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Lateral flow test performance in children for SARS-CoV-2 using anterior nasal and buccal swabbing: sensitivity, specificity, negative and positive predictive values

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ABSTRACT

Objective To determine if the sensitivity of the lateral flow test is dependent on the viral load and on the location of swabbing in the respiratory tract in children.

Design Phase 1: Routinely performed reverse transcriptase PCR (RT-PCR) using nose and throat (NT) swabs or endotracheal (ET) aspirates were compared with Innova lateral flow tests (LFTs) using anterior nasal (AN) swabs. Phase 2: RT-PCR-positive children underwent paired AN RT-PCR and LFT and/or paired AN RT-PCR and buccal LFT.

Setting Tertiary paediatric hospitals.

Patients Children under the age of 18 years. Phase 1: undergoing routine testing, phase 2: known SARS-CoV-2 positive.

Results Phase 1: 435 paired swabs taken in 431 asymptomatic patients resulted in 8 positive RT-PCRs, 9 PCR test failures and 418 negative RT-PCRs from NT or ET swabs. The test performance of AN LFT demonstrated sensitivity: 25% (4%–59%), specificity: 100% (99%–100%), positive predictive value (PPV): 100% (18%–100%) and negative predictive value (NPV): 99% (97%–99%).

Phase 2: 14 AN RT-PCR-positive results demonstrated a sensitivity of 77% (50%–92%) of LFTs performed on AN swabs. 15/16 paired buccal LFT swabs were negative.

Conclusion The NPV, PPV and specificity of LFTs are excellent. The sensitivity of LFTs compared with RT-PCR is good when the samples are colocated but may be reduced when the LFT swab is taken from the AN. Buccal swabs are not appropriate for LFT testing. Careful consideration of the swabbing reason, the tolerance of the child and the requirements for test processing (eg, rapidity of results) should be undertaken within hospital settings.

Trial registration number NCT04629157.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lateral flow tests (LFTs) for SARS-CoV-2 are a rapid, easily accessible method of testing for SARS-CoV-2 infection, but they are known to have lower sensitivity than reverse transcriptase PCR (RT-PCR) tests when tested on nose and throat specimens.

WHAT THIS STUDY ADDS

⇒ This study demonstrates that the specificity, positive and negative predictive values of LFTs performed on anterior nasal swabs are very good compared with nose and throat or endotracheal specimens in asymptomatic children but the sensitivity appears low. The sensitivity of LFTs may improve with specimens both taken from the same location (in this study anterior nares) but buccal swabbing is not an appropriate LFT specimen. This study demonstrates the low prevalence of SARS-CoV-2 in asymptomatic children during the study period.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Testing for SARS-CoV-2 in children continues in community and hospital settings. This study demonstrates the performance of LFTs compared with RT-PCR in different patient populations and using different swabbing locations in children. Consideration of the indication and impact of swabbing a child and the timeliness of results should be weighed when developing policies around SARS-CoV-2 testing in children.

Check for updates

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INTRODUCTION

The COVID-19 pandemic made swabbing for viral reverse transcriptase PCR (RT-PCR) and lateral flow tests (LFTs) a routine part of life, including for many children. RT-PCR is well-recognised as being the most sensitive test for detecting SARS-CoV-2 with the sensitivity of LFTs appearing to increase with an increased viral load.[1 2] RT-PCR testing of asymptomatic children for SARS-CoV-2 remains commonplace hospital practice before aerosol generating procedures (AGPs) and at the time of admission to hospital. In the UK, widespread community testing has ceased, meaning that more children may be attending hospital unaware that they are infected with SARS-CoV-2. The correlation between SARS-CoV-2 antigen positivity and potential transmissibility is believed to be higher than relying on the detection of SARS-CoV-2 RNA which can remain positive for an extended period.[3]

We have previously demonstrated that anterior nasal swabs are more acceptable to children than nose and throat swabs which are associated with a significantly higher pain score.[4] Repeated swabbing procedures are likely to be poorly tolerated by children, particularly those under 5 years of age. This makes it important to understand the impact of the swabbing method on test results. The aim of this study was to determine if the sensitivity of the LFT is dependent on the viral load and on the location of swabbing in the respiratory tract. The viral load, measured on RT-PCR using the cycle threshold (CT) value, was compared with the result of the Innova LFT using swabs taken from the anterior nares and the buccal mucosa.

METHODS

This study was performed with approval from the Research Ethics Committee (London City and East: 292509) and Health Research Authority (20/HRA/6152). All children participating in the study had consent to be included either given by themselves or their parents, depending on their age and understanding.

There were four participating centres, all of which were UK specialist paediatric hospitals. Innova LFTs (Innova Medical Group, Monrovia, California, USA) were used for all LFTs reported and tests were performed according to manufacturer's instructions. RT-PCR tests were performed within the local hospital according to manufacturer and hospital protocols and the CTs were detected using either the Cephid GeneXpert or ABI7500 Fast using Viasure SARS-CoV-2 Real-Time PCR Detection Kit (Pro-Lab Diagnostics, UK) with a positive CT of 44 being used. A CT value of 44 was chosen as within the UK this is the agreed value for reporting a positive result. Each centre had their own protocol for processing the tests. Two centres ran PCR analysis initially without CT value estimation. If the test was presumed positive based on single gene analysis, it was repeated with testing for two SARS-CoV-2 gene loci and with CT analysis. Two centres assessed CT value on all study tests with two gene loci analysis. The lowest CT value of the two tested genes was reported. The laboratory staff were not aware of the LFT results before the RT-PCR test was analysed and brief clinical details were sent with the swab. Recruitment was undertaken between March 2021 and December 2021, and the full study protocol is available in the online supplemental information.

Phase 1

From January to June 2021, children under 18 years of age undergoing routine nose and throat or endotracheal (ET) aspirate RT-PCR for SARS-CoV-2 were invited to undergo a simultaneous anterior nasal swab for LFT for comparison with the CT value found at PCR. Children undergoing repeated swabbing were eligible to be included for each routine RT-PCR test that was performed during the study period. A 2-week pilot study was undertaken between November and December 2020,[4] and the results were used to guide feasibility and perform a power calculation for the study. This indicated that 24 positive swabs were required to answer the primary study question and on the basis of the initial study design and current prevalence that 5400 paired swabs were required to achieve this. High recruitment numbers were required as there was a relatively low prevalence of SARS-CoV-2 in children attending hospital for swabbing, even during peaks of community prevalence; during the 2-week pilot study in two centres, 324 paired swabs were performed and therefore the study design appeared feasible. Recruitment to phase 1 of the study was substantially lower than during the pilot study with fewer patients and families willing to undergo additional tests and therefore the study design was changed.

Phase 2

From July to December 2021, children under 18 years of age with confirmed SARS-CoV-2 on nose and throat RT-PCR were approached and consented to undergo an anterior nasal swab for RT-PCR and an anterior nasal swab or buccal swab or both for LFT within 72 hours of the RT-PCR-positive nose and throat swab.

Statistical analysis

Two-sided Fisher's exact test was used to compare categorical variables of positive and negative RT-PCR and LFT for phase 1 of the study and are described using relative risk (%, 95% CI). Sensitivity, specificity, positive and negative predictive values are calculated using the Wilson-Brown method. A comparison between the CT value at RT-PCR and the LFT result in phase 2 of the study was performed using logistic regression, and the results are described using ORs (95% CI). Statistical significance was taken as $p < 0.05$. This study is reported according to the STARD guidelines[5] used for reporting diagnostic accuracy studies.

RESULTS

A total of 470 paired swabs were performed during the study. Four hundred and thirty-five were performed during phase 1 of the study and 35 during phase 2 of the study.

Phase 1

During phase 1 of the study, 8/435 (2%) had a positive RT-PCR, 9/435 (2%) had a RT-PCR test failure and 2/435 (0.5%) had an initial LFT test failure. The LFT test failures were immediately repeated on a second cassette with the same extraction solution and were successful. The relative risk of (RR) test failure was significantly lower with LFTs than RT-PCR (RR 0.98 (95% CI 0.96 to 0.99), $p=0.004$). 68/435 (16%) had their RT-PCR sample taken as an ET aspirate, and all results of these tests (RT-PCR and LFT) were negative. 431/435 (99%) swabs were performed on asymptomatic children. The correlation between RT-PCR and LFT test results are shown in table 1.

Phase 2

Twenty-nine of the 35 anterior nasal swabs taken in the second phase of the study were RT-PCR positive. Of children swabbed in the second phase, 43% (15/35) were asymptomatic. Six patients with negative RT-PCR tests also had negative LFT tests.

Table 1 Matrix of RT-PCR and LFT findings along with calculated sensitivity, specificity, positive and negative predictive values

	RT-PCR positive	RT-PCR negative	% (95% CI)
LFT positive	2	0	PPV 100% (18% to 100%)
LFT negative	6	418	NPV 99% (97% to 99%)
% (95% CI)	Sensitivity 25% (4% to 59%)	Specificity 100% (99% to 100%)	

LFT, lateral flow test; NPV, negative predictive value; PPV, positive predictive value; RT-PCR, reverse transcriptase PCR.

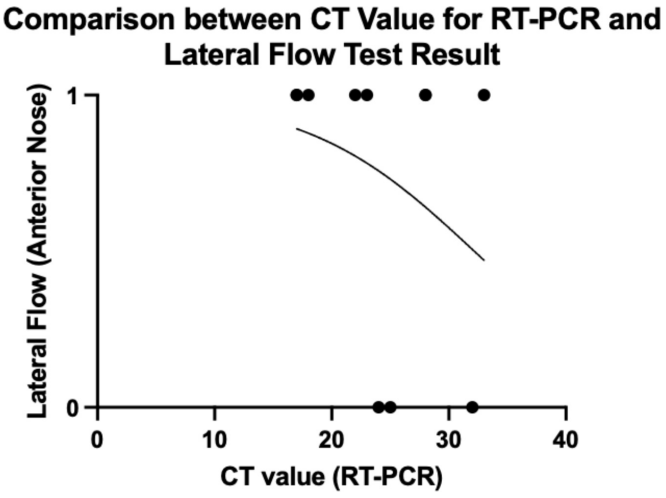

Figure 1 Comparison between cycle threshold (CT) value for reverse transcriptase PCR (RT-PCR) and the result of lateral flow test (1—positive, 0—negative).

Fourteen paired swabs taken from the anterior nose were compared using RT-PCR and LFT, and one was negative for both. Ten of 13 LFTs were positive, demonstrating an overall sensitivity of 77% (95% CI 50% to 92%). There was no significant correlation demonstrated between CT value (provided in 11 samples) and LFT result (OR 0.87 (95% CI 0.62 to 1.12), p=0.32) (figure 1).

Twenty-one paired swabs compared anterior nasal RT-PCR and buccal LFT, and 16 were RT-PCR positive. Fifteen out of 16 positive RT-PCR swabs were negative on buccal LFT and one was positive.

DISCUSSION

Testing has been a cornerstone of controlling the spread of SARS-CoV-2 globally and remains important in healthcare settings. The use of LFTs for near-patient testing was a novel development resulting from the SARS-CoV-2 pandemic. The speed of introduction and initial paucity of real-world data was a source of significant disagreements in the scientific community as to their role and utility in detection of infection and control of spread of infection. It was in this context that this study was conducted.

Lateral flow testing is recommended for asymptomatic individuals coming into contact with clinically vulnerable people, those who are isolating and those who are working in high-risk environments.[6] Furthermore, many children being admitted to hospital and those undergoing an AGP are routinely tested for SARS-CoV-2. The UK Health and Security Agency (UKHSA) recommends that rapid or near-patient testing should be available for risk mitigation of infection prevention and control,[7] and the Royal College of Paediatrics and Child Health 'Recovery of Elective Surgery Guidance'[8] has recommended the use of a rapid test, rather than RT-PCR, before an AGP because of the ability to perform it just before the procedure. Despite this guidance, many centres continue to use RT-PCR as the screening test before procedures and at the time of admission to hospital.

This study demonstrates that the Innova LFT test failure rate is much lower than has been previously reported and the false positive rate is very low and consistent with previous studies.[9] It also highlights that the negative predictive value of the test is extremally high in asymptomatic children within hospital settings, primarily a reflection of the low prevalence of SARS-CoV-2 in asymptomatic children in this setting. A recent systematic review reviewed the sensitivity and specificity of eight different brands of LFT and demonstrated that the specificity of the majority of these tests is above 96%.[2] In-keeping with the findings of phase 2 of this study, the sensitivity of LFTs are shown to be over 70% for the majority of tests studied too.[2]

This study demonstrates that buccal swabbing for SARS-CoV-2 is not an appropriate means of testing for the virus. The correlation between RT-PCR and LFT when both swabs are taken from the same location appears to be higher than when the PCR is taken from the nose and throat and the LFT from the anterior nares, although this study does not demonstrate this conclusively. However, it is suggestive that swabbing the anterior nares is less sensitive than swabbing the nose and throat. The method of swabbing chosen may depend on the importance of having an absolutely accurate result and on how well the child tolerates the swab.

When contemplating the approach to testing asymptomatic children for SARS-CoV-2 within hospital settings, the reasons for selecting a test should be considered. For adults undergoing surgery, there is evidence that recent infection within the preceding 7 weeks increases the risk of adverse outcomes,[10] but the same has not been shown in children.[11] As RT-PCR tests continue to detect viral particles for an extended period following acute infection, there may be an advantage of using RT-PCR in preoperative adults to detect recent asymptomatic infection. Children are more likely than adults to have asymptomatic SARS-CoV-2 infection[12] but as the risks of adverse outcome after surgery do not appear to be increased in children following asymptomatic SARS-CoV-2 infection, the requirements of the test differ. For children undergoing AGP when they are asymptomatic and SARS-CoV-2 positive, the primary risk is that of transmission to healthcare staff rather than an increased risk of adverse outcome to the child. The requirement of a test is, therefore, to detect those children who are infectious with SARS-CoV-2 prior to undergoing AGP. The viral load of a person infected with SARS-CoV-2 changes substantially during the incubation period of the virus before symptoms start.[13] Current UK recommendations highlight that testing should be performed as close to the time of the procedure as possible but it is acceptable to test up to 72 hours before an AGP[8] which many continue to enable time for an RT-PCR test to be processed. The advantage of a point-of-care test (POCT) such as LFT is that they can be performed just before a procedure. Modelling performed by UKHSA demonstrates that the risk of performing an AGP on an asymptomatic SARS-CoV-2-positive child is reduced by performing an LFT within 6 hours before the procedure compared with an RT-PCR test performed 36 hours before the procedure.[8] The high negative predictive value and specificity of the Innova LFT demonstrated in this study highlights that there would be very low numbers of children requiring to be cancelled 'on the day' due to either a true positive or a false positive test.

Strengths and limitations of the study

This study demonstrates the 'real-life' applicability of LFTs within a clinical environment. Tests were performed by clinical staff within their routine roles and therefore demonstrates the clinical applicability of the approach to testing. The study is significantly limited by the inability to achieve adequate power to determine a more precise sensitivity of the LFTs compared with PCR. Variability in PCR testing processes (platforms) is likely to also have played a role in the inability to identify a relationship between the LFT result and the CT value at PCR.

CONCLUSIONS

The Innova LFT is shown to have a positive and negative predictive value and specificity over 99% compared with RT-PCR. When RT-PCR and LFT are performed on swabs from the same region, the sensitivity is good—approximately 77%. The sensitivity of LFT performed on anterior nares swab compared with nose and throat PCR appears to be reduced, although this study does not demonstrate this conclusively. Careful consideration of the reason for swabbing, the tolerance of the child to swabbing and the requirements for test processing (eg, rapidity of results) should be undertaken within hospital settings.

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Contributors RH, EW, TF, HW, SD, SP, AF, NG and SK conceived and designed the study. RH, LR, CK, ES and MR undertook the delivery of the study, recruiting patients, inputting data and overseeing trial quality in individual centres. RH wrote the manuscript and all authors reviewed, edited and approved the manuscript before submission. RH is the guarantor of this paper.

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Competing interests Public Health England, now UK Health and Security Agency, supplied the lateral flow tests used within the study and contributed £100,000 towards the running of the study.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by 20/HRA/6152City and East NHS REC: 292509 Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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The LAVA (Lateral flow Antigen Validation and Applicability) 2 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 diagnosis is the reverse transcriptase polymerase chain reaction (RT-PCR), performed on a sample from the respiratory tract. 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. 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 net result is that the high sensitivity and specificity of RT-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 infectious individuals, for example people who are about to undergo a high-risk aerosol generating procedure (AGP), enter a crowded emergency room, or for rapid investigation of localised outbreaks.

Alternative methods and modalities of testing have 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 device (LFD)(4). Antigen based tests 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 we have shown 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, 4) it is intended to detect patients who are infectious and will not detect those who are still shedding non-infective viral particles. 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. Laboratory testing suggests that the analytical sensitivity is approximately 50% overall, but this increases to approximately 90% when the viral load is >60,000 (RT-PCR cycle threshold <27)(5). Contrary to some media reports, the performance of lateral flow antigen tests to detect people harbouring live, culturable virus, has been shown to be good compared to PCR, where results can be positive when there is no viable virus detected(6). The specificity of antigen tests has consistently been reported to be very high, with recent clinical screening in a schools setting showing a specificity of 99.6%(5). 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 LFDs to defeat COVID-19 are evident, with several clinical scenarios below demonstrating areas where comparing the performance of RT-PCR to LFD could improve the care of children and improve the safety of staff in hospital. However, evaluation of the performance of these tests in clinical settings is essential before full implementation in routine clinical practice is advised in order to understand opportunities and limitations.

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(7). 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 also their parents who are likely to be infected as a household contact. Additionally, early identification of children with COVID-19 using LFD has the potential to guide the level of PPE required by staff delivering routine care.

Children being admitted for elective procedures are currently also routinely tested for COVID-19 using RT-PCR 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 influenza like illness (ILI) symptoms often precludes an anaesthetic being performed. In this population, LFDs could be used to identify children who were incubating SARS-CoV-2 at the time of RT-PCR swab but were not shedding the virus, who have subsequently progressed to shedding the virus (Table 1). Performing a LFD potentially enables infectious children to be identified immediately prior to a procedure and can be used to guide decisions around whether the case should proceed and the use of PPE in children if the procedure goes ahead.

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 PCR test. It can be seen that half of individuals testing negative for the virus will be infectious and shedding virus 72hours after the swab, the maximum recommended interval between preoperative test and procedure.

Finally, LFDs 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. LFDs may be utilised as a screening tool for infectious patients in these areas to support the use of appropriate PPE.

Validation and usability in a controlled clinical setting is recommended prior to use in the wider community. Mathematical analysis of LFD results 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 2).

	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 2. 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. The case for screening tests becomes much stronger as prevalence increases, leading to higher positive predictive values (PPV). NPV: negative predictive value.

This study aims to determine the sensitivity of a lateral flow antigen test for children with a high viral load which can be performed using an anterior nose swab in children who are undergoing any routine testing for SARS-CoV-2. The relationship between viral load and infectivity is clear, with multiple publications demonstrating the association between high viral load and the ability to replicate the virus in culture conditions(8-10). However, there is not a clear cycle threshold (CT) value (a measure of viral load) which determines infectivity.

Differences in assays and machines used to perform RT-PCR mean that the CT value associated with a particular viral load are not completely uniform. Data from Public Health England show that, in laboratory conditions, over 90% of individuals with a CT <28 are antigen positive. However, it is likely that over 25% of people with a CT value <31 have virus which can be cultured, this drops to under 10% in those with a CT value of 35 or over(8). Research into the association of CT value, viral load and infectivity is ongoing, with some early results now being available(11).

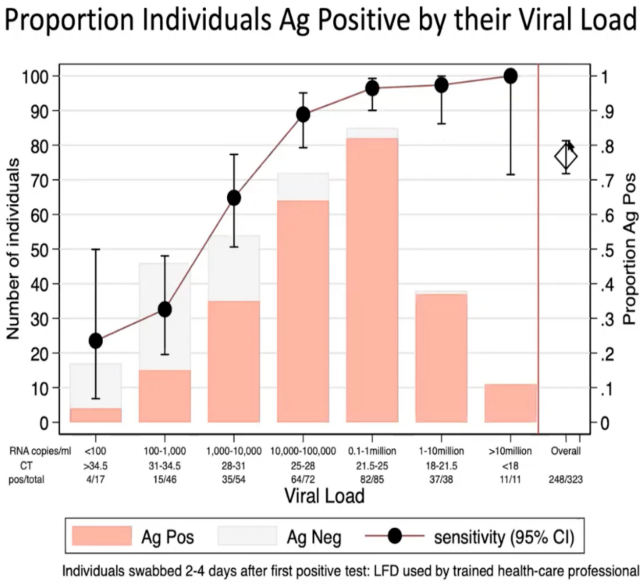

Figure 1. Association between viral load and antigen positivity (Data and figure supplied by PHE).

Pilot study

322 lateral flow device (LFD) tests were performed, 186 in Alder Hey Children's Hospital, 136 in Manchester Children's hospital during a recruitment period of 2 weeks in each centre. 316 LFD results were paired with an RT-PCR result.

297/322 tests were performed on children who were asymptomatic with only 25 being performed on symptomatic children. 15/322 (5%) of swabs were performed on children being admitted through A&E, 189/322 (59%) were performed on children who were undergoing routine pre-operative swabbing or undergoing peri-operative swabbing as part

of the study. 118/322 (36%) underwent lateral flow testing simultaneously with routine screening for COVID-19 whilst an inpatient on ICU, HDU or the transplant ward.

Symptom	Number (%) n=25
Fever	8 (32%)
Cough	13 (52%)
Shortness of Breath	8 (32%)
Wheeze	3 (12%)
Runny nose	6 (24%)
Ear Ache	1 (4%)
Lethargy	5 (20%)
Off feeds / reduced intake	2 (8%)
Myalgia	1 (4%)
Abdominal pain	2 (8%)
Vomiting	3 (12%)
Rash	1 (4%)
Headache	2 (8%)
Seizures	2 (8%)

Table 1. Symptoms in symptomatic children undergoing lateral flow testing

Patients and parents pain scores were collected whenever possible. Children who had a parental pain score recorded for the anterior nasal swab but not for the nose and throat swab were excluded from analysis as these children were undergoing peri-operative swabbing and had already undergone a swab before their operation. Children who found the previous swab too distressing may have opted not to have the peri-operative swab so inclusion of these scores could skew the data. Analysis was performed by testing for normality, which was not found, and therefore paired Wilcoxon Rank Sum Test was performed. The median child reported pain score for the anterior nasal swab was 0/10 (IQR 0-2) and for the nose and throat swab was 4/10 (IQR 2-4). The median parent reported pain score for the anterior nasal swab was 0/10 (IQR 0-2) and for the nose and throat swab was 6/10 (IQR 2-8).

Version 5 IRAS: 292509
3rd June, 2021

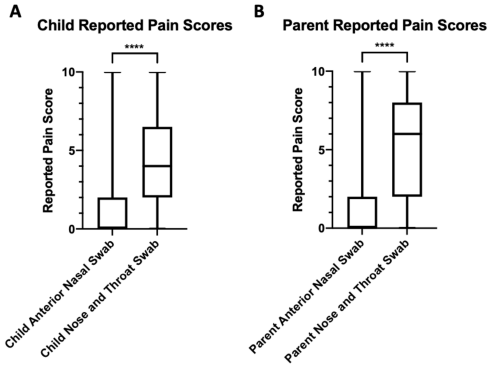

Figure 1. A: Child reported pain scores using Wong-Baker Faces Scoring chart, B: Parent reported pain scores.

The median time to test result was 0 days (IQR 0-0) for LFD and 1 day (IQR 1-1) for PCR (Figure 2).

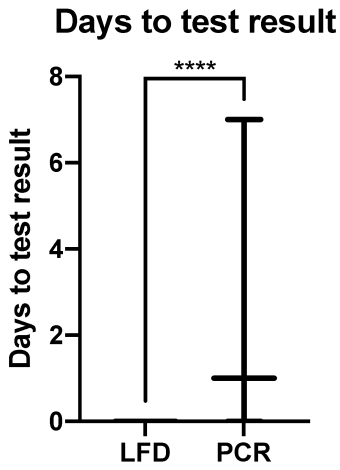

Figure 2. Time between swab and finalised test result being available.

The correlation between LFD finding and PCR findings are displayed in table 2. These data suggest that the sensitivity of the swab for all-comers in hospital is 25%. However, when we assess the test using a threshold of a CT of <35 as a marker of moderate and high viral load, we see in this small data set that the sensitivity is good. The specificity 100%. The positive predictive value is 100% and the negative predictive value 99% (calculated without including prevalence data). The patient who was LFD and PCR positive was symptomatic, the three patients who were LFD negative but PCR positive were asymptomatic. Cycle Threshold (CT)

values were measured on two different devices, the Cephid and the Roche 8800 platform. Standardisation between the two different machines has not been undertaken as part of this project.

		PCR			
		Positive	Negative	Test Failure	Not Performed
LFD	Positive	1	0	0	0
	Negative	3	307	1	6
	Test failure	0	4	0	0
		Positive, CT<35	Positive, CT ≥35 or negative	Test Failure	Not Performed
LFD	Positive	1	0	0	0
	Negative	0	310	1	6
	Test Failure	0	4	0	0

Table 2. Lateral Flow Device results.

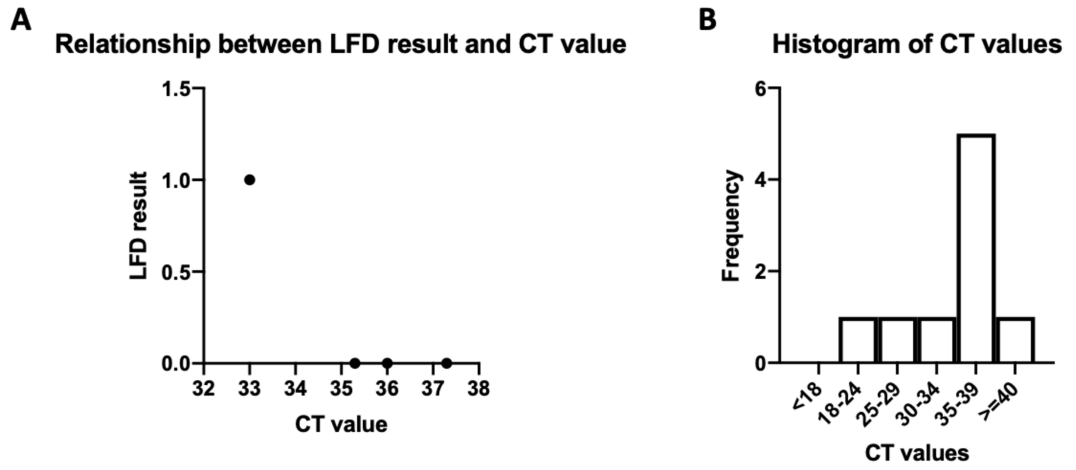

Figure 3. A: Relationship between LFD result and CT value B: Distribution of CT values

During the two week study period, the CT values of all patients with a positive RT-PCR test at Alder Hey were collected in order to inform the normal distribution of PCR results. Whilst this may differ depending on whether prevalence is increasing or decreasing they are useful

to help to inform a power calculation. The findings show that the majority of children have high CT values. Approximately half of the positive results were in patients undergoing screening/admission swabs as inpatients and half were in patients attending for pre-operative screening. There was a range of CT values in both groups.

Lessons Learnt for LAVA 2 study

- Recruitment: The method of recruitment used for this study was time consuming for the research nurses and was difficult for patients to undertake remotely and return. We have developed electronic consent forms using Redcap which can be emailed to patients and completed online without needing to be emailed back.
- Recruitment: The uptake of the study was generally good. The concerns which parents expressed related to being concerned that an additional swab may be distressing to their child and the risk that an elective surgical procedure may be cancelled. We have shown that the anterior nasal swabs are associated with no or very mild pain in the vast majority of cases, making them an acceptable test. Given the parental concern about cancellation of elective procedures we will always gain consent *before* performing a peri-operative swab.
- Training: Training of the use of lateral flow devices was effectively performed and very few test failures were observed. The roll-out of staff testing using the same device means that all staff are now trained in the use of the kit and reduces the burden of staff training for LAVA 2.
- Data collection: Data collection using the power-app at Alder Hey had positive and negative aspects. Real-time data collection meant that it wasn't time consuming or difficult, however, issues with connectivity meant that at times it was frustrating. A new data collection form has therefore been developed which can be completed contemporaneously or retrospectively but photographs are still requested. These can be uploaded in retrospect to the patient record.

3. Research Questions:

All outcome measures relate to comparison of the Innova Lateral Flow Device (LFD) performed on an anterior nasal swab compared to RT-PCR performed on a nose and throat swab or ET aspirate.

1. Primary Outcome Measure:
    a. The association of LFD result (positive/negative) taken from an anterior nares swab compared to RT-qPCR finding (Cycle Threshold Values)
    b. The association of LFD result (positive/negative) taken from a buccal swab compared to RT-qPCR finding (Cycle Threshold Values)
2. Secondary Outcome Measures - to be addressed through previous iterations of the protocol:
    a. The sensitivity of the LFD compared to RT-PCR with CT <28
    b. The sensitivity of the LFD compared to RT-PCR with CT <33
    c. The overall sensitivity of the LFD compared to RT-PCR.
    d. The overall specificity of the LFD compared to RT-PCR.
    e. Sub-analysis
        i. The sensitivity of the LFD compared to RT-PCR performed on nose and throat swabs (overall and high viral load).
        ii. The sensitivity of the LFD compared to RT-PCR performed on endotracheal (ET) aspirates (overall and high viral load).
        iii. The sensitivity of the LFD compared to RT-PCR performed on anterior nasal swabs (overall and high viral load).
        iv. The sensitivity of the LFD compared to RT-PCR performed on buccal swabs (overall and high viral load).
        v. Comparison of the sensitivity of LFD to RT-PCR in nose and throat swabs taken in awake patients and those taken under anaesthetic.

4. Rationale for this work:

As the prevalence of COVID-19 is once again high in the UK, the need for effective testing strategies is essential. Whilst combined nose and throat RT-PCR currently remains the gold standard diagnostic test, alternative testing modalities may provide benefits that cannot be realised using RT-PCR. Point of care testing has the potential to rapidly identify infective patients that may improve the management of the patient and enable better protection of staff. LFDs have been shown to have a very high specificity when used in clinical and non-clinical settings(5). Laboratory testing has shown that the sensitivity of the test is high when the viral load is high, but when low numbers of

plaque forming units are present, the sensitivity is reduced. Whilst this means that the LFD is not a diagnostic test, it does mean that it has utility in detecting patients who pose a risk to infecting others. This is particularly important in in children undergoing an aerosol generating procedure. These children are currently having a nose and throat swab performed up to 72 hours pre-operatively when attending for an elective procedure, or upon admission if they are admitted as an emergency. Current Personal Protective Equipment (PPE) is determined by the level of prevalence of COVID-19 in the UK, along with the patient's pre-operative swab result(12). The time delay between a swab being performed and the operation taking place means that a person who was incubating but not shedding the virus could be doing so at the point that they undergo anaesthetic. Using a LFD immediately pre-operatively may allow this to be detected and enables the protection of healthcare workers by either delaying the procedure or wearing aerosol precaution PPE. It may also protect other children in the hospital by ensuring that other AGPs such as extubation are performed in theatre, rather than in the recovery area. Conversely, we know that a person can remain PCR positive for a long time after they have had the virus, when they have very low levels of virus within their body. This may mean that children's operations are delayed due to a positive swab when they no longer pose a risk to those around them. Utilising an LFD rather than PCR in this situation may result in children not being inappropriately delayed for surgery.
Stratification of risk using LFDs could be implemented in other areas of the hospital. Performing an LFD test when children are admitted enables rapid identification of children who pose the most risk to those around them if positive, ensuring that they are isolated appropriately and that PPE is used, reducing the risk to healthcare workers and other patients in hospital.

Furthermore, lateral flow tests are being used in schools. By matching lateral flow with RT-PCR in children's hospitals, understanding of the utility of lateral flow testing in schools at times of increased prevalence will be increased.

Innova LFD tests can be performed on nose and throat swabs or anterior nasal swabs. Anterior nasal swabs are being used in this study for two reasons. Firstly, as shown in the pilot study, they are far more acceptable for children compared to nose and throat

swabs. This means that, particularly for children who have to undergo repeated swabbing, they are much more amenable and less distressed by the experience. Secondly, LFDs may not only have a role in clinical settings but also in community settings such as schools. Repeated acquisition of a nose and throat swab as a screening tool in schools will be untenable for both children and staff. Testing the devices using a swab which is more acceptable and can safely be obtained by an untrained individual is essential before they move into mainstream use.

5. Design

This will be a multi-centre comparative study of a medical device.

6. Basic Demographic Data/subjects

Any child < 18 years of age who has had a positive RT-PCR swab within the past 72 hours within a participating centre.

7. Methods

Inclusion criteria:

1. Child with a recent positive PCR (within the past 72 hours) which was not paired with a lateral flow test.

Exclusion criteria:

None.

Training and Engagement

Training and engagement of staff in this study is key to its success and therefore sufficient emphasis should be placed on the role out of training for both using the LFD and recording the results of the study in each site. The method of delivery of training will differ according to site set-up but linking with the laboratory, the point of care team, nurse educators and nursing and clinical leads within each department is essential. Training will be delivered by the study team in person, whenever practical to do so, to each site who will then disseminate training locally. A detailed SOP and video is available for performing and training for using the lateral flow test.

Version 5 IRAS: 292509
3rd June, 2021

Identification of participants

Children who have had a positive RT-PCR within the last 72 hours (whether or not it was paired with a lateral flow test) will be identified either by a member of the direct care team or by a member of the research team.

Information and consent

The pilot study has shown that the anterior nasal swab is associated with significantly less pain than the nose and throat swab and is therefore a very acceptable test. Buccal swabs are routinely performed in clinical practice and are also generally viewed as acceptable swab types for children and young people. Parents will be approached by the direct care team, or a member of the research team to discuss the study with the family. If the family are happy to receive further information they will request the patient or parent's email address. They will then be approached by email link to read the PIS and consent to gain consent for entry into the study.

Consent for all patients will be taken electronically using a study and patient-specific weblink which is emailed to the parent/guardian or patient. This will be based in RedCap and has been created by the Liverpool Clinical Trials Unit. Each centre will have access to their own patients' records and completed forms will be held securely on the University of Liverpool's server.

Details taken within the electronic consent will enable accurate identification of each child using their local hospital number so that as they move through the hospital system these details will be able to be used to accurately identify which children are consented to be in the study, rather than using a study number to do so. The consent form will be held on the Redcap system, be emailed, given or posted to parents and will be placed into the patient notes.

Procedures

Children will undergo three additional swabs - two which will be taken from the anterior nose for the lateral flow test and the RT-PCR and one from the cheek just inside the mouth for the lateral flow test.

Prior to performing the anterior nasal swab, any nasal mucous will be removed by wiping or blowing the nose if a child is coryzal and then a thin swab will be rubbed on the inside of the nose, below the level of the inferior turbinate. 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). The buccal swab will be rubbed inside the cheek for approximately 10 seconds.

Children who have had a recent positive RT-PCR in the previous 72 hours which was not already paired with a lateral flow test will be approached. Participating in the study will entail having an additional two anterior nasal swabs for the lateral flow test and for RT-PCR.

Study process (Participant):

After a child, their family or both, have agreed to participate in the study they will undergo two anterior nasal swabs and a buccal swab. If the child or family do not want 3 additional swabs performing, they can opt just to have a lateral flow test swab performed as a buccal or anterior nasal swab. If the repeat PCR swab is negative the family will still be asked to continue isolation on the basis of the initial positive swab, in line with government guidance. The family will be informed of the results of the LFD or the PCR if they are positive.

Children who have been discharged from the hospital and whose swab is then returned positive, or who have had a swab performed as an outpatient which is positive, will be approached by the study team to return for the repeat RT-PCR swab and lateral flow swab. Families will be asked to travel by private transport to avoid infecting others. They will be offered re-imbursement for their travel costs from their home address to and from the hospital.

Swabbing process:

The anterior nasal swabs will be taken one after the other - one used for the lateral flow test and one used for RT-PCR. The buccal swab can be taken before or after the anterior nasal swabs. The healthcare worker taking the swab should wear PPE in line with hospital policy for performing a nose and throat swab for a positive patient. The anterior nasal swabs will be rolled 5 times on the inside of each nostril to ensure that mucous and cells are collected and can be performed by members of the direct care team or research team. The buccal swab will be rubbed inside the cheek for approximately 10 seconds.

The lateral flow assay should be performed as soon as possible after the sample is taken and is performed at room temperature. Gloves should be changed before taking the specimen tubes out of the bag to avoid contamination of the other tubes, if the swab has already been taken. The antigen is extracted from the swab by adding 6 drops of extraction solution to the labelled 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 and 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:

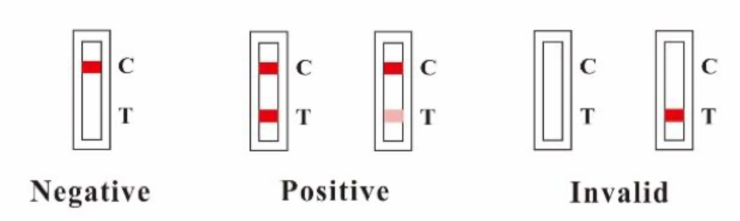

Figure 5. Results using Lateral Flow Devices

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. There is enough extraction fluid to perform a second test in the event of a test failure,

therefore this is recommended in this event. Both tests should be recorded within RedCap or on the CRF and the specimens will be disposed of in clinical waste.

Clinical Data Collection(13):

This will be performed using a RedCap based system which is being built and validated by the Liverpool Clinical Trials Unit. Consent for the study will be taken through a patient and family portal into the system. Nursing and medical staff who have been trained to perform LFD swabs and the LFD assay will be given access to the system. They do not need to be GCP trained in order to do this. This will maximise recruitment of patients from within one centre and will ensure that the flow of data about each patient is accurate, whilst still ensuring that the patients and their family remain fully informed about the study. The system will allow for patient lists to be made to enable accurate identification of patients who are recruited to the study e.g. lists of children attending for outpatient swabbing for each day which will be complied by an administrator. The data from the LFD will be recorded in the patient notes and contemporaneously in the data collection tool.

The following data will be held about the patient:

First 3 letters of postcode:

Hospital number

Sex

DOB

Reason for swab

1. Screening for elective admission / procedure
2. Peri-operative swab
3. Symptomatic child of member of staff
4. New admission to hospital
5. Routine screening of inpatient
6. Clinical suspicion of SARS-CoV-2 in inpatient
7. Known positive

Symptoms

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

Date swabs taken:

Specimen type used for lateral flow device: Buccal, Anterior Nasal

Lateral flow device result: positive / negative / test failure

If test failure, reason for failure:

Absent control line

Unable to confidently differentiate whether result line is present or absent

Other, Details:

Unknown

Second lateral flow assay result if initial test failure: positive / negative / test failure

If test failure, reason for failure:

Absent control line

Unable to confidently differentiate whether result line is present or absent

Other, Details:

Unknown

Specimen used for RT-PCR: Anterior nasal, Nose and throat swab or ET aspirate:

If nose and throat - taken awake or under general anaesthetic

RT-PCR swab result:

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

Cycle threshold of RT-PCR if positive:

Machine used to determine CT:

Laboratory used to determine CT:

The results of RT-PCR will be entered by a research administrator into the database.

Correlation assay in participating centres

A correlation assay will be performed using the PCR assays and machines used to analyse nose and throat swabs in participating centres. This will enable external quality assurance to be undertaken and will enable differences in CT values to be accounted for. 2x7 specimens of inactivated SARS-CoV-2 along with a negative control will be given to participating laboratories in logarithmic increments of PFUs/ml to determine the CT value associated with known viral load. The specimens will be provided by E Adams at the University of Liverpool. A comparison of CT values can then be undertaken between centres and standardised to enable appropriate comparison.

8. Data Monitoring

The steering committee will operate under the terms of reference stated within Appendix 1. They will oversee the data monitoring process and will undertake any required reviews, within some or all centres that are required.

Data monitoring will be devised and undertaken by the sponsor who will report to the steering committee.

9. Data Management

Data will be held on a central server at the University of Liverpool. Each site will have their own logins which only give users access to the data for their centre. Patients will be asked to consent for their data being held for the duration of the study and archived for 10 years after completion of the study. No patient identifiable data will be published or be made available and the data will not be used for any other activity unless permission is given by the participant. Data will be archived by Alder Hey Children's NHS Foundation Trust.

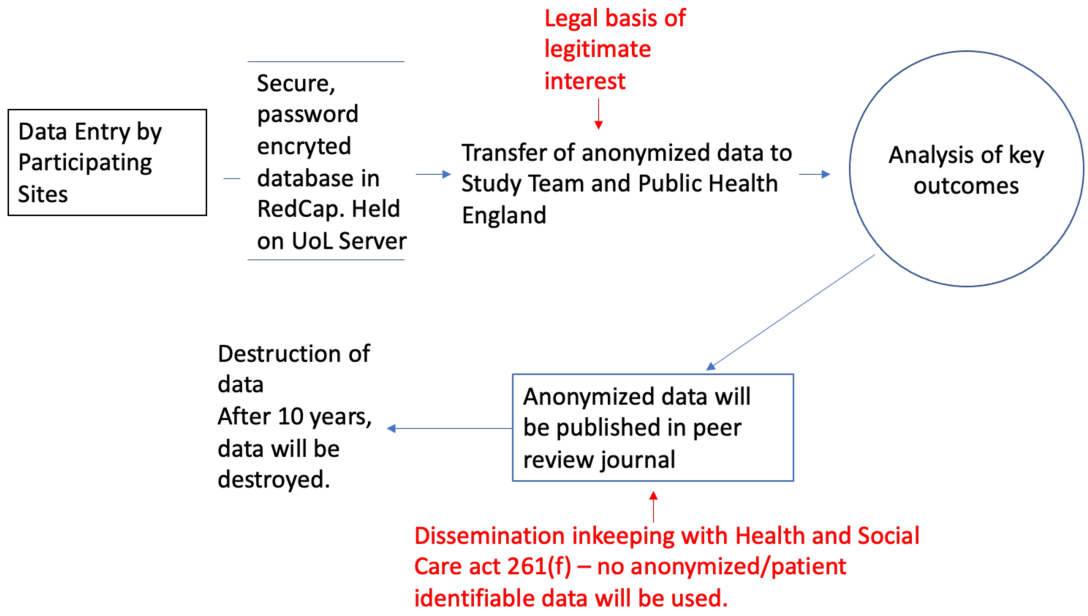

Figure 6. Data Flow and Data Management

10. Site engagement and Recruitment Target

Updated after steering group meeting

A steering group meeting undertaken 6 weeks into the trial has found that recruitment has been slower than anticipated in the 3 recruiting centres due to now resolved problems with the RedCap eConsent system and reduced uptake of the study compared to the pilot study. Current prevalence of <0.2% is lower than the prevalence seen during the pilot period of the study when prevalence was ~0.5%. This means that to recruit 24 positive patients, 12,000 patients in total would need to be recruited. This is not feasible within the constraints and funding of the study. The steering group has therefore decided to focus on the primary aim of the study

which is to determine the relationship between CT value and LFD result, both taken with an anterior nasal swab. The results collected to date will be used to describe the specificity of the test. Known positive patients will be approached to enable selective recruitment of known positive patients. The number of RT-PCR positive paired swabs required to answer the primary study question remains 24. 24 paired swabs for buccal and 24 paired swabs for anterior nasal samples will be required. Some patients may have both swab types performed and therefore contribute to the numbers for both of these targets. Five centres will be requested to recruit 7 patients each. Prior to closure of the study, the CT values of the results will be reviewed to ensure that the range of CT values required to answer the study question are represented.

11. Statistical Analysis

An interim analysis of the primary outcome will be performed by the PHE mathematical modelling team 6 weeks and 9 weeks after the first centre has started to recruit patients to monitor whether the expected number of positive patients have been recruited and that these patients are displaying the expected range and distribution of CT values required to answer the study question. If an insufficient number of positive patients have recruited, proportional to overall recruitment, the steering group will meet to discuss whether it will be necessary to increase the recruitment target. When sufficient numbers of positive patients have been recruited in order to answer the study question, the study will be terminated early.

Demographic details of patients included in the study and their patient group will be described.

The primary outcome will be analysed by using 'corrected' CT values based on external quality control findings and evaluating these compared to the findings of the lateral flow device (positive or negative). Results will be analysed using univariate logistic regression analysis and will be displayed using a ROC curve to display sensitivity.

Statistical significance will be taken as $p<0.05$ to determine the relationship between the two variables.

Secondary outcomes will be analysed using a 2x2 contingency table determined by the outcome which is being examined. McNemar's test will be used to determine the Sensitivity and Specificity of the test under the conditions being assessed.

Prevalence will be used to determine the PPV of the LFD in each of the sensitivity/specificity secondary outcomes described above.

12. 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.

13. Dissemination

Anonymised results of this work will be shared with Public Health England and will be published in an open-access peer reviewed journal.

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Version 5 IRAS: 292509
3rd June, 2021

Appendix 1.

The Lateral Flow Antigen Applicability and Validity (LAVA) 2 study Steering Group Terms of Reference

Purpose
The purpose of the steering group is to carry out the following tasks:

- To determine the Primary and Secondary aims of the study
- To critically review the study methodology
- To monitor recruitment at each site
- To monitor for any safety issues
- To oversee data monitoring within the study period and undertake reviews of processes within centres in collaboration with the CI and PI when data discrepancies are detected within the audit process.
- To be responsible for the analysis of the data and the production of any reports or publications or both resulting from this study.

Membership

- The steering group will be made up of a multi-disciplinary team of stakeholders in the testing of children for COVID-19 in the UK in clinical settings.

Defining Roles/Duties

- Simon Kenny will act as chair of the steering committee
- PIs for each centre will report on their centre's experience of the study and identify any successes and challenges which are important for the group when the study is ongoing.
- The CI will have oversight of the running of the study at each site and is responsible for determining and communicating any problems which arise
- Nick Gent and Hannah Williams will undertake determining the sample size required to answer the study question and statistical analysis of the results of the study.

Quorum
Meetings can be held if either the chief investigator or Professor Kenny are able to attend with a minimum of three members of either the PIs or the steering group.

Meetings

- The steering group will meet virtually, in part or in full, on alternate weeks during the study period to identify any problems and to review the data monitoring which has been occurring.
- Additional meetings will be called in the event of a serious breach of protocol (identified by the PI or CI) and 48 hours' notice will be given in the event of this occurring.
- Minutes of the meetings will be taken and distributed to the study group as a whole.

Authority
Decisions will be taken by the group as a whole, with a process to meet consensus whenever possible. Professor Kenny makes the final decision if an occasion arises when there is not consensus within the group.

Reporting
Minutes will be taken for each meeting and will be shared amongst the group within a week of each meeting.

Dissolving the Steering Group

- The group will be dissolved when the study is completed, written up and published.