 (23, 47)	1.46 (1.22, 1.74)	83	65 (56, 73)	88 (69, 98)
No (low or very low band)	3	22	-	-	-	17	-	-
						Overall accuracy = 69% (61, 76)		

PPA; Positive percentage agreement. NPA; Negative percentage agreement. LR; Likelihood ratio. PPV; Positive predictive value. NPV; Negative predictive value. CI; confidence interval. N/A; Non-applicable.

For the MeMed BV score, scores in the high bacterial band had a PPV of 86% (95%CI 76–92) and an LR of 3.23 (95%CI 2.24–4.68) for bacterial infection. Scores in the moderate bacterial band had a PPV of 25% (95%CI 11–45) and a LR of 0.33 (95%CI 0.15–0.73), with most patients in this band having either viral infection (16/28 [57%]) or non-infective illness (5/28 [18%]). Scores in the moderate viral band had an NPV of 91% (72–99) and LR of 0.09 (0.02–0.39), and those in the lowest band (i.e. high viral) had an NPV of 50% (95%CI 42–58) and LR of 0.99 (95%CI 0.06–16) although there were only 2 patients with scores in this band. Overall PPA for MeMed BV score was 96% (95%CI 90–99), and NPA was 34% (95%CI 23–47). Overall accuracy for bacterial infection (with high and moderate bacterial bands considered positive and moderate and high viral bands considered negative) was 69% (95%CI 61–76). Clinical details for patients adjudicated as bacterial but with a MeMed BV viral scores in the moderate or high band (i.e. false negatives) are provided in the supplementary appendix.

Overall accuracy and likelihood bands for viral versus non-viral infection

Measures of diagnostic accuracy overall and according to TriVerity viral score likelihood band and MeMed score likelihood band (for FA) are shown in Tables 3a 3b (measures for CA are shown in the supplementary table S7a and S7b).

For the TriVerity viral score, scores in the very high viral band had a PPV value of 91% (95%CI 75–198) and LR of 10.8 (95%CI 3.4–34.0) for viral infection. Scores in the high viral band (26 [15%] patients) had a PPV of 69% (95%CI 48–86) and a LR of 2.50 (95%CI 1.15–5.44). Scores in the low band had an NPV of 71% (55–84) and LR of 0.39 (0.22–0.73), and those in the very low band had a negative predictive value of 90% (95%CI 70–99) and a likelihood ratio of 0.12 (95%CI 0.0–0.50) for viral infection. Of those patients classified as very high or high likelihood of viral infection, 51 (88%) of 58 had a respiratory virus detected, and an additional 4 (7%) had *Mycoplasma*

Table 3a
Measures of diagnostic accuracy for viral infection for **TriVerity Viral score** compared with clinical adjudication according to likelihood band and overall, for forced adjudication (all patients), n = 169.

TriVerity score Band	Yes viral	No viral	PPA (95%CI)	NPA (95%CI)	LR (95%CI)	% of results in band	PPV (95%CI)	NPV (95%CI)
Very high	29	3	36 (26, 48)	97 (91, 99)	10.75 (3.41, 33.95)	19	91 (75, 98)	N/A
High	18	8	23 (14, 33)	91 (83, 96)	2.50 (1.15, 5.44)	15	69 (48, 86)	N/A
Moderate	16	13	20 (12, 30)	85 (76, 92)	1.37 (0.70, 2.67)	17	55 (36, 74)	45 (26, 64)
Low	15	46	81 (71, 89)	52 (41, 62)	0.36 (0.22, 0.60)	36	N/A	75 (63, 86)
Very Low	2	19	98 (91, 100)	21 (13, 31)	0.12 (0.03, 0.49)	12	N/A	90 (70, 99)
Total	80	89						
Overall result								
Yes (high or very high band)	47	11	73 (61, 84)	86 (76, 93)	5.07 (2.88, 8.94)	41	81 (69, 90)	79 (69, 87)
No (low or very low band)	17	65	-	-	-	59	-	-
						Overall accuracy = 80% (72, 86)		

PPA; Positive percentage agreement. NPA; Negative percentage agreement. LR; Likelihood ratio. PPV; Positive predictive value. NPV; Negative predictive value. CI; confidence interval. N/A; Non-applicable.

Table 3b
Measures of diagnostic accuracy for viral infection for **MeMed BV score** compared with clinical adjudication according to likelihood band and overall, for forced adjudication (all patients), n = 169.

MeMed score Band	Yes viral	No viral	PPA (95%CI)	NPA (95%CI)	LR (95%CI)	% of results in band	PPV (95%CI)	NPV (95%CI)
High viral	2	0	3 (0.3, 9)	100 (96, 100)	5.43 (0.26, 111.46)	1	100 (16, 100)	N/A
Moderate viral	12	11	15 (8, 25)	87 (79, 94)	1.19 (0.55, 2.54)	14	52 (31, 73)	N/A
Equivocal	13	7	16 (9, 26)	92 (84, 97)	2.02 (0.85, 4.81)	12	65 (41, 85)	35 (15, 59)
Mod bacterial	18	10	78 (67, 86)	11 (6, 20)	1.96 (0.96, 3.99)	17	N/A	36 (19, 56)
High bacterial	35	59	56 (45, 67)	68 (57, 77)	0.65 (0.48, 0.86)	56	N/A	63 (52, 73)
Total	80	87						
Overall result								
Yes (high or very high band)	14	11	21 (12, 33)	86 (77, 93)	1.52 (0.74, 3.12)	17	56 (35, 76)	57 (47, 66)
No (low or very low band)	53	69				83		
						Overall accuracy = 56% (48, 64)		

PPA; Positive percentage agreement. NPA; Negative percentage agreement. LR; Likelihood ratio. PPV; Positive predictive value. NPV; Negative predictive value. CI; confidence interval. N/A; Non-applicable.

pneumoniae detected by PCR. One further patient had a viral pneumonitis pattern on chest CT but was PCR negative for viruses. Overall PPA for the TriVerity viral score was 73% (95%CI 61–84), and NPA was 86% (95%CI 76–93). Overall accuracy for viral infection (with very high and high bands considered positive and very low and low bands considered negative) was 80% (95%CI 72–86).

For the MeMed BV score, scores in the high viral band had a PPV of 100% (95%CI 16–100) and LR of 5.43 (95%CI 0.26–111), although there were only 2 patients in this band. Scores in the moderate viral band had a PPV of 52% (95%CI 31–73) and a LR of 1.19 (95%CI 0.55–2.54). Scores in the moderate bacterial band had a NPV of 36% and LR of 2.02 (0.85–4.81), and those in the lowest band (i.e. high bacterial) had a NPV of 63% (95%CI 52–73) and LR of 0.65 (95%CI 0.48–0.86). Overall PPA for viral infection for MeMed BV was 21% (95%CI 12–33), and NPA was 86% (77–93). Overall accuracy for viral infection (with high and moderate viral bands considered positive and moderate and high bacterial bands considered negative) was 56% (95%CI 48–64). When analysing performance for viral infection using bacterial (including bacterial/viral co-infection) versus viral infection, MeMed BV score had a PPA of 27% (95% CI 14–43), an NPV of 96% (95%CI 90–99%) and an overall accuracy of 73% (95%CI 64–81%).

ROC Curves

ROC curves with AUC and 95%CI for bacterial (including co-infection with viruses) Vs non-bacterial infection and viral Vs non-viral infection for Triverity bacterial and viral scores and MeMed BV score are shown in Fig. 3a-d, for forced adjudication (ROC curves for

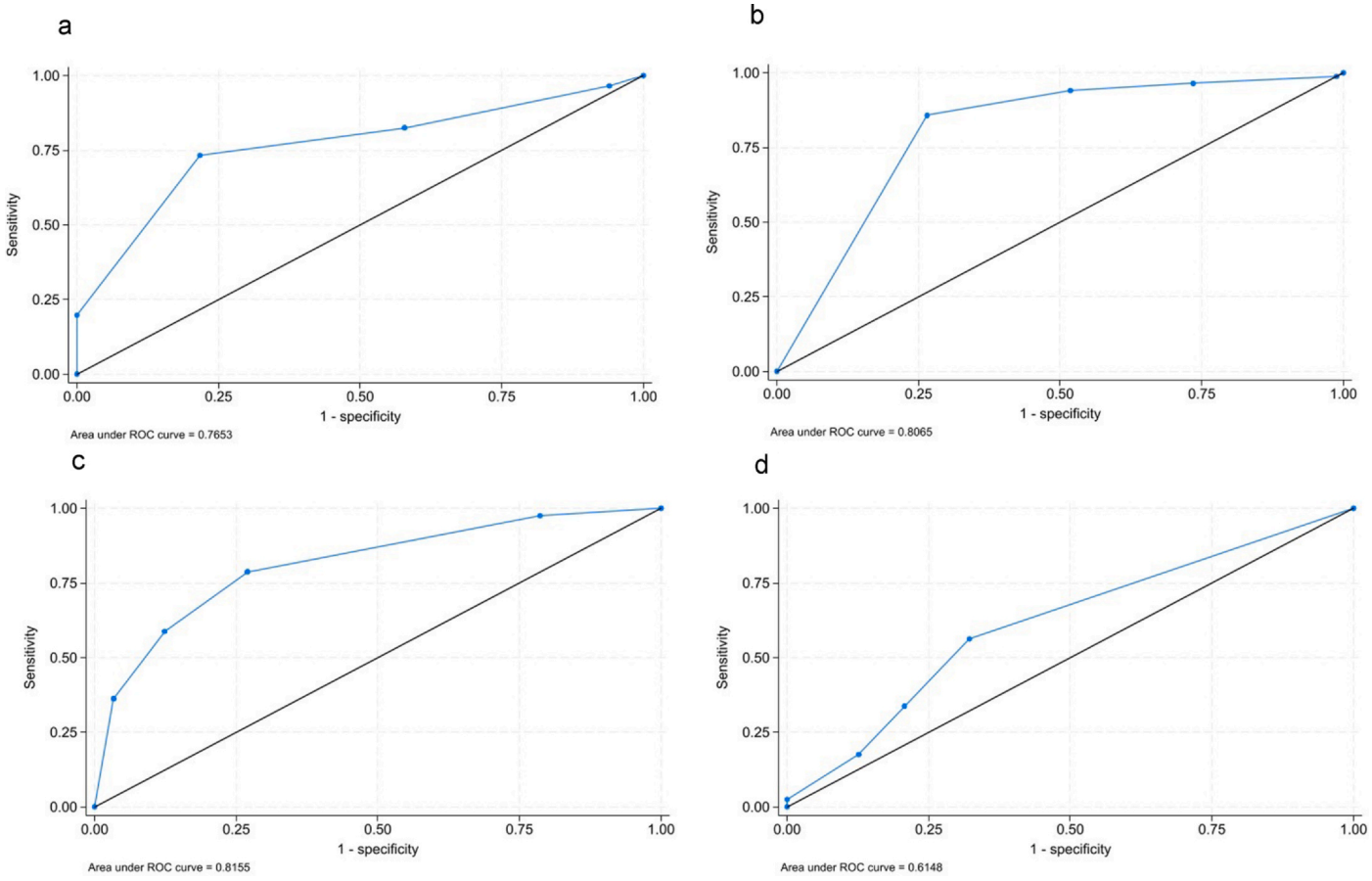

Fig. 3. a-d. ROC curves for bacterial Vs non-bacterial infection for TriVerity bacterial score (a) and MeMed BV score (b) and for viral Vs non-viral infection for TriVerity viral score (c) and MeMed BV score (d), for forced adjudication (all patients).

Table 4
Actual antibiotic use according to adjudicated aetiology and potential antibiotic use according to different cut-off values for Triverity Bacterial and MeMed BV scores.

	All patients n = 169	Bacterial n = 60	Viral n = 54	Co-infection, n = 26	Non-infective n = 29
Treated with Antibiotics (actual)	152 (90%)	60 (100%)	47 (87%)	26 (100%)	19 (66%)
Inflammatix bacterial score > 20	119 (70%)	53 (88%)	26 (48%)	18 (69%)	22 (76%)
Inflammatix bacterial score > 30	81 (48%)	49 (82%)	9 (16%)	14 (54%)	9 (35%)
MeMed BV score > 35	142/167 (85%)	58/58 (100%)	43 (80%)	24 (92%)	18 (62%)
MeMed BV score > 65	123/167 (74%)	56/58 (97%)	30 (56%)	23 (88%)	14 (48%)

consensus adjudication are shown in the supplementary appendix). For bacterial infection, the Triverity bacterial score performed with an AUC of 0.77 (95%CI 0.70 to 0.84) and the MeMed BV score with an AUC of 0.81 (95%CI 0.74–0.87), difference of 0.04, p = 0.388. For viral infection, the Triverity viral score performed with an AUC of 0.82 (95%CI 0.75 to 0.88) and the MeMed BV score with an AUC of 0.61 (95%CI 0.54–0.69). When analysing viral infection as bacterial (including bacterial/viral co-infection) versus viral infection MeMed BV had an AUC of 0.81 (95%CI 0.73–0.89).

Potential antibiotic reduction

Table 4 demonstrates the actual antibiotic use in patients according to adjudicated aetiological status and potential use according to different cut-offs for Triverity bacterial score and MeMed BV score. Overall, 152 (90%) of 169 patients were treated with antibiotics. Theoretically, using a Triverity bacterial score of > 30 (high and very high bacterial bands), 81 (48%) patients would have been treated and using a MeMed BV cut-off of > 65 (high and moderate bacterial bands), 123 (74%) of 167 patients would have been treated. For the 54 patients adjudicated as viral aetiology alone, 47 (87%) were treated with antibiotics. Using a TriVerity bacterial score of > 30, 9 (16%) of these patients would have been treated and using a MeMed BV score of > 65, 30 (56%) would have been treated. Conversely, for patients adjudicated as bacterial or bacterial/viral co-infection, using a Triverity bacterial score of > 30, 13 (27%) of 86 patients would not have received antibiotics and using a MeMed BV score of > 65 2 (2.4%) of 84 would not have received antibiotics.

Discussion

This study is the first, to our knowledge, to directly compare the diagnostic accuracy of two different rapid, multi-target, host response tests and the first to compare protein-based and gene expression (mRNA) based tests. We have shown that, in a cohort of hospitalised adults with ARI containing a complex mix of bacterial, viral, co-infection, and non-infective aetiologies, both tests were able to distinguish bacterial from non-bacterial infection with similar levels of overall accuracy but with quite different performance characteristics. The bacterial score of the Triverity test had lower PPA (i.e. sensitivity) and NPV compared with MeMed BV score as it misclassified several patients adjudicated as bacterial (including bacterial/viral co-infection), as viral. However, Triverity had a higher NPA (i.e. specificity) and a higher PPV, as MeMed BV misclassified the majority of viral infections as bacterial. The viral score of the TriVerity test was superior to MeMed BV for detecting viruses in terms of PPA (i.e., sensitivity), and both had comparable NPA (i.e., specificity); accepting that the MeMed BV test is not able to differentiate between viral and other non-bacterial aetiologies. In addition, as MeMed BV cannot distinguish bacterial/viral co-infection from bacterial, we also evaluated performance for viral infection by analysing bacterial (including bacterial/viral infection) versus viral infection, and PPA remained very low (27%), albeit with an improvement in NPA. The principal use case for host response testing is to guide antibiotic use with a view to reducing unnecessary use and prevention. Therefore, potential antibiotic reductions were greater with the Triverity test compared with MeMed BV. However, this was at the cost of potentially missing more bacterial infections, which is concerning.

Diagnostic accuracy assessments of host response tests are always hampered by the lack of a gold standard diagnostic test and the inherent inaccuracy of using clinical adjudication as the reference standard.[25] The estimates for performance measures in this study were consistently higher with CA (supplementary appendix) compared to FA, supporting this. FA is the measure we have principally reported, as it is arguably more relevant to clinical practice, analysing all patients including those that are most difficult to categorise. However, even with CA, it is likely that some adjudications are incorrect, potentially leading to underestimates of performance. ARI may also represent an infection syndrome that is particularly difficult to clinically adjudicate with any certainty, given the issues of colonisation of upper airways with potentially pathogenic bacteria, infrequent detection of bacteria from blood or pleural fluid, PCR detection of some viruses (particularly Rhinovirus) that may not be related to the current ARI, and uncertainty when viruses are detected as to whether high biomarkers or radiological consolidation represent bacterial super-infection or are caused by the viruses alone.[12,29,30] In fact, many bacterial cases that had low or very low TriVerity bacterial scores (i.e. false negatives) had high viral scores and were PCR positive for viruses but were adjudicated as bacterial/viral coinfection due to high levels of biomarkers (CRP and/or PCT) and/or radiological consolidation. It is possible that these cases may have actually represented viral infection alone without a bacterial component.

Diagnostic testing, including host response tests, are not used in isolation and should be regarded as adjuncts to clinical decision-making, to be used alongside clinical, radiological and other laboratory data to make antibiotic and other decisions, and the pre-test probability of bacterial infection must always be considered when interpreting results. In addition, the clinical adjudication of a case as bacterial, even when correct, may not equate to gaining benefit from antibiotic use as in many bacterial infectious syndromes such as exacerbation of COPD, the gains from antibiotics are known to be marginal.[31,32] The true test of the utility of host response testing to guide antibiotics will require prospective interventional trials adequately designed and powered to assess the impact both on antibiotic use and on clinical recovery (i.e. safety), as has become the standard in other antibiotic stewardship intervention trials.[33]

The results of our study are comparable to other studies evaluating earlier versions of the TriVerity test using laboratory methods, although none of these evaluated exclusively ARI cohorts, evaluating cohorts of patients presenting to ED and containing a mixture of infection syndromes.[19,20,34]

The accuracy of MeMed BV has been assessed in a number of studies and although the reported levels of PPA (i.e. sensitivity) for bacterial infection across these studies are broadly comparable to our study, levels of NPA (i.e. specificity) and also NPV were much lower in our study.[16,17,35–38] There are several likely explanations for this including that most accuracy studies of MeMed BV have evaluated children in an ED or urgent care setting, with a low prevalence of bacterial infection and a high prevalence of viral infection,

compared to our hospitalised adult cohort where bacterial infection was present in over 50% of patients.[17,35–38] In addition, studies of MeMed BV have generally excluded large numbers of patients from analysis with less certain adjudication status and have also removed patients with scores in the equivocal band when calculating ROC curves and other measures of accuracy.[16,35–38] Furthermore the MeMed BV test is unable to distinguish viral from non-infected patients and so in cohorts, including non-infected patients, the accuracy for viral infection will be low when evaluated against true viral infection rather than viral and non-infected patients combined.

Our study has also highlighted a particular issue with host response testing and atypical bacteria. Our cohort included several cases of *Mycoplasma pneumoniae* infection which were adjudicated as bacterial, but in most cases the TriVerity bacterial score was low and the viral score high. This has been noted in other studies of host response testing and likely represents a shared immune response of atypical intracellular bacterial with viruses.[39,40] As *M. pneumoniae* infection is frequently associated with pneumonia and requires treatment with specific antibiotics active against intracellular bacteria, generating a host response test result suggestive of a viral infection is problematic and argues for the need to perform pathogen detection and host response testing in combination.

The host response tests that we evaluated have some similarities and differences in the presentation of results. Both tests separate scores into bands in order to stratify results into levels of likelihood and both include 'moderate' or 'equivocal' bands where the interpretation is uncertain. It is not known how physicians and other prescribers will view the usefulness of such bands and what impact they will have on prescribing. Unlike MeMed BV, TriVerity has two separate scores for bacterial and viral infection, which can be viewed as independent of each other. This potentially allows for the delineation of bacterial and bacterial/viral co-infection and also for the identification of patients with non-infectious aetiology (i.e., non-bacterial and non-viral), which is not possible with MeMed BV as other all non-bacterial aetiologies, including non-infectious conditions and healthy status, are included within the viral score of < 35.

This study has several strengths. It is a large, well-characterised cohort of adults with ARI and includes a mixture of aetiologies including many patients PCR positive for rhinovirus. In addition, transparent pre-specified methods were used to independently adjudicate the cohort, consistent with regulatory guidelines.[27] Finally, the use of FA, where all patient results are included in the analysis, for the presentation of results provides reassurance that measures of performance are not over-estimated.

This study also has a number of limitations**.** It is a retrospective sub-cohort of the parent study using convenience sampling with just under 30% of patients from the parent study included in this study. However, comparison of baseline data between the two cohort shows that the patients in this study are highly representative of the entire study cohort. The TriVerity test also includes a severity score as its third output giving potential added value beyond distinguishing bacterial and viral infection. Unfortunately, we were not able to assess the accuracy of this due to the low event rate for the outcomes it has been validated against (intensive care unit admission and death) in this cohort. As already discussed, the use of clinical adjudication as the reference standard is a potential weakness in some ways although this is the accepted reference standard for host response testing studies, and we ensured that this process was as robust and reproducible as possible. In addition, the prominent use of CRP for clinical adjudication in ARI (where confirmation of bacterial infection is infrequent) introduces considerable bias when assessing host response that themselves include CRP, such as MeMed BV. It is likely that this could lead to overestimates of performance compared with the 'true' aetiology, however no suitable alternatives for adjudication are currently available. Assessing the usability and acceptability of diagnostic tests along with potential barriers and facilitators to adoption are critically important to the successful deployment of point-of-care tests, and we did not address this formally in this study. In addition, we performed MeMed BV testing using the high-throughput DiaSorin Liaison XL immunoanalyzer, but a new instrument has been developed (MeMed Key) to allow testing at the point-of-care, and we did not assess this.

In conclusion, in this hospitalised cohort of adult patients with ARI the TriVerity test demonstrated moderate to high sensitivity and specificity for both bacterial and viral infection, whereas MeMed BV demonstrated high sensitivity for bacterial infection but very low specificity, with most viral infections classified as bacterial. Potential antibiotic reductions were subsequently greater with TriVerity but at the potential cost of more untreated bacterial infections. Impact studies of both tests are now needed, performed in clinical areas at the point-of-care and including assessments of efficacy, safety, usability, and cost-effectiveness.

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Author contributions

TWC conceived of and designed the study, assisted in the statistical analysis and drafted the manuscript. EBD assisted with the design of the study, analysed the samples, and assisted with drafting the manuscript. ART and NJB assisted with drafting of the manuscript. HEM performed the statistical analysis and assisted with the drafting of the manuscript.

Data availability

All de-identified participant data analysed and presented in this study are available from the corresponding author following publication, on reasonable request.

Declaration of Competing Interest

TWC has received speaker fees, honoraria, travel reimbursement, and equipment and consumables at discount or free of charge for the purposes independent of research, outside of this submitted study, from BioFire diagnostics, BioMerieux, Cepheid and QIAGEN. He has received consultancy fees from Cepheid, Synairgen research, Roche, Janssen, Biofire diagnostics and BioMerieux. He has received honoraria for participation in advisory boards from Cepheid, Roche, Janssen, Shionogi, GSK, Seqirus and Sanofi. He is a member of an independent data monitoring committee for a trial sponsored by Roche. He has acted as the UK chief investigator for a study sponsored by Janssen. All other authors declare no competing interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106360.

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