










RESEARCH ARTICLE

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

[version 1; peer review: 1 approved with reservations]

Ana I Cubas-Atienzar ¹, Fiona Bell ², Rachel L. Byrne ¹, Kate Buist¹, David J. Clark³, Michael Coccozza⁴, Andrea M. Collins^{1,5,6}, Luis E. Cuevas¹, Annelyse Duvoix ⁴, Nicholas Easom ⁷, Thomas Edwards ¹, Daniella M. Ferreira ¹, Tom Fletcher¹, Elisabetta Groppelli³, Angela Hyder-Wright^{1,5,6}, Ewelina Kadamus⁴, Daniela E. Kirwan³, Konstantina Kontogianni ¹, Sanjeev Krishna^{3,8-10}, Diana Kluczna⁷, Julian Mark², Josephine Mensah-Kane⁴, Elisha Miller ², Elena Mitsi¹, Donna Norton⁷, Emma O'Connor⁴, Sophie I. Owen ¹, Tim Planche^{3,8}, Samuel Shelley⁴, Henry M. Staines³, David Tate¹¹, Caitlin R. Thompson¹, Gemma Walker⁷, Christopher T. Williams¹, Dominic Wooding¹, Joseph R. A. Fitchett^{4,12}, Emily R. Adams¹

¹Liverpool School of Tropical Medicine, Liverpool, UK

²Yorkshire Ambulance Service NHS Foundation Trust, Yorkshire, UK

³Institute for Infection & Immunity, St George's University of London, London, UK

⁴Mologic COVID-19 Diagnostics Development Unit, Bedford, UK

⁵Liverpool University Hospitals National Health Services Foundation Trust, Liverpool, UK

⁶National Institute of Health Research Comprehensive Research Network North West Coast, Liverpool, UK

⁷Hull Royal Infirmary, Hull University Teaching Hospitals, Hull, UK

⁸St George's University Hospitals NHS Foundation Trust, London, UK

⁹Institut für Tropenmedizin, Universitätsklinikum Tübingen, Tübingen, Germany

¹⁰Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon

¹¹Northumbria Healthcare NHS Foundation Trust, North Tyneside General Hospital, Northumbria, UK

¹²Harvard T.H. Chan School of Public Health, Boston, MA, USA

V1 First published: 28 May 2021, 6:132
<https://doi.org/10.12688/wellcomeopenres.16842.1>

Latest published: 28 May 2021, 6:132
<https://doi.org/10.12688/wellcomeopenres.16842.1>

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

Open Peer Review

Reviewer Status ?

Invited Reviewers

1

version 1

28 May 2021

?

report

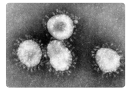
reaction (RT-qPCR) results from individuals with and without COVID-19 symptoms. Participants had attended hospitals in Merseyside, hospital and ambulance services in Yorkshire, and drive-through testing facilities in Northumberland, UK.

Results: The limit of detection of the Mologic COVID-19 Ag-RDT was 5.0×10^2 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



This article is included in the [Coronavirus \(COVID-19\)](#) collection.

1. **Janneke H. H. M. van de Wijert** 

University of Liverpool, Liverpool, UK

Utrecht University, Utrecht, The Netherlands

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Emily R. Adams (emily.adams@lstmed.ac.uk)

Author roles: Cubas-Atienzar AI: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Writing – Original Draft Preparation; Bell F: Methodology; Byrne RL: Methodology, Writing – Review & Editing; Buist K: Methodology; Clark DJ: Methodology, Writing – Review & Editing; Cocozza M: Resources; Collins AM: Investigation, Writing – Review & Editing; Cuevas LE: Formal Analysis, Investigation, Writing – Review & Editing; Duvoix A: Methodology, Resources, Writing – Review & Editing; Easom N: Investigation, Methodology, Writing – Review & Editing; Edwards T: Methodology, Writing – Review & Editing; Ferreira DM: Funding Acquisition, Investigation, Supervision, Writing – Review & Editing; Fletcher T: Writing – Review & Editing; Gropelli E: Methodology; Hyder-Wright A: Investigation, Supervision; Kadamus E: Resources; Kirwan DE: Writing – Review & Editing; Kontogianni K: Methodology, Writing – Review & Editing; Krishna S: Writing – Review & Editing; Kluczna D: Methodology; Mark J: Methodology; Mensah-Kane J: Resources, Writing – Review & Editing; Miller E: Methodology; Mitsi E: Writing – Review & Editing; Norton D: Methodology; O'Connor E: Writing – Review & Editing; Owen SI: Methodology, Writing – Review & Editing; Planche T: Writing – Review & Editing; Shelley S: Resources; Staines HM: Writing – Review & Editing; Tate D: Investigation, Writing – Review & Editing; Thompson CR: Methodology, Writing – Review & Editing; Walker G: Methodology; Williams CT: Methodology, Writing – Review & Editing; Wooding D: Methodology; Fitchett JRA: Conceptualization, Investigation, Resources, Writing – Review & Editing; Adams ER: Conceptualization, Funding Acquisition, Investigation, Supervision, Writing – Review & Editing

Competing interests: JF, MC, AD, EK, JMK, EOC, SS, and KW are employees of Mologic.

Grant information: The work was supported by the Wellcome Trust through an Epidemic Preparedness coronavirus grant [220764] and institutional support for St. George's University of London [204809]. This work was also supported by the St George's Hospital Charity. AC, AHW, DMF and EM were funded by Pfizer [WI2558862-2] for clinical enrollment and collection of samples in Merseyside. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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How to cite this article: Cubas-Atienzar AI, Bell F, Byrne RL *et al.* **Accuracy of the Mologic COVID-19 rapid antigen test: a prospective multi-centre analytical and clinical evaluation [version 1; peer review: 1 approved with reservations]** Wellcome Open Research 2021, 6:132 <https://doi.org/10.12688/wellcomeopenres.16842.1>

First published: 28 May 2021, 6:132 <https://doi.org/10.12688/wellcomeopenres.16842.1>

Introduction

Responding to the coronavirus disease-19 (COVID-19) pandemic has necessitated urgent development of rapid diagnostics¹. 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 diagnosis⁴, it has many drawbacks, including long turnaround times often more than 24 hours⁵, the need for dedicated staff and expensive platforms and reagents⁶. 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^{7,8}. Ag-RDTs are less sensitive than RT-qPCR in detecting infections with low viral loads but detect most individuals with high-viral loads ($>10^6$ genomic virus copies/ml), who are considered the most likely to transmit the infection⁹⁻¹⁴. 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 results¹⁵.

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^{7,16-19}.

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 B.1.1.7 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 SARS-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, (XPCOV2/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 fluids 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 (Mpbs 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 Xpert 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. 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/REMRQ0001/2020²⁰". 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 1.0×10^6 plaque forming units (pfu)/ml to 1.0×10^1 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 (gcn) of the serial dilutions were quantified as follows: viral RNA was extracted using QIAmp 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 RNA²¹. 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 gcn/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) guidance²².

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 1.0×10^4 pfu/ml (for interference).

Viral cultures were tested at 1.0×10^5 pfu/ml and bacterial cultures at 1.0×10^6 colonies forming units (cfu)/ml. Quantified cell culture supernatants were obtained from Zeptomatrix corporation (Zeptomatrix 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 # 0810037CF), parainfluenza 1 (PIV1) (cat # 0810014CF), parainfluenza 2 (PIV2) (cat # 0810015CF), parainfluenza 3 (PIV3) (cat # 0810016CF), parainfluenza 4 (PIV4) (cat # 0810060CF), human coronavirus

229E (hCoV) (cat # 0810229CF), hCoV-NL63 (cat # 0810228CF) and hCoV-OC-43 (cat # 0810024CF), human metapneumovirus A (hMPV A) (cat # 0810161CF), human metapneumovirus B (hMPV B) (cat # 0810162CF), *Haemophilus influenza* (cat # 0801679), *Streptococcus pneumoniae* (cat # 0801439), *Streptococcus pyogenes* (cat # 0801512), *Candida albicans* (cat # 0801504), *Bordetella pertussis* (cat # 0801459), *Legionella pneumophila* (cat # 0801645) and *Pneumocystis jirovecii* (cat # 0801698).

Statistical analysis

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)²³ 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 statistics²⁴. 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)²⁵.

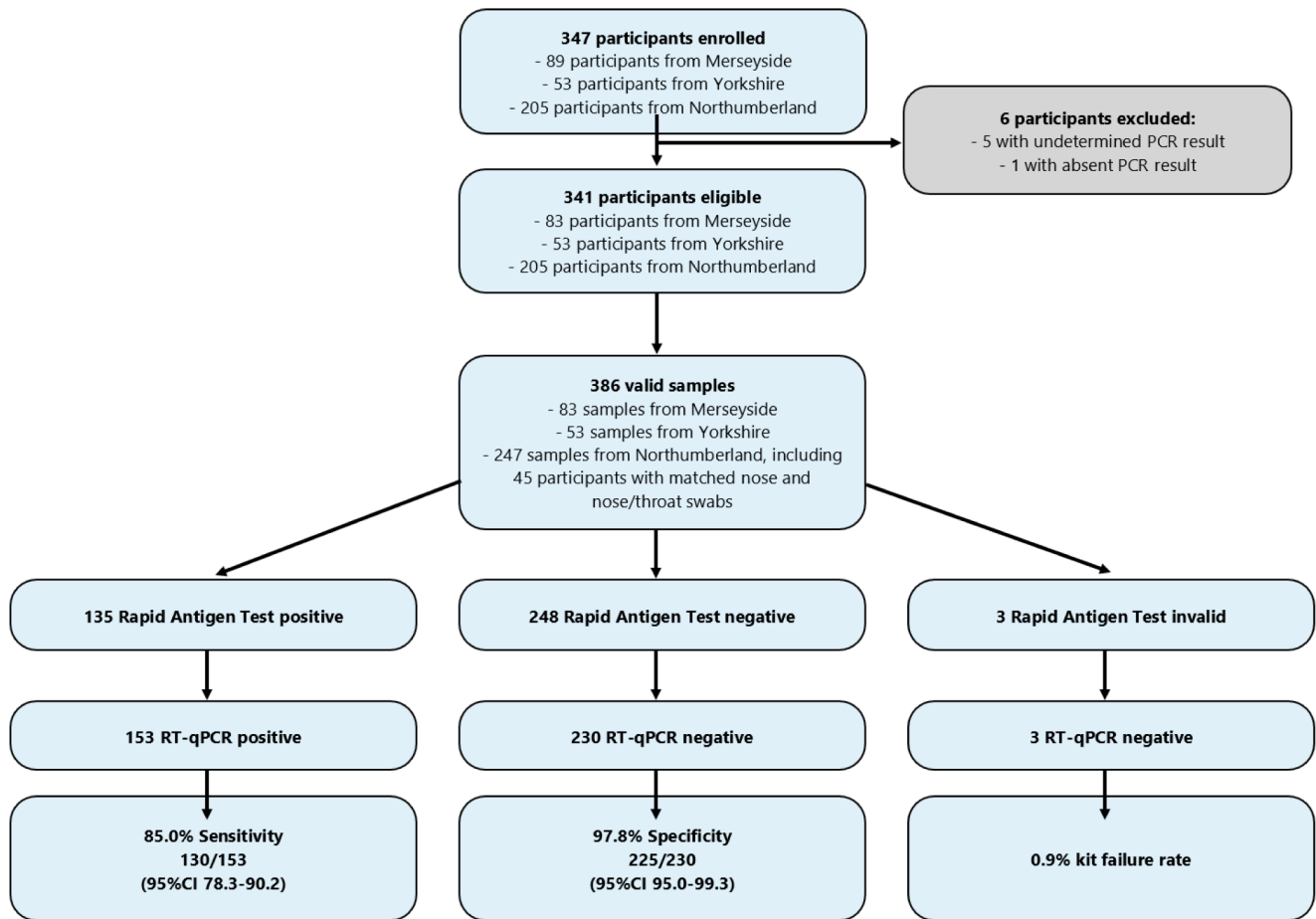


Figure 1. Flow diagram of participant enrolment, eligibility, and results.

Table 1. Characteristics of the population of study.

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

Abbreviations: standard deviation (SD), interquartile range (IQR).

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 five (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=17.1) 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),

Table 2. Mologic COVID-19 antigen test performance by site and setting.

Site/setting	Total	TP	TN	FP	FN	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
Merseyside ^a Hospital admission	83	45	28	1	9	83.3% (70.7-92.1)	96.6% (82.2-99.9)	75.7% (63.1-85.0)	97.8% (86.7-99.7)
Northumberland ^b Drive-through centers	247	74	157	4 ^c	12 ^d	86.0% (76.9-92.6)	97.5% (93.8-99.3)	92.9% (88.6-95.7)	94.9% (87.5-98.0)
Yorkshire (all settings)	53	11	40	0	2	84.6% (54.6-98.1)	100% (91.2-100)	95.2% (84.8-98.6)	100% (71.5-100)
Yorkshire/ Hospital admission	32	7	24	0	1	87.5% (47.4-99.7)	100% (85.8-100)	96.0% (79.7-99.9)	100% (59.0-100)
Yorkshire/ Ambulance service	21	4	16	0	1	80.0% (28.4-99.5)	100% (79.4-100)	94.1% (71.3-99.9)	100% (39.8-100)
Overall	383	130	225	5	23	85.0% (78.3-90.2)	97.8% (95.0-99.3)	90.7% (87.0-93.5)	96.3% (91.6-98.4)

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 paired 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 $\kappa=0.89$ (almost perfect agreement). The agreement between 'nose only', 'nose/throat' and RT-qPCR was 83.3%,

with $\kappa=0.75$ (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 LLOD using SARS-CoV-2 live virus was 5.0×10^2 pfu/ml using pooled swab matrix. These equals to approximately 1.1×10^6 gc/ml (Table S1, *Extended data*²⁵). The dilution 1.0×10^2 pfu/ml was detected in 17/20 (85%) replicates and the dilution 2.5×10^2 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*²⁵).

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).

	Total	Mologic +	Mologic –	Sensitivity
Ct value <20				
Merseyside (all strains)	9	9	0	100% (66.4-100)
Merseyside (B1.1.7)	5	5	0	100% (47.8-100)
Northumberland	52	51	1	98.1% (89.7-100)
Yorkshire ^a	1	1	0	100% (2.50-100)
All	62	61	1	98.4% (91.3-100)
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 (LOD = 5.0×10^2 pfu/ml, approximately 1.2×10^6 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 contacts²⁶.

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

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

	Total	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Swab type									
Nasal/Throat	151	57	84	2	8	87.7% (77.2-94.5)	97.7% (91.9-99.7)	96.6% (87.8-99.1)	91.3% (84.6-95.3)
Throat	83	45	28	1	9	83.3% (70.7-92.1)	96.6% (82.2-99.9)	97.8% (86.7-99.7)	75.7% (63.0-85.0)
Nasal ^a	149	28	113	2	6	82.4% (65.5-93.2)	98.3% (93.9-99.8)	93.3% (77.8-98.3)	95.0% (90.1-97.5)
Sampling strategy									
Self-testing	247 ^b	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.

	Total	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Presentation									
Asymptomatic ^a	71	19	47	0	5	79.2% ^c (57.9-92.9)	100% (92.5-100)	100%	90.4% (81.2-95.4)
Symptomatic ^b	285	99	163	5	18	84.6% ^d (76.8-90.6)	97.0% (93.2-99.0)	95.2% (89.3-97.9)	90.1% (85.5-93.3)
Days post symptom onset									
0–5 days ^e	86	46	30	2	8	85.2% (72.9-93.4)	93.8% (79.2-99.2)	95.8% (85.7-98.9)	78.9% (66.3-87.7)
>5 days	41	26	11	0	4	86.7% (69.3-96.2)	100% (71.5-100)	100%	73.3% (52.5-87.3)
6–10 days	25	18	5	0	2	90.0% (68.3-98.8)	100% (47.8-100)	100%	71.4% (40.2-90.3)
11–24 days	16	8	6	0	2	80.0% (44.4-97.5)	100% (54.1-100)	100%	75.0% (46.5-91.2)

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.

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

4-5.5 times higher than those for other common respiratory infectious diseases as influenza because of an increased probability of hospitalization and mortality²⁷⁻³⁰.

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)¹⁰. 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 (Abbot, US)³¹. 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, cautions 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 $1.1-2.4 \times 10^6$ gcn/ml fulfilled the prioritization criteria of WHO²⁶ 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 10^8 to 10^{11} gcn/ml in the most contagious patients^{32,33} 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 (Ct <25)¹⁴, 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 B.1.1.7 VOC. The VOC strain B.1.1.7 currently comprises approximately 95% of new SARS-CoV-2 infections in England and has now been identified in at least 82 countries³⁴. The B.1.1.7 VOC may cause false positive results in RT-qPCR tests that target the S gene³⁵. 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 B.1.1.7 VOC has been performed so far and found that the five Ag-RDTs evaluated were all able to detect the variant³⁶ The present work

reports clinical diagnostic accuracy of an Ag-RDT among infected individuals with the B.1.1.7 VOC strain and we observed that the Ag-RDT had comparable sensitivity to detect the B.1.1.7 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-RDTs³⁷. Previous studies comparing the performance of Ag-RDTs in N and NP swabs have reported similar performance³⁷. 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 testing³⁸. 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%)³⁹⁻⁴². 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^{42,43}, 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 B.1.1.7 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 B.1.1.7.

To conclude, the Mologic Ag-RDT fulfills the WHO target diagnostic accuracy²⁴ 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

Dryad: Accuracy of the Mologic COVID-19 rapid antigen test-a prospective multi-centre analytical and clinical evaluation. <https://doi.org/10.5061/dryad.0vt4b8gzq²⁵>.

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

Dryad: Accuracy of the Mologic COVID-19 rapid antigen test-a prospective multi-centre analytical and clinical evaluation. <https://doi.org/10.5061/dryad.0vt4b8gzq²⁵>.

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

We thank all the participants that agreed to take part in the study and the professionals that helped with the sampling. We thank the Nationwide Association of Blood Bikers for their support in the logistics throughout the development of the Mologic rapid antigen test. We thank the Yorkshire Ambulance Service for their contribution to the study. We would like to thank LSTM Global Health Trials Unit, specially Jim Read and Kelly Byrne for their support on data management.

References

1. FIND: SARS-CoV-2 diagnostic pipeline. Cited 23rd March 2021. [Reference Source](#)
2. Kretzschmar ME, Rozhnova G, Bootsma MCJ, et al.: **Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study.** *Lancet Public Health.* 2020; 5(8): e452–e459. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Kucharski AJ, Klepac P, Conlan AJK, et al.: **Effectiveness of isolation, testing, contact tracing, and physical distancing on reducing transmission of SARS-CoV-2 in different settings: a mathematical modelling study.** *Lancet Infect Dis.* 2020; 20(10): 1151–1160. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
4. Corman V, Bleicker T, Brünink S, et al.: **Diagnostic detection of 2019-nCoV by real-time RT-qPCR.** 2020. Cited 23rd March 2021. [Reference Source](#)
5. **Weekly statistics for NHS Test and Trace (England): 7 January to 13 January 2021.** GOV.UK. 2021. Cited 23rd March 2021. [Reference Source](#)
6. Abduljalil JM: **Laboratory diagnosis of SARS-CoV-2: available approaches and limitations.** *New Microbes New Infect.* 2020; 36: 100713. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
7. World Health Organization (WHO): **Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays.** Interim Guide. 2020. Cited 23rd March 2021. [Reference Source](#)
8. Adams ER, Augustin Y, Byrne RL, et al.: **Rapid development of COVID-19 rapid diagnostics for low resource settings: accelerating delivery through transparency, responsiveness, and open collaboration.** *medRxiv.* 2020. [Publisher Full Text](#)
9. Weiss G, Bellmann-Weiler R: **Rapid antigen testing and non-infectious shedding of SARS-CoV-2.** *Infection.* 2021; 1–2. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
10. Iglói Z, Velzing J, Van Beek J, et al.: **Clinical evaluation of the Roche/SD Biosensor rapid antigen test with symptomatic, non-hospitalized patients in a municipal health service drive-through testing site.** *medRxiv.* 2020. [Publisher Full Text](#)
11. Mina MJ, Parker R, Larremore DB: **Rethinking Covid-19 Test Sensitivity — A Strategy for Containment.** *N Engl J Med.* 2020; 383(22): e120. [PubMed Abstract](#) | [Publisher Full Text](#)
12. **Options for the use of rapid antigen tests for COVID-19 in the EU/EEA and the UK.** *Eur Cent Dis Prev Control.* 2020; Cited 23rd March 2021. [Reference Source](#)
13. Pekosz A, Cooper CK, Parvu V, et al.: **Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture.** *medRxiv.* 2020. [Publisher Full Text](#)
14. Singanayagam A, Patel M, Charlett A, et al.: **Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020.** *Euro Surveill.* 2020; 25(32): 2001483. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Bonačić Marinović AA, Koopmans M, Dittrich S, et al.: **Speed versus coverage trade off in targeted interventions during an outbreak.** *Epidemics.* 2014; 28–40. [PubMed Abstract](#) | [Publisher Full Text](#)
16. Mak GC, Cheng PK, Lau SS, et al.: **Evaluation of rapid antigen test for detection of SARS-CoV-2 virus.** *J Clin Virol.* 2020; 129: 104500. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Nagura-Ikeda M, Imai K, Tabata S, et al.: **Clinical evaluation of self-collected saliva by quantitative reverse transcription-PCR (RT-qPCR), Direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification, and a rapid antigen test to diagnose COVID-19.** *J Clin Microbiol.* 2020; 58(9): e01438–20. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Scohy A, Anantharajah A, Bodéus M, et al.: **Low performance of rapid antigen detection test as frontline testing for COVID-19 diagnosis.** *J Clin Virol.* 2020; 129: 104455. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Weitzel T, Legarraga P, Iruretagoyena M, et al.: **Head-to-head comparison of four antigen-based rapid detection tests for the diagnosis of SARS-CoV-2 in respiratory samples.** *bioRxiv.* 2020. [Publisher Full Text](#)
20. Patterson EI, Prince T, Anderson ER, et al.: **Methods of inactivation of SARS-CoV-2 for downstream biological assays.** *J Infect Dis.* 2020; 222(9): 1462–1467. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Byrne RL, Kay GA, Kontogianni K, et al.: **Saliva offers a sensitive, specific and non-invasive alternative to upper respiratory swabs for SARS-CoV-2 diagnosis.** *medRxiv.* 2020. [Publisher Full Text](#)
22. Food and Drug Administration (FDA): **In Vitro/corona diagnostics EUAs.** Antigen template for test developers. 2020; Cited 23rd March 2021. [Reference Source](#)
23. Public Health England (PHE): **Investigation of novel SARS-CoV-2 variant Variant of Concern 202012/01.** Technical briefing 5. 2021; Cited 12th April;. [Reference Source](#)

24. McHugh ML: **Interrater reliability: The kappa statistic.** *Biochem Med (Zagreb)*. 2012; **22**(3): 276–82.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
25. Atienzar C, Isabel A, Bell F, *et al.*: **Accuracy of the mologic COVID-19 rapid antigen test—a prospective multi-centre analytical and clinical evaluation.** *Dryad, Dataset*, 2021.
<http://www.doi.org/10.5061/dryad.0vt4b8gzq>
26. (WHO) WHO, R&D Blueprint WH (HQ): **Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.1.0.** 2020; Cited 23th March 2021.
[Reference Source](#)
27. Filgueiras PS, Corsini CA, Almeida NBF, *et al.*: **COVID-19 Rapid Antigen Test at hospital admission associated to the knowledge of individual risk factors allow overcoming the difficulty of managing suspected patients in hospitals COVID-19 Rapid Antigen Test facilitates the management of suspected patients on hospital admission.** *medRxiv*. 2021.
[Publisher Full Text](#)
28. Bartsch SM, Ferguson MC, McKinnell JA, *et al.*: **The Potential Health Care Costs And Resource Use Associated With COVID-19 In The United States.** *Health Aff (Millwood)*. 2020; **39**(6): 927–935.
[PubMed Abstract](#) | [Publisher Full Text](#)
29. Bai G, Zare H: **Hospital Cost Structure and the Implications on Cost Management During COVID-19.** *J Gen Intern Med*. 2020; **35**(9): 2807–2809.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
30. Molinari NAM, Ortega-Sanchez IR, Messonnier ML, *et al.*: **The annual impact of seasonal influenza in the US: measuring disease burden and costs.** *Vaccine*. 2007; **25**(27): 5086–96.
[PubMed Abstract](#) | [Publisher Full Text](#)
31. Pollock NR, Jacobs JR, Tran K, *et al.*: **Performance and Implementation Evaluation of the Abbott BinaxNOW Rapid Antigen Test in a High-throughput Drive-through Community Testing Site in Massachusetts.** *J Clin Microbiol*. 2021; **59**(5): e00083–21.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Kawasuji H, Takegoshi Y, Kaneda M, *et al.*: **Transmissibility of COVID-19 depends on the viral load around onset in adult and symptomatic patients.** *PLoS One*. 2020; **15**(12): e0243597.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
33. Zou L, Ruan F, Huang M, *et al.*: **SARS-CoV-2 Viral Load in Upper Respiratory Specimens of Infected Patients.** *N Engl J Med*. 2020; **382**(12): 1177–1179.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
34. Davies NG, Abbott S, Barnard RC, *et al.*: **Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England.** *Science*. 2021; **372**(6538): eabg3055.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. U.S. Food and Drug Administration (FDA): **Genetic Variants of SARS-CoV-2 May Lead to False Negative Results with Molecular Tests for Detection of SARS-CoV-2 - Letter to Clinical Laboratory Staff and Health Care Provider.** Cited 12th April 2021.
[Reference Source](#)
36. GOV.UK: **SARS-CoV-2 lateral flow antigen tests: evaluation of VUI-202012/01.** 2020; Cited 12th April 2021.
[Reference Source](#)
37. Lindner AK, Nikolai O, Kausch F, *et al.*: **Head-to-head comparison of SARS-CoV-2 antigen-detecting rapid test with self-collected anterior nasal swab versus professional-collected nasopharyngeal swab.** *medRxiv*. 2020.
[Publisher Full Text](#)
38. Wölfel R, Corman VM, Guggemos W, *et al.*: **Virological assessment of hospitalized patients with COVID-2019.** *Nature*. 2020; **581**(7809): 465–469.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Bullete O, Lorente P, Leiva A, *et al.*: **Panbio™ rapid antigen test for SARS-CoV-2 has acceptable accuracy in symptomatic patients in primary health care.** *J Infect*. 2021; **82**(3): 391–398.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Bullete O, Lorente P, Leiva A, *et al.*: **Evaluation of the Panbio™ rapid antigen test for SARS-CoV-2 in primary health care centers and test sites.** *medRxiv*. 2020.
[Publisher Full Text](#)
41. Albert E, Torres I, Bueno F, *et al.*: **Field evaluation of a rapid antigen test (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 diagnosis in primary healthcare centres.** *Clin Microbiol Infect*. 2021; **27**(3): 472.e7–472.e10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Torres I, Poujois S, Albert E, *et al.*: **Evaluation of a rapid antigen test (Panbio™ COVID-19 Ag rapid test device) for SARS-CoV-2 detection in asymptomatic close contacts of COVID-19 patients.** *Clin Microbiol Infect*. 2021; **27**(4): 636.e1–636.e4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
43. Baggio S, L'Huillier AG, Yerly S, *et al.*: **SARS-CoV-2 viral load in the upper respiratory tract of children and adults with early acute COVID-19.** *medRxiv*. 2020.
[Publisher Full Text](#)

Open Peer Review

Current Peer Review Status: ?

Version 1

Reviewer Report 12 July 2021

<https://doi.org/10.21956/wellcomeopenres.18577.r44823>

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? **Janneke H. H. M. van de Wiggert** 

¹ Department of Clinical Infection, Microbiology and Immunology, Institute of Infection, Veterinary, and Ecological Sciences, University of Liverpool, Liverpool, UK

² Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

General comments:

The results described in this manuscript are useful and the in vitro work is elegant. My main concern is the heterogeneity of the study populations and study procedures (sample collection procedures and reference standards), in combination with a small sample size, in the clinical study. My recommendation would be to limit the clinical analyses to symptomatic patients. The asymptomatic participants in this study are problematic for several reasons: 1) the sensitivity estimate for this group is very imprecise (95% confidence interval 57.9%-92.9%) due to the small sample size of 62 individuals; 2) there seems to be significant heterogeneity within this group, which matters because the a priori probability of testing positive is much higher for asymptomatic household contacts of index cases than for asymptomatic people who are tested for other reasons; and 3) in the case of exposed contacts, the timing of exposure is unclear. The latter is problematic because all SARS-CoV-2 tests, including RT-PCR reference standards, have limited sensitivity on the first 4 days after infection. Symptoms typically develop at least 5 days after infection and this limitation, therefore, does not – or to a lesser extent - apply to the symptomatic group. To summarise, reliably determining clinical diagnostic test performance in asymptomatic people requires more careful data collection than was done in this study.

The UK's COVID-19 vaccination programme was initiated during the study period. Breakthrough infections do occur and diagnostic performance of Ag-RDTs might differ by vaccination status. It is unclear whether any of the study participants had been vaccinated at the time of sampling.

The last sentence of the abstract overstates the value of the Mologic Ag-RDT. As with all other Ag-RDTs that have been evaluated thus far, they are very useful in some settings but not sufficiently sensitive in other settings. For example, RT-PCR testing and/or repeat or confirmatory testing strategies are required in hospitalised patients or in carers who are in close contact with

vulnerable people. And in all settings, people testing negative on an Ag-RDT should be urged to get retested if symptoms develop or worsen after the sample for the first test was taken. When Ag-RDTs are used in random testing situations (e.g. to provide access to nightclubs/events or for travel), one should always be aware that false-negative Ag-RDT results will occur and that these can spark an outbreak – we have seen plenty of examples in recent weeks. Some of these caveats are mentioned in the discussion, but they should also be mentioned in the abstract and they deserve more attention in the discussion.

Specific comments:

- Abstract: The end-date of the study is reported as 15 Feb 2021 in the abstract but as 11 Mar 2021 in the main text – which date is correct? Explain the study populations more clearly in the abstract (in terms of asymptomatic but exposed, asymptomatic not exposed, symptomatic) and report sensitivity for these groups separately or – even better – limit the manuscript to symptomatic individuals (see general comments above). Add a brief statement about the caveats of Ag-RDTs, including the Mologic Ag-RDT (see general comments above) in the conclusions.
- Introduction: Explain the currently available evidence for transmission probabilities by viral load in more detail. Also, be clear which statements about transmission probabilities are hypothesised but not yet proven. For example, Ct cut-offs are indeed widely used (for good reasons) but the direct links between specific Ct value cut-offs and transmission probabilities are still uncertain. Describe the diagnostic accuracies of Ag-RDTs that are currently widely used in the UK so that the reader can compare the diagnostic accuracies of the Mologic test to those of other widely used tests.
- Methods:
 - Study populations: Were all patients attending the Liverpool A&E departments or admitted to the Yorkshire hospitals routinely tested for SARS-CoV-2 during the study period even if they sought care for non-COVID reasons? If yes, please state this; readers outside the UK do not have that information. If no, please explain the reasons why they were tested. Do I understand correctly that all Northumberland NHS staff and their household members were either symptomatic or had been exposed to a COVID case in their household? In other words, none of them were asymptomatic and not exposed (as far as they knew at the time)?
 - Sample collection methods: At all three sites, why was the sampling method for the Ag-RDT not matched with the one used for the RT-PCR? There is so much heterogeneity.
 - Sample testing: I am assuming that the people who read the results of the Mologic test were also blinded to the results of the RT-PCR reference test? You do not describe where the RT-PCR and Ag-RDT tests were done and within what time frames after sampling.
 - Analytical evaluation: Many of the readers of this paper are likely not laboratory scientists. I think that they will find it challenging to understand your description of the analytical evaluation. Can you walk us through it more clearly? It would help if you could add explanations such as: “We added virus stock dilutions to a liquid from NP swabs of SARS-CoV-2 negative individuals to mimic real world swab material (referred to as matrix)”. Etc. It is unclear to me whether the concentrations in pfu/ml were determined by adding virus stock dilutions (without matrix) or ‘virus-in-matrix’

dilutions to cells. I think that I understood how you linked the Mologic LLOD to pfu/ml and genomic copies/ml. However, I did not understand how you normalised the Ct values of the different RT-PCR platforms that were used (as you undoubtedly know, Ct values of different PCR platforms or even different laboratories using the same PCR platform are not always directly comparable), and how you subsequently translated these normalised Ct values to genomic copies/ml.

- It is not clear to me from the methods what information you collected from each participant, at which time point (at the same time as collecting the samples?), and how. Judging from the many missing values for symptom-onset, I suspect that you extracted the information from medical records? Why did you not extract information about COVID vaccination? Why was symptom-onset missing for so many participants?
- Results and discussion:
 - The second sentence of the discussion (about the LLOD) should be moved to the results.
 - See general comments for further feedback.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Infectious disease epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
