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RESPIRATORY MEDICINE

Utility of the FebriDx point-of-care test for rapid triage and identification of possible coronavirus disease 2019 (COVID-19)

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Abstract

Objectives: The Coronavirus disease 2019 (COVID-19) pandemic is straining health-care resources. Molecular testing turnaround time precludes having results at the point-of-care (POC) thereby exposing COVID-19/Non-COVID-19 patients while awaiting diagnosis. We evaluated the utility of a triage strategy including FebriDx, a 10-minute POC finger-stick blood test that differentiates viral from bacterial acute respiratory infection through detection of Myxovirus-resistance protein A (MxA) and C-reactive protein (CRP), to rapidly isolate viral cases requiring confirmatory testing.

Methods: This observational, prospective, single-center study enrolled patients presenting to/within an acute care hospital in England with suspected COVID-19 between March and April 2020. Immunocompetent patients ≥ 16 years requiring hospitalisation with pneumonia or acute respiratory distress syndrome or influenza-like illness (fever and ≥ 1 respiratory symptom within 7 days of enrolment, or inpatients with new respiratory symptoms, fever of unknown cause or pre-existing respiratory condition worsening). The primary endpoint was diagnostic performance of FebriDx to identify COVID-19 as a viral infection; secondary endpoint was SARS-CoV-2 molecular test diagnostic performance compared with the reference standard COVID-19 Case Definition (molecular or antibody detection of SARS-CoV-2).

Results: Valid results were available for 47 patients. By reference standard, 35 had viral infections (34/35 COVID-19; 1/35 non-COVID-19; overall FebriDx viral sensitivity 97.1% (95%CI 83.3-99.9)). Of the COVID-19 cases, 34/34 were FebriDx viral positive (sensitivity 100%; 95%CI 87.4-100); 29/34 had an initial SARS-CoV-2 positive molecular test (sensitivity 85.3%; 95%CI 68.2-94.5). FebriDx was viral negative when the diagnosis was not COVID-19 and SARS-Cov-2 molecular test was negative (negative predictive value (NPV) 100% (13/13; 95%CI 71.7-100)) exceeding initial SARS-CoV-2 molecular test NPV 72.2% (13/19; 95%CI 46.4-89.3). The diagnostic specificity of FebriDx and initial SARS-CoV-2 molecular test was 100% (13/13; 95%CI 70-100 and 13/13; 95%CI 85.4-100, respectively).

Conclusions: FebriDx could be deployed as part of a reliable triage strategy for identifying symptomatic cases as possible COVID-19 in the pandemic.

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1 | INTRODUCTION

Acute respiratory infection (ARI) is the most common reason patients seek healthcare worldwide. Uncomplicated ARIs in the outpatient setting are often of viral origin [acute bronchitis (90%), pharyngitis (85%), and sinusitis (98%)] or are self-limiting and tend to resolve without antibiotics.¹ Reliable differentiation between uncomplicated and self-limiting viral from bacterial ARIs remains challenging, primarily due to the non-specific overlapping clinical manifestations which can be present in both clinical scenarios, and secondly because many patients are carriers of or are colonised with bacterial or viral pathogens.^{2,3}

The coronavirus disease 2019 (COVID-19) pandemic is putting an extraordinary strain on healthcare resources. To date, molecular reverse transcriptase polymerase chain reaction (rRT-PCR) has been used for screening and initial diagnosis⁴⁻⁶ despite long turnaround times that can take upwards of 48 hours from sample collection to result.⁷ Some patients, including those with high likelihood COVID-19 infection who present symptomatic (eg, fever, cough, shortness of breath or sudden onset of anosmia, ageusia or dysgeusia) with recent exposure (within 14 days) and chest imaging consistent with COVID-19 infection (eg, ground-glass opacities, multifocal organising pneumonia and architectural distortion in a peripheral distribution),^{5,8} test negative on the initial rRT-PCR test which requires multiple, between 2 and 4 subsequent tests, to return an eventual positive result.⁹⁻¹¹ Variations of sampling techniques, viral load at the time of testing may impact sensitivity and false negative rates due to insufficient quantity of viral ribonucleic acid (RNA) to meet the manufacturer's test kit limit of detection (LOD).¹² Additionally, analytical sensitivity of the manufacturer test kit may impact sensitivity such as a limit of detection (LOD) that is too high would result in patients with SARS-CoV-2 testing negative and thereby increasing the false negative rate.^{12,13} Molecular tests are also impacted by efficiency of viral sample transfer to the test and can differ depending on sampling technique (oropharyngeal vs. nasopharyngeal).^{14,15} This may offer an explanation as to why early data from China reported test sensitivities ranging from 66% to 70% which results in false negative rates ranging from 34% to 30%.^{12,16} Studies comparing rRT-PCR to a composite of radiological plus clinical findings (signs/symptoms, epidemiological evidence of exposure) have reported that chest imaging improves initial diagnosis of COVID-19 and is associated with fewer false negatives; Ai and colleagues reported a decrease in false negative diagnosis from 25% (initial rRT-PCR) to 3% (chest imaging) whereas Long et al, found that false negative diagnosis decreased from 16.7% (initial rRT-PCR) to 2.8% (chest imaging) when chest imaging was included in the initial diagnosis of COVID-19.^{9,17} Therefore, a comprehensive clinical diagnosis inclusive of clinical exam (symptoms/signs), laboratory findings, confirmatory testing (ie, rRT-PCR), and chest imaging (chest computed tomography (CT)) may also be considered as indicators of COVID-19 to reduce false negative rate of molecular testing.¹⁷⁻²⁰

Rapid host response assays have been proposed for initial triage as components of a comprehensive COVID-19 diagnostic strategy

What's known

- COVID-19 rRT-PCR long turnaround time and marginal sensitivity have led to delays in test results, diagnosis, and thus have exposed non-COVID-19 patients to cross-infection.
- FebriDx, a rapid, point-of-care diagnostic test, that identifies bacterial, viral infection (or no infection) and may be able to improve time to diagnosis and cohorting practices.

What's new

- FebriDx was found to be highly sensitive (97.1%-100%) and specific (100%) for identifying COVID-19 infections.
- FebriDx identified all patients with bacterial infection (eg, lower respiratory tract infection or pneumonia) which could reduce exposure to patients with suspected COVID-19 whilst awaiting rRT-PCR results.
- FebriDx testing (10 minutes) can improve time to initial triage and isolation when compared with rRT-PCR (48 hours).

that also includes molecular and antibody testing. This is in an effort to streamline patients for confirmatory testing, quarantine and facilitate hospitalisation or discharge.²¹ FebriDx, (Lumos Diagnostics, Sarasota, FL, USA) is a point-of-care (POC) immunoassay that rapidly (10 minutes) assesses a host immune response to an ARI and differentiates viral from bacterial infection through detection of both Myxovirus resistance protein A (MxA) and C-reactive protein (CRP) from a fingerstick blood sample.

C-reactive protein (CRP) is a non-specific, acute-phase protein that is upregulated due to acute inflammation, including response to infection and is predominately produced by the liver in response to inflammatory cytokines such as interleukin (IL)-6.²²⁻³⁰ MxA is an intracellular protein that is exclusively induced by type I interferon (IFN) and not by other cytokines expressed during bacterial infection (eg, IFN-gamma, IL-1, tumour necrosis factor (TNF)-alpha).³¹ Type I IFNs are produced by many different cell types, specifically monocytes and macrophages, in response to a wide range of viral infections and are found to be elevated in the presence of most acute viral infections.³²⁻³⁴ Therefore, MxA is upregulated in response to an acute viral infection and remains low in bacterial infections.^{32,33} It is hypothesised that SARS-CoV-2 may initially suppress type I IFN production causing loss of viral containment early in of infection followed by an influx of neutrophils, macrophages and excessive production of type I IFN.³⁵ However, considering that MxA is exclusively expressed by type 1 IFNs and similar viruses such as Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) and SARS-CoV have been found to elevate MxA, it is likely that MxA would also increase in response to SARS-CoV-2 infection.³⁶

FebriDx, utilises monoclonal anti-MxA and anti-CRP antibodies to detect elevated levels of MxA and CRP, respectively. MxA elevation with or without an elevation in CRP is consistent with a viral infection. An elevation in CRP without MxA is consistent with a bacterial infection that may require antibiotic therapy. Two multicenter trials demonstrated that FebriDx had high accuracy for bacterial infections (sensitivity 87%-90% [95% CI 59%-100%]; specificity 93%-94% [95% CI 88%-98%]) as well as viral infection (sensitivity 90%-97% [95% CI 75%-96%]; specificity 76%-83% [95% CI 66%-89%]) to differentiate viral from bacterial and non-infectious conditions in patients presenting to General Practice offices as well as Hospital or Emergency Department (ED) settings, with non-specific ARI symptoms.^{32,33}

Therefore, we hypothesised that FebriDx would provide an early indication of a host immune response in suspected COVID-19 cases presenting to or within the hospital. Identifying patients as having a bacterial or viral infection or non-infectious condition could significantly decrease time to presumed diagnosis and allow for appropriate isolation from the outset. The primary objective was to assess the FebriDx assay ability to identify COVID-19 patients as viral infections in order to inform clinical management strategies and initial isolation procedures until confirmatory testing results are available.

2 | METHODS AND MATERIALS

2.1 | Study design and patients

Patients presenting between March 16 and April 3, 2020 to the emergency department (ED) or within a hospital ward of Kettering General Hospital, a 600-bed acute care hospital serving a population of 330 000 of middle (mainly)-to low income Caucasians in Kettering, England, with suspected COVID-19 infection were prospectively screened for eligibility. Patients were considered to be eligible if they were 16 years or older, met the Public Health England (PHE) criteria for swab testing for COVID-19 which included the requirement of hospital admission and having either clinical or radiological evidence of pneumonia or acute respiratory distress syndrome or influenza-like illness (fever $\geq 37.8^{\circ}\text{C}$ and at least one of the following respiratory symptoms, which must be of acute onset (within 7 days of enrollment): persistent cough (with or without sputum), hoarseness, nasal discharge or congestion, shortness of breath, sore throat, wheezing, sneezing), or inpatients with new respiratory symptoms or fever without another cause or worsening of a pre-existing respiratory condition.⁶ Patients were not eligible if they did not consent to participate, did not meet PHE criteria for COVID-19 swab testing, received a live vaccine or antivirals in the last 14 days, had respiratory symptoms for more than 7 days, or were taking immunosuppressive therapy or systemic corticosteroids. Consent was obtained from patients prior to inclusion in the study. The POC FebriDx test was offered by study physicians at the time nurses performed the nasal and pharyngeal swab for viral PCR testing (SARS-CoV-2, Influenza A, Influenza B and Respiratory Syncytial Virus

(RSV)). Study physicians preformed, interpreted and documented the FebriDx test results at the time of testing and were blinded of the SARS-CoV-2 rRT-PCR test results which were not available for 2-4 days following the FebriDx test. Standard routine blood tests (eg, complete blood cell count (CBC), CRP and procalcitonin (PCT)) were also performed and unblinded to study and treating physicians. SARS-CoV-2 rRT-PCR testing was repeated, based on availability as tests were very limited early in the COVID-19 pandemic, if there was strong clinical suspicion of COVID-19 infection and an initial test was negative. SARS-CoV-2 antibody testing was performed in patients with negative SARS-CoV-2 rRT-PCR test results and who had (i) clinical features suggestive of Covid-19 infection⁶ (ii) epidemiological evidence of recent exposure (within 14 days of enrollment) to COVID-19, or (iii) diagnostic evidence (eg, a combination of radiological changes consistent with pneumonia/COVID-19 such as multifocal ground-glass opacities progressing to multifocal organising pneumonia/consolidation, and intra/interlobular septal thickening in a predominantly peripheral distribution or acute respiratory distress syndrome (ARDS) consistent with the European Centre for Disease Prevention and Control (ECDC) and the United States Centers for Disease Control and Prevention (CDC) Case Definitions of Possible (clinical evidence) or Probable (clinical plus epidemiological evidence or diagnostic evidence) COVID-19.^{5,8} Eligibility and workflow are summarised in Figure 1.

2.2 | Reference method case definitions

Patients were categorised as having a final diagnosis of Bacterial Infection,^{37,38} COVID-19 Viral Infection,^{5,8} Non-COVID-19 Viral Infection^{37,38} and Non-Infectious conditions^{37,38} were based on the following Case Definitions (Figure 1). Patients were considered to have a final diagnosis (ie, Case Definition) of COVID-19 Viral Infection if SARS-CoV-2 was detected by rRT-PCR or if antibodies to SARS-CoV-2 were detected. The Case Definition for a Non-COVID-19 Viral Infection was defined as the absence of bacterial pathogen or positive rRT-PCR detection of a non-SARS-CoV-2 respiratory viral pathogen and laboratory findings consistent with viral infection such as lymphocytosis, PCT $< 0.1 \text{ ng/mL}$,³⁷ CRP $< 40 \text{ mg/L}$.³⁸

Patients were considered to have a final diagnosis (ie, Case Definition) of Bacterial lower respiratory tract infection (LRTI)/Pneumonia if found to have negative molecular viral testing (SARS-CoV-2, RSV, Influenza) and one of the following: 1) chest imaging with new onset focal consolidation and CRP $\geq 100 \text{ mg/L}$ ³⁸ and PCT $\geq 0.25 \text{ ng/mL}$ ³⁷ (bacterial pneumonia) or 2) No consolidation on chest imaging and CRP ≥ 40 ³⁸ mg/L and PCT $\geq 0.1 \text{ ng/mL}$ ³⁷ (bacterial LRTI) with or without positive bacterial culture.

The Case Definition for a Non-Infectious condition was defined as having clear evidence of alternative diagnosis with absence of a detected pathogen, chest imaging that was negative for consolidation/infiltrates consistent with infection, Procalcitonin $< 0.1 \text{ ng/mL}$.³⁷ Diagnostic performance of FebriDx was measured against the reference method as defined by the Case Definitions was confirmed

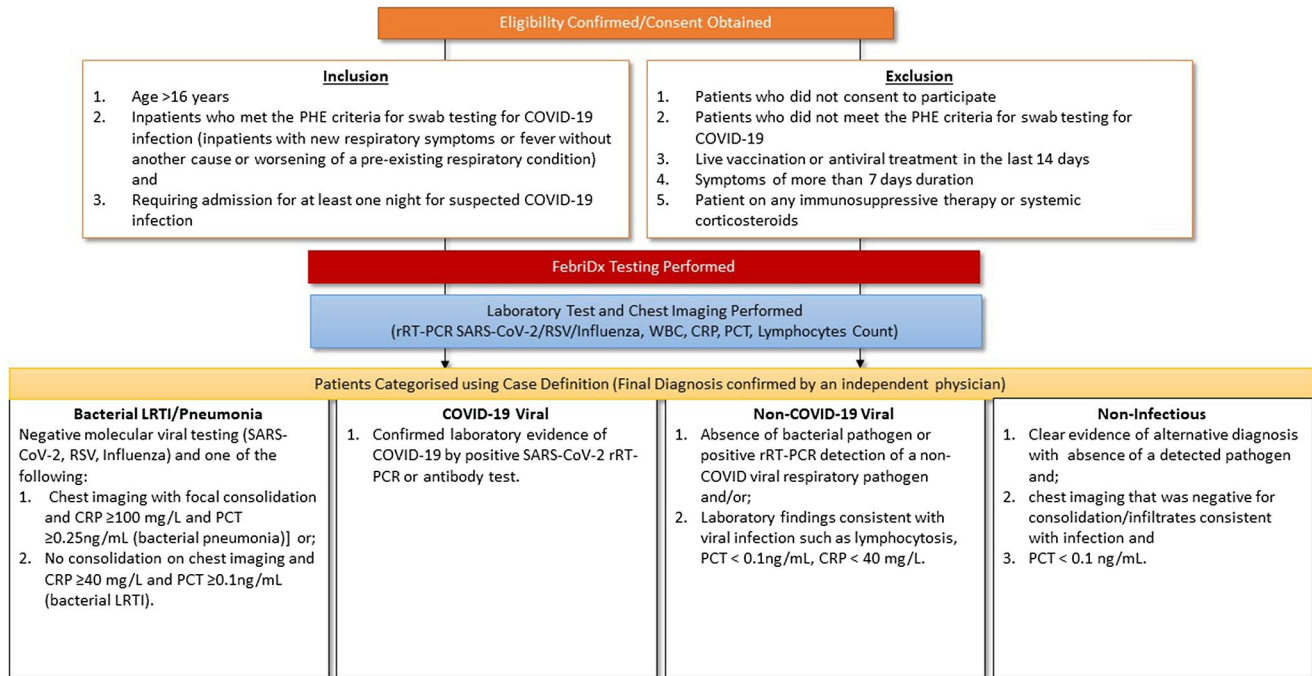


FIGURE 1 Eligibility criteria, workflow and case definitions for final diagnosis categorisation

by an independent physician who was blinded to the FebrileDx results (Figure 1).

2.3 | FebrileDx point-of-care testing and interpretation

The FebrileDx test procedure is simple, performed in approximately 30 seconds and results are available in 10 minutes. The tip of the finger is cleaned and lanced with a built-in, retractable lancet. A small amount of blood (~5 μ L) is collected from a fingerstick sample via a built-in collection tube and transferred to the test strip by rotating the collection tube. A buffer solution activates the test. The test is visually interpreted by the presence or absence of lines that represent viral or bacterial pathogen has induced a host immune response or if no host immune response is present (<https://www.febrileDx.com/how-to-use#testing>). Presence of MxA is represented by a red line, presence of CRP is represented by a black line and the control line is blue. A blue control line needed to be present in order for a test result to be valid.

FebrileDx was performed according to the manufacturer package insert and a second physician was consulted to verify the results of all viral negative tests as well as those where the physician performing the test requested help interpreting the colour saturation of the FebrileDx test lines. FebrileDx results were not shared with treating physicians at the time of testing and were blinded to study staff analysing the final diagnosis. Consistent with previous studies,^{32,33} an elevation of MxA (red line) with or without elevation in CRP (black line) was interpreted as a viral infection; and an elevation in CRP without MxA was interpreted as a bacterial infection. The absence

of an elevation in either CRP or MxA was interpreted as negative (blue line).

2.4 | Primary endpoint

Evaluate the diagnostic performance (Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV)) of FebrileDx to identify patients with COVID-19, defined as detection of SARS-CoV-2 by rRT-PCR or antibody test, as a viral infection (+MxA) in hospitalised patients with ARI symptoms (Figure 1).

2.5 | Secondary endpoints

Evaluate the diagnostic performance of FebrileDx to identify Bacterial Infection in hospitalised patients with suspected ARI. The diagnostic performance of the initial SARS-CoV-2 rRT-PCR test as compared with the Case Definition for COVID-19 (ie, positive SARS-CoV-2 rRT-PCR or antibody test).

2.6 | Statistical analysis

Sample size was not prespecified. The data were summarised using descriptive statistics and results are reported as medians and interquartile ranges or means and standard deviations, as appropriate. Categorical variables are summarised numerically and percentages. Diagnostic Sensitivity, Specificity, PPV, NPV as well as positive and negative likelihood ratios (LRs) and clinical utility indices (CUI) are

reported as point estimates and 95% confidence intervals. CUIs are the degree to which a diagnostic test is useful in clinical practice and considers prevalence and performance in the calculation. Tests with CUIs ≥ 0.81 are considered to have excellent clinical utility, 0.64-0.80 good utility and 0.49-0.63 fair utility.³⁹

3 | RESULTS

A total of 75 consecutive patients were screened for eligibility and 26 patients were deemed ineligible due to history of symptoms being longer than 7 days in duration ($n = 25$) and immunosuppression ($n = 1$). FebriDx testing was performed on 49 patients, test results were obtained for 48/49 patients as testing was not possible in one patient due to an inability to obtain enough blood on the first attempt. A second attempt was not deemed appropriate as this patient was elderly, frail, and clinically unstable at the time of testing. A second patient with a diagnosis that met the ECDC/CDC Case Definition for Probable COVID-19 based on clinical features and diagnostic evidence, in whom SARS-CoV-2 was not detected by rRT-PCR, died before the diagnosis could be verified by SARS-CoV-2 or antibody testing. The final diagnosis by reference method could not be confirmed as bacterial, viral, or non-infectious, and therefore this patient was removed from the analysis. Data from 47 patients were included for final analysis (Figure 2). Of the 47 patients with FebriDx and final diagnosis categorised by reference method Case Definitions, 34 had a final diagnosis of COVID-19 (prevalence of 72%); patients with COVID-19 were more likely to be male, febrile, older than 65 and have classic radiological features of COVID-19 (eg, ground-glass opacities, multifocal organising pneumonia, or architectural distortion in

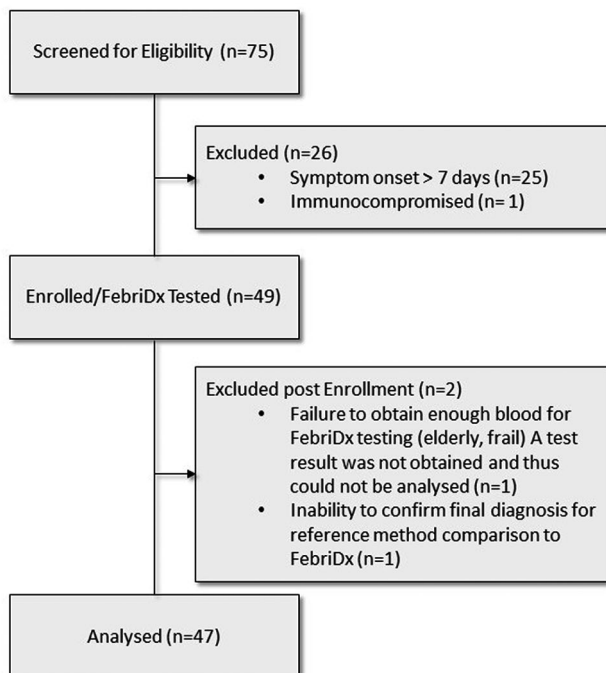


FIGURE 2 Screened and enrolled patients

a peripheral distribution). Non-infectious patients tended to have lower procalcitonin, leucocytes and standalone conventional CRP levels compared with patients with COVID-19, non-COVID-19 viral infection, and bacterial infection. Although standalone conventional CRP and leucocyte counts were higher in bacterial infection compared with COVID-19, the inter quartile range had substantial overlap. Procalcitonin did not differ between COVID-19 and bacterial infections. The majority of patients in the study were discharged home, 27 with viral diagnosis (26 with COVID-19 and one with a non-COVID-viral diagnosis), seven with bacterial infection, and two with non-infectious conditions. Patients who died in the hospital due to complications associated with COVID-19 were more likely to be older than 65 years of age (range 68-96 years), male (6/8), and febrile (8/8). Cohort characteristics are described in Table 1.

The FebriDx test results were positive for a viral infection in all cases that had a final diagnosis of COVID-19 Viral infection (SARS-CoV-2 detected by rRT-PCR or antibody testing) by reference standard (Figure 3). In all cases where FebriDx was negative for a viral infection (13/47), SARS-CoV-2 rRT-PCR was also negative. Therefore, when considering COVID-19 cases alone ($n = 34$), FebriDx identified all positive cases and ruled out all Non-COVID-19 cases ($n = 13$). In one case of LRTI, it was not possible to determine the exact cause of infection and a viral infection could not be excluded despite negative viral tests (FebriDx test, rRT-PCR for SARS-CoV-2, influenza, and RSV). Procalcitonin, CRP and leucocyte count were slightly elevated or normal (PCT 0.06 ng/mL CRP 25 mg/L, leucocyte count $9.4 \times 10^3/\text{microL}$). In addition to the laboratory data, a final diagnosis of Non-COVID-19 Viral was deemed clinically appropriate based on the patient's history of clinical improvement without antibiotics and discharge home without complications after a 1-day admission. FebriDx diagnostic performance is, therefore, reported for all viral infections ($n = 35$) as well as for COVID-19 only ($n = 34$).

In secondary endpoint analyses, SARS-CoV-2 was detected by rRT-PCR in (31/34) cases with a final diagnosis of COVID-19 Viral infection; 85.3% (29/34) were detected on the first rRT-PCR test (test to confirmation, 48 hours), 5.8% (2/34) were detected on the second rRT-PCR test (test to confirmation, 96 hours), and 8.8% (3/34) SARS-CoV-2 were not detected by rRT-PCR despite clear clinical evidence and/or diagnostic evidence of COVID-19 that was consistent with the ECDC/CDC Case Definition for Probable COVID-19. Of the three patients that met the ECDC/CDC Case Definition for Probable COVID-19, but where SARS-CoV-2 was not detected by rRT-PCR, 100% (3/3) developed SARS-CoV-2 antibodies and FebriDx was viral positive. FebriDx was positive for bacterial infection in 100% (8/8) bacterial infections as defined by the reference method Case Definition for Bacterial Infection. Diagnostic performance for primary and secondary endpoints is summarised in Table 2.

4 | DISCUSSION

Our findings demonstrate that FebriDx can be used to rapidly identify suspected cases of COVID-19 in symptomatic patients presenting to

TABLE 1 Cohort characteristics

Characteristic no. (%)	Total cohort (n = 47)	Viral (COVID-19/Non-COVID-19) (n = 35)	Bacterial (n = 8)	Non-infectious (n = 4)
Sex no. (%)				
Male	32 (68.1)	26 (74.2)	4 (50.0)	2 (50.0)
Female	15 (31.9)	9 (32.4)	4 (50.0)	2 (50.0)
Age – years, no. (%)				
<65	22 (46.8)	17 (48.6)	4 (50.0)	1 (25.0)
≥65	25 (53.2)	18 (51.4)	4 (50.0)	3 (75.0)
Range	24-96	24-96	28-94	50-82
Mean (±SD)	63.7 (±18.9)	62.7 (±18.2)	62.5 (±24.0)	72 (±14.9)
Median [IQR]	67 [53-77]	66 [53-74]	69 [49-78]	78 [69-80]
Symptom Onset – days				
Range	2-7	2-7	2-5	2-7
Mean (±SD)	3.8 (±1.9)	4 (±1.9)	2.9 (±1.2)	3.8 (±2.2)
Median [IQR]	3 [2-5]	3 [2-5]	2 [2-3]	3 [3-4]
Fever (≥37.8°C) no. (%)				
Yes	40 (85.1)	33 (94.3)	6 (75.0)	1 (25.0)
No	7 (14.9)	2 (5.7)	2 (25.0)	3 (75.0)
Final disposition no. (%)				
Home	36 (75.6)	27 (77.1) ^a	7 (87.5)	2 (50.0)
Died	11 (23.4)	8 (22.9) ^b	1 (12.5)	2 (50.0)
Final diagnosis no. (%)				
COVID/Viral	34 (72.3)	34 (97.1)	0 (0)	0 (0)
Non-COVID/Viral	1 (2.1)	1 (2.9)	0 (0)	0 (0)
Bacterial	8 (17.0)	0 (0)	8 (100)	0 (0)
Non-infectious	4 (8.5)	0 (0)	0 (0)	4 (100)
FebriDx results no. (%)				
Viral	34 (72.3)	34 (97.1)	0 (0)	0 (0)
Bacterial	11 (23.4)	1 (2.8)	8 (100)	2 (50.0)
Non-infectious	2 (4.2)	0 (0)	0 (0)	2 (50.0)
Procalcitonin (ng/mL)				
Range	0.05-13.6	0.05-0.33	0.08-0.68	0.09-0.09
Median [IQR]	0.17 [0.08-0.33]	0.17 [0.08-0.33]	0.24 [0.14-0.41]	0.09 [0.09-0.09]
C-reactive protein (mg/L)				
Range	5-310	5-304	32-310	5-34
Median [IQR]	73 [37-116]	74 [50-116]	94 [60-152]	17 [11-23]
Leucocyte count				
Range	2.6-19.1	2.6-13.6	10.5-19.1	9.7-15.7
Median [IQR]	9 [5-11]	7 [4-10]	13 [11-16]	12 [10-14]
Lymphocyte count				
Range	0.4-8.4	0.4-8.4	0.6-6.4	1.2-6.4
Median [IQR]	1 [0.7-1.4]	0.8 [0.7-1.3]	1.4 [0.8-1.7]	2 [1.6-3.3]

Abbreviations: IQR, interquartile range; SD, standard deviation.

^aTwenty-seven patients with viral diagnosis were discharged home; 26 had COVID-19, 1 had a non-COVID-19 viral diagnosis.

^bMortality in the viral diagnosis group was related to complication of COVID-19.

and within the hospital with a high degree of accuracy. Additionally, our study found that rRT-PCR was highly specific for SARS-CoV-2 and also noted a lower false negative rate compared with previous

studies (15% vs. 30% respectively),^{12,16} however, the delay in time to confirmatory rRT-PCR test results (48 hours) and need for repeat tests (two to three repeats) were similar to other studies.⁹⁻¹¹

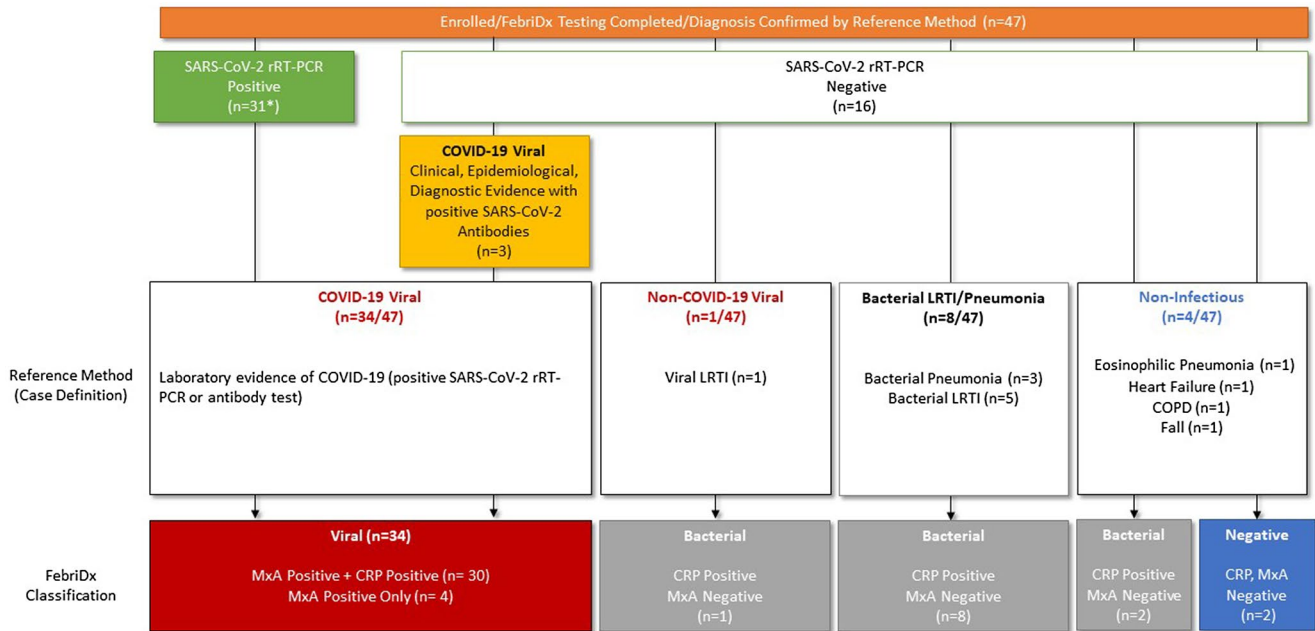


FIGURE 3 Final diagnosis categorisation

Based on the diagnostic performance characteristics of Febrile Dx demonstrated in our study (Table 2), we propose that in the COVID-19 pandemic, patients presenting with signs and symptoms of ARI and suspected of COVID-19 infection should be tested with Febrile Dx test as part of the initial diagnostic triage process. Those testing “viral positive” (+MxA), should be cohorted away from “viral negative” (-MxA) while awaiting confirmatory rRT-PCR results for SARS-CoV-2 and other respiratory pathogens (eg, panels including influenza, RSV, human metapneumovirus etc) This would help avoid unnecessary exposure while awaiting confirmatory rRT-PCR testing. Furthermore, if the Febrile Dx result is “viral negative” an alternative diagnosis such as bacterial infection or non-infectious conditions such as acute exacerbation of chronic obstructive pulmonary disease, asthma or heart failure, should be considered at the outset.

Future viral outbreaks and seasonal infections could be managed, ideally, by optimising all available diagnostic tools (eg, clinical assessment, host response, molecular testing, antibody testing etc).²¹ Pulia et al proposed “Multi-tiered Screening and Diagnostic Strategy” that incorporates a comprehensive approach that could be used in the SARS-CoV-2 pandemic and potentially as a general strategy in future pandemics.²¹ The strategy proposes that after initial screening (eg, clinical signs/symptoms of the suspected infection), such as the initial screening performed in our study, patients could be (i) be quickly tested for a viral, bacterial or absent immune response to an infection, followed by (ii) rapid confirmatory pathogen-specific testing; and (iii) rapid antibody testing could be performed in patients that present with greater than 7 days of symptom onset to confirm a recent or past infection. Although Febrile Dx should not be used as a surrogate for pathogen-detection tests, it can be applied to rapidly categorise patients as having bacterial or viral infections or

non-infectious conditions as part of the diagnostic triage process.⁴⁰ This would allow bacterial infections/non-infectious conditions to be cohorted separately from suspected viral infections. Those with viral infections would go on to have confirmatory testing to improve cohorting within the viral category, whereas antibiotics could be considered for patients positive for bacterial infection. Repeat rRT-PCR testing could be considered in high-risk patients who test viral positive on Febrile Dx but have a negative initial SARS-CoV-2 rRT-PCR.

A study by Clark and colleagues from University Hospital Southampton, Southampton, England, also evaluated the diagnostic accuracy of Febrile Dx in hospitalised adults who presented with suspected COVID-19 regardless of duration of symptom onset.⁴⁰ Of the 248 patients who underwent Febrile Dx and SARS-CoV-2 rRT-PCR, 118 had SARS-CoV-2 detected (prevalence 48%). Diagnostic sensitivity, specificity, NPV and PPV were 93%, 86%, 86% and 93%, respectively. Despite some methodological differences their results were comparable to our study. Clark et al, also found CRP to be elevated in both COVID and Non-COVID-19 cases (median (range) 83 mg/L (32-136 mg/L); 33 (9-114 mg/L), respectively).⁴⁰ Although Clark et al found the difference to be statistically significant, the considerable overlap of standalone conventional CRP may make it difficult to differentiate viral from bacterial infection as a standalone test.⁴⁰ The same appears to apply to procalcitonin and leucocyte count in our study (Table 1).

Febrile Dx MxA confers the diagnostic sensitivity and specificity needed to differentiate elevated CRP associated with viral versus bacterial infection and may help to avoid mixing non-COVID-19 with COVID-19 whilst awaiting the results of rRT-PCR that can take up to 48 hours in the hospital/ED settings. In our study seven patients, who were symptomatic for ARI but in the end did not have COVID-19, were inadvertently exposed to SARS-CoV-2 due

TABLE 2 FebriDx and rRT-PCR diagnostic performance characteristics following initial screening (eg, clinical signs/symptoms of COVID-19)

	Primary endpoint(s)	Primary endpoint(s)	Secondary endpoint	Additional endpoint
	FebriDx viral versus case definition (Viral-all)	FebriDx viral versus case definition (COVID-19 Viral)	Initial SARS-CoV-2 rRT-PCR versus case definition (COVID-19 Viral)	FebriDx bacterial versus case definition (Bacterial)
Sensitivity	97.1%	100%	85.3%	100%
TP/TP + FN	34/35	34/34	29/34	8/8
[95% CI]	[83.3-99.9]	[87.4-100]	[68.2-94.5]	[59.8-100]
Specificity	100%	100%	100%	92.3%
TN/FP + TN	12/12	13/13	13/13	36/39
[95% CI]	[70.0-100]	[71.7-100]	[71.7-100]	[78.0-98.0]
PPV	100%	100%	100%	72.7%
TP/TP + FP	34/34	34/34	29/29	8/11
[95% CI]	[87.4-100]	[87.4-100]	[85.4-100]	[39.3-92.3]
NPV	92.3%	100%	72.2%	100%
TN/FN + TN	12/13	13/13	13/18	36/36
[95% CI]	[62.0-99.6]	[71.7-100]	[46.4-89.3]	[88.0-100]
+LR	NA	NA	NA	13.0
SE/(1-SP)	0.97/0	1/0	0.85/0	1/0.077
[95% CI]	[NA]	[NA]	[NA]	[4.5-38.6]
-LR	0.03	0	0.15	NA
(1-SE)/SP	0.03/1	0/1	0.15/1	0/0.92
[95% CI]	[0.004-0.02]	[0.0-0.2]	[0.07-0.33]	[NA]
+CUI	0.97	1.0	0.85	0.73
SE*PPV	0.97*1	1*1	0.85*1	1*0.727
[95% CI]	[0.92-1.0]	[1.0-1.0]	[0.74-0.97]	[0.46-0.99]
-CUI	0.92	1.0	0.72	0.92
SP*NPV	1*0.92	1*1	1*0.72	0.92*1
[95% CI]	[0.83-1.0]	[1.0-1.0]	[0.58-0.87]	[0.87-0.98]

Abbreviations: CUI, clinical utility index; FN, false negative; FP, false positive; LR, likelihood ratio; NA, not applicable; NPV, negative predictive value; PPV, positive predictive value; rRT-PCR, reverse transcriptase polymerase chain reaction; SE, sensitivity; SP, specificity; TN, true negative, TP, true positive.

to the unintended mixed cohorting that occurred whilst awaiting swab rRT-PCR results. As our study was intended to evaluate diagnostic accuracy of FebriDx as part of an initial triage strategy, the FebriDx test results were not used to make decisions regarding cohorting until after the study was concluded and the results were analysed. Based on the high NPV of FebriDx in our setting, it is possible that the unintended exposure of non-COVID-19 patients could have been avoided if FebriDx was utilised part of the initial triage of ARI patients with suspected COVID-19. Utilising FebriDx for enabling cohorting decisions could have avoided exposure in these cases.

Our study is not without limitations. Based on the urgent need to improve testing turnaround times and patient isolation strategies at our hospital, it was not possible to design and perform a multi-centre trial that included a control group. Antibody testing was not available for all patients enrolled, however, antibody confirmation

would be ideal for determining definitive COVID-19 infection after 14 days of symptom onset, especially in cases that have a high clinical suspicion but where SARS-CoV-2 is not detected by rRT-PCR testing. Due to the lack of a gold standard test for COVID-19 infection, the current assessment of the performance characteristics of rRT-PCR may suffer from an incorporation bias. We attempted to mitigate this by including serology evidence of COVID-19 infection. Our reference method COVID-19 Case Definition required objective laboratory evidence (eg, SARS-CoV-2 rRT-PCR or antibody testing) to confirm the diagnosis of COVID-19 for FebriDx comparison. One patient who met the EDCD/CDC Probable Case Definition based on clinical (shortness of breath) and diagnostic criteria (consolidation on chest imaging consistent with pneumonia) for COVID-19 was excluded due to lack of laboratory evidence (ie, rRT-PCR or serology) to confirm the diagnosis. FebriDx was viral positive in this patient and diagnostic performance was minimally impacted by the

exclusion of this patient due to incomplete reference method test results. Additionally, all patients in our study were symptomatic for acute respiratory infection that was likely related to COVID-19 due to the high prevalence (72%) of disease at the time of enrollment. Thus, the triage strategy proposed cannot be generalised to asymptomatic population, however, studies and diagnostic tests that can rapidly identify asymptomatic and presymptomatic patients with COVID-19 are of critical importance. Patient enrollment took place at the peak of the COVID-19 outbreak in our region and pre-test probability for COVID-19 positive patients was relatively high. This strategy allowed us to obtain a maximum number of potential COVID-19 infections to evaluate if FebriDx identified COVID-19 as a viral infection. Therefore, diagnostic accuracy results must be taken in the context of the high disease prevalence (72%) at the time of enrollment. Most notably, as the prevalence of COVID-19 decreases, the positive predictive value of FebriDx will also decrease. Conversely, lower prevalence will have the opposite effect on negative predictive value which would increase in times of lower prevalence. It is possible the one patient who was categorised as non-COVID-19 Viral infection/CRP positive FebriDx had a common respiratory virus (eg, Human Metapneumovirus, Parainfluenza) or atypical bacteria (ie, *Mycoplasma pneumoniae*) that was not identified by our routine testing. Future studies should include a molecular respiratory panel consisting of common respiratory infection pathogens. Finally, patients presenting in our hospital with COVID-19 symptoms were generally immunocompetent adults and our study only included immunocompetent adults. Therefore, additional studies would be required to assess this strategy in children as well as immunocompromised patients.

5 | CONCLUSION

According to overwhelming data reports from the PHE, CDC and ECDC, as well as based on experience in our own clinical setting, the predominant virus causing hospitalisation amongst adults at present, seems to be SARS-CoV-2. Based on our study findings, we provide evidence that FebriDx could be deployed as part of the initial diagnostic triage strategy for identification of symptomatic COVID-19 patients presenting in a hospital setting.

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DISCLOSURES

Authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPLIANCE WITH ETHICS GUIDELINES

Prior to enrollment, the study was submitted for review to the Kettering General Hospital Ethics Committee and granted approval by the Research Committee Chair. Informed Consent was obtained from all participants and failure to consent was considered an exclusion criterion.

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