Accuracy of the Mologic COVID-19 rapid antigen test: a prospective multi-centre analytical and clinical evaluation

[version 1; peer review: awaiting peer review]

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Abstract
Background: The coronavirus disease 2019 (COVID-19) pandemic has highlighted the reliance on antigen detection rapid diagnostic tests (Ag-RDTs). Their evaluation at point of use is a priority.
Methods: Here, we report a multi-centre evaluation of the analytical sensitivity, specificity, and clinical accuracy of the Mologic COVID-19 Ag-RDT by comparing to reverse transcriptase polymerase chain
reaction (RT-qPCR) results from individuals with and without COVID-19 symptoms. Participants had attended hospitals in Merseyside, hospital and ambulance services in Yorkshire, and drive-through testing facilities in Northumberland, UK.

**Results:** The limit of detection of the Mologic COVID-19 Ag-RDT was $5.0 \times 10^2$ pfu/ml in swab matrix with no cross-reactivity and interference for any other pathogens tested. A total of 347 participants were enrolled from 26th of November 2020 to 15th of February 2021 with 39.2% (CI 34.0-44.6) testing RT-qPCR positive for SARS-CoV-2. The overall sensitivity and specificity of the Mologic Ag-RDT compared to the reference SARS-CoV-2 RT-qPCR were 85.0% (95% CI 78.3-90.2) and 97.8% (95.0-99.3), respectively. Sensitivity was stratified by RT-qPCR cycle threshold (Ct) and 98.4% (91.3-100) of samples with a Ct less than 20 and 93.2% (86.5-97.2) of samples with a Ct less than 25 were detected using the Ag-RDT. Clinical accuracy was stratified by sampling strategy, swab type and clinical presentation. Mologic COVID-19 Ag-RDT demonstrated highest sensitivity with nose/throat swabs compared with throat or nose swabs alone; however, the differences were not statistically significant.

**Conclusions:** Overall, the Mologic test had high diagnostic accuracy across multiple different settings, different demographics, and on self-collected swab specimens. These findings suggest the Mologic rapid antigen test may be deployed effectively across a range of use settings.

**Keywords**
COVID-19, SARS-CoV-2, Rapid Antigen Test (Ag-RDT), Epidemic preparedness and response, Diagnostics

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Introduction

Responding to the coronavirus disease-19 (COVID-19) pandemic has necessitated urgent development of rapid diagnostics\(^1\). Rapid detection of infection, prompt isolation, and systematic contact tracing are critical to break the spread of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)\(^2,3\).

Although reverse transcriptase polymerase chain reaction (RT-qPCR) is the reference standard for COVID-19 diagnosis\(^4\), it has many drawbacks, including long turnaround times often more than 24 hours\(^4\), the need for dedicated staff and expensive platforms and reagents\(^8\). Delays in RT-qPCR reporting can decrease the impact of infection control measures to reduce the spread of SARS-CoV-2, especially when contact tracing is delayed. In contrast, antigen detection using rapid diagnostic tests (Ag-RDTs) can provide rapid local results and the relatively small investment in resources and expertise required make it easier to implement globally\(^7,14\). Ag-RDTs are less sensitive than RT-qPCR in detecting infections with low viral loads but detect most individuals with high-viral loads (>10\(^9\) genomic virus copies/ml), who are considered the most likely to transmit the infection\(^6,15\). During outbreaks rapid results are paramount, and a diagnostic test with lower sensitivity than RT-qPCR, yet faster time to result, could be more effective than more accurate but substantially slower test results\(^5\).

Despite the increase in use of Ag-RDTs as an alternative for RT-qPCR, independent diagnostic accuracy data are currently lacking for many rapid antigen tests on the market. Evaluation of Ag-RDTs before proceeding to clinical implementation is particularly important because their performance is highly variable, with sensitivity ranging from 0% to 95% in the literature\(^5,16–19\).

Here we describe a multi-centre study to evaluate the analytical sensitivity, specificity and clinical performance of a novel Ag-RDT (Mologic COVID-19 Rapid Antigen Test, Mologic Ltd, UK) among individuals with and without COVID-19 symptoms attending secondary care facilities in Merseyside, Yorkshire ambulance services, and drive-through testing facilities in Northumberland, UK.

Methods

Clinical evaluation: study design, settings and participants

This was a prospective evaluation of consecutive participants enrolled from the 26th of November 2020 to 11th of March 2021 in three different settings: (a) In Merseyside, consecutive enrolment of adults with symptoms suggestive of COVID-19 or asymptomatic individuals with SARS-CoV-2 NHS RT-qPCR positive tests attending the Accident and Emergency Department of the Royal Liverpool University (RLUH), Whiston University (WUH) and Aintree University Hospitals (AUH). All patients meeting the described criteria in this period were enrolled in the study.

Participants were tested using one sample for RT-qPCR testing, obtained as a nasopharyngeal (NP) swab in Amies media (Copan, Italy), and a second sample, obtained as a throat swab (T) with no media (Copan, Italy) for the Ag-RDT. Samples were obtained by healthcare professionals and swabs were transported and processed at the Liverpool School of Tropical Medicine (LSTM) by trained laboratory researchers. Mologic Ag-RDTs were performed upon arrival on the dry swabs, while NP swabs in Amies were aliquoted and stored at -80°C until RNA extraction. RNA was extracted using QIAamp Viral RNA Mini Kit (Cat # 52904, Qiagen, Germany) and RNA was screened using TaqPath COVID-19 kit (ThermoFisher, UK) (Cat # A8067) in the Quant Studio ™ thermocycler, model 5 (96-well plate) (ThermoFisher, UK). The kit contains all primers and probes needed for the detection of SARS-CoV-2 targets (ORF1, N and S gene) and the internal extraction control (MS2).

PCR amplification protocol consisted of an initial incubation step at 25°C for 2 minutes, a reverse transcription step at 53°C for 10 minutes, initial denaturation step of 95°C for 2 minutes, followed by 40 cycles of denaturation for 3 seconds at 95°C, annealing for 30 seconds at 60°C.

The Thermo Fisher RT-qPCR was used to detect the presence of the SARS-CoV-2 B1.1.7 strain, with samples positive by the N and ORF1a probes but negative by the S probes classified as presumptive B.1.1.7.

(b) In Northumberland, NHS staff (clinical and non-clinical) and their families who wished to be tested for SAR-CoV-2 were enrolled. Staff rang a dedicated COVID-19 telephone line to arrange a test if they or a member of their household had symptoms suggestive of COVID-19 and receive appointments to attend an outdoor drive-through testing centre. Verbal informed consent was obtained at the time of the phone call. Two swabs were taken at the time of the appointment, one for the standard RT-qPCR and one for the Ag-RDT. The swab for the RT-qPCR was obtained by drive-through staff as a combined nasal throat (NT) swab in Sigma-Virocult® (MWE, UK) and processed using with Xpert Xpress SARS-CoV-2/FLU/RSV kit designed to amplify sequences of E and N2 genes, (XPCCOV2/FLU/RSV-10) in the GeneXpert DX system models XVI and IV (Cepheid, USA). The Xpert Xpress test consists of two main components: (i) the Xpert plastic cartridge, which contains liquid sample-processing and PCR buffers to detect SARS-CoV-2 genes (E and N2 genes) and lyophilized real-time RT-PCR reagents, and (ii) the GeneXpert instrument, which controls intracartridge fluidics and performs real-time RT-PCR analysis . Swabs for the Ag-RDTs were self-collected as NT and/or nasal (N) swab using iClean® swabs (Mbs inc., Canada). Ag-RDTs were performed by health care professionals within the drive-through COVID-19 test centre. Feedback of results was based on the RT-qPCR and Ag-RDT results were not used for patient management.

(c) In Yorkshire, adults were enrolled when admitted to the hospital via the Emergency Department or the Acute Admissions Unit at the Hull Royal Infirmary (HRI), with or without symptoms of COVID-19. Patients were being assessed before...
hospital admission by ambulance paramedics, who were assessing whether a patient warranted hospital admission. Participants underwent two swabs, one for RT-qPCR testing, taken as a combined NT using Remel M4RT swabs (ThermoFisher, UK) as part of their routine care, and a second swab for the Ag-RDT obtained as combined NT swab or N swab using iClean® swabs. Swabbing was conducted by healthcare professionals and swabs were tested within 24 hours. RNA extraction and RT-qPCR was performed using the Hologic Aptima SARS-CoV-2 Assay kit that targets ORF1ab gene (Hologic, USA, PRD-06419) in Hologic Panther (model Panther Fusion®) and/or with Xpress Xpress SARS-CoV-2/FLU/RSV kit (XPCOV2/FLU/RSV-10) in the GeneXpert DX system models XVI and IV (Cepheid, USA). Similar to Northumberland, results for the Ag-RDTs were not used for patient’s management.

Ethics statement

Ethical approval was obtained from the National Health Service South Central Oxford Committee (20/SC/0169) for samples collected in Merseyside; while samples tested in Yorkshire and Northumberland were considered to constitute a service evaluation by Hull University Teaching Hospitals and the Pathology Governance Committee of Northumbria Healthcare NHS Foundation Trust, as they were considering whether to introduce RDTs into routine use at that time. The project in Northumbria was subjected to the Health Research Authority (HRA) website’s decision tool ‘Is my study research?’ in reference to the UK Policy Framework for Health and Social Care Research, which defined it as ‘not research’. Therefore, NHCT-IR&D, HRA and Research Ethical Approvals (REC) were not required. Participant consent was written in Merseyside and verbal in Yorkshire and Northumberland. In Yorkshire, verbal consent was documented securely in Trust servers and in Northumbria verbal consent was documented on paper in the ‘Mologic trial forms’.

Mologic SARS-CoV-2 Ag-RDT testing protocol

The Mologic COVID-19 Ag-RDT detects the nucleoprotein of SARS-CoV-2. The testing was performed following the manufacturers’ instructions for use. Briefly, patients’ swabs were immersed into 350 µl of proprietary extraction buffer and 80 µl were pipetted onto the cassette without additional processing. Results were scored using a proprietary score card with scores ranging from 10 (very strong positive) to 0 (negative). Results were read by two operators blind to each other’s results. Discrepant results were read by a third operator as a tie breaker.

Analytical evaluation

Lower limit of detection (LLOD) using SARS-CoV-2 dilution.

The SARS-CoV-2 virus used in this study is the clinical isolate named “SARS-CoV-2/human/Liverpool/REMRQ0001/2020”. In total, the stock was passaged fourth times in Vero E6 cells (C1008; African green monkey kidney cells) before being used for the serial dilutions. Pooled nasal swabs from 56 volunteers were used as a matrix; each nasal swab was immersed into 350 µl of Mologic proprietary extraction buffer and these were pooled and aliquoted in volumes of 350 µl. Ten-fold serial dilutions of the SARS-CoV-2 stock were made using Dulbecco’s Modified Eagles Medium (DMEM) supplemented with 4.5g/L glucose and L-Glutamine (Lonza, US) culture media as a diluent. The pooled nasal aliquots were spiked with the serial dilution with a final concentration of 1.0 × 10⁶ plaque forming units (pfu)/ml to 1.0 × 10⁶ pfu/ml.

The LLOD was determined in two steps: 1) a tentative LLOD, where every dilution was tested in three (SGUL) or five (LSTM) replicates and 2) a final LLOD, where two-fold dilutions above and below of the tentative LLOD were performed and tested in 20 replicates. The genome copy numbers (gen) of the serial dilutions were quantified as follows: viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, UK) and tested using the RT-qPCR kit Genesig (Primer Design, UK cat # Z-Path-COVID-19-CE). RT-qPCR assays were carried out using the Rotor-Gene Q (model 5Plex, Qiagen, Germany), with a ten-fold serial dilution using quantified specific in vitro-transcribed RNA². The kit contains all primers and probes needed for the detection of SARS-CoV-2 targets and the internal extraction control. PCR amplification protocol consisted of a reverse transcription step at 55°C for 10 minutes, initial denaturation step at 95°C for 2 minutes, followed by 45 cycles of denaturation for 10 seconds at 95°C, annealing for 60 seconds at 60°C.

A total of five replicates were tested for each standard curve point and extracted RNA from each culture dilution was tested in triplicate. The gen/ml was calculated from the mean cycle threshold (Ct) value of the replicates. The LLOD was determined as the lowest concentration for which 19 of 20 replicates (95% positives) were positive by two operators as per the US Food and Drug Administration (FDA) guidance².

Analytical specificity and interference analysis. The specificity and pathogen interference characteristics were investigated by testing samples with known respiratory pathogens. Specificity testing aimed to assess whether the Ag-RDT reacted with other respiratory pathogens. Interference testing assessed whether the test reported false negative results when SARS-CoV-2 was present in a specimen together with other microorganisms.

Each pathogen in these assessments was tested in triplicate without SARS-CoV-2 (for specificity) and with SARS-CoV-2 at 1.0 × 10⁶ pfu/ml (for interference).

Viral cultures were tested at 1.0 × 10⁶ pfu/ml and bacterial cultures at 1.0 × 10⁷ colonies forming units (cfu)/ml. Quantified cell culture supernatants were obtained from Zeptometrix corporation (Zeptometrix corporation, USA) containing adenovirus 5 (cat # 0810020CF), adenovirus 68 (cat # 0810300CF) enterovirus (cat # 0810228CF), rhinovirus (cat # 0810012CFN), respiratory syncytial virus (RSV) (cat # 0810040ACF), influenza A virus H1N1 (cat # 0810036CF), influenza A virus H3N2 (cat # 0810252CF), influenza B virus (cat # 081037CF), parainfluenza 1 (PIV1) (cat # 0810014CF), parainfluenza 2 (PIV2) (cat # 0810015CF), parainfluenza 3 (PIV3) (cat # 0810016CF), parainfluenza 4 (PIV4) (cat # 0810060CF), human coronavirus
Sensitivity, specificity, positive (PPV), and negative (NPV) predictive values were calculated with 95% confidence intervals (CIs) by comparing the Ag-RDT results to the RT-qPCR, as the reference standard. Sub-analyses of diagnostic performance were performed by Ct-value ranges, sampling strategy (self-testing or professional testing), swab type (N, T or NT swab), clinical presentation (symptomatic or asymptomatic) and symptoms onset using nonparametric statistics. In Merseyside, the sensitivity was further analysed by RT-qPCR Spike gene target failure (SGTF) to investigate whether the presence of the B.1.1.7 variant of concern (VOC) modified the performance of the Ag-RDT in the subset of samples tested with the RT-qPCR the Spike gene and TaqPath COVID-19 (ThermoFisher, UK). The level of agreement between tests was determined using Cohen’s Kappa statistics. Statistical analysis was performed on SPSS v26. Statistical significance was set to P <0.05. The manuscript was prepared in accordance with the EQUATOR Network’s STARD guidelines.

Results
Clinical evaluation
A total of 347 participants were enrolled between the 26th of November 2020 and the 11th of March 2021. Of these, 136 (39.2%, CI 34.0-44.6) tested positive by SARS-CoV-2 RT-qPCR. Five RT-qPCR had undetermined and one had no available RT-qPCR results and thus these samples were excluded from the analysis. Participant enrolment, eligibility, and results are summarized in Figure 1 and patient demographics are described in Table 1. Clinical presentation was not available for 27 participants and onset of symptoms was not available for 141 symptomatic patients (17 with paired N and NT swab data).
Eighty-nine participants with suspected SARS-CoV-2 infections were recruited in Merseyside. The mean age was 61.3 (standard deviation (SD=15.6)) years, with 35 (39.3%) being female. Five participants (6%) were asymptomatic and 84 (94%) had symptoms. The onset of symptoms ranged from 0 to 24 days, with a median of seven days (IQR 4–10). Data of six participants was excluded due to undetermined or missing RT-qPCR results. Three Ag-RDTs with positive results initially categorised as false positives (as the LSTM RT-qPCR was negative) were re-classified as true positives as the NHS RT-qPCR result was positive and the patients were symptomatic.

Two hundred and five participants were recruited in Northumberland. Paired N and NT swabs were collected from 45 participants and unpaired NT and N swabs were collected in 61 and 102 participants, respectively. The mean age of the participants was 38 (SD=11.5) years, with 160 (82.5%) being female. One hundred and twenty fine (60.9%) participants presented clinical symptoms, 53 (25.9%) were asymptomatic and 27 (13.2%) had no clinical presentation recorded. The onset of symptoms ranged from one to four days, with a median of one day (IQR 1–2). Three NT swabs gave invalid Ag-RDTs results. The Ag-RDTs failure rate for Northumberland was 1.6% (three participants, CI 0.3-4.7).

Fifty-three participants were recruited in Yorkshire, of which 32 were enrolled at hospital admission and 21 in the ambulance service. N swabs were collected in five and combined NT in 48 individuals. The mean age of the participants was 68.7 (SD=17.2) years, with 20 (38%) being female. Three (6%) participants were asymptomatic and 50 symptomatic (94%). Onset of symptoms ranged from two to 14 days, with a median of four days (IQR 2–12).

The overall Ag-RDT failure rate was 0.9% (N=3, CI 0.2-2.7), which included three failed tests out of the 383 tests performed. The clinical accuracy of the Ag-RDT per site and setting is presented in Table 2. The overall sensitivity of the Ag-RDT RT-qPCR was 85.0% (CI 78.3-90.2) against the SARS-CoV-2 RT-qPCR with a specificity and 97.8% (CI 95.0-99.3), with PPV and NPV of 96.3% (CI 91.6-98.4) and 90.7% (CI 87.0-93.5), respectively.

### Table 1. Characteristics of the population of study.

<table>
<thead>
<tr>
<th></th>
<th>Merseyside</th>
<th>Northumberland</th>
<th>Yorkshire</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>89</td>
<td>205</td>
<td>53</td>
<td>347</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>35 (39.3%)</td>
<td>160 (82.5%)</td>
<td>20 (37.7%)</td>
<td>215 (61.9%)</td>
</tr>
<tr>
<td>Male</td>
<td>54 (60.7%)</td>
<td>34 (17.5%)</td>
<td>33 (62.3%)</td>
<td>121 (34.9%)</td>
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<tr>
<td>Unknown</td>
<td>0</td>
<td>11 (5.4%)</td>
<td>0</td>
<td>11 (3.2%)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>61.3 (SD ±15.6)</td>
<td>38.0 (SD ±11.5)</td>
<td>68.7 (SD ±17.2)</td>
<td>51.0 (SD ±19.5)</td>
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<tr>
<td>Range</td>
<td>22-95</td>
<td>18-63</td>
<td>20-99</td>
<td>18-99</td>
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<tr>
<td>Unknown</td>
<td>3 (3.3%)</td>
<td>29 (14.2%)</td>
<td>0</td>
<td>32 (9.2%)</td>
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<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>6 (6.7%)</td>
<td>53 (30.2%)</td>
<td>3 (5.6%)</td>
<td>62 (17.9%)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>83 (93.3%)</td>
<td>125 (60.9%)</td>
<td>50 (94.4%)</td>
<td>258 (74.4%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>27 (13.2%)</td>
<td>0</td>
<td>27 (7.8%)</td>
</tr>
<tr>
<td><strong>Onset of symptoms (days)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>7 (IQR 4-10)</td>
<td>1 (IQR 1-2)</td>
<td>4 (IQR 2-12)</td>
<td>4 (IQR 1-7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>19 (22.9%)</td>
<td>82 (66.4%)</td>
<td>40 (80.0%)</td>
<td>141 (55.0%)</td>
</tr>
<tr>
<td>0–5</td>
<td>22 (26.1%)</td>
<td>42 (33.6%)</td>
<td>7 (14.0%)</td>
<td>71 (27.2%)</td>
</tr>
<tr>
<td>6–10</td>
<td>27 (32.5%)</td>
<td>0</td>
<td>0</td>
<td>27 (10.5%)</td>
</tr>
<tr>
<td>11–24</td>
<td>14 (16.9%)</td>
<td>0</td>
<td>3 (6.0%)</td>
<td>17 (10.5%)</td>
</tr>
<tr>
<td>PCR positive</td>
<td>54 (60.7%)</td>
<td>69 (33.6%)</td>
<td>13 (24.5%)</td>
<td>136 (39.2%)</td>
</tr>
</tbody>
</table>

Abbreviations: standard deviation (SD), interquartile range (IQR).
Table 2. Mologic COVID-19 antigen test performance by site and setting.

<table>
<thead>
<tr>
<th>Site/setting</th>
<th>Total</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>NPV (95% CI)</th>
<th>PPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yorkshire (all settings)</td>
<td>53</td>
<td>11</td>
<td>40</td>
<td>0</td>
<td>2</td>
<td>84.6% (54.6-98.1)</td>
<td>100% (91.2-100)</td>
<td>95.2% (84.8-96.2)</td>
<td>100% (71.5-100)</td>
</tr>
<tr>
<td>Yorkshire/Hospital admission</td>
<td>32</td>
<td>7</td>
<td>24</td>
<td>0</td>
<td>1</td>
<td>87.5% (47.4-99.7)</td>
<td>100% (85.8-100)</td>
<td>96.0% (79.7-99.9)</td>
<td>100% (59.0-100)</td>
</tr>
<tr>
<td>Yorkshire/Ambulance service</td>
<td>21</td>
<td>4</td>
<td>16</td>
<td>0</td>
<td>1</td>
<td>80.0% (28.4-99.5)</td>
<td>100% (79.4-100)</td>
<td>94.1% (71.3-99.9)</td>
<td>100% (39.8-100)</td>
</tr>
<tr>
<td>Overall</td>
<td>383</td>
<td>130</td>
<td>225</td>
<td>5</td>
<td>23</td>
<td>85.0% (78.3-90.2)</td>
<td>97.8% (95.0-99.3)</td>
<td>90.7% (87.0-93.5)</td>
<td>96.3% (91.6-98.4)</td>
</tr>
</tbody>
</table>

Abbreviations: true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), positive predictive value (PPV), negative predictive value (NPV), confidence interval (CI).

a Three of four FP with Mologic Ag-RDT tested positive by NHS RT-qPCR and thus were classified as true positive.
b Includes repeat N and NT swab data of 42 participants.
c Includes repeat N and NT false positive results from one participant.
d Includes repeat N and NT false negative results from four participants.

respectively. Sensitivity and specificity per site were 83.3% (CI 70.7-92.1) and 96.6 (CI 82.2-99.9) in Merseyside; 86.0% (CI 76.9-92.6) and 97.5% (CI 93.8-99.3) in Northumberland; and 84.6% (CI 54.6-98.1) and 100% (CI 91.2-100) in Yorkshire. Sensitivity and specificity per setting were 83.9% (CI 72.3-91.9) and 98.1% (CI 89.9-99.9) at hospital admission, 86.0% (CI 76.9-92.6) and 97.5% (CI 93.8-99.3) in drive-through centres; and 80.0% (CI 28.4-99.5) and 100% (CI 79.4-100) in ambulance service.

Sensitivity was stratified by RT-qPCR Ct (Table 3) and ranged from 98.4% (CI 91.3-100) in samples with Ct <20, to 85.0% (CI 78.3-90.2) with Ct <45. The sensitivity of the Ag-RDT was statistically higher in samples with a Ct <20 when compared to samples with Ct <33 (P = 0.009) and Ct <45 (P <0.002); and in samples with a Ct <25 when compared to samples with Ct <45 (P <0.023). Sensitivity on samples with presumptive B1.1.7 VOC strain was 76.9%, (CI 56.4-91.0), which was not statistically different to other strains (P = 0.351).

The clinical accuracy attained by sampling strategy (self-testing or professional testing) and swab type (N, NT or T swab) are shown in Table 4. There were no statistically significant differences in the diagnostic accuracy for swabs taken by professionals and self-swabbing (P = 0.422). The sensitivity of the Ag-RDT was comparable at detecting positive cases by N (82.4% (65.5 - 93.2), NT (87.7%, CI 77.2-94.5) and T (83.3%, CI 70.1-92.1) swabs (P = 0.432 comparing N to T swabs and P = 0.337 comparing N to NT swabs).

The agreement between ‘nose only’ and ‘nose/throat’ swabs was 95.2%, with k=0.89 (almost perfect agreement). The agreement between ‘nose only’, ‘nose/throat’ and RT-qPCR was 83.3%, with k=0.75 (strong agreement). The agreement increased with increasing viral loads and decreasing Ct values. One sample with a SARS-CoV-2 RT-qPCR Ct of 19.2 was positive by the NT swab but negative by the N swab RT-qPCR and one sample with a negative RT-qPCR result was positive by the NT swab but negative by the N swab.

The Ag-RDT had insignificant lower sensitivity in detecting SARS-CoV-2 in the 62 asymptomatic individuals (79.2%, CI 57.9-92.9) compared to the 258 symptomatic participants (84.6%, CI 76.8-90.6, P = 0.361) (Table 5). Sensitivity at a Ct <33 among asymptomatic and symptomatic participants was 94.4% (CI 72.7-99.9, 17/18) and 85.1% (95% CI 76.7% to 91.4%, 86/101), respectively (P = 0.259). Results were also stratified by symptoms onset. There was no difference on test performance among participants with symptoms onset ≤5 days and >5 days (P = 0.564).

Analytical evaluation

The final LOD using SARS-CoV-2 live virus was 5.0 × 10⁵ pfu/ml using pooled swab matrix. These equals to approximately 1.1 × 10⁶ gcn/ml (Table S1, Extended data†). The dilution 1.0 × 10⁵ pfu/ml was detected in 17/20 (85%) replicates and the dilution 2.5 × 10⁴ was detected in 18/20 (90%).

Mologic’s Ag-RDT did not cross-react with the non-SARS-CoV-2 pathogens tested and SARS-CoV-2 was detected in all replicates when tested in the presence of other pathogens (Table S2, Extended data†).

Discussion

Results of this multi-centre diagnostic accuracy study and analytical assessment show that the Mologic COVID-19
Table 3. Analysis by subgroup of cycle threshold (Ct).

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mologic +</th>
<th>Mologic −</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ct value &lt;20</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merseyside (all strains)</td>
<td>9</td>
<td>9</td>
<td>0</td>
<td>100% (66.4-100)</td>
</tr>
<tr>
<td>Merseyside (B1.1.7)</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100% (47.8-100)</td>
</tr>
<tr>
<td>Northumberland</td>
<td>52</td>
<td>51</td>
<td>1</td>
<td>98.1% (89.7-100)</td>
</tr>
<tr>
<td>Yorkshire a</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>100% (2.50-100)</td>
</tr>
<tr>
<td>All</td>
<td>62</td>
<td>61</td>
<td>1</td>
<td>98.4% (91.3-100)</td>
</tr>
</tbody>
</table>

| **Ct value <25** |       |           |           |                   |
| Merseyside (all strains) | 23 | 21 | 2 | 91.3% (71.9-98.9) |
| Merseyside (B1.1.7) | 11 | 9 | 2 | 81.8% (48.2-97.7) |
| Northumberland | 75 | 70 | 5 | 93.3% (85.1-97.8) |
| Yorkshire a | 5 | 5 | 0 | 100% (47.8-100) |
| All | 103 | 96 | 7 | 93.2% (86.5-97.2) |

| **Ct value <33** |       |           |           |                   |
| Merseyside (all strains) | 43 | 37 | 6 | 86.0% (72.1-94.7) |
| Merseyside (B1.1.7) | 23 | 18 | 5 | 78.3% (56.3-92.5) |
| Northumberland | 82 | 73 | 9 | 89.0% (80.2-94.9) |
| Yorkshire a | 6 | 5 | 1 | 83.3% (35.9-99.6) |
| All | 131 | 115 | 16 | 87.8% (80.9-92.9) |

| **Ct value <45** |       |           |           |                   |
| Merseyside (all strains) | 54 b | 45 b | 9 | 83.3% (70.7-92.1) |
| Merseyside (B1.1.7) | 26 | 20 | 6 | 76.9% (56.4-91.0) |
| Northumberland | 86 c | 74 c | 12 | 86.0% (76.9-92.6) |
| Yorkshire a | 7 | 6 | 1 | 85.7% (42.1-99.6) |
| All | 147 | 125 | 22 | 85.0% (78.2-90.4) |

a Cepheid results only, as Hologic Panther does not report a Ct value.
b Ct value was not available for the three samples that were RT-qPCR NHS positive and RT-qPCR LSTM negative.
c Includes paired N and NT swab data of 42 participants.
d Indication of B1.1.7 strain only available from Merseyside site, and given study was active in 2021 may represent an underestimation.

Ag-RDT offers a high degree of analytical performance (LLOD = 5.0 \times 10^5 \text{ pfu/ml}, approximately 1.2 \times 10^6 \text{ gc/ml in swab matrix}) with a high sensitivity (85.0%) and specificity (97.8%) among participants, fulfilling the LOD, sensitivity and specificity criteria described in the World Health Organization (WHO) Target product profile for the use of Ag-RDT in suspected COVID-19 cases and their close contacts\textsuperscript{26}.

This is the first reported evaluation that we know of an Ag-RDTs within an ambulance service. Here, the results show that SARS-CoV-2 rapid antigen testing in ambulance services can be used to identify COVID-19 patients in prehospital settings with high sensitivity (80.0%, CI 28.4-99.5) and specificity (100%, CI 79.4-100) which can help to rule in the need for isolation precautions at hospital arrival. This can be very impactful to reduce transmission among hospital staff and patients in high prevalence scenarios and extend early detection to pre-hospital care.

Accuracy of the Ag-RDT was also investigated at hospital admission and sensitivity and specificity in this scenario were 83.3% (CI 70.7-92.1) and 96.6% (CI 82.2-99.9) in Merseyside...
and 87.5% (CI 47.4-99.7) and 100% (CI 85.8 -100.0) in Yorkshire. The implementation of Ag-RDTs in hospital admission and/or preadmission would facilitate the management of suspected patients, providing faster results for a more efficient triage. Time to result for SARS-CoV-2 RT-qPCR ranges from 24-48 hours, time where hospitals need to decide the safest manner to allocate the suspected patients, with a great chance of accommodating COVID-19 negative patients with positive patients. Knowledge of the diagnosis upon patient admission is critical to provide appropriate treatment, reduce hospital internal costs and in-hospital transmission. Hospital costs for COVID-19 patients have been estimated to be

Table 4. Analysis by subgroup of testing strategy and swab type.

<table>
<thead>
<tr>
<th>Swab type</th>
<th>Total</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal/Throat</td>
<td>151</td>
<td>57</td>
<td>84</td>
<td>2</td>
<td>8</td>
<td>87.7% (77.2-94.5)</td>
<td>97.7% (91.9-99.7)</td>
<td>96.6% (87.8-99.1)</td>
<td>91.3% (84.6-95.3)</td>
</tr>
<tr>
<td>Throat</td>
<td>83</td>
<td>45</td>
<td>28</td>
<td>1</td>
<td>9</td>
<td>83.3% (70.7-92.1)</td>
<td>96.6% (82.2-99.9)</td>
<td>97.8% (86.7-99.7)</td>
<td>75.7% (63.0-85.0)</td>
</tr>
<tr>
<td>Nasal</td>
<td>149</td>
<td>28</td>
<td>113</td>
<td>2</td>
<td>6</td>
<td>82.4% (65.5-93.2)</td>
<td>98.3% (93.9-99.8)</td>
<td>93.3% (77.8-98.3)</td>
<td>95.0% (90.1-97.5)</td>
</tr>
</tbody>
</table>

Sampling strategy

| Self-testing   | 247   | 74  | 157 | 4   | 12  | 86.0% (76.9-92.6)  | 97.5% (93.8-99.3)  | 94.9% (87.5-98.0)  | 92.9% (88.6-95.7)  |
| Professional testing | 136  | 56  | 68  | 1   | 11  | 83.6% (72.5-91.5)  | 98.6% (92.2-100)  | 98.3% (88.9-99.8)  | 86.1% (78.3-91.4)  |

Abbreviations: true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), positive predictive value (PPV), negative predictive value (NPV).

a Nasal swab refers to anterior nares.
b Includes paired N and NT swab data of 42 participants.

Table 5. Analysis by onset of symptoms and clinical presentation.

<table>
<thead>
<tr>
<th>Presentation</th>
<th>Total</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>71</td>
<td>19</td>
<td>47</td>
<td>0</td>
<td>5</td>
<td>79.2% (57.9-92.9)</td>
<td>100% (92.5-100)</td>
<td>100%</td>
<td>90.4% (81.2-95.4)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>285</td>
<td>99</td>
<td>163</td>
<td>5</td>
<td>18</td>
<td>84.6% (76.8 -90.6)</td>
<td>97.0% (93.2-99.0)</td>
<td>95.2% (89.3-97.9)</td>
<td>90.1% (85.5-93.3)</td>
</tr>
</tbody>
</table>

Days post symptom onset

<table>
<thead>
<tr>
<th>Days post symptom onset</th>
<th>Total</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5 days</td>
<td>86</td>
<td>46</td>
<td>30</td>
<td>2</td>
<td>8</td>
<td>85.2% (72.9-93.4)</td>
<td>93.8% (79.2-99.2)</td>
<td>95.8% (85.7-98.9)</td>
<td>78.9% (66.3-87.7)</td>
</tr>
<tr>
<td>&gt;5 days</td>
<td>41</td>
<td>26</td>
<td>11</td>
<td>0</td>
<td>4</td>
<td>86.7% (69.3-96.2)</td>
<td>100% (71.5-100)</td>
<td>100%</td>
<td>73.3% (52.5-87.3)</td>
</tr>
<tr>
<td>6–10 days</td>
<td>25</td>
<td>18</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>90.0% (68.3-98.8)</td>
<td>100% (47.8-100)</td>
<td>100%</td>
<td>71.4% (40.2-90.3)</td>
</tr>
<tr>
<td>11–24 days</td>
<td>16</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>80.0% (44.4-97.5)</td>
<td>100% (54.1-100)</td>
<td>100%</td>
<td>75.0% (46.5-91.2)</td>
</tr>
</tbody>
</table>

Abbreviations: true positives (TP), true negatives (TN), false positives (FP), false negatives (FN), positive predictive value (PPV), negative predictive value (NPV).
a Includes paired N and NT swab of nine participants.
b Includes paired N and NT swab of 33 participants.
c Sensitivity for asymptomatic participants with Ct<33 was 94.4% (95% CI 72.7 - 99.9) (n=18).
d Sensitivity for symptomatic participants with Ct<33 was 85.1% (95% CI 76.7-91.4) (n=101).
e Includes paired N and NT data of 16 participants.
4-5.5 times higher than those for other common respiratory infectious diseases as influenza because of an increased probability of hospitalization and mortality.\textsuperscript{27-30}

The performance of the Ag-RDT in drive-through centres was evaluated in Northumberland, the sensitivity and specificity in this setting were 84.6\% (54.6-98.1) and 100\% (91.2 -100.0), being comparable to those reported in other studies in drive-through centres. In a study in the Netherlands, sensitivity and specificity were 84.9\% (CI 79.1-89.4) and 99.5\% (CI 98.7-99.8) among 970 symptomatic adults using the Ag-RDT Standard Q (SD Biosensor, Korea).\textsuperscript{31} A high throughput drive-through community testing site in Massachusetts found similar results of 84.1\% (CI 77.4 -89.4) sensitivity and 99.6\% (99.1 -99.9) of specificity among 1385 adults using the Ag-RDT kit Abbott Binax NOW (Abbott, US).\textsuperscript{32} Results of Ag-RDT in drive-through centres suggest that the use of these would provide a good method to identify the majority of infected people (>84\%), enabling faster detection of infected cases and implementation strategies than with RT-qPCR. However, caution against sole use of Ag-RDTs should be taken because of the potential of missing positive cases; a negative test cannot always rule out the infection but could be secured through a triage system that guides patients to the proper testing algorithm such as follow-up testing by RT-qPCR for negative cases and people at or in contact with high-risk individuals.

The LLOD obtained here using SARS-CoV-2 live virus of approximately 1.1-2.4 x 10^6 gc/ml fulfilled the prioritization criteria of WHO\textsuperscript{33} and suggests that the Ag-RDT will be useful at identifying individuals with high viral loads, who are the more likely to be highly contagious as with other similar antigen tests. Viral loads have been estimated to range from 10^4 to 10^{11} gc/ml in the most contagious patients\textsuperscript{34,35} the LLOD obtained in the analytical evaluation correlates well with the sensitivity obtained in the clinical evaluation; the Ag-RDT had a sensitivity of 93.2\% (83.7-95.8) for detecting infected individuals with high viral loads and sufficient viral loads to allow virus culture (CI <25)\textsuperscript{36} which has been suggested as a proxy for transmissibility. Whilst a model to delineate a threshold for infectiousness has not been defined, the findings here suggest that screening based on the Ag-RDT in this population would have a high sensitivity for ruling in individuals with high viral loads and a probability of being more infectious. The sensitivity of the Ag-RDT was also investigated for its ability to detect the SARS-CoV-2 strain B1.1.7 VOC. The VOC strain B1.1.7 currently comprises approximately 95\% of new SARS-CoV-2 infections in England and has now been identified in at least 82 countries\textsuperscript{37}. The B1.1.7 VOC may cause false positive results in RT-qPCR tests that target the S gene\textsuperscript{38}. Only the TaqPath RT-qPCR assay used in this study targeted the S gene and since this test is designed to detect multiple genetic targets; the overall test sensitivity should have not been impacted. Rapid evaluation of the accuracy of novel diagnostic tests for identifying this new emerging strain is a priority to secure effective detection and contact tracing. Only one published assessment on Ag-RDTs to evaluate the detection of B1.1.7 VOC has been performed so far and found that the five Ag-RDTs evaluated were all able to detect the variant\textsuperscript{39}.

The present work reports clinical diagnostic accuracy of an Ag-RDT among infected individuals with the B1.1.7 VOC strain and we observed that the Ag-RDT had comparable sensitivity to detect the B1.1.7 VOC to the other variants.

Results in this study have shown that the Ag-RDT benefits from equivalent performance between self-swabbing and swabs taken by professionals, which aligns well with other studies comparing self-swabbing and professionally taken swabs on other marketed Ag-RDTs.\textsuperscript{40} Previous studies comparing the performance of Ag-RDTs in N and NP swabs have reported similar performance\textsuperscript{41}. It is noteworthy to mention that most commercialised Ag-RDT are manufactured to be used with NP swabs. The use of NT swabs has advantages as they are simpler and safer to collect than the more invasive NP swabs. The collection of NP swabs presents challenges as they require the expertise of a health-care professional and the process can be uncomfortable, especially in children, decreasing acceptability for repeat testing.\textsuperscript{42} An Ag-RDT that is used on N and NT swab specimens has a great potential to be deployed for self-testing. The use of a self-test for the detection of SARS-CoV-2 can improve access to COVID-19 diagnosis, enabling more widespread and frequent testing.

Studies have noted lower sensitivity in asymptomatic (48.1\%-56.5\%) individuals compared to symptomatic ones (71.4\%-80.4\%)\textsuperscript{43-45}. Since Ag-RDT sensitivity is largely related to viral load, these differences might reflect dissimilarities in the kinetics of SARS-CoV-2 viral load in the upper respiratory tract. While it is well known that SARS-CoV-2 load peaks around the time of symptoms onset\textsuperscript{46,47}, the timing is uncertain in asymptomatic cases.

Our study has several limitations as different RT-qPCR methods, with alternative genome targets, were used across sites; this might cause misleading comparisons of Ct-values between sites. Therefore, to minimize the variance across sites, we performed the Ct-values subanalysis using categories as Ct <20, Ct <25, Ct <33 and Ct <45. Overall performance of the Mologic Ag-RDT without the stratification by Ct value meets or exceeds the target product profiles for the US FDA, WHO, and the UK Medicines and Healthcare products Regulatory Agency. For specificity, the study gold standard does not account for possible false negative RT-qPCR due to suboptimal sampling. Cases where COVID-19 could not be clinically excluded were not removed from analysis and considering high background prevalence in study sites may underestimate the specificity of the Mologic Ag-RDT. The number of samples tested with the samples that were infected with presumptive B1.1.7 VOC as per S gene drop was small as only Merseyside used the Spike gene as RT-qPCR target but considering the study timeline concentrating from December 2020 to early 2021 in the UK, the likelihood is that the majority of samples included in this analysis will be B1.1.7.

To conclude, the Mologic Ag-RDT fulfills the WHO target diagnostic accuracy\textsuperscript{48} with a high sensitivity (93.2\%-98.4\%) for detecting individuals with high viral load infections (Ct <20 and Ct <25). The Mologic Ag-RDT has replicated diagnostic
accuracy in a variety of settings important for COVID-19 control (hospitals, ambulatory services, and drive-through centres) and on self-collected swab specimens. Although further research is underway on additional use cases, the Mologic Ag-RDT has shown promise as a candidate for wider testing strategies.

Data availability

Underlying data

This project contains the following underlying data:
- Underlying_data.xlsx (raw study data file)
- Readme_file.xlsx (data dictionary for raw study data)

Extended data

This project contains the following extended data in the file ‘Extended data.docx’:
- Table S1 (details of the limit of detection)
- Table S2 (details of cross-reactivity organisms and interference)

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Acknowledgements

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References