The LAVA (Lateral flow Antigen Validation and Applicability) Study for COVID-19

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2. **Background**

During the ongoing COVID-19 pandemic, testing of children for COVID-19 has become an area of substantial need and intense scrutiny. The current gold-standard method for SARS-CoV-2 detection is the real time reverse transcriptase polymerase chain reaction (RT-PCR), performed on a sample from the respiratory system. This diagnostic test identifies fragments of viral RNA which are specific to SARS-CoV-2 and amplifies them. It can detect even relatively low levels of RNA in people who have contracted the virus but have not yet developed a high viral load or symptoms. It can also detect RNA after the live virus has cleared, but fragments of the virus' RNA remain. This test has a high analytical sensitivity and specificity. However, obtaining an adequate specimen is more difficult in children as the test is uncomfortable and not always well tolerated. This means that the diagnostic sensitivity is approximately 80%(1). Nevertheless, RT-PCR currently remains the most accurate method of detection of SARS-CoV-2. However, logistical issues around RT-PCR mean that the availability and usability of the test is reduced. This is due to a combination of factors including testing site capacity, processing capacity and the time taken to process and report the results by centralised laboratories. The

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net result is that the high sensitivity and specificity of PCR is offset by the time lag for result reporting. In acute hospitals, these delays can lead to poor patient flow through clinical areas, overuse of personal-protective equipment (PPE) or late recognition of nosocomial transmission. Thus, rapid testing is of particular importance in identifying highly infective individuals, for example people who are about to undergo a high-risk aerosol generating procedure, enter a crowded emergency room, or in rapid investigation of localised outbreaks.

Alternative methods and modalities of testing have therefore been explored, in particular the use of anterior nasal swabs to detect the SARS-CoV-2. Salivary RT-PCR is also under investigation(2, 3) but is affected by some of the limitations of performing RT-PCR on any sample, as described above. An alternative method of detection of SARS-CoV-2 is the use of antigen testing using a lateral flow assay (LFA)(4). Antigen-detection diagnostic test are designed to directly detect SARS-CoV-2 proteins produced by replicating virus in respiratory secretions. Most antigen rapid diagnostic tests use a sandwich immunodetection method using a simple-to-use lateral flow test format similar to a pregnancy test. Antigen detection tests have several advantages compared to RT-PCR; 1) it can be performed on an anterior nasal swabs- a specimen which is much more acceptably obtained in children-, 2) the results can be rapidly processed using a commercial kit at the bedside, with a result being available within 30 minutes of the sample being taken, and 3) it is less expensive to perform an antigen test compared to RT-PCR. The trade-off for ease-of-use and rapid turnaround time of antigen tests is a decrease in sensitivity compared to RT-PCR, particularly if the person has a low viral load (e.g. they are early or late in the disease) because the levels of antigens present in the upper respiratory tract might fall below the threshold for detection. The analytical sensitivity appears to be approximately 50% overall, but this increases during periods of anticipated infectivity when the viral load is >60,000 (RT-PCR cycle time threshold <27) to approximately 90%. The specificity of antigen tests has consistently been reported to be very high (>97%). Thus, if the antigen test is positive then it is very likely that it is a true result, and the person does have COVID-19. Antigen-detection tests are therefore primarily being used to detect infectivity of an individual, rather than being used as a clinical diagnostic test for COVID-19.

The potential application of LFAs to defeat Covid-19 are evident, with several clinical scenarios below demonstrating areas where comparing the performance of RT-PCR to LFA

could improve the care of children and improve the safety of staff in hospital. However, evaluation of the use of these tests in clinical settings is essential before full implementation in routine clinical practice is advised.

At present, all children are tested for COVID-19 with RT-PCR when they are admitted to hospital, regardless of their symptoms. During the winter months, when more children with respiratory symptoms will seek medical care, current guidance recommends isolation or cohorting of these patients within hospital until their SARS-CoV-2 test is back(5). However, the majority of children are likely to have an alternative cause for their symptoms, such as respiratory syncytial virus, adenovirus or influenza. Early identification of infectious children with COVID-19 using a point of care test would allow for more effective cohorting or isolation to occur and potentially reduce the spread of COVID-19 in hospital settings, not just from children but their parents too who are likely to be infected as a household contact. Besides, early identification of children with COVID-19 using LFA will aid compliance with stringent use of PPE for healthcare workers caring for these children.

Children being admitted for elective procedures are currently also routinely tested for COVID-19 with a nasopharyngeal swab prior to admission. This is performed to reduce the chance of them harbouring SARS-CoV-2 at the time of having a high-risk aerosol generating procedure (AGP), where the risk of transmission of SARS-CoV-2 to staff is increased. Generally, these children are asymptomatic, as the presence of ILI symptoms often precludes an anaesthetic being performed. In this population, LFAs could be used to identify children who were incubating SARS-CoV-2 at the time of swabbing but were not shedding the virus who have subsequently progress to start shedding the virus (Table 1). Performing LFA potentially enables infectious children to be identified and can be used to guide decisions around proceeding with a procedure and the use of PPE in children when the procedure goes ahead.

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Hours after test	2.5 quantile	Median	97.5 quantile
1	0.726	0.766	0.804
3	2.228	2.298	2.363
6	4.493	4.586	4.68
12	9.017	9.159	9.295
24	17.961	18.135	18.319
32	23.748	23.929	24.125
48	34.587	34.814	35.037
60	41.961	42.212	42.427
72	48.647	48.895	49.123
84	54.633	54.867	55.095

Table 1. The proportion of people who are incubating SARS-CoV-2 at the time of a negative swab, who go on to start shedding the virus at the displayed time-points after the test.

Finally, LFAs could be used in 'high risk' hospital areas, particularly intensive care and high dependency units where high-risk AGPs are commonly performed and where children are more likely to have symptoms which are in-keeping with COVID-19. Surveillance of SARS-CoV-2 using RT-PCR is being performed routinely in some units, but a 24-48 hour delay in the result has minimal impact on practice around AGPs. LFAs may be utilised as a screening tool for infectious patients in these areas to support the use of appropriate PPE.

Further use of these tests could also be considered within health care worker (HCW) screening for infectivity after known exposures to Covid-19 cases, to limit the number of HCW absences due to potential exposure both outside and inside the hospital. They could potentially also be used in the community, in settings such as schools and sports clubs, to identify infectious individuals. Validation and usability in a controlled clinical setting is recommended prior to use in the wider community. Mathematical analysis of LFAs shows that, due to their high specificity, the negative predictive value is good in times of both high and low prevalence, even when the sensitivity of the test is low (Table 1).

	PREVALENCE 0.50%				PREVALENCE 2%			
SPECIFICITY	98.00%		99.70%		98.00%		99.70%	
SENSITIVITY	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
100.00%	20.1%	100.0%	62.6%	100.0%	50.5%	100.0%	87.2%	100.0%
90.00%	18.4%	100.0%	60.1%	100.0%	47.9%	99.8%	86.0%	99.8%
80.00%	16.7%	99.9%	57.3%	99.9%	44.9%	99.6%	84.5%	99.6%
70.00%	15.0%	99.9%	54.0%	99.9%	41.7%	99.4%	82.6%	99.4%
60.00%	13.1%	99.8%	50.1%	99.8%	38.0%	99.2%	80.3%	99.2%
50.00%	11.2%	99.7%	45.6%	99.8%	33.8%	99.0%	77.3%	99.0%

Table 1. Negative and positive predictive value of performing a point of care test with high specificity and different levels of sensitivity at times of high and low prevalence.

3. Research Questions:

1. What are the technical and practical considerations of performing lateral flow antigen assays in children in hospital?
   a) What are the practical considerations of performing lateral flow assays in children?
   b) What is the test acceptability compared to combined nasal and throat swabs?
2. How many paired tests need to be performed to determine non-inferiority of LFA to RT-PCR.

4. Objectives and Specific Aims:

This study is a two-centre pilot study which will address the following aims:

Aim 1. To determine the technical failure rate of LFAs performed on nasal swabs and compare this to the failure rate of combined nasal and throat RT-PCR. Reasons for LFA test failure will be reviewed, with recommendations for training or improvements to be made before proceeding to the second stage.

Aim 2. To compare the acceptability of both RT-PCR and nasal swab LFAs using parental, and when appropriate child, feedback on the acceptability of both tests using a Likert Scale assessment of discomfort during collection of the test specimens.

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Aim 3. To determine how many paired tests of RT-PCR and LFA need to be performed to assess for non-inferiority of LFA for all patients.

5. Rationale for this work:

As the prevalence of COVID-19 rises once again in the UK, the need for effective testing strategies is essential. Whilst combined nasal and throat RT-PCR currently remains the gold standard diagnostic test, alternative testing modalities may provide benefits which cannot be realised using RT-PCR. Point of care testing has the potential to rapidly identify infective patients which may improve the management of the patient and enable better protection of staff. The first part of the work described involves 'troubleshooting' the test in two pilot sites. This will enable identification for causes of test failure and enable the discomfort of both tests (nasopharyngeal swabbing and nasal swabbing) to be assessed.
The evaluation will then go on to identify specific clinical processes which could be impacted most significantly by the introduction of LFA:

Pre-operative screening: Prior to an elective procedure occurring a child currently undergoes a routine combined nasal and throat swab for RT-PCR. This has a very good diagnostic sensitivity and specificity for SARS-CoV-2 and can detect SARS-CoV-2 in children who have started shedding the virus but who are not yet symptomatic. However, a small proportion of children will have been infected with the virus but still be in the early incubation period at the time of the swab. In this situation, the swab will be negative for SARS-CoV-2 as the virus is not being shed, but there is the potential for shedding to commence between the time of the swab and the time of the procedure. If this were to occur, the person would have become potentially infectious to others. By 24 hours after a swab has been taken in people who are incubating the virus, 20% of those people will be shedding the virus and therefore be infectious. By 72 hours, just over 50% of those people will be shedding the virus. As LFA becomes substantially more sensitive as a test for individuals who are infectious, it is reasonable for it to be performed at the time of attending for surgery to assess for infectivity of the individual. This may impact on the service in several ways. Firstly, the availability of LFA may mean that the time between the combined nasal and throat RT-PCR swabbing and the procedure can be more safely limited to 72 hours, rather than the current 'best practice' recommendation which is that

swabbing is performed within 24 hours of the procedure whenever possible during times of high ($\geq 2\%$) prevalence. This will allow more 'give' in the system and potentially make access to services more equitable. Secondly, assessment of infectivity just before a high-risk AGP is planned to be performed, could affect care in two ways; Firstly, it may be decided by the clinicians, family, or both, to delay a case when the LFA is positive, as would occur if the nasopharyngeal swab was positive and it was clinically appropriate to defer the procedure. Secondly, if the case went ahead, airborne PPE would be worn by theatre staff in-line with Public Health England guidance for 'amber' and 'red' pathways(6). To validate the LFA results, a second nasopharyngeal swab would be taken when the patient is anaesthetised to limit discomfort of the repeated procedure.

Acute new admissions of children with symptoms which are in keeping with COVID-19:
Many children are expected to be admitted through accident and emergency departments with symptoms which could be consistent with COVID-19 during the winter period. The use of LFAs will be assessed in this symptomatic population group as the early identification of a child with COVID-19 will enable much better isolation or cohorting of patients to be undertaken and will reinforce the need for stringent PPE use. A negative result from LFA is not intended to change the care of children with symptoms of COVID-19 from current practice when the patient should remain cohorted and PPE should be worn until at least the result of the RT-PCR is known.
Early testing of LFAs in the community which has been led by Public Health England has suggested that test failure may occur more commonly in children with rhinorrhoea. Assessing the use of LFAs in children in hospital with symptoms which are in keeping with COVID-19 will enable the test performance to be determined in these children, as well as in asymptomatic children.

Screening of children on intensive care and high dependence units and transplant wards:
Many units are undertaking routine screening using nasopharyngeal RT-PCR of patients for COVID-19 on ICU and HDU as these children are often undergoing prolonged high-risk AGPs and therefore the risk of spread of COVID-19 is potentially higher. Many units are also screening children on transplant wards on a weekly basis to attempt to mitigate any

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potential outbreaks of SARS-CoV-2 early and to reduce the risk to those patients. Anecdotally, children and families are refusing these frequent tests because of the distress that results from combined nasal and throat swabbing. Performing LFA on an anterior nasal swab may enable early identification of infectious children but is likely to be much more acceptable to children compared to the combined throat and nasal RT-PCR.

This pilot study intends to assess the practical aspects of performing LFAs as a point of care test and determine the acceptability of the test by asking about the discomfort experienced with the test. Comparison of RT-PCR and LFA will be used to perform a power calculation to determine the number of tests which need to be performed in order to determine non-inferiority of LFA.

6. Design

This will be a prospective two-centre pilot study.

7. Basic Demographic Data/subjects

Any child < 18 years of age who undergoes a combined nose and throat swab for RT-PCR for COVID-19 within the three specified groups as routine care in a participating centre will be offered the opportunity to enter the study.

8. Methods

Inclusion criteria:

1. Children aged 0 - <18 years undergoing combined nose and throat swabbing for an elective diagnostic or surgical procedure.
2. Children aged 0 - <18 years undergoing combined nose and throat swabbing on admission to hospital when there is a differential diagnosis of COVID-19.
3. Children aged 0 - <18 years undergoing routine combined nose and throat swabbing during a stay on the intensive care unit, the high dependency unit or a transplant ward.

Exclusion criteria:

None.

Consent

All children and families will provide informed consent before inclusion in the study. For children who are attending for pre-operative screening for COVID-19: The administrative staff who confirm the procedure with the family pre-operatively will request an email address to send parent and age-appropriate child information leaflets to. A research nurse associated with the study will call the family after 24 hours to discuss the study and if the family are happy to participate, the family will be asked to email back a signed copy of the consent form. When consent has been gained, the details of the CRF which can be completed in advance will be performed and the nurses who perform the pre-operative swabbing will be informed. The patient will be asked to confirm consent verbally before the pre-operative swab and the swab that is performed upon admission.

For children who are admitted from A&E: These children and their families will be approached by GCP trained members of the direct care team for enrolment in the study. They will be provided with patient information leaflets and have a verbal discussion about the study. Due to the more urgent nature of performing the swab they will be asked for consent after a minimum of 30 minutes.

For children who are inpatients on ICU, HDU or the transplant ward: These children and their families will either be approached by the direct care team or by the research nurses for inclusion in the study. They will be asked whether they consent after 24 hours and if so, the consent will be valid for the two week study duration.

Procedures

Children will undergo an additional swab which will be taken from the anterior nose prior to the routinely performed combined nose and throat swab being taken.

Prior to performing the anterior nasal swab, any nasal mucous will be removed by wiping or blowing the nose and then a swab the size of a cotton bud will be rubbed on the inside of the nose, below the level of the inferior turbinate to a maximum of 2.5cm. It will be rolled 5 times along the mucosa inside each nostril. This may tickle or cause some children to sneeze but is very unlikely to be painful. There is a risk of the swabbing causing a nosebleed, but this is no more likely to occur than during other routine swabs that are currently taken in clinical practice for surveillance of other infections such as Methicillin-resistant *Staphylococcus aureus* (MRSA).

Children attending for an elective procedure currently routinely undergo combined nose and throat swabbing a few days before their procedure. If they are participating in the study they will be offered the opportunity to have an anterior nasal swab taken at the time of their pre-operative swab and at the time of admission for their elective procedure. To enable a comparison of the anterior nasal swab taken at admission with RT-PCR, an additional combined nose and throat swab will be taken whilst the child is anaesthetised.

Timeline

This pilot study will be undertaken in two sites - Alder Hey Children's Hospital, Royal Manchester Children's Hospital.

Study process (Participant):

After a child, their family or both, have agreed to participate in the study they will undergo an anterior nasal swab. The routine combined nose and throat swab can then be taken. For children who are undergoing nasal swabbing at the time of admission for a procedure, their additional combined nose and throat swab will be performed when they are anaesthetised.

A child who has multiple subsequent routine combined nose and throat swabs could be included in the study for each swab.

The family will be informed of the results at the time, if they wish to know them.

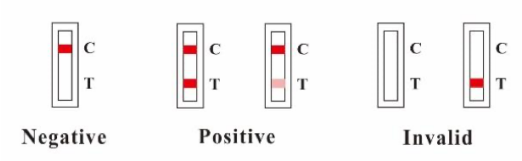
Study process (Recruiting healthcare worker, part of the direct care team):

The anterior nasal swab prior to the routine combined nose and throat swab. It will be rolled 5 times on the inside of each nostril to ensure that mucous and cells are collected.

The test should be performed as soon as possible after the sample is taken and is performed at room temperature. The specimen is extracted by adding 6 drops of

extraction solution to the extractor tube then inserting the swab into the tube and pressing the swab against the wall of the tube. The swab should be rotated for about 10 seconds. The swab head should be squeezed through the walls of the tube nand removed and disposed of in biohazard waste. The nozzle cap should be placed onto the extraction tube and 2 drops of extraction solution should be dropped into the sample well of the test cartridge. The test should be read at 30 minutes, although a strong positive result may be visible before this.

Results will be displayed as follows:



The baseline demographics will be documented, a photograph will be taken of the lateral flow assay result and the results recorded. If the test fails this will be documented and the reasons for this will be elicited whenever possible.

The person undergoing lateral flow assay will be informed of the results (positive, negative, test failure).

In the event of a positive lateral flow assay result:

The child and their family or the healthcare worker will be informed of the result of the lateral flow assay, whether positive, negative or the test has failed.

If the result is positive and they have attended for pre-operative screening, they will be asked to follow government guidance about isolation until the results of the RT-PCR are returned. The RT-PCR results supersede the results of the lateral flow assay and therefore if the RT-PCR is negative and the person has no new symptoms which would be in-keeping with COVID-19 that have developed since the test, they will be able to come out of isolation.

If the child has a positive LFA result on the day of admission for an elective procedure, the results will be discussed with the family. It would generally be anticipated that the procedure could go ahead, with the theatre team in appropriate PPE for a SARS-CoV-2 positive patient and the theatre processes followed for this. Children who have been

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SARS-CoV-2 positive who have undergone surgery do not appear to have experienced significant adverse effects(7). However, discussion between the family, anaesthetic and surgical team is appropriate, and it may be decided to delay the procedure until a later date. If this occurs, it is recommended that the child undergoes a second nasopharyngeal swab to confirm the result and that they follow government advice on isolation.

If a child who is being newly admitted or is currently an inpatient has a positive lateral flow result, the family should be informed and if appropriate, household members should isolate in line with government guidance until the RT-PCR result is returned. Healthcare professionals caring for the patient should be meticulous in their use of appropriate PPE.

Clinical and Salivary Data Collection(8):

Written consent to participate: Y/N

If N - please record patient's reasons or concerns:

Recruiting Centre:

First 3 letters of postcode:

Local Hospital Number

Sex

Age (d) (m) (y)

Symptoms and duration (d)

Fever

Cough

Shortness of breath

Wheeze

Runny nose

Sore throat

Ear ache

Lethargy

Off feeds / reduced oral intake

Myalgia

Abdominal pain

Vomiting

Diarrhoea

Rash

Headache

Seizures

Reduced consciousness

Asymptomatic

Patient group:

Elective admission for surgery or diagnostic procedure

Diagnostic swab due to clinical suspicion of COVID-19 - New admission from ED

Screening swab for current inpatient

Date of Lateral flow antigen test:

Time of nasal swab collection:

Lateral flow assay result: positive / negative / test failure

Date of lateral flow assay result:

Time of lateral flow assay result:

If test failure, reason for failure:

Absent control line

Unable to confidently differentiate whether result line is present or absent

Other, Details:

Unknown

Photograph of lateral flow assay:

Parental assessment of the level of discomfort of the nasal test: /10

Parental assessment of the level of discomfort of the nasopharyngeal test: /10

Child assessment of the level of discomfort of the nasal test: /10

Child assessment of the level of discomfort of the nasopharyngeal test: /10

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Nasopharyngeal RT-PCR swab result:

Date swab result returned:

Time swab result returned:

Results of swab: SARS-CoV-2 detected / SARS-CoV-2 not detected

Cycle threshold of RT-PCR if positive:

9. Outcome measures

As a pilot study there is not a single primary outcome measure. The followings measures will be assessed:

Failure rates for LFA and RT-PCR will be described using percentages.

Reasons for LFA test failure will be described and where possible the reasons for this will be elicited by examining for correlation between the presence of symptoms (e.g. runny nose) and age.

Time from Sample acquisition to result for both lateral flow assay and RT-PCR.

Child reported Likert scale score for discomfort from each test. The results will be tested for normality and if normally distributed compared using student t test and if non-parametric will be compared using Mann-Whitney U test. The same will be performed for parental Likert scale scores.

Reasons for people refusing to participate will be described.

Concordance between results RT-PCR and LFA tests will be assessed in the following manner;

	LFA - Positive	**LFA - Negative**
RT-PCR: Positive		

RT-PCR - Negative		

Power calculations will be undertaken to determine the number of tests that need to be performed to determine non-inferiority of LFA compared to RT-PCR. Variations in prevalence levels, age, sex and cycle threshold will be considered within this calculation.

10. Data Management

Patient data will be collected in paper format in the departments where lateral flow assays are being undertaken. These will be transferred to the study team to perform the follow-up data collection. Data will be sent from Royal Machester Children's Hospital to the Alder Hey study team in an anonymised format at the end of the pilot study period. Data will be transferred from an nhs.net/nhs.uk email address only and will be held on hospital databases. Anonymised data will be shared with Public Health England to enable rapid policy decisions to be made.

11. Approvals required for this work

The work described in this protocol will be undertaken following Research and Ethics Committee review. Sponsorship will be provided by Alder Hey Children's Hospital.

12. Dissemination

Anonymised results of this work will be shared with Public Health England. The results will be used to design a full study to assess the validity of LFA compared to RT-PCR.

13. Funding

The lateral flow antigen tests will be provided by Public Health England. Tests and machine readers will be provided free of charge to participating hospitals in exchange for data submission. Test and trace will provide funding for two full-time research nurses for both centres for two weeks

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1. Mohammadi A, Esmaeilzadeh E, Li Y, Bosch RJ, Li J. SARS-CoV-2 Detection in Different Respiratory Sites: A Systematic Review and Meta-Analysis. medRxiv. 2020.
2. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva or Nasopharyngeal Swab Specimens for Detection of SARS-CoV-2. N Engl J Med. 2020;383(13):1283-6.
3. Chong CY, Kam KQ, Li J, Maiwald M, Loo LH, Nadua KD, et al. Saliva is not a useful diagnostic specimen in children with Coronavirus Disease 2019. Clin Infect Dis. 2020.
4. CEBM. What tests could potentially be used for the screening, diagnosis and monitoring of COVID-19 and what are their advantages and disadvantages? 2020 [Available from: https://www.cebm.net/wp-content/uploads/2020/04/CurrentCOVIDTests_descriptions-FINAL.pdf.
5. RCPCH. National guidance for the management of children with bronchiolotis and lower respiratory tract infections during COVID-19 2020 [Available from: https://www.rcpch.ac.uk/resources/national-guidance-management-children-bronchiolitis-during-covid-19#recommendations---on-admission-to-paediatric-ward---hdu.
6. PHE. COVID-19: infection prevention and control guidance2020. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/893320/COVID-19_Infection_prevention_and_control_guidance_complete.pdf.
7. Nepogodiev D, Bhangu A, Glasbey JC, Li E, Omar OM, Simoes JFF, et al. Mortality and pulmonary complications in patients undergoing surgery with perioperative SARS-CoV-2 infection: an international cohort study. The Lancet. 2020;396(10243):27-38.
8. NICE. Evidence standards framework for SARS-CoV-2 and anti-SARS-CoV-2 antibody diagnostic tests. 2020.