Letter to the Editor – ACCEPTED MANUSCRIPT

Title:

Diagnostic accuracy and utility of SARS-CoV-2 antigen lateral flow assays in medical admissions with possible COVID-19

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Text:

Dear Editor,

The scale-up of SARS-CoV-2 antigen lateral flow assays (LFAs) has caused much controversy, with concerns about lower sensitivity in asymptomatic individuals and when assays are performed by operators without healthcare training.[1,2] The proposed benefits of SARS-CoV-2 antigen LFAs are high specificity, fast turnaround times for results (under 30 minutes) and ease of scalability.[3] These assays are of potential utility for rapidly identifying SARS-CoV-2 in patients who fit the COVID-19 case definition and require hospital admission as prompt isolation prevents nosocomial transmission. Isolation rooms are often limited and capacity easily overwhelmed, necessitating the cohorting of patients with proven COVID-19. Even using rapid platforms, SARS-CoV-2 RT-PCR turnaround times are often too slow to inform patient placement from emergency departments (EDs).[4] SARS-CoV-2 LFAs could help improve flow of patients from the ED into 'COVID-19 positive' cohorts and reduce pressure on limited hospital isolation rooms. However, little data exists on their diagnostic accuracy in this setting.

We therefore evaluated diagnostic accuracy of the Innova SARS-CoV-2 Antigen Rapid Qualitative Test (Lotus Global Company, London, UK) compared to SARS-CoV-2 RT-PCR from nasopharyngeal swabs (NPS) in adult admissions who met the COVID-19 case definition at a busy acute hospital in the UK.[5] The Innova LFA was performed as per the manufacturer's instructions by appropriately trained health-care assistants in the ED. A second NPS was simultaneously sent for SARS-CoV-2 RT-PCR. Between the 17th November 2020 and 31st December 2020, 728 patients presenting to the ED met the COVID-19 case definition and had valid Innova LFA and RT-PCR results. Baseline characteristics are shown in Table 1A. 55·1% were male and median age was 67·5 years. 264 patients tested positive by Innova LFA. Those testing positive were younger (median age 65 vs 71, p=0.038), more unwell (NEWS of 5 vs 3, p<0.001) and more often febrile on arrival (Temperature >38°C in

41·9% vs 15·8%, p<0.001) than those with negative LFA results. Overall, admission SARS-CoV-2 RT-PCR was positive in 38·5% (280/728).

Compared to SARS-CoV-2 RT-PCR as the reference standard, the Innova LFA had sensitivity of 86·4% (242/280, 95% Confidence Interval [CI] 81·9-90·0) and specificity of 95·1% (426/448, 95%CI 92·6-96·7) (Table 1B). 22/448 (4·9%) patients with a negative SARS-CoV-2 RT-PCR on admission had a positive LFA. 8 of these 22 patients reported a positive COVID-19 test result up to 14 days prior to admission and 5/22 subsequently had a positive PCR result within 5 days of admission. 13/22 had chest radiograph features consistent with 'classic/probable COVID-19' as reported by a radiologist. Only 5/22 patients had no PCR or radiological evidence of COVID-19. 1/5 patients reported a confirmed household contact and only 2/5 left hospital with a diagnosis other than COVID-19. This suggests the lower than expected specificity of Innova LFA is likely to be a result of an imperfect reference standard, and specificity would be higher if using a clinical and RT-PCR based composite reference standard.[6]

38 patients had negative Innova LFAs but positive PCR results. 20/38 had cycle threshold (Ct) values available, with median Ct values of 29 (IQR 27-35). Innova LFA results were available a median 3·2 hours after arrival (IQR 2·0-4·3, n=681) compared to 13·8 hours (IQR 9·9-18·2, n=679) for RT-PCR. 57·1% (n=35) had chest radiographs which were reported as typical for COVID-19. Of those with symptom duration recorded, 77.3% (17/22) were symptomatic for at least 7 days prior to attending the ED.

Accounting for the inadequacy of a single SARS-CoV-2 RT-PCR as a reference standard, we found the Innova SARS-CoV-2 Antigen Rapid Qualitative Test had good specificity in patients with symptoms of COVID-19 presenting to hospital. Sensitivity in this setting was high (86.4%) when compared to pre-clinical evaluation studies.[1] Furthermore, results were mostly available within a few hours of

presentation, allowing transfer of patients to COVID-19 cohort areas and reducing demand for isolation rooms whilst awaiting PCR results. Placing patients in the 'right bed' first-time is also likely to reduce delays in care and increase efficiency, and allows isolation rooms to be prioritised for individuals requiring admission with suspected COVID-19 but negative LFA results. Of the 38 patients with COVID-19 (based on SARS-CoV-2 RT-PCR) who were 'missed' by the Innova LFA, median Ct values were reasonably high, and correspond to viral loads associated with lower sensitivity in previous studies.[1] However, sensitivity of the Innova LFA appears lower than some other SARS-CoV-2 viral antigen LFAs .[7] Importantly, individuals requiring admission with suspected COVID-19 should not be moved out of isolation on the basis of a negative SARS-CoV-2 viral antigen LFA results.

In summary, the Innova LFA can be used to rapidly identify COVID-19 cases amongst hospital admissions meeting the COVID-19 case definition with good diagnostic accuracy, and rapidly identify patients that can be allocated to COVID-19 cohort areas. Based on these data, this application of COVID-19 LFAs has now been recommended by NHS England.[8]

Declarations:

Funding: This study received no specific grant from any funding agency in the public, commercial or not-for-profit sector. The manufacturer (Lotus Global Company, London, UK) had no role in the study conception, design, data analysis or manuscript preparation.

Approval: The study was approved by the London North West University Hospitals Trust Research and Development Committee, and given the SARS-CoV-2 antigen lateral flow assay was implemented as part of routine clinical care and this was a retrospective review using routinely collected clinical data, they deemed formal ethical approval was not required.

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Conflicts of interest: The authors declare that they have no competing interests.

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Table 1: Baseline Characteristics and Diagnostic Performance

A – Baseline Characteristics	LFA Negative	LFA Positive	Total	p-value
N	464	264	728	
Age on arrival (years) median (IQR)	71 (53·5, 83) (n=464)	65 (49·5, 80) (n=264)	67·5 (52, 82) (n=728)	0·038
Age over 65 years, n (%, 95%CI)	260 (56·0%, 51·5 - 60·6)	125 (47·3%, 41·3 - 53·4)	385 (52·9%, 49·3 - 56.5)	0·024
Female Sex, n (%)	211 (45·5%, 40·9 - 50·0)	116 (43·9%, 38·0 - 49·9)	327 (44·9%, 41·3 - 48·5)	0·69
Male Sex, n (%)	253 (54·5%, 50·0 - 59·1)	148 (56·1%, 50·1 - 62·0)	401 (55·1%, 51·5 - 59·7)	
NEWS, median (IQR)	3 (1, 6) (n=422)	5 (3, 7) (n=230)	4 (2, 6) (n=652)	<0·001
Pulse, median (IQR)	94 (82, 111) (n=426)	96 (84, 108) (n=229)	95 (82, 110) (n=655)	0·66
Systolic BP, median (IQR)	136 (120, 151) (n=421)	135·5 (122·5, 149) (n=228)	136 (121, 151) (n=649)	0·93
Diastolic BP, median (IQR)	78 (68, 88) (n=421)	80 (71, 89) (n=228)	79 (70, 88) (n=649)	0·10
Respiratory rate, median (IQR)	20 (18, 27) (n=425)	24 (20, 32) (n=228)	22 (18, 28) (n=653)	<0·001
SpO2 <94%, n (%, 95%CI)	55 (12·9%, 9·8 - 16·1)	68 (29·7%, 23·8 - 35·6)	123 (18·8%, 15·8 - 21·8)	<0·001
Required Supplemental Oxygen, n (%, 95%CI)	72 (16·9%, 13·3 - 20·4)	69 (29·9%, 24·0 - 35·8)	141 (21·4%, 18·3 - 24·6)	<0·001
Temperature >38°C, n (%, 95%CI)	67 (15·8%, 12·3 - 19·2)	96 (41·9%, 35·5 - 48·3)	163 (24·9%, 21·6 - 28·2)	<0·001
B – Diagnostic Performance	**LFA Negative**	**LFA Positive**	**Total**	
N	464	264	728	
SARS-CoV2 RNA Detectable on RT-PCR, n (%)	38	242	280	Sensitivity = 86·4% (95%CI 81·9-90·0)
SARS-CoV2 RNA Undetectable on RT-PCR, n (%)	426	22	448	Specificity = 95·1% (95%CI 92·6-96·7)
	NPV = 91·8% (95%CI 87·7-94·5)	PPV = 91·8% (95%CI 87·7-94·5)		

Table 1 footnotes:

A - Baseline characteristics and SARS-CoV-2 RT-PCR results for patients testing positive and negative by the Innova Lateral Flow Antigen (LFA) Assay. For observations on arrival, 9·6 to 10·9% of data were missing. Pair-wise comparisons were performed using chi-squared tests for proportions, t-tests for means and Wilcoxon rank sum for median. *P-values are shown for the comparison between the LFA positive and LFA negative groups. IQR=Inter-quartile range, CI=Confidence Interval, NEWS=National Early Warning Score, SpO2=Oxygen Saturations.

B - Diagnostic performance of the Innova Lateral Flow Antigen (LFA) Assay compared to a single SARS-CoV-2 RT-PCR from nasopharyngeal swab on admission· PPV = Positive Predictive Value, NPV = Negative Predictive Value, CI = confidence interval.