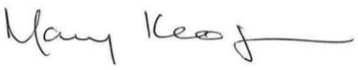
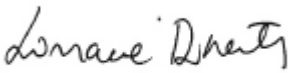
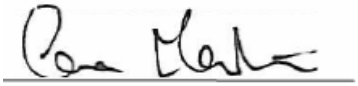
Title: COVID 19 Antigen Test Validation Summary Report

Date: 4th June 2021

Report produced by the HSE COVID-19 Antigen Testing Working Group

COVID 19 Antigen Test Validation Project Summary Report – Final Approval

	TITLE	SIGNATURE	DATE
Clinical Review/ Approved by:	Prof Mary Keogan Clinical Lead National Clinical Programme for Pathology & Consultant Immunologist		04/06/2021
Clinical Review/ Approved by:	Dr Lorraine Doherty, National Clinical Director in Health Protection Public Health		04/06/2021
Scientific Review / Approved by:	Prof Cara Martin Scientific Lead for HSE Antigen Validation Project & Assistant Professor in Molecular Pathology Trinity College Dublin		04/06/2021

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Acknowledgements:

The work was supported by the RADT Validation testing team- Project Manager: Dr Barbara Hughes, Scientists: Anita Martin, Christina Rushe, and Orin Fagan and Research Nurses: Sheila Sugrue, Sophie Trainor and Mary Mc Mahon.

We thank the staff at Beaumont Hospital COVID19 Testing Pod, Letterkenny University Hospital, University Hospital Limerick, University Hospital Galway, Citywest Hotel COVID-19 Community Testing Centre, Croke Park Handball Alley COVID-19 Community Testing Centre and The National Show Centre COVID-19 Community Testing Centre for facilitating the project.

We thank the DAFM scientists and the meat processing plants for supporting and facilitating the validation project.

Finally, we thank all the participants for volunteering to take part in this validation project.

Executive Summary

The Irish Antigen Project Evaluation Working Group was established in October 2020, to perform independent and site-specific validations in Ireland. It has undertaken desktop evaluations to identify assays suitable for further evaluation, and site-specific evaluations in acute hospitals, in meat processing plants and in community swabbing centres. The validation has focussed on symptomatic individuals in line with intended use, with one of the assays also extensively validated in a cohort of asymptomatic individuals. A total of seven antigen test have been validated/verified, 6 lateral flow tests (LFT's) and one microfluidic device with reader.

The key findings from this validation work are as follows:

- Reverse transcription polymerase chain reaction (RT-PCR) is the gold standard test for detection of SARS-CoV-2. It is the most sensitive technology for detection of SARS-CoV-2 virus components in a sample when the virus components are present at low levels. The currently available antigen diagnostic tests (ADTs) require higher levels of virus components in the sample to generate a positive result, when compared to RT-PCR.

- Although the threshold for virus detection with currently available SARS-CoV-2 ADTs is higher than for RT-PCR tests, ADTs may have applications in specific contexts, due to their suitability for deployment in different settings, faster turnaround times or because there is a clinical preference for a test that only detects the virus when it is present at higher levels.

- A preference for detection of virus only when present at high levels could arise if there was evidence that SARs-CoV-2 was only significant in clinical or public health terms when present at high levels. Based on current evidence, people can be seriously ill and can be infectious or be about to become infectious - even when the virus is only detectable at low levels. Therefore testing systems that detect virus at the lowest possible level are generally preferred.

- When tested in a real-world setting, validation of the actual performance of ADTs within their intended use, in accordance with manufacturer's instructions highlighted significant differences from manufacturers claimed test performance. This finding is in line with international experience, and highlights the importance of defining the performance characteristics of the ADTs compared to the gold standard method to make good decisions about how those tests should be used with maximum benefit and the least harm.

- The validation work focussed on verification of the performance of several ADTs as a diagnostic test, for use with both nasopharyngeal and nasal collected sample types in symptomatic individuals. The PanBio COVID-19 Rapid Test (Nasal) was evaluated as an asymptomatic screening test for individuals working in meat processing plants.

- Where a public health risk assessment indicates likely utility of ADTs, for example in a suspected outbreak or among vulnerable populations, testing a series of samples from suspected cases by a method such as ADT, that is readily deployed and provides near real time results can be valuable, even when that method does not reliably detect all infected or infectious people. PCR remains the most sensitive method for detecting infectious virus, however if deploying ADTs in an outbreak setting, nasopharyngeal ADTs with the highest sensitivity, demonstrated by our independent validations, would be preferred over ADTs that use nasal sampling.

- Well characterised ADTs may also have a role as a supplement to RT-PCR testing in the event of circumstances in which PCR capacity is not adequate to meet requirements. In such circumstances symptomatic people with a not-detected ADT would require further testing, either with PCR, or a second ADT 2-3 days later.

- Among asymptomatic workers in meat processing plants with positive RT-PCR results for SARS-CoV-2, 51.9% had a positive test with the validated nasal ADT test (i.e. a test sensitivity of 51.9%). For use with asymptomatic individuals, the sensitivity of the Abbot Panbio COVID-19 Ag Rapid Test (Nasal) even taking into account higher viral RNA levels, is below the minimum requirements set out by WHO and ECDC.

- The lower sensitivity of an individual test can be compensated for to some extent by frequent testing. Modelling studies by HIQA have predicted that if this test is employed in a serial screening programme in meat processing plants, with supervised self-swabbing and ADTs performed by trained individuals, once or twice a week this would be a viable alternative to the current procedure of monthly RT-PCR testing in terms of reducing spread of COVID-19. However, this is modelling data which implies full compliance with a testing regimen of once or twice per week, (by the food and business organisation (FB0) organising the testing, supervised self-swabbing and the workers being tested) which in the real-world setting may not be achievable. We know from the validation work and subsequent implementation of ADTs at meat processing plants that there are significant practical and logistical issues around implementation and operationalising of ADTs in this setting.

- A single ADT, even under optimal conditions of use, will not detect a significant proportion of people who would be identified by RT-PCR as infected and potentially infectious for others. On that basis it is not recommended as a single stand-alone test and is not the preferred method to maximise detection of infected or infectious people.

- ADTs are highly specific, which means that people who test positive by ADT almost always test positive also by RT-PCR. However, it is important to stress that even with a highly specific test (antigen or RT-PCR) the proportion of all positive tests that are false positives increases as number of infected people in the population tested declines. A test system with 99% specificity, is expected to generate an equal number of false positive and true positive test results, if only 1% of the people tested truly has the infection. If we consider the current estimated prevalence in Ireland which is 0.1%, only 1 in 10 positive results will be true positives. Hence as the

prevalence of infection in the population tested decreases, ADT positive results will require confirmation with PCR, to prevent inappropriate isolation or broader public health actions on the basis of false positive ADTs.

- The validation data presented in this report for the different ADTs evaluated in symptomatic individuals should not be generalised to asymptomatic cohorts where the low prevalence of infection, and lower viral loads will affect sensitivity. All validation was undertaken in adults, and should not be extrapolated to children, where lower viral loads may affect the performance characteristics of the test.

- Further real-world studies are required to determine if detection of infectious people by wider deployment of ADT testing has net benefits in the context of potential false reassurance and behaviour change as a result of failure to detect other people who are infectious and to understand the impact of false positive results. Such pilots should include post pilot PCR testing, as well as logistics and cost-benefit analysis

- There may be other settings in which a test that detects some infected or infectious people that would otherwise go undetected can be useful.

- The quality of the sample is a key determinant of the quality of the result. All samples were taken from symptomatic groups by trained swabbers, and the asymptomatic validation was performed by supervised self -swabbing. All testing in this validation project was performed by scientists trained to perform the tests. The results should not be generalised to a setting of self-testing as the clinical performance of tests /kits and interpretation is heavily dependent on the competence of the operator.

- Safe delivery of testing by any method including ADT requires appropriate clinical governance and quality management in relation to implementation, training, competency assessment, testing, resulting, public health reporting and logistical and operations to ensure the appropriate quality assurance standards are met. The logistics of performing ADT in large numbers need careful consideration, with trained individuals able to perform between 50 and 80 tests per day, excluding sampling.

Recommendations

1. RADT tests vary in performance characteristics, and so should not be considered interchangeable.

2. Based on the validation data, the ADT Working Group recommends that the use of ADT be considered as a diagnostic test, in symptomatic people when:

    a. A Public Health risk assessment determines that the rapidity of result availability is a useful adjunct to the available PCR capacity.

    b. In vulnerable communities where follow up of those with positive results is likely to be challenging, often as an adjunct to PCR testing.

    c. As a supplement to RT-PCR based testing in the event of circumstances in which PCR capacity is not adequate to meet requirements. In such circumstances symptomatic people with a not-detected ADT would require further testing, either with PCR, or a second ADT 2-3 days later.

3. For the purposes of asymptomatic screening, a single, stand-alone ADT is not recommended, as a significant proportion of people who are infected, and infectious to others will not be detected.

4. A recent modelling exercise by HIQA, the Health Information and Quality Authority of Ireland, has suggested that with respect to workers in meat processing plants, which are high risk work environments, ADT-based testing of supervised self-collected nasal samples once or twice a week with RT-PCR confirmation of positive results may offer benefit in terms of a potentially increased detection of cases, reduction in infectious person-days circulating, and a reduced overall cost relative to the current practice of monthly RT-PCR testing (5). The ADT evaluated in asymptomatic people in this report is suitable for use where this approach (supervised self-collected nasal samples) is implemented, but close evaluation of the impact of this screening should be undertaken to ensure these potential gains are realised.

5. Clinical governance and quality management of all aspects of sampling and analysis, as well as careful assessment of logistical and operational aspects, including indemnity, of the testing programme should be in place before initiation.

Plain English Summary

COVID-19 is caused by a virus. When someone catches this infection, the virus starts to multiply in their airways including in the nose and throat. When someone first gets infected, there is not enough virus in the nose and throat for any test to find. As the virus multiplies it gets to the stage where there is enough virus to find with a test. It can take from 1 day up to a few days for the virus to reach the level where you can find it.

There are two main types of test to check for the virus. The first type of method which we have had since the start of the pandemic looks for virus genes and is called a PCR test. The second type of method that came a bit later looks for virus proteins and is called antigen testing.

Both types of test are carried out on a swab sample taken from the back wall of the person's nose and throat (nasopharyngeal sample) or on a sample from deep within the nose (deep nasal swab). For each type of test there are many different versions or brands of the test from many different companies.

This report compares some of the newer antigen test methods with the PCR test methods that the HSE has been using as its main test method since the start of the pandemic. The main questions we want to answer are

1) How good are the antigen tests we examined, at finding infection when we compare them to the test we normally use, the PCR test. In other words, how sensitive is the test.

2) How reliable are the antigen tests we examined compared to the normal test we use, the PCR test, when there is no infection there. In other words, how specific is the test.

The way we have answered these questions is by asking people for their permission to take two samples at the same time when they were being tested. One of the samples was tested by PCR as normal, and the other by one of several different antigen tests we looked at. All the tests were carried out by scientists who were trained in how to do the test carefully and in how to read the result.

We describe how good the antigen test is at finding the virus, when compared to the PCR test result. If the antigen test is positive in everyone who has a positive PCR result we say it is 100% sensitive.

If the antigen test is negative in people who also had a negative PCR result we say the antigen test is 100% specific.

One weakness of comparing an antigen test to a PCR test is that no test (including the PCR test) is perfect. However, the PCR test is the best test available at the moment and so it is what is called "the gold standard". The usual way to assess a newer type of test like an antigen test is to compare them with the gold standard test.

The PCR test can find traces of virus in people who are no longer at risk of spreading the virus to other people. It is possible to allow for this by looking at a measure of how strong the genetic trace of virus in the samples is. This measure is the Ct (full text for first mention) value. If the Ct value is lower it means there is more virus in the sample. If the Ct value is higher, it means that there is less virus in the sample. If the Ct value is lower than 25, the person is very likely

to be infectious for other people and if the Ct value is between 25 and 30, the person is quite likely to be infectious for other people. If the Ct value is above 30 the person could be in the very early stage of infection, and about to become infectious, or they may have recovered but just have a trace of virus genes left. To take account of this, the report shows how the antigen tests work compared to PCR tests that are positive at different Ct values.

The study we have done has looked at seven different antigen tests, and we have looked at different ways in which they can be used. Four of the antigen tests we looked at were tested on the best type of sample – a nasopharyngeal sample, while three of the antigen tests were tested on nasal swab samples, which cause less discomfort during sampling.

We also looked separately at people who had symptoms of COVID at the time of testing (symptomatic) and people who had no symptoms of COVID at the time of testing (asymptomatic). This is because the virus is easier to find in people who have symptoms, and most tests were developed to test people with symptoms.

Antigen tests for use on nasopharyngeal samples from people with symptoms of COVID-19

For people who were **symptomatic** we looked at four different antigen tests that used **nasopharyngeal collected samples**. All of these tests worked well in individuals who had symptoms of COVID-19. None of the four tests picked up all of the PCR detected cases, however all four were very good at picking up those cases that were strongly positive on PCR (Ct values of less than 25)- in other words they had a sensitivity ranging from 91-100% for cases that were strongly positive by PCR. When the PCR was negative, the antigen test was almost always negative.

Antigen tests for use with deep nasal swabs on people with symptoms of COVID-19

For people who had Covid-19 type **symptoms** we looked at four different antigen tests that used **nasal collected samples**. None of the four tests we looked at picked up all of the PCR detected cases, however all four were relatively good at picking up those cases that were strongly positive on PCR (Ct values of less than 25)- with a sensitivity ranging from 85-96% for cases that were strongly positive by PCR.

When the PCR was negative, the antigen test was usually negative - all four antigen tests had a high degree of specificity at 98 to 100%.

When these antigen tests (either those for nasopharyngeal or deep nasal swab sample types) are used by trained scientists to test people with symptoms of Covid-19 infection we can be confident that a positive result means that the virus is there (they are highly specific).

Antigen tests (either nasopharyngeal or nasal) are not as reliable as PCR to rule out infection. If the antigen test does not find the virus in someone with symptoms, a PCR test is needed in order to be confident that the virus is not there.

Antigen tests in people with no symptoms of COVID-19

For people who are **asymptomatic** (people with no symptoms), we looked at the PanBio COVID-19 Rapid Test for Nasal samples on nasal samples that people took themselves (self-collected) under supervision, and the antigen test was then performed by a trained scientist.

When we compared the PanBio COVID-19 Rapid Test for Nasal samples to PCR the test was 52% sensitive. This means the antigen test picked up half of the cases that were positive by PCR. The sensitivity of the antigen test was better (80% sensitive), at picking up those strongly positive PCR tests (Ct value of less than 25).

The test has a high degree of specificity in self-collected samples (99.9%).

In summary, the antigen test assessed detects about half of the asymptomatic people who are PCR positive when the samples were self-collected under supervision and the testing was performed by trained scientists. The test detected about 4 out of 5 asymptomatic people who are strongly positive on PCR, and likely to be infectious.

Limitations

- This project did not look at how antigen tests perform when they are used by people who have limited training in carrying out tests. However, there is other evidence that kits do not perform as well when used by people with limited training compared with trained scientists.
- All validation was performed on adults, and the results cannot be extrapolated to children, who have lower viral loads.

Overall summary

It is really important before starting to use any new test or technology that it is tested in the way it is intended to be used. This is why projects like this are important, to get real life data on how the different antigen tests will work when used in real life situations.

The antigen tests for COVID-19 evaluated in this project are very specific when used by trained scientists. If they give a positive result in people with COVID type symptoms, it is almost always a real positive. Hence, antigen tests are highly specific.

The antigen tests are a bit different from each other in terms of how good they are at detecting people who are PCR positive.

- Even when performed by a trained scientist none of them found everyone who showed up as positive by PCR test.
- All of them are positive in the majority of symptomatic people who are positive by PCR, particularly when viral loads are high, and this is true for nasopharyngeal samples and deep nasal samples.
- In people with no symptoms (asymptomatic) the antigen test kit assessed here finds virus in 5 in 10 people who are PCR positive, and finds 8 in 10 strongly PCR positive samples in people who do not have symptoms, when using supervised, self-collected deep nasal swabs with the test performed by trained scientists. This means that even where there is a high viral load antigen tests in this study could miss 2 in 10 infectious cases where people have no symptoms.

1.0 Introduction

Widespread vaccination against COVID-19 is now underway in the European Union, however due to the issues around supply of vaccines in the short to medium term, COVID19 testing and tracing remains a core element of the public health response to COVID-19.

Clinical samples

The diagnosis of current COVID-19 infection is based on detection of the virus SARS-CoV-2 or components of SARS-CoV-2 in clinical samples. A good quality clinical sample is critical to the performance of any test for SARS-CoV-2. Nasopharyngeal swabs are generally regarded as the optimal sample type on which to perform tests for SARS-CoV-2. However deep nasal (mid-turbinate) swabs are also widely used in children and in others in whom collection of a nasopharyngeal sample may be unacceptable to the person offered testing. Testing of deep nasal samples is somewhat less likely to detect SARS-CoV-2 virus than testing of nasopharyngeal samples but the differences are small when using a test method that can detect SARS-CoV-2 at low levels. Use of deep nasal swabs may be particularly useful when recurrent testing at intervals is being considered because the sample collection method is often better tolerated and repeated testing may compensate for any reduction in the ability of the test to detect virus in a single sample. Saliva samples have also been used for testing (1). Provision of saliva samples may be preferred by some patients to either nasopharyngeal or deep nasals swabs. While saliva samples have been used for SARS-CoV-2 detection they are substantially less likely to support detection of SARS-CoV-2 than nasopharyngeal samples even with methods that can detect SARS-CoV-2 at low levels (1).

Analysis of samples

Culture of SAR-CoV-2 virus is labour intensive and associated with risk and is not used for routine diagnosis. Therefore, for practical purposes clinical laboratory diagnosis of current infection is based on detection of components of the virus in swabs or fluids collected from the nose, throat or mouth. The basis for testing can be detection of virus nucleic acid or virus protein. Virus nucleic acid is generally detected by reverse-transcriptase polymerase chain reaction RT-PCR. RT-PCR can detect nucleic acid in samples containing 10s to 100s of virus particles per microlitre.

The virus protein is detected using antibody-based technology (immunoassays). In this context the proteins detected are referred to as antigens and the tests are therefore referred to as antigen detection tests (ADTs). ADTs detect specific SARS-CoV 2 antigens, usually the nucleoprotein, which is the most abundant protein in SARS-CoV-2. Samples generally need to contain thousands of virus particles per microlitre to produce a positive result by antigen detection therefore if a person has low SARS-CoV-2 RNA viral load SARS-CoV-2 antigen may not be detected.

There are many different products and platforms for both RT-PCR and for ADTs and the performance characteristics differ by product and platform. Performance characteristics of test systems are also critically dependent on sample quality, the skill of the operator and the quality management system within which testing is performed.

Key properties of any test are how specifically it detects the target that it is intended to detect (analytical specificity) and how low the threshold for detection of that target is (analytical sensitivity). RT-PCR is generally accepted as the gold standard for testing because a high-quality RT-PCR test has a lower threshold for detection of SARS-CoV-2 virus than other methods currently available. It is noted however that detection of SARS-CoV-2 nucleic acid in a sample by RT-PCR does not equate to that person being infectious for others. Conversely, detection of SARS-CoV-2 antigen is likely to indicate replicating virus and can identify many people who are potentially infectious.

The analytical specificity and sensitivity are important determinants of the clinical specificity (percentage of people without infection with virus not detected) and clinical sensitivity (percentage of people with infection with virus detected).

Public health and clinical utility of test results

The clinical specificity and clinical sensitivity together with the characteristics of the group of people to whom the test is applied are key determinants of the negative predictive value (percent of people with virus not detected who do not have the infection) and the positive predictive value (percent of people with a positive test who do have infection)

The negative predictive value (NPV) and positive predictive value (PPV) of the test as applied to a particular group of people tested are the critical values. This is because in practice public health and other clinical decisions on each individual are made on the basis of the test being reported as detected or not detected. A test with a specificity of 99% has a high positive predictive value if applied to group where there is an overall high likelihood of infection (for example people who are symptomatic during a period of intense community transmission) (2). The same test will have a low positive predictive value if applied to a group where there is an overall low likelihood of infection (for example people who are asymptomatic during a period of low community transmission) (2).

The role of ADTs

While RT-PCR remains the gold standard test for SARS-CoV-2 detection, as outlined above, the use of ADTs can play a role in our public health response to COVID19 as they can rapidly confirm SARS-CoV-2 in many infectious individuals, for example in symptomatic cases within a stated number of days of onset of symptoms. They can also detect SARS-CoV-2 in some pre-symptomatic and asymptomatic cases but this is outside the specified intended use of many of the ADTs currently on the market. However, it should be noted this is an evolving field and ADT manufacturers are increasingly extending their claims to include asymptomatic individuals.

Antigen tests are currently designed to be performed on nasopharyngeal or nasal swab specimens placed directly into the assay extraction buffer or reagent. Tests take between 15 and 30 minutes to perform and generate a result and most assays are CE marked for performance by trained personnel. The main potential benefits identified are that ADTs may be easier to deploy in some settings that many RT-PCR platforms, and they can generate results more quickly than many RT-PCR platforms and reagent costs are lower. Used in this way, ADTs may help reduce further transmission

through early detection of highly infectious cases, enabling rapid isolation of cases, and early contact tracing and restriction of movements of contacts.

The clinical sensitivity and specificity of ADTs depends on sample quality, the performance characteristics of individual assays, and the population/circumstances in which they are used. A range of ADTs are available, with variation observed in sensitivity overall (3). The World Health Organisation (WHO) have set a minimum performance criteria of ≥80% sensitivity and ≥97% specificity (2), with the ECDC highlighting a target closer to ≥90% sensitivity and ≥97% specificity, especially in low prevalence environments (4). There are further recommendations around use of ADTs in different circumstance and where there are different pre-test disease probabilities, including the requirement for confirmatory RT-PCR or in some instances repeated antigen testing every 2-3 days (2,4). A recent modelling exercise by HIQA the Health Information and Quality Authority of Ireland, has suggested that with respect to workers in meat processing plants, ADT-based testing of supervised self-collected nasal samples once or twice a week with RT-PCR confirmation of positive results may offer benefit in terms of a potentially increased detection of cases, reduction in infectious person-days circulating, and a reduced overall cost relative to the current practice of monthly RT-PCR testing (5).

There has been increasing interest in the use of ADT's in a self-test model. SARS-CoV-2 diagnostic self-tests require individuals to collect a specimen from their nose/throat and conduct the test and interpret the results according to the instructions provided. At the time of writing this report there were only a few ADTs available (CE marked) for self-testing for COVID-19 (3, 6). Some assays have indications for use with self-collected samples collected under supervision of health professional and the actual testing performed by trained professionals. Importantly the self-tests currently in use in some EU/EEA countries are regulated by each country's national regulatory system (6).

In Ireland the Health Products Regulatory Authority has responsibility for regulation of medical devices. Rapid tests for SARS-CoV-2 are in-vitro diagnostic (IVD) devices with respect to the In Vitro Diagnostic Medical Devices Directive 98/79/EC as amended (IVDD) (7) which has been transposed into Irish law by the European Communities (In vitro Diagnostic Medical Devices) Regulations 2001 SI 304/2001 as amended (IVD Regulations) (8). These require that devices perform safely while achieving the purpose intended by the medical device manufacturer. Medical devices which are appropriately CE marked (i.e. have undergone the appropriate conformity assessment) may be freely placed on the European market. The classification and intended purpose of an IVD influences the level of specific assessment of a medical device's conformity with the legal requirements that is conducted. For instance, for general category IVDs, a manufacturer self-declares that their device conforms to the requirements. In order to obtain the performances claimed by the manufacturer for the device, the test should be used according to the instructions for use (IFU) provided by the manufacturer. Within the legislative framework, 'device for self-testing' means any device intended by the manufacturer to be able to be used by lay persons in a home environment. Such devices intended by the manufacturer

for self-testing, require an assessment by a notified body as part of the conformity assessment process, whereas, other types of tests for SARS-CoV-2 do not require a notified body assessment prior to CE marking.

The impact of shifting the responsibility of sample collection, performing and interpreting test results from health professionals and laboratories to individuals is an important consideration in relation to use of ADT's. There is considerable uncertainty regarding the performance of ADTs in this contact with potential for errors in use leading to reduced detection of SARS-CoV-2 infected people which could make the Public Health response measures such as contract tracing and quarantine of contacts even more challenging (6).

Chronology of ADT Evaluation and Recommendations

In October 2020 the Irish Antigen Project Evaluation Working Group was established, to perform independent and site-specific validations in Ireland. It has undertaken desktop evaluations to identify assays suitable for further evaluation, and site-specific evaluations in acute hospitals, in meat processing plants and in community swabbing centres. The validation has focussed on symptomatic individuals in line with intended use, with one of the assays also extensively validated in a cohort of asymptomatic individuals. A total of seven antigen test have been validated/verified, 6 lateral flow tests (LFT's) and one microfluidic device with reader. This report summarises the results of this validation work. Individual reports have been written for each assay (7-13).

On November 19th, 2020, the European Centres for Disease Control (ECDC) recommended that EU Member States perform independent and setting-specific validations of RADTS before their implementation (4). Furthermore, the ECDC concurs with the validation model for rapid antigen tests presented by FIND (14), which specifically states that the performance of the new test should be compared to the current gold standard RT-PCR.

On the 29th January 2021, interim guidance and recommendations were issued around the use of ADTs in Ireland to support the public health response to COVID 19, specifically in the acute hospital setting and for managing community-based outbreaks (15).

On the 17th February, 2021, the EU commission published a common list of COVID-19 rapid antigen tests, including those whose test results can be mutually recognised within member states, and a common standardised set of data which should be included in COVID-19 test result certificates, to allow member states to use these COVID-19 rapid antigen tests in line with their own countries testing strategies (3).

On the 1st April 2021, a report published by the COVID-19 Rapid Testing Group (16) established by the Minister for Health, Ireland, outlines a number of recommendations for widespread evaluations of the use of rapid testing in various settings. This report suggests that rapid tests, such as lateral flow antigen tests and loop-mediated isothermal amplification tests, should be evaluated to complement existing national HSE Public Health PCR testing programmes, preferably through the use of self-administered sampling (nasal or saliva) (16).

2.0 Objectives

Analytical sensitivity and specificity measures provided by the kit manufacturers do not necessarily reflect the actual sensitivity and specificity of the test when employed in different settings. There is rarely sufficient information on the population studied, inclusion and exclusion criteria, and the comparator PCR assays used to allow the performance characteristics of assays to be comprehensively evaluated and compared. Therefore, evaluation in the "real-world" setting, with consistency of methods across assays to be compared, is an essential part of implementation of any new diagnostic test. The purpose of the ADT validation project was to support the implementation of antigen testing by the HSE nationally in line with the national guidance on the use of antigen testing (15) in the following settings or scenarios:

(A) In the Acute Hospital Setting

- In evaluation of symptomatic patients in Emergency Departments and in ambulances arriving at Emergency Department pending admission to the Emergency Department;
- To support early diagnosis in hospital outbreaks, including testing of symptomatic health care workers;
- In identification of infectious cases in outbreaks;
- In situations where ADTs can reduce pressures on the hospital's capability for rapid PCR testing.

(B) Use in community outbreak response and control in vulnerable populations

ADT's that have been verified/validated by the antigen validation team have been made available to acute hospitals, public health teams involved in management of outbreaks and the National Ambulance Service for testing of samples from symptomatic individuals in unscheduled care.

(C) Meat Processing Plants:

A separate piece of work was conducted to evaluate the use antigen testing among asymptomatic workers in food production plants as part of a risk management strategy to complement the serial (monthly) PCR testing underway within these plants. One specific ADT (the Panbio COVID-19 Ag Rapid Test Device (Nasal)), using self-collected mid-turbinate nasal swabs was validated in asymptomatic workers within meat processing plants.

3.0 Study Design

For simplicity the overall design of the evaluation is divided into two parts. Part 1 evaluation/verification on symptomatic individuals and Part 2 validation in asymptomatic individuals.

Part 1 (Symptomatic Cohort):

Individuals presenting for SARS COV2 testing at one of several community swabbing centres in the Dublin area (CityWest, Croke Park, and Swords) and healthcare workers presenting for testing at Beaumont Hospital staff testing pod were invited to participate. In line with HSE guidelines, participants provided written informed consent to participate in the antigen validation project. Following the sampling for the standard PCR test (nasopharyngeal swab/oropharyngeal), a second swab (either a nasopharyngeal or a bilateral mid-turbinate deep nasal swab -depending on assay under evaluation) was specifically taken for the antigen test. Samples were taken by trained swabbers and the antigen tests were performed on-site by trained scientists, following individual kit manufacturer's instructions. Seven ADTs were evaluated in this cohort by comparing with the gold standard RT PCR (Table 1).

Part 2 (Asymptomatic Cohort):

Workers in 18 different meat processing plants across Ireland, in which serial testing by RT PCR was underway, were invited to participate in the Antigen Validation Project. In line with HSE guidelines, participants provided written informed consent to participate in the antigen validation project. The antigen testing project was run alongside the HSE serial testing underway within the plant between the 6th January 2021 and the 1st February 2021. Individuals who consented to participate in the study were given information on how to self-collect a nasal swab sample. The individuals then proceeded to self-collect a nasal swab specimen under supervision immediately prior to their NPS swab being taken by a trained swabber for PCR. Trained scientists from the Department of Food, Agriculture and the Marine, performed the testing on site. The validation consisted of a head-to-head comparison of one ADT only, the Abbott Panbio COVID-19 Ag Rapid Test Device (Nasal), with RT PCR at a single time point. It is important to note that repeat testing was not evaluated in this validation. A total of 5,111 participants were included in this validation.

Test performance: The performance of the various ADT's is estimated based on sensitivity and specificity. Sensitivity is the probability that the test result will be positive when the disease is present (true positive rate). Specificity is the probability that a test result will be negative when the disease is not present (true negative rate). The cycle threshold (Ct value) is the number of PCR cycles that must be run for detection of the viral RNA. In general, the higher the Ct value, the less virus present in the sample. However, Ct values do not represent accurate quantification of the quantity of virus present and a given sample may be give different Ct values when tested on different platforms or when tested repeatedly on the same platform (17). With this in mind, we analysed the performance data for each ADT at different Ct cut-offs to account for different levels of SARS-CoV-2 present. The cut-off's we applied were at Ct ≤25 and Ct≤30. This is on the basis that people who test positive a lower Ct values are much more likely to be infectious than those

positive at higher Ct values. Noting the recognised limitations detailed below, this approach is in line with international evidence and practice at the time of writing this report.

The public health and clinical utility of an assay is determined by the way it is used, the population to whom it is applied, and the action taken based on the results, and not solely on the sensitivity and specificity.

3.1 Antigen Diagnostic Tests Evaluated:

The following ADT's were evaluated as outlined in the table below.

Table 1: List of antigen tests validated/verified

Assay Name	Manufacturer	Assay Type	Sample type evaluated	Patient Cohort	Reference RT PCR Assay
MoLab mö-screen Corona Antigen Test	MyBio	LFT	Nasopharyngeal	Symptomatic	Flowflex and Euroimmuno PCR
Sars CoV2 Antigen Test	Roche Diagnostics	LFT	Nasopharyngeal	Symptomatic	RealStar® SARS-CoV-2 RT-PCR Assay (Altona), cobas® SARS-CoV-2 Test (Roche), GSD NovaPrime® SARS-CoV-2 (COVID-19) RT PCR
BIOSYNEX COVID-19 Ag BSS	BIOSYNEX	LFT	Nasopharyngeal	Symptomatic	Thermofisher TaqPath COVID 19, cobas® SARS-CoV-2 Test , Serosep Respbio, Logix Smart COVID 19
PanBio COVID-19 Rapid Test (Nasopharyngeal)	Abbott	LFT	Nasopharyngeal	Symptomatic	Thermofisher TaqPath COVID 19, cobas® SARS-CoV-2 Test , Logix Smart COVID 19 , RealStar® SARS-CoV-2 RT-PCR Assay (Altona), GSD NovaPrime® SARS-CoV-2 (COVID-19)
PanBio COVID-19 Rapid Test (Nasal)	Abbott	LFT	Bi-lateral mid turbinate nasal swab	Symptomatic and Asymptomatic	RealStar® SARS-CoV-2 RT-PCR Assay (Altona), cobas® SARS-CoV-2 Test , Thermofisher TaqPath COVID 19, Flowflex, ViroBoar V2, GSD Novaprime
CLINITEST Rapid COVID-19 Antigen Test	Siemens	LFT	Bi-lateral mid turbinate nasal swab	Symptomatic	Thermofisher TaqPath, GSD NovaPrime® SARS-CoV-2 (COVID-19), cobas® SARS-CoV-2 Test
LumiraDx SARS-CoV-2 Ag Test (Microfluidic device)	LumiraDX	Microfluidic chip with Reader	Bi-lateral mid turbinate nasal swab	Symptomatic	GSD NovaPrime, RealStar® SARS-CoV-2 RT-PCR Assay (Altona), Thermofisher TaqPath, Flowflex

3.2 Study Limitations:

As with all "real-world" evaluations there were limitations in the design and process. These limitations are inherent for all ADT evaluations.

- Participants were included on the basis of consent.
- As the ADTs are performed before the result of the PCR is known, it was not possible to select participants to specifically obtain the range of Ct values, which would normally be included in comparisons of PCR platforms.
- Given the delay in obtaining PCR results (24 hours), and the limited stability of the sample for ADT, it was not possible to retest the ADT when discordant results were obtained.
  Evaluation was carried out in adults, >18 years of age, who consented to provide a second swab. Results should not be extrapolated to children, where lower viral loads may affect sensitivity.
- One of the key challenges in carrying out these types of validations, in asymptomatic populations where the prevalence of infection is low, is getting sufficient numbers of positive cases. Our validation of the PanBio COVID-19 Rapid Test [Nasal] is among one of the largest studies conducted on over 5,000 asymptomatic individuals with a direct comparison with PCR. However, despite the very large number of people tested, the target of comparing 100 PCR positive individuals was not achieved.
- It is also acknowledged that comparison of antigen testing with PCR has limitations; both techniques detect different viral targets, use different technologies, limits of detection vary across different assays and there remains significant uncertainty as to the validity of using Ct value cut-offs from a single sample in isolation to judge whether a person is an infectious case. A low level of SARS-CoV-2 (high Ct value) may represent either pre-symptomatic infection, a poor-quality sample from an infectious person or residual SARS-CoV-2 RNA in a person who is no longer infectious.
- As no true test of infectiousness is currently available, and RT-PCR is recognised by the WHO as the "gold standard" for diagnosis of COVID 19, comparison with RT-PCR is the accepted method for verification/validation of alternative assays (2, 14). Furthermore, analysis of the data using Ct ≤25 and Ct≤30 as indicators of infectiousness, is in line with international evidence. However, it is fully acknowledged that Ct values can differ across different assays, with different limits of detection and this is a limitation of this approach.

4.0 Summary Results

We evaluated the performance of seven ADT's compared to the gold standard test RT PCR. Four of the ADTs evaluated were for use with nasopharyngeal samples and were evaluated/verified specifically in symptomatic individuals. Three of the ADTs evaluated were for use with bilateral nasal swabs and were also evaluated/verified specifically in symptomatic individuals. One assay, the PanBio COVID-19 Rapid Test NPS Test [Nasal] was validated in a cohort of asymptomatic meat plant workers. Summaries of the validation reports are available for each assay in Appendix 1 (7-13).

For all seven ADTs evaluated the specificity was excellent, ranging between 98-100%, and above the threshold recommended by the WHO/ECDC of 97% (Table 2 and Table 3). Overall, as expected, the performance data in terms of clinical sensitivity was higher in the ADT's which were used with nasopharyngeal swab samples (Table 2) than those used with nasal swab samples (Table 3). Nasopharyngeal swabs are the specimens which are most likely to yield a positive result when tested for SARS-CoV-2 RNA, however in scenarios of frequent testing or where self-swabbing may be required, nasal swabs are more acceptable.

Of note for several of the ADT's evaluated the "real world" performance in terms of sensitivity was lower than that claimed by the manufacturers (Table 2 and Table 3), highlighting the importance of conducting "real world" evaluations of these tests in different settings.

The WHO recommends rapid antigen tests that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity, while the ECDC recommends where COVID 19 prevalence is low, one should aim to use tests with a performance closer to RT-PCR, i.e. ≥90% sensitivity and ≥97% specificity (2, 4). All of the ADT's evaluated for use with nasopharyngeal swab samples on symptomatic individuals meet the minimum criteria in terms of sensitivity (≥80%) and specificity (≥97%) set out by the WHO (2). The overall sensitivities of those ADT's used with nasopharyngeal swab samples ranged from 80% to 90% (Table 2), increasing to 90.9%-100% for cases with presumably higher viral loads with Ct's ≤ 25. While there were differences between the four different ADT's evaluated on nasopharyngeal swab samples, it should be noted that the sample number of PCR positives for verification was low and confidence intervals overlapped.

The performance of the ADT's for use with nasal swab samples was generally lower than nasopharyngeal swab samples when used on symptomatic individuals. The overall sensitivities of those ADT's used with nasal swab samples on **symptomatic individuals** ranged from 75.6% to 86.8% (Table 3), increasing to 84.7% - 95.5% for samples with low values (Ct's ≤ 25). The Lumira DX assay had the highest sensitivity overall of the assays evaluated for use with nasal swabs. However, it is an instrument-based assay, more suited to small test numbers in a clinical environment, rather than a high test numbers or a community setting for example. An additional validation exercise was carried out on the PanBio COVID-19 Rapid Test [Nasopharyngeal] assay, for use with bilateral mid-turbinate nasal collected swabs, to assess if

this assay could be used specifically with nasal collected samples. This was an off-label use and not recommended by the manufacturers but the rationale for doing this was because of the supply of the PanBio COVID-19 Rapid Test [Nasopharyngeal] assay, available in Ireland from the EU antigen tender. The sensitivity of 75.6% of the assay used in this was lower, but not significantly lower, than that of the PanBio COVID-19 Rapid Test [Nasal] (79%). Users did report some challenges with using the swabs in this way, so this approach may not be suitable for all settings.

An evaluation of the PanBio COVID-19 Rapid Test [Nasal] was also conducted among asymptomatic workers in meat processing plants across Ireland. The specificity of the assay in this cohort was excellent, however the sensitivity was significantly lower among asymptomatic individuals (51.9%) than symptomatic individuals (78.6%) (Table 4). The performance was higher at Ct ≤ 25 where presumably there was a high viral load with a sensitivity of 79.5 %, but still well below the 91% sensitivity observed for the symptomatic group. This is consistent with findings generally on antigen test performance in asymptomatic individuals (18). Our data indicates that there remains a risk of missing a substantial proportion of potentially infectious cases, including 20% of those with Ct ≤25 and 31% of those with Ct ≤30. Therefore, use of this assay in settings such as testing of asymptomatic high-risk populations is not recommended as a standalone test, but as suggested by the ECDC, to compensate for this low sensitivity, the test could potentially be used in a testing regimen that includes frequent repeat testing every 2-3 days and or with confirmatory RT-PCR (4), although this repeat testing regimen was not validated in this study. A recent modelling exercise by HIQA the Health Information and Quality Authority of Ireland, has suggested that ADT-based testing of supervised self-collected samples once or twice a week with RT-PCR confirmation of positive results may be a viable alternative to the current approach of once monthly RT-PCR serial testing of workers in meat processing plants (5).

Table 2: ADT Assay Validation Summary on Symptomatic Cohorts for assays used with nasopharyngeal samples

	MoLab mö-screen Corona Antigen Test (MyBio)	Sars CoV2 Antigen Test (Roche)	BIOSYNEX COVID-19 Ag BSS	PanBio COVID-19 Rapid Test NPS (Abbott)
Sample	Nasopharyngeal	Nasopharyngeal	Nasopharyngeal	Nasopharyngeal
Manufacturer claimed sensitivity	**97.3% (71/73**)	**95.5 %** (95 % CI: 91.8 % - 97.8 %] for Ct values ≤ 30	**96% (99/103)** [95%CI: 93.6-98.4%]	**93.3% (56/60)** [95%CI: 83.8%; 98.2%]
Overall Sensitivity	**95% (38/40)** [95%CI; 0.8261 to 0.9950]	**86.8% (33/38)** [95%CI; 0.7220 to 0.9472]	**88.1% (37/42)** [95% CI: 0.7454 to 0.9527]	**80% (32/40)** [95% CI: 0.6499 to 0.8976]
Sensitivity Ct≤25	**100% (34/34)** [95% CI; 0.8793 to 1.0000]	**100% (28/28)** [95%CI; 0.8570 to 1.000]	**92.1% (35/38)** [95%CI; 0.7848 to 0.9800]	**90.9% (30/33)** [95% CI:0.7566 to 0.9763]
Sensitivity Ct ≤30	**95% (37/39)** [95% CI; 0.8221 to 0.9948]	**94.1% (32/34)** [95%CI; 0.7993 to 0.9935]	**88 % (37/42)** [95% CI: 0.7454 to 0.9527]	**80% (32/40)** [95% CI: 0.6499 to 0.8976]
Specificity	**100% (120/120)** [95% CI:0.9627 to 1.0000]	**100% (126/126)** [95% CI: 0.9644 to 1.0000]	**100% (110/110)** [95% CI: 0.9595 to 1.0000]	**100% (176/176)** [95% CI: 0.9743 to 1.0000]
Overall Specificity * Includes symptomatic, asymptomatic, close contacts	**100% (324/324)** [95%CI; 0.9859 to 1.0000]	**99.5% (240/241)** [95%CI; 0.9745 to 1.000]	**100% (587/587)** [95%CI: 0.9922 to 1.0000]	**100% (222/222)** [95% CI: 0.9795 to 1.0000]

Table 3: ADT Assay Validation Summary on Symptomatic Cohorts for assays used with nasal samples

	PanBio COVID-19 Rapid Test Nasal (Abbott)	PanBio COVID-19 Rapid Test NPS Test [Nasopharyngeal] (Abbott)	CLINITEST Rapid COVID-19 Antigen Test	LumiraDx SARS-CoV-2 Ag Test (Microfluidic device)
Sample	Mid-turbinate nasal	*NPS test - bilateral mid turbinate nasal collected swabs* ***(off label)***	Mid-turbinate nasal	Mid-turbinate nasal
Manufacturer claimed sensitivity	**98.1% **** [95% CI: 93.2-99.8%] **91.1% (NPS)** [95% CI: 84.2-95.6%]	**93.3% (56/60) (NPS)** [95%CI: 83.8%; 98.2%]	**97.25% (106/109)** [95% CI: 92.17% to 99.43%]	**97.6% (81/83)****
Overall Sensitivity	**78.6% (33/42)** [95%CI;0.6385 to 0.8851]	**75.6%% (90/119)** [95% CI: 0.6715 to 0.8250]	**79.4 % (27/34**) [95%CI; 0.6290 to 0.8995]	**86.8% (46/53)** [95% CI; 0.7485 to 0.9376]
Sensitivity Ct≤25	**91% (31/34)** [95% CI: 0.7628 to 0.9771]	**84.7% (89/105)** [95% CI:7657 to 0.9050]	**89.7% (26/29)** [95%CI; 0.7281 to 0.9722]	**95.5% (42/44)** [95% CI; 0.8403 to 0.9958]
Sensitivity Ct ≤30	**85% (33/39)** [95% CI: 0.6989 to 0.9314]	**81.6% (89/109)** [95% CI: 0.7327 to 0.8787]	**84.4% (27/32)** [95%CI; 0.6777 to 0.9361]	**93.6% (44/47)** [95% CI; 0.8219 to 0.9845]
Specificity	**100% (218/218)** [95%CI: 0.9792 to 1.0000]	**99.42% (517//520)** [95% CI:0.9823 to 0.9989]	**98% (150/153)** [95%CI; 0.9438 to 0.9959]	**98.2% (269/274)** [95% CI: 0.9568 to 0.9934]
Overall Specificity * Includes symptomatic, asymptomatic, close contacts	**100% (318/318)** [95%CI: 0.9856 to 1.0000]	**99.46% (558/561)** [95% CI: 0.9836 to 0.9990	**98.8% (246/249)** [95%CI; 0.9635 to 0.9976]	**98.5% (401/407)** [95%CI; 0.9674 to 0.9940]

Table 4: PanBio COVID-19 Rapid Test Nasal (Abbott) in Asymptomatic Cohort compared to Symptomatic Cohorts

	Asymptomatic	Symptomatic
Assay	PanBio Nasal test Nasal Swab (Self-collected)	PanBio Nasal test Mid-turbinate nasal swab collected by trained swabber
Overall Sensitivity	**51.9 % (41/79)** [95%CI; 0.4105 to 0.6257]	**78.6% (33/42)** [95%CI;0.6385 to 0.8851]
Sensitivity Ct ≤ 25	**79.5% (35/44)** [95% CI; 0.6528 to 0.8907]	**91.1% (31/34)** [95% CI: 0.7628 to 0.9771]
Sensitivity Ct ≤ 30	**68.9% (40/58)** [95% CI: 0.5614 to 0.7943]	**84.6% (33/39)** [95% CI: 0.6989 to 0.9314]
Specificity	**99.9% (5030/5032)**	**100% (218/218)** [95%CI: 0.9792 to 1.0000]

5.0 Conclusions

The WHO recommends rapid antigen tests that meet the minimum performance requirements of ≥80% sensitivity and ≥97% specificity, while ECDC suggests where COVID 19 prevalence is low, aiming to use tests with a performance closer to RT-PCR, i.e. ≥90% sensitivity and ≥97% specificity (2, 4).

Overall, all seven ADT's evaluated by this group when used with symptomatic individuals, met the minimum requirements set out by the WHO in terms of 80% sensitivity and ≥97% specificity. There were some differences in the performance of the different ADT's and using different sampling approaches. Generally, tests using nasopharyngeal collected swab samples were superior in terms of clinical sensitivity to nasal collected swabs, when swabs were collected by trained healthcare professionals.

For use with asymptomatic individuals, the sensitivity of the Abbot Panbio COVID-19 Ag Rapid Test (Nasal) even taking into account higher viral RNA levels, is below the minimum requirements set out by WHO and ECDC. There remains a risk of missing a substantial proportion of potentially infectious cases, including 20% of those with Ct ≤25 and 31% of those with Ct ≤30. In light of this, and based on the recommendations outlined by the ECDC (4), consideration for the use of this assay in settings such as testing of asymptomatic high-risk populations should be in the context of frequent

antigen testing 2-3 times per week and or with confirmatory RT-PCR (3). It is important to note that repeat testing has not been validated in this study, validation is required in the real world setting to assess the effectiveness of this approach. A recent modelling exercise by HIQA the Health Information and Quality Authority of Ireland, has suggested that ADT-based testing of supervised self-collected samples once or twice a week with RT-PCR confirmation of positive results may be a viable alternative to the current approach of once monthly RT-PCR serial testing of workers in meat processing plants (5).

Different cases for use will be more suited to different antigen tests. Higher sensitivity can be achieved with antigen tests using a nasopharyngeal swab, which would be a preferable approach for outbreak management for example. Some assays are more suited to being performed by trained professionals in a clinical environment. For example, the LumiraDx ADT which was the highest performing assay for use with nasal samples, uses a specific reader and requires specific training and expertise that would be better suited to a clinical environment. However, consideration should be given to the specificity of the assay in settings where false positives may pose a risk to the individual, requiring PCR confirmation.

A small number of the assays we evaluated have positive and negative controls included with the test or can be supplied as an adjunct. These assays are as follows; PanBio COVID-19 Rapid Test (Abbott); LumiraDx SARS-CoV-2 Ag Test (LumiraDx); and the Sars CoV2 Antigen Test (Roche Molecular Diagnostics). This is beneficial for user training and competency assessment, and importantly for batch verification of different kit lot numbers and batches. Depending on the use case, this is an important consideration, so for example, outside of the laboratory setting where controls would not be readily available this is a very important component of the quality assurance and governance around the testing process.

The specificity of currently available ADTs is very high, but when used in asymptomatic individuals in a low prevalence setting, the positive predictive value is low. Clearly as COVID-19 vaccination rolls out and the disease prevalence drops, use of ADT's needs careful consideration in relation to the impact of false positives on any use case. Where the pre-test probability of infection is high, positive results are likely to be true positives; however, when the pre-test probability is low, the proportion of false positives may exceed that of true positives (2,4). It is important to stress that even with a highly specific test (antigen or RT-PCR) the proportion of all positive tests that are false positives increases as number of infected people in the population tested declines. A test system with 99% specificity, is expected to generate an equal number of false positive and true positive test results, if only 1% of the people tested truly has the infection (2, 4). Similarly, if we consider the current estimated prevalence in Ireland which is 0.1%, only 1 in 10 positive results will be true positives. Hence as the prevalence of infection in the population tested decreases, it will be important when considering the use ADTs in different setting that the impact of false positives and indeed false negatives are carefully considered. For example, false positives, will require a period of self-isolation, and the associated

contact tracing and community measures may be impacted. False negatives could give a false sense of security leading to individuals changing their behaviours. It is important to recognise that different scenarios may prioritise different aspects of assay performance and where confirmatory PCR may be required. Further real-world studies are required to determine if the wider deployment of ADT testing has net benefits in the context of potential false reassurance and behaviour change as a result of failure to detect other people who are infectious and to understand the impact of false positive results.

Furthermore, our experience from these pilot validation studies and the subsequent roll out in the meat processing plants and management of outbreaks, has highlighted significant operational, practical and logistical challenges in introducing ADT's at scale. Any case use needs to carefully consider each of these challenges, and a risk assessment of the process should be performed in advance of any implementation. Operationally these challenges include systematic changes to all processes and systems such as referral, scheduling, swabbing, testing, result reporting, contact tracing, and system reporting. Logistically, there is a requirement for training of personnel to do the antigen testing. If the self-test model is being considered, appropriate training, competency assessment and quality assurance needs careful consideration, and the test performance in this setting needs to be evaluated. Evidence from the UK has shown a significant reduction in the performance of one specific ADT in terms of sensitivity when the same ADT is performed as a self-test, rather than by trained healthcare professionals or laboratory scientists (19). Moreover, adherence to self-testing among staff using ADTs within the NHS, in the care home setting, has been shown to be very poor, with reports of only 8.6% of staff in care homes, achieving more than 75% adherence to the ADT serial testing protocol, highlighting a big disconnect between the prescribed testing regime and the 'real-life' context of use (20). Irrespective of what the use case is for ADT's, appropriate clinical governance and quality management around the testing and operating to appropriate quality assurance standards is essential.

Lastly, the validations carried out by this group were performed in participants > 18 years old, and findings should not be extrapolated to children, where lower viral loads may affect sensitivity. The validation data presented here was carried out with trained professionals performing the antigen testing, and the findings should not be extrapolated to self-testing where the sensitivity is likely to be affected. Lastly, the validation data presented for the different ADT's evaluated in symptomatic individuals should not be generalised to asymptomatic cohorts where the low prevalence of infection, and lower viral loads will affect sensitivity.

8.0 References

1. Czumbel LM, Kiss S, Farkas N, Mandel I, Hegyi A, Nagy Á, *et al*. Saliva as a Candidate for COVID-19 Diagnostic Testing: A Meta-Analysis. Front Med . 2020 Aug 4;7:465. https://www.frontiersin.org/article/10.3389/fmed.2020.00465/full

2. World Health Organization (WHO). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. Geneva: WHO; 2020 [updated 11 September 2020; cited 8 November 2020]. Available from: https://www.who.int/publications/i/item/antigen-detection-in-the-diagnosis-of-sars-cov-2infection-using-rapid-immunoassays

3. EU health preparedness: A common list of COVID-19 rapid antigen tests, including those of which their test results are mutually recognised, and a common standardised set of data to be included in COVID-19 test result certificates. 17th February 2021. https://ec.europa.eu/health/sites/default/files/preparedness_response/docs/covid-19_rat_common list_en.pdf

4. European Centre for Disease Prevention and Control. Options for the use of rapid antigen tests for COVID-19 in the EU/EEA and the UK. 19 November 2020. ECDC: Stockholm; 2020.

5. Advice to HSE: Potential impact of different serial testing scenarios using RADTs to detect SARS-CoV-2 in meat processing plant workers. 30th April 2021. https://www.hiqa.ie/sites/default/files/2021-04/RADT-in-MPP_Advice-to-HSE.pdf

6. European Centre for Disease Prevention and Control. Considerations on the use of self-tests for COVID-19 in the EU/EEA –17 March 2021. ECDC: Stockholm; 2021.

7. National Clinical Programme for Pathology. Validation Summary Report for the MoLab Point of Care Corona Antigen Test VER-REP/2021/1 Version 1

8. National Clinical Programme for Pathology. Validation Summary Report for the Panbio COVID-19 Ag Rapid Test Device (Nasal) VER-REP/2021/2 Version 5

9. National Clinical Programme for Pathology. Validation Summary Report for the CLINITEST Rapid COVID-19 Antigen Test VER-REP/2021/4 Version 1

10. National Clinical Programme for Pathology. Validation Summary Report for the SARS-CoV-2 Rapid Antigen Test (Roche) VER-REP/2021/5 Version 1

11. National Clinical Programme for Pathology. Validation Summary Report for the BIOSYNEX COVID-19 Ag BSS VER-REP/2021/6 Version 1

12. National Clinical Programme for Pathology. Validation Summary Report for the LumiraDx SARS-CoV-2 Ag test REP/2021/7 Version 1

13. National Clinical Programme for Pathology. Validation Summary Report for the Panbio COVID-19 Ag Rapid Test Device (Nasopharyngeal) VER-REP/2021/8 Version 1

14. Foundation for Innovative New Diagnostics (FIND). Comparative evaluation of lateral flow assay tests that directly detect antigens of SARS-CoV-2. Geneva: FIND; 2020 [cited 10 November 2020]. Available from: https://www.finddx.org/wp-content/uploads/2020/04/20200421-COVID-Ag-RDT-Evaluation-Synopsis.pdf.

15. Interim guidance on use of Antigen Detection Tests in the public health system in Ireland. HPSC January 2021.

16. COVID-19 Rapid Testing Group. Safe Sustainable Re-opening: The Role of Rapid SARS-CoV-2 Testing: Report of the COVID-19 Rapid Testing Group 2021 https://www.gov.ie/en/publication/f50f0-report-of-the-covid-19-rapid-testing-group/

17. Carroll Anne, McNamara Eleanor. Comparison and correlation of commercial SARS-CoV-2 real-time-PCR assays, Ireland, June 2020. Euro Surveill. 2021;26(6):pii=2002079. https://doi.org/10.2807/1560-7917.ES.2021.26.6.2002079

18. Cochrane Database of Systematic Reviews (Dinnes et al). Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection 2021 [Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD013705.pub2/full

19. Tim Peto, UK COVID-19 Lateral Flow Oversight Team. COVID-19: Rapid Antigen detection for SARS-CoV-2 by lateral flow assay: a national systematic evaluation for mass-testing. medRxiv 2021.01.13.21249563; doi: https://doi.org/10.1101/2021.01.13.21249563

20. Elisabeth Mahase. Covid-19: Lateral flow tests in care homes failed to stop outbreaks, finds study BMJ 2021;373:n1025 doi: https://doi.org/10.1136/bmj.n1025 (Published 20 April 2021)

Appendix 1: Summary Reports for each of the ADT validated.

Note full validation reports for each assay are available (7-13)

1.0 Validation Summary Report for MoLab mö-screen Corona Antigen Test [VER-REP/2021/1]

This report describes the verification of the performance of the MoLab mö-screen Corona Antigen Test. Verification of assay performance in line with intended use in symptomatic people has been completed. As only 1/203 asymptomatic people tested were PCR positive, the assay cannot be considered validated for use in asymptomatic people. As antigen testing must be completed before the PCR result is known, adequate validation in this population is challenging. However, sensitivity obtained in symptomatic people cannot be extrapolated to the asymptomatic population.

Manufacturer's Claimed Performance Characteristics of the test in symptomatic population

Relative Sensitivity: 97.3% (71/73)

Relative Specificity: >99.9% (130/130)

Accuracy: 99.0% (201/203)

Verification of Performance (symptomatic population):

Sensitivity 95 % (38/40) [95%CI; 0.8261 to 0.9950]

Specificity 100% (324/324) [95%CI; 0.9859 to 1.0000]

* 2 false negatives Ct 27.17 and Ct 28.7

Validation of Performance (asymptomatic population):

This work could not be completed due to low positivity rate in the population tested.

The population included 102 asymptomatic close contacts and 101 asymptomatic individuals tested as part of surveillance.

No comment can be made on assay sensitivity in this population (1/1 PCR positive subject was ADT positive)

Specificity was excellent at 100%. [203/203 (100%: 95% CI 0.9776 to 1.0000].

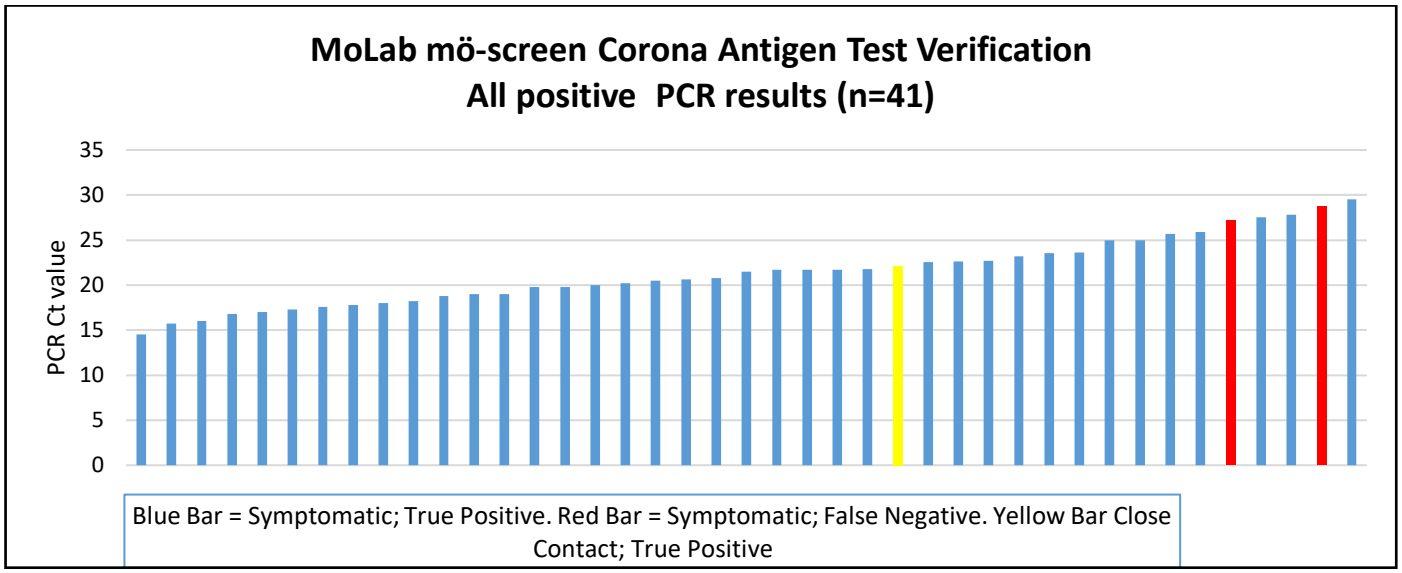

Figure 1: Ct data plotted for all PCR positive cases in the overall study evaluation cohort (n=41) PCR positive cases.

Recommendation:

When used with fresh nasopharyngeal swabs, this assay has excellent sensitivity in symptomatic individuals, and excellent specificity.

All samples with a Ct </= 26 were detected.

Further validation is required to establish sensitivity in asymptomatic populations, where viral loads may be lower.

2.0 Validation Summary Report for Panbio COVID-19 Ag Rapid Test Device (Nasal Swabs) [VER-REP/2021/2]

This report describes the verification and validation of the performance of the **Panbio COVID-19 Ag Rapid Test Device** for use with **Nasal Swabs**. Verification of the assay performance in line with its intended use in symptomatic people has been completed. This verification has specifically focussed on symptomatic individuals in the acute hospital setting and community swabbing centres.
A validation has been completed on asymptomatic people working in the food processing industry.

As antigen testing must be completed before the PCR result is known, adequate validation in this population is challenging. It is acknowledged that comparison of antigen testing with PCR has limitations; both techniques detect different viral targets and use different technologies, however as no test of infectiousness is currently available, and as PCR is recognised by the WHO as the "gold standard" for diagnosis of COVID-19, comparison with this assay is the accepted method for verification/validation of alternative assays. The clinical utility of an assay is determined by the way it is used, and the action taken on the basis of the results, and not solely on the sensitivity and specificity.

Manufacturer's Claimed Performance Characteristics of the test in symptomatic population

Sensitivity: 98.1% (95% CI: 93.2-99.8%)

Specificity: 99.8% (95% CI: 98.6-100.0%)

Manufacturer's Claimed Performance Characteristics of the test in asymptomatic population

Sensitivity: 66% (95% CI 51.2-78.8%)

93.8% (95%CI: 79.2%; 99.2%) Ct ≤ 30

Specificity: 100% (95% CI: 99.2%; 100.0%)

(1) Verification of Performance (symptomatic population):

Sensitivity 78.6% (33/42) [95%CI; 0.6385 to 0.8851]

Specificity 100% (218/218) [95%CI: 0.9792 to 1.0000]

Ct	Sensitivity
≤ 25	91.1% (31/34) [95% CI: 0.7628 to 0.9771]
≤ 30	84.6%(33/39) [95% CI: 0.6989 to 0.9314]
≤ 35	80.5% (33/41) [95%CI; 0.6573 to 0.9003]

≤ 40	78.6% (33/42) [95%CI; 0.6385 to 0.8851]

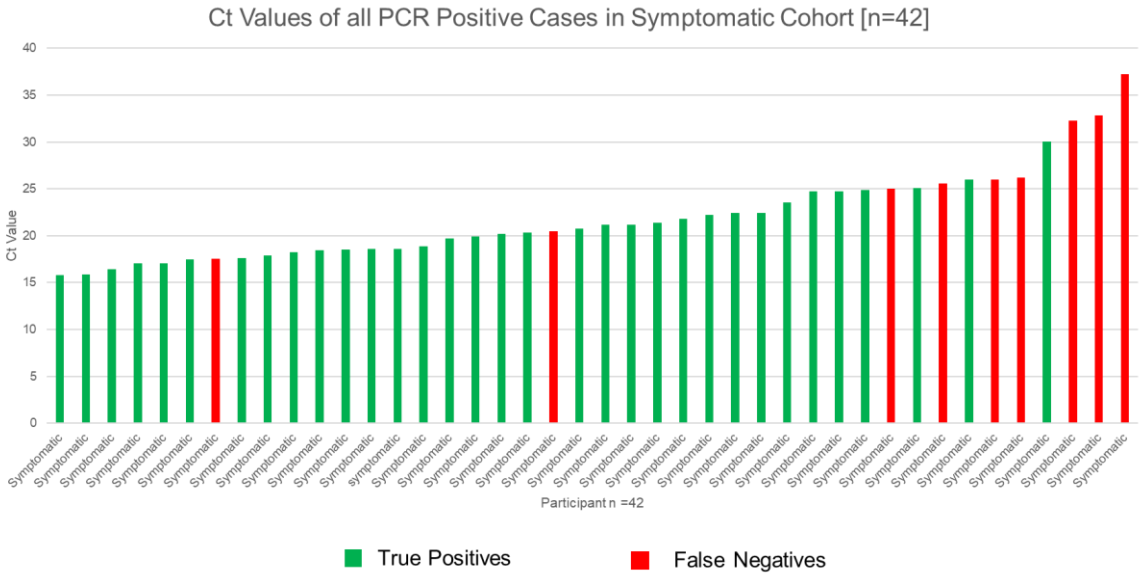

Figure 1: Ct values for all PCR positive cases within the symptomatic group. Those cases with an antigen negative PCR positive result are highlighted in red.

(2) Validation of Performance (asymptomatic population):

A validation exercise has been completed for the use of the **Panbio COVID-19 Ag Rapid Test Device** for use with **Nasal Swabs (self-collected)** in an asymptomatic population of workers from the food processing industry.

Sensitivity: 51.9 % (41/79) [95%CI; 0.4105 to 0.6257]

Specificity: 99.9% (5030/5032)

Ct Value	Sensitivity	95% CI
Ct ≤25	80 % (35/44)	0.6528 to 0.8907
Ct ≤30	69% (40/58)	0.5614 to 0.7943
CT≤35	56.2% (41/73)	0.4609 to 0.6822

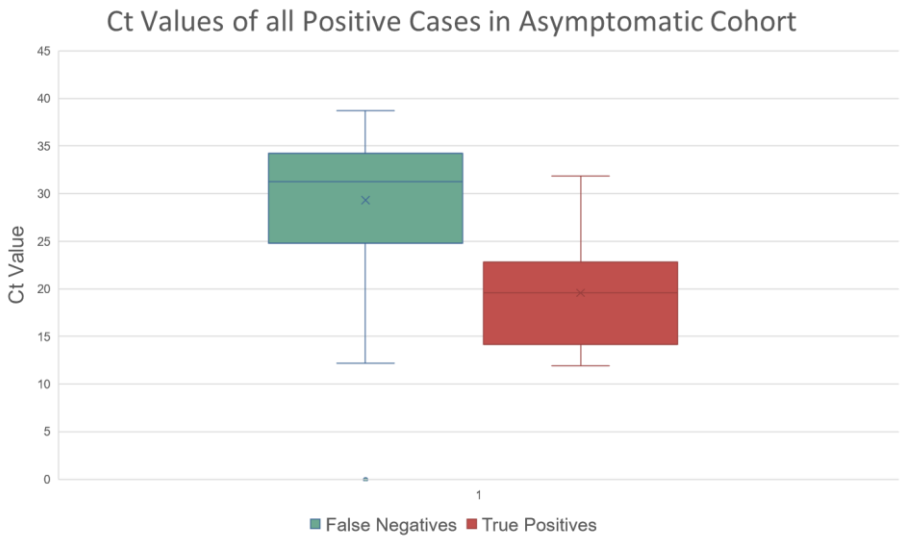

Figure 2: The Ct range and median for all PCR positive cases within the asymptomatic group, highlighting the difference between those that were antigen positive and antigen negative.

Recommendations:

1. When used with fresh nasal swabs, this assay has adequate sensitivity in symptomatic individuals, and excellent specificity. This assay is suitable for use in settings when nasopharyngeal swabs are not acceptable.
2. For use with asymptomatic individuals the sensitivity of the Abbot Panbio COVID-19 Ag Rapid Test (Nasal) even taking into account higher viral RNA levels, is below the minimum requirements set out by WHO and ECDC. There remains a risk of missing a substantial proportion of potentially infectious cases, including 20% of those with Ct ≤25 and 31% of those with Ct ≤30. Therefore, use of this assay in settings such as testing of asymptomatic high-risk populations is not currently recommended as a standalone test. Further work is ongoing to assess the potential role of this assay with frequent testing in high-risk settings, where sensitivity limitations may be overcome by frequent testing.
3. It is essential that Infection Prevention and Control measures are not adjusted solely on the basis of a Not Detected PanBio Nasal Antigen Test. Use in an asymptomatic cohort should only be in a manner demonstrated to compensate for the lower performance compared to PCR, in line with ECDC recommendations. Any such use should be part of ongoing audit of Antigen Testing. Modelling work is expected to refine recommendations as to the frequency of testing required to achieve a stated reduction in chains of transmission, and when available an updated validation report will be produced.
4. Validations were performed in participants > 18 years old, and findings should not be extrapolated to children, where lower viral loads may affect sensitivity.

3.0 Validation Summary Report for Panbio COVID-19 Ag Rapid Test Device for Nasopharyngeal Swabs [VER-REP/2021/8]

This report describes the verification and validation of the performance of the **Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal Swabs**. Verification of the assay performance in line with its intended use in symptomatic people has been completed. This verification has specifically focussed on symptomatic individuals in community swabbing centres.

A validation exercise was also conducted using the **Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal Swabs** but collecting a bilateral mid-turbinate nasal swab. The purpose of this was to explore the possibility of using the **Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal Swabs** for use with nasal sampling, so there might be a wider use application for the supply of the **Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal Swabs** available from the EU antigen tender.

A separate report has been issued for the **Panbio COVID-19 Ag Rapid Test Device** for **Nasal Swabs**

As antigen testing must be completed before the PCR result is known, adequate validation in this population is challenging. It is acknowledged that comparison of antigen testing with PCR has limitations; both techniques detect different viral targets and use different technologies, however as no test of infectiousness is currently available, and as PCR is recognised by the WHO as the “gold standard” for diagnosis of COVID-19, comparison with this assay is the accepted method for verification/validation of alternative assays. The clinical utility of an assay is determined by the way it is used, and the action taken on the basis of the results, and not solely on the sensitivity and specificity.

Manufacturer’s Claimed Performance Characteristics of the test in <u>symptomatic</u> population

Sensitivity: 93.3% (95% CI: 83.8-98.2%)

Specificity: 99.4% (95% CI: 97.0-100%)

(1) Verification of Performance of Panbio COVID-19 Ag Rapid Test Device for Nasopharyngeal **(symptomatic population):**

Sensitivity: 80% (32/40) [95%CI; 0.6499 to 0.8976]

Specificity: 100% (176/176) [95%CI: 0.9743 to 1.0000]

Overall specificity: 100% (222/222) [95% CI: 0.9795 to 1.0000]*

* includes 176 symptomatic, 7 asymptomatic, 39 close contacts

Ct	Sensitivity
≤ 25	90.9% (30/33) [95% CI: 0.7566 to 0.9763]
≤ 30	80.0 % (32/40) [95% CI: 0.6499 to 0.8976]

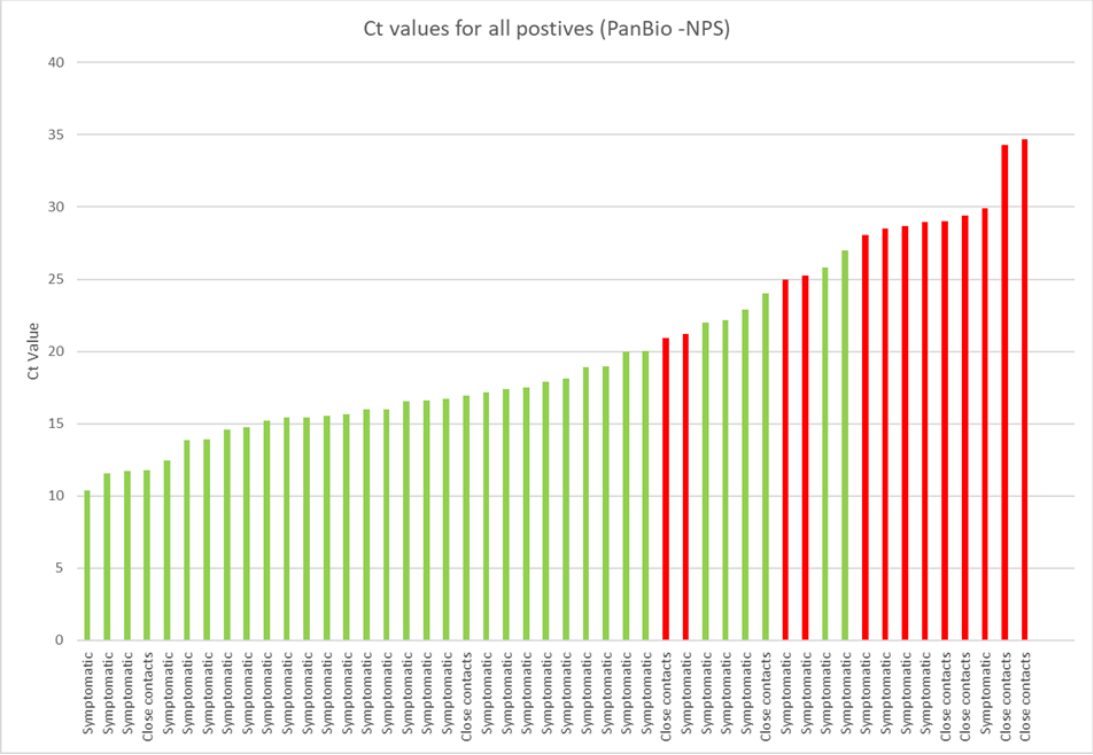

Figure 1: Ct values for all PCR positive cases within the symptomatic and close contact group. Those cases with an antigen negative PCR positive result (false negatives) are highlighted in red.

(2) Validation of the Performance of Panbio COVID-19 Ag Rapid Test Device for **Nasopharyngeal** used with bilateral **mid turbinate nasal collected swabs** (**symptomatic population):**

Sensitivity 75.63 % (90/119) [95%CI; 0.6715 to 0.8250]

Specificity 99.42% (176/176) [95%CI: 0.9823 to 0.9989]

Ct	Sensitivity
≤ 25	84.5 % (89/105) [95% CI:0.6715 to 0.8250]
≤ 30	81.6 % (89/109) [95% CI: 0.7327 to 0.8787]

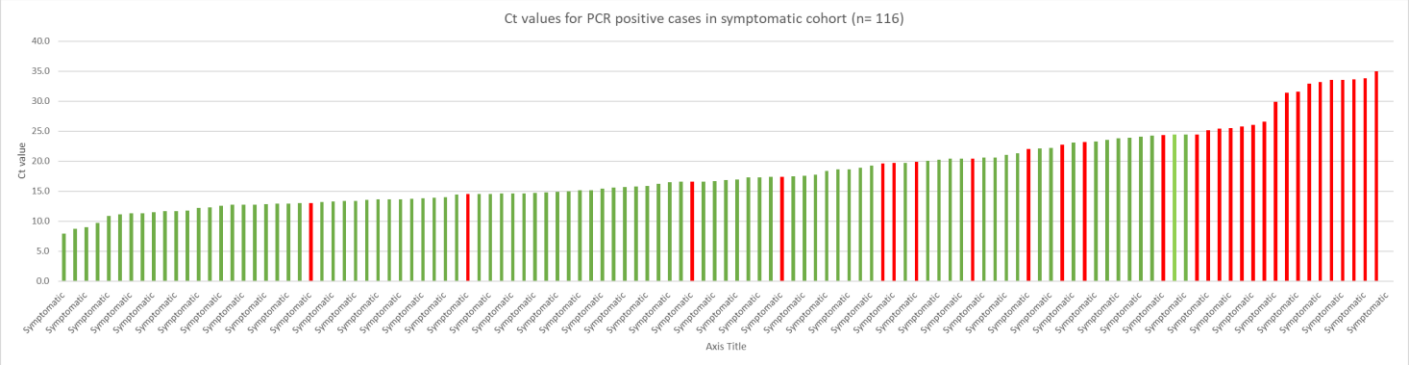

Figure 2: Ct values for all PCR positive cases within the symptomatic group tested with Abbot Panbio COVID-19 Ag Rapid Test Device (Nasopharyngeal) with mid-turbinate collected nasal swabs. Those cases with an antigen negative PCR positive result (false negatives) are highlighted in red.

Recommendation:

1. The **Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal** swabs has an excellent specificity at 100% and adequate sensitivity of 80% for a Ct ≤ 30 and 90.9% for a Ct ≤ 25. This assay can be recommended for use in symptomatic individuals suspected as having COVID 19 in line with national guidelines.
2. **Validation of the Panbio COVID-19 Ag Rapid Test Device** for **Nasopharyngeal** swabs used with bilateral mid turbinate collected nasal swabs, also demonstrated excellent specificity of 99.4% and adequate sensitivity of 81.6% for a Ct ≤ 30. This is lower than some of the other assays with nasal samples as intended use, and is at the lower end of the minimum guidelines set out by the WHO. Therefore, we would recommend that where other better performing antigen tests for nasal samples are available such as the Abbott PanBio COVID-19 Ag Rapid Test (Nasal), these should be used preferentially to the Abbot Panbio COVID-19 Ag Rapid Test (nasopharyngeal) test with nasal samples.

4.0 Validation Summary Report for SARS-CoV-2 Rapid Antigen Test (Roche Molecular Diagnostics) [VER-REP/2021/5]

This report describes the verification of performance of the Roche **SARS-CoV-2 Rapid Antigen Test** for use with **nasopharyngeal swabs**. Verification of assay performance in line with intended use in symptomatic individuals has been completed. This verification has specifically focussed on symptomatic individuals in referred for COVID-19 testing in a community swabbing centre, in Dublin.

Manufacturer's Claimed Performance Characteristics of the test in symptomatic population

Relative Sensitivity: 95.5 % (95 % CI: 91.8 % - 97.8 %) for Ct values ≤ 30

Relative specificity: 99.2 % (95 % CI: 98.2 % - 99.7 %)

Verification of Performance (symptomatic population):

Sensitivity: 86.8% (33/38) [95% CI: 0.7220 to 0.9472]

Specificity: 100% (126/126) [95% CI: 0.9644 to 1.0000]

Overall Specificity: 99.5% (240/241) [95%CI; 0.9745 to >0.9999] *

* includes 126 symptomatic, 43 asymptomatic, 72 close contacts

	Sensitivity	95% CI
Ct ≤ 25	100.0% (28/28)	0.8570 to 1.0000
Ct ≤30	94.1% (32/34)	0.7993 to 0.9935
Ct ≤ 35	89.2% (33/37)	0.7471 to 0.9630

Validation of Performance (asymptomatic population):

This work could not be completed due to low positivity rate in the population tested. Specificity was excellent in this population.

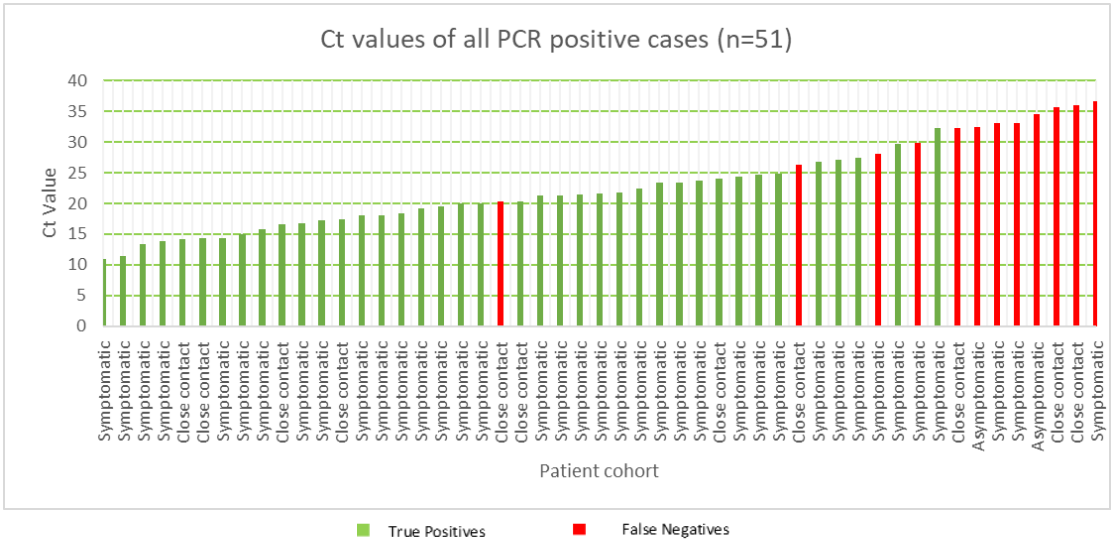

Figure 1: Ct values for all PCR positive cases within the study cohort (n=51), including symptomatic, close contacts and asymptomatic individuals. Those cases with an antigen negative PCR positive result are highlighted in red.

Recommendation:

The SARS-CoV-2 Antigen Assay (Roche) Test used with fresh nasopharyngeal swabs has an excellent specificity at 99.5% and excellent sensitivity of 94.1% for a Ct ≤ 30. In the symptomatic group, all samples with a Ct <25 were detected.

This assay can be recommended for use in symptomatic individuals suspected as having COVID 19 in line with national guidelines.

5.0 Validation Summary Report for the CLINITEST Rapid COVID-19 Antigen Test for use with Nasal Swabs. [VER-REP/2021/4]

This report describes the verification of performance of the **CLINITEST Rapid COVID-19 Antigen Test** for use with **Nasal Swabs**. Verification of assay performance in line with intended use in symptomatic individuals has been completed. This verification has specifically focussed on symptomatic individuals referred to a community swabbing centre for COVID 19 testing.

Manufacturer's Claimed Performance Characteristics of the test in symptomatic population

CLINITEST Rapid COVID-19 Antigen Test (Nasal swabs)
Relative Sensitivity: 97.25% (95% Cl: 92.17% to 99.43%)

Relative Specificity: 100% (95% CI: 97.16% to 100%)

Accuracy: 98.73% (95%CI: 96.35% to 99.74%)

Verification of Performance (symptomatic population):

Sensitivity 79.4% (27/34) [95%CI; 0.6290 to 0.8995]

Specificity 98% (150/153) [95%CI; 0.9438 to 0.9959]

Overall Specificity (all cohorts) 98.8% (246/249) [95%CI; 0.9635 to 0.9976]

	Sensitivity	95%CI
Ct ≤25	89.7% (26/29)	0.7281 to 0.9722
Ct ≤30	84.4% (27/32)	0.6777 to 0.9361
Ct ≤35	81.8% (27/33)	0.6523 to 0.9177

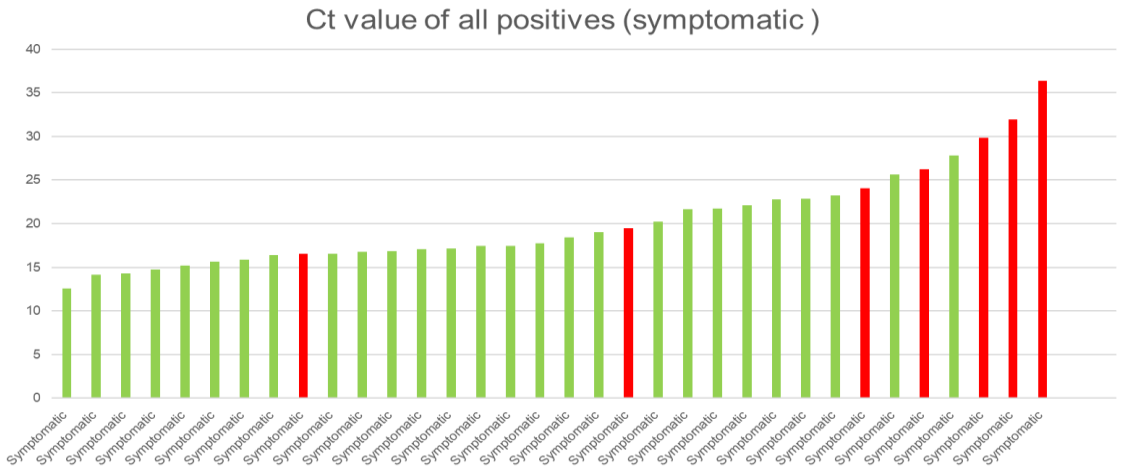

Figure 1: Ct values for all PCR positive cases within the symptomatic group. Those cases with an antigen negative PCR positive result are highlighted in red.

Validation of Performance (asymptomatic population):

This work could not be completed due to low positivity rate in the population tested. Specificity was excellent in this population.

Recommendation:

When used with fresh nasal swabs, this assay has adequate sensitivity for use in symptomatic individuals, and adequate specificity. In certain settings, PCR confirmation of positive results may be required, particularly in a healthcare setting. This assay is not recommended for rapid evaluation in the ED, because of the risks associated with a false positive result in this setting.

This assay is suitable for use in settings when nasopharyngeal swabs are not acceptable.

6.0 Validation Summary Report for the BIOSYNEX COVID-19 Ag BSS Assay for use with nasopharyngeal swabs [VER-REP/2021-6]

This report describes the verification of performance of the **BIOSYNEX COVID-19 Ag BSS Assay** for use with **nasopharyngeal swabs**. Verification of assay performance in line with intended use in symptomatic individuals has been completed. This verification has specifically focussed on symptomatic individuals in referred for COVID-19 testing in a community swabbing centre, in Dublin.

Manufacturer's Claimed Performance Characteristics of the test in symptomatic population

Sensitivity: 96% (95%CI: 93.6-98.4%)

Specificity: 100% (95%CI: 100%-100%)

Accuracy: 98% (95%CI: 96.4-99.6%)

Verification of Performance (symptomatic population):

Sensitivity: 88.1% (37/42) [95% CI: 0.7454 to 0.9527]

Specificity: 100% (110/110) [95% CI: 0.9595 to 1.0000]

Overall specificity: 100% (587/587) [95%CI: 0.9922 to 1.0000]*.

* includes symptomatic, asymptomatic, and close contacts.

Performance BIOSYNEX COVID-19 Ag BSS test with different Ct cut-offs in symptomatic group

	Sensitivity	95% CI
Ct ≤ 25	92.1 % (35/38)	0.7848 to 0.9800
Ct ≤ 30	88 % (37/42)	0.7454 to 0.9527

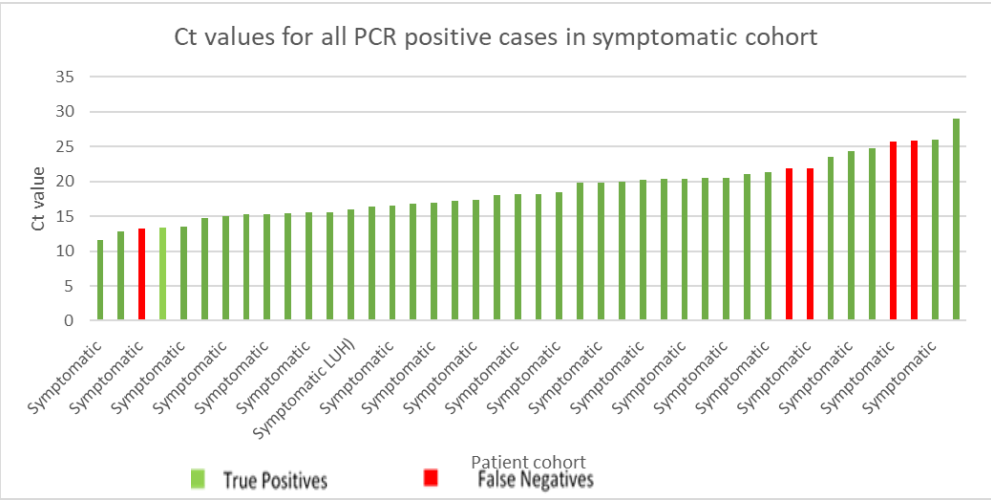

Figure 1: Ct values for all PCR positive cases within the study cohort (n=42). Those cases with an antigen negative PCR positive result are highlighted in red

Validation of Performance (asymptomatic population):

This work could not be completed due to low positivity rate in the population tested. Specificity was excellent in this population.

Recommendation:

The **BIOSYNEX COVID-19 Ag BSS Assay** used with fresh nasopharyngeal swabs has an excellent specificity at 100 % and but sensitivity of 88% for a Ct ≤ 30. This assay is within the minimum threshold required by WHO, but just below that recommended by the ECDC, in terms of sensitivity and specificity. Of concern is all (5/5) false negatives in the symptomatic population were in people whose PCR results showed Ct values ≤26 with one of the five false negative results having a PCR Ct values <20. If considered for use, further setting specific evaluation is recommended, to ensure that this assay addresses the clinical need.

7.0 Validation Summary Report for the LumiraDx SARS-CoV-2 Ag test [VER-REP/2021/7]

This report describes the verification of performance of **LumiraDx SARS-CoV-2 Ag test** for use with **nasal swabs**. Verification of assay performance in line with intended use in symptomatic individuals has been completed. This verification has specifically focussed on symptomatic individuals in referred for COVID-19 testing in a community swabbing centre, and a testing Pod at a hospital in Dublin.

Manufacturer's Claimed Performance Characteristics of the test

Symptomatic Individuals

Relative Sensitivity: 97.6 % (95 % CI: 91.6 % - 99.3 %) (Nasal)

Relative specificity: 96.6 % (95 % CI: 92.7 % - 98.4 %) (Nasal)

Relative Sensitivity: 97.5% (95 % CI: 87.1% - 99.6%) (Nasopharyngeal)

Relative specificity: 97.7 % (95 % CI: 94.7% - 99.0% (Nasopharyngeal)

Asymptomatic Individuals

Relative Sensitivity: 82.4% (95 % CI: 59.0- 93.8%) (Nasal)

Relative specificity: 99.3% (95 % CI: 97.5- 99.8%) (Nasal)

Relative Sensitivity: 80.0% (49.0-94.3%) (Nasopharyngeal)

Relative specificity: 98.4% (95.3-99.4) (Nasopharyngeal)

Verification of Performance (symptomatic population; nasal swabs):

Sensitivity: 86.8% (46/53) [95% CI: 0.7485 to 0.9376]

Specificity: 98.2% (269/274) [95% CI: 0.9568 to 0.9934]

Overall Specificity: 98.5% (401/407) [95%CI; 0.9674 to 0.9940]*

*includes 327 symptomatic, 30 asymptomatic, 113 close contacts

	Sensitivity	95% CI
Ct ≤ 25	95.5% (42/44)	0.8403 to 0.9958
Ct ≤30	93.6% (44/47)	0.8219 to 0.9845

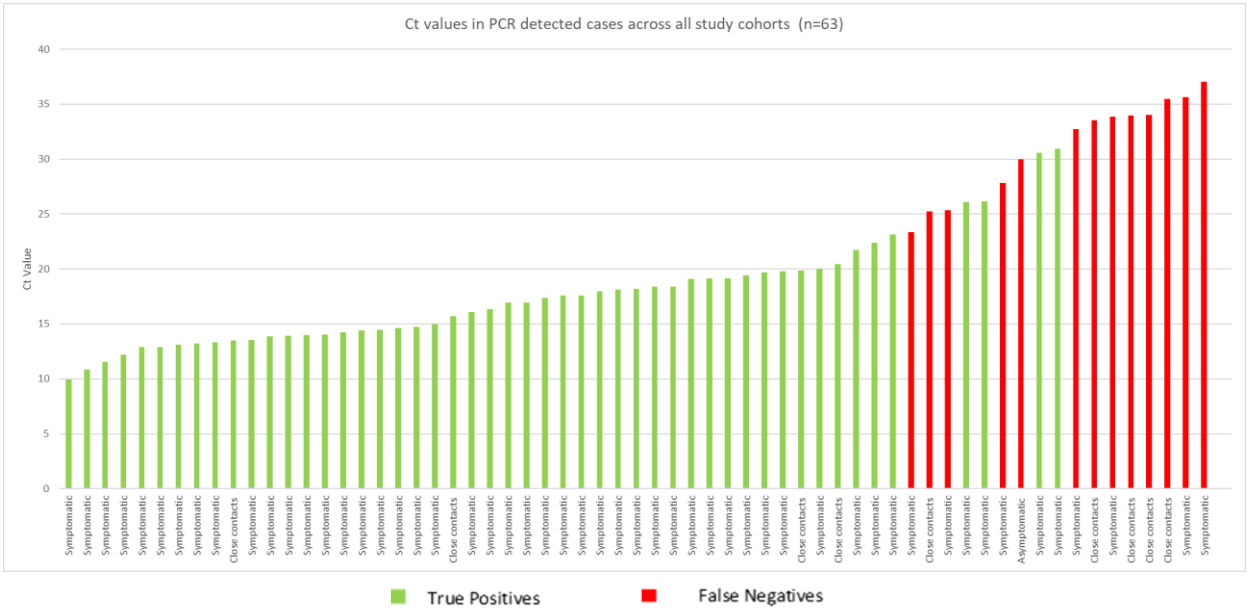

Figure 1: Ct values for all PCR positive cases within the entire study cohort (n=63), including symptomatic, close contacts and asymptomatic cases. Those cases with an antigen negative PCR positive result are highlighted in red.

Recommendation: The **LumiraDx SARS-CoV-2 Ag test** used with fresh **nasal swabs** has adequate specificity at 98.2% and good sensitivity of 93.6% for a Ct ≤ 30 and 95.5% for Ct <25. This assay can be recommended for use in symptomatic individuals suspected as having COVID 19 in line with national guidelines. Consideration needs to be given for the specific settings in which it might be deployed, as it is instrument based, it is better suited to certain environments and has specific requirements in this regard. Similarly, consideration of the issue of false positives need to be factored in for deployment in certain settings where the risk might be greater.