Performance of a SARS-CoV-2 Antigen Pool Test to aid diagnosis of acute COVID-19 at the point of care

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Coronavirus disease (COVID-19) is an infectious disease caused by a novel coronavirus - Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In 2020, the World Health Organization (WHO) classified the disease as a pandemic[1]. Widespread population testing has been required to control the spread of infection and this includes molecular and antigen-based tests. The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, headache, conjunctivitis, sore throat, diarrhoea, loss of taste or smell, or a rash on skin or discoloration of fingers or toes.

An accurate diagnosis is essential to identify and manage SARS-CoV-2-infected patients and for the implementation of effective infection control measures. Currently, the gold standard for SARS-CoV-2 diagnosis is reverse transcription polymerase chain reaction (RT-PCR) using nasopharyngeal, throat, or saliva swabs. However, these tests are labour intensive, require expensive machinery, are often only available in specialised laboratories and can take up to 24–48 hours to provide a result to the patient [2,3]. Rapid point of care antigen testing can provide results in under 30 minutes, however lateral flow technologies have limited sensitivity and may miss significant infections [4,5]. The LumiraDx SARS-CoV-2 Antigen (Ag) Test is a highly-sensitive, microfluidic immunofluorescence assay for use with the LumiraDx point of care Platform for the qualitative detection of the nucleocapsid protein antigen in individual samples from nasal or nasopharyngeal swabs in 12 minutes. The high sensitivity (97.6%) and specificity (96.6%) make it ideal for point of care use [6].

The test has been further developed to allow for pooling of swab samples taken from multiple subjects. The LumiraDx SARS-CoV-2 Ag Pool Test aids in the diagnosis of current SARS-CoV-2 infection by detection of SARS-CoV-2 antigen when testing large groups in low disease prevalence settings. Groups of 1 to 5 subjects can be tested using a single test strip, which can enable physicians, employers, or schools to verify infection quickly among a pool of up to 5 subjects so that, where needed, proper treatment can begin and initiate isolation precautions helping prevent further spread of infection.

Methods

The performance of the LumiraDx SARS-CoV-2 Ag Pool Test was established with direct anterior nares nasal swabs. Swabs were collected from asymptomatic and symptomatic individuals during the 2020 pandemic (October 7th – November 7th 2020). Subjects were either presenting with symptoms of COVID-19 or were key workers being screened for infection. Prospective collection of 30 positive pools (150 individual subjects) was conducted in the USA at a drive through testing site. Each subject self-collected 2 nasal swabs using the dual anterior nares method. The swabs were placed into separate, clean tubes and transferred to the testing area. One swab was immediately placed into the LumiraDx extraction buffer and tested on the LumiraDx SARS-CoV-2 Ag Test. Once identified as positive, the second swab from the positive subject was randomly allocated into one of 30 pools, therefore each pool contained one positive swab plus four negative swabs. The Pooling process was then followed according to the LumiraDx SARS-CoV-2 Ag Pool Test instructions for use.

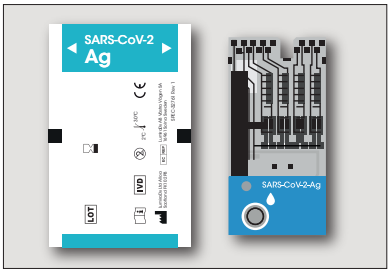
For the negative pools, 145 subjects in the UK also simultaneously collected two anterior nares nasal swabs using the dual anterior nares method, of which one swab was tested immediately on the LumiraDx SARS-CoV-2 Ag test and the second included in one of 29 negative pools. The Pooling process was then followed according to the Pooling instructions for use. The reference method was the LumiraDx SARS-CoV-2 Antigen test (individual subject test). All specimens were tested freshly collected and not frozen.

Ethics approval was obtained for the US study by Toolbox Medical Innovations, who were the appointed Clinical Research Organiation (CRO) conducing the study, through WCG IRB under protocol number CS-1211-01 (WCG IRB 20201775). Approval for sample collection for UK subjects followed the LumiraDx UK Stirling Site Employees protocol. Written informed consent was obtained from all participants prior to enrolment. In addition, study protocols complied with the Declaration of Helsinki (2013). All data collected from enrolled Subjects was anonymized, which complies with the relevant sections of Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices, ICH GCP E6 (R2), and General Data Protection Regulation (GDPR) (Regulation (EU) 2016/679) also apply.

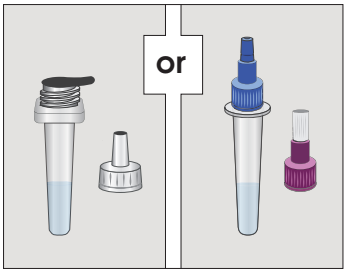
Outline of the Pooling Test process is shown below:

LumiraDx SARS-CoV-2 Ag Pool Test Kit Components

Test Strip



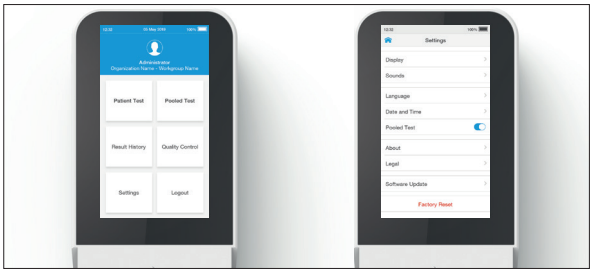
Extraction Vial and Dropper Lids



Instrument set up

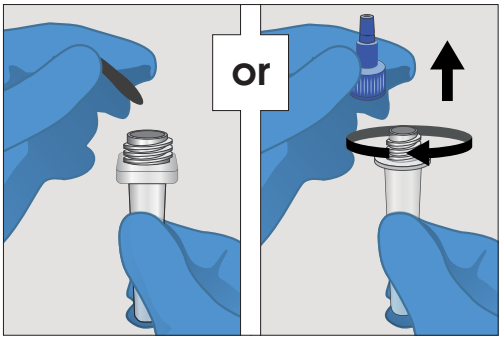
Check that 'Pooled Test' is available on Instrument home screen.

Enable 'Pooled Test' in the Instrument settings menu.



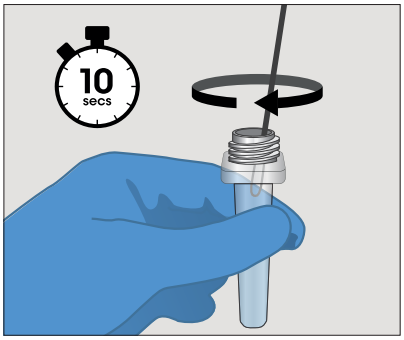
Preparing the sample

Collect 1 to 5 individual patient swab samples (either all nasal swabs or all nasopharyngeal swabs) and place in dry tubes before following steps 1 – 4 of **Running the Test.** The swabs must be processed in the extraction vial within 1 hour of collection. **Collection and Handling:** Proper sample collection and handling of nasal and nasopharyngeal swabs is required to ensure accurate results (refer to product insert). Additional training or guidance is recommended if operators are not experienced with sample collection and handling procedures.



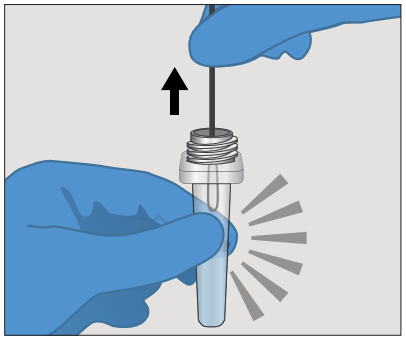
1. Remove seal

Remove the seal or blue screw cap from top of **Extraction Vial** containing the **Extraction Buffer**.



2. Soak Swab

Place and soak the **Patient Swab** in the **Extraction Buffer** for 10 seconds, then stir well by rotating the swab against the side of the vial 5 times.

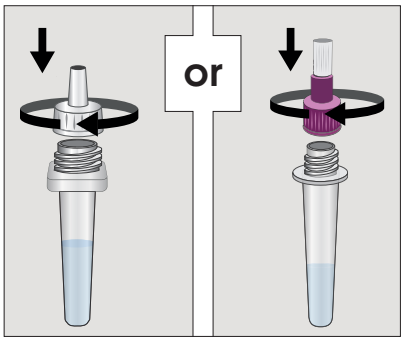


3. Squeeze Swab

Remove the **Patient Swab** while squeezing the **Extraction Vial** to remove the liquid from the swab. Discard the swab in biohazard waste.

4. Repeat steps 2-3

Repeat steps 2 and 3 sequentially for up to 4 more swabs into the same Extraction Buffer Vial.



5. Attach Dropper Lid

Firmly attach the clear or purple **Dropper Lid** to the top of the **Extraction Vial. The extracted sample must be used (see Step 5 and 6 below) within 5h of preparation when stored at room temperature.**

Results

The performance of the LumiraDx SARS-COV-2 Ag Pool Test for each subject was compared to the results from the simultaneously collected nasal swabs tested individually with the LumiraDx SARS-COV-2 Ag Test. Analysis showed Positive Percent Agreement (PPA) of 100% (CI 88.6-100%) and Negative Percent Agreement of 96.6% (CI 82.8%-99.4%) (Table 1) [7,8].

Table 1. Performance measures, and 95% confidence intervals, as calculated with the Wilson Score method.

		Single Swab			Measure	Estimate	95% Confidence Interval (CI)	
		POS	NEG	TOTAL	PPA	100%	88.6%	100.0%
LumiraDx SARS-CoV-2 Ag Test	POS	30	1	31	NPA	96.6%	82.8%	99.4%
	NEG	0	28	28	PPV	96.8%	83.8%	99.4%
	TOTAL	30	29	59	NPV	100%	87.9%	100.0%
					Prevalence	50.8%	38.4%	63.2%
					OPA (% agreement)	98.3%	91.0%	99.7%

Conclusion

The LumiraDx SARS-CoV-2 Ag Pool Test is a rapid microfluidic immunofluorescence assay for the qualitative detection of the nucleocapsid protein antigen, in nasal or nasopharyngeal swab specimens pooled from up to 5 individuals suspected of COVID-19 within the first twelve days of symptom onset or up to 5 asymptomatic individuals. Used with the LumiraDx Platform the test offers a rapid, scalable and cost-effective screening solution for potentially infectious individuals. Pool Testing is the method of testing specimens in a "pool" (or group) rather than individually and is useful when testing high volumes of clinical specimens for infectious diseases or for screening groups when prevalence of infection is low. A positive pool result indicates that at least one subject in the group is positive for the SARS-CoV-2 antigen and the whole group should be tested individually. A negative pool indicates that none of the subjects had detectable SARS-CoV-2 antigen.

The study demonstrated a high level of agreement with the Pool test results and the results from the individual LumiraDx SARS-CoV-2 antigen test, which has been shown to have a PPA of 97.6% and NPA of 96.6% versus RT-PCR.

The LumiraDx SARS-CoV-2 Ag Pool Test is the first commercial point of care antigen Pooling Test and demonstrates high sensitivity compared to the individual test, saving time and cost. The LumiraDx SARS-CoV-2 Pool Ag Test takes just 12 minutes to report the results.

Pool testing can improve access to testing by significantly reducing resources (tests, operators, instruments) required on a per patient basis, and is useful to quickly and easily scale up testing while minimising resources required. Pool testing can be an appropriate strategy to help facilitate the reopening of places of work, sports, and cultural or social events.

References:

1. World Health Organisation www.who.int
2. Peto T. COVID-19: Rapid Antigen detection for SARS-CoV-2 by lateral flow assay: a national systematic evaluation for mass-testing. medRxiv. 2021;2021.01.13.21249563.
3. Yamayoshi S, Sakai-Tagawa Y, Koga M, et al. Comparison of Rapid Antigen Tests for COVID-19. Viruses. 2020;12:
4. Toptan, T., Eckermann, L., Pfeiffer, A.E., et al., Evaluation of a SARS-CoV-2 rapid antigen test: Potential to help reduce community spread? J Clin Virology. 135: Feb 2021.
5. Prince-Guerra, J., Almendares, O., Nolen, LD., Evaluation of Abbott BinaxNOW Rapid Antigen Test for SARS-CoV-2 Infection at Two Community-Based Testing Sites - Pima County, Arizona, November 3-17, 2020. MMWR Morb Mortal Wkly Re 2021 Jan 22;70(3):100-105.
6. Drain, P., Ampajwala M, Chappel C, et al., A Rapid, High-Sensitivity SARS-CoV-2 Nucleocapsid Immunoassay to Aid Diagnosis of Acute COVID-19 at the Point of Care: A Clinical Performance Study. Infect Dis Ther. 2021 Jun;10 (2):753-761.
7. LumiraDx Data on File. S-CLIN-REP-00026 SARS-CoV-2 Antigen Positive Pooling Clinical Study Report.
8. LumiraDx Data on File. S-CLIN-REP-00027 SARS-CoV-2 Antigen Positive Pooling Clinical Study Report.

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