# Introduction Of An External Quality Assessment Scheme For The Molecular Detection Of SARS-CoV-2 Organised Jointly By The UK NEQAS Consortium and NIBSC

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Protecting and improving the nation's health

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## INTRODUCTION

SARS-CoV-2 is the seventh known Coronavirus and causes the disease Covid-19. SARS-CoV-2 is structurally similar to SARS-CoV, which was responsible for the 2003 outbreak of a similar disease SARS. Both bind to the ACE2 receptor, however SARS-CoV-2 binds with a much higher affinity (Ai *et al.,* 2020) resulting in a deadlier disease that is able to spread more rapidly in the population.
Covid-19 is spread through human transmission via close contact of an infected individual or through respiratory droplets whilst sneezing or coughing. The main symptoms exhibited by an infected individual includes fever, loss of taste and smell and a continuous cough. In extreme cases individuals may develop a lethal inflammatory response of extreme acute respiratory syndrome, pneumonia or kidney failure which is ultimately fatal if not treated instantly. Those who are in the high-risk group for Covid-19 include individuals with severe lung conditions, receiving cancer treatment, have heart conditions, kidney disease, diabetes and respiratory conditions such as asthma.

WHY WE NEED A COVID-19 EQA

When Tedros Adhanom Ghebreyesus the Director-General of the World Health Organisation came out and said 'test, test, test', this was seen as the most effective method to try to contain and limit the spread of COVID-19 in the population. The testing method widely adopted by testing labs was molecular methods such as RT-PCR, due to its ability in early detection of SARS-CoV-2 in suspected cases (Udugama *et al.,* 2020)
This led to the rapid development of many commercial and in-house assays and without them being thoroughly validated, it was recommended and encouraged by WHO for testing laboratories to enrol in an external quality assessment (EQA) for molecular detection of SARS-CoV-2 (CV) .
On April 1st the numbers of virus tests conducted in the UK was 11,924 and by the time our first pilot EQA was sent out on 21st May we were testing 105,655 in the UK (Official UK Coronavirus Dashboard, 2020).
One study suggests that the sensitivity of RT-PCR for SARS-CoV-2 detection may be as low as 59% (Lang *et al*., 2020), which makes the introduction of CV EQA even more vital in reducing the possibility of false-negative results. Further impacting on patient care or potentially spreading the virus in the population.
The introduction of a Covid-19 EQA plays an essential role in controlling the spread of Covid-19 within the population, as it provides great confidence in obtaining the correct sample test results. Therefore having a progressive positive impact on patient-centred care.

## METHODS

Three freeze-dried specimens (6327, 6328 and 6329), two positive and one negative for SARS-CoV-2 were dispatched on the 21st May 2020 for this first pilot studies. Participants were given further instructions on the testing and reporting results for this exercise via email communications and also advised to report on the state of the specimens on receipt. An online EQA specialist tool, Wolfson EQA Software was used to collect results from participating laboratories.

The positive specimens were prepared by National Institute of Biological Standards and Control ( NIBSC) as their 2019 novel coronavirus (SARS-CoV-2) working reagent for nucleic acid amplification testing (NAT) and consisted of a series of recombinant viruses which together encode the entire genome of SARS-CoV-2.

Specimen 6327: Represented a simulated nasopharyngeal swab from a 40-year-old diabetic female complaining of fever and sore throat for the last 5 days.

Specimen 6328: Represented a simulated nasopharyngeal swab from a 82-year-old male in a residential home with cough, headache and exhaustion for the last 7 days.

Specimen 6329: Represented a simulated nasopharyngeal swab from a 20-year-old university fresher with shortness of breath, diarrhoea and fever for the last 4 days.

These specimens were dispatched frozen on ice to one hundred and ten laboratories in the UK that had expressed interest to participate in this first pilot studies of "Molecular detection of SARS-CoV-2 EQA" with a request to test each specimen for the presence of SARS-CoV-2 using molecular methods.

## RESULTS

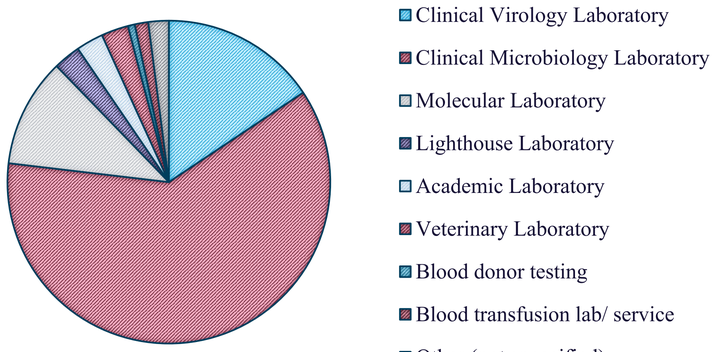


**Figure 1: Type of Laboratories Participating in Pilot EQA**

- A total of 110 laboratories expressed interest in this initial pilot study, of which 86 (77.3%) returned results before the closing date.
- 98.3% of participants returned the intended results:
  100% of participants reported intended results for specimen 6327 which was positive for SARS-CoV-2
  95.7% of participants reported intended results for specimen 6328 which was negative for SARS-CoV-2
  99.1% of participants reported intended results for specimen 6329 which was positive for SARS-CoV-2
- Figure 1 shows that the majority of participants were based within Clinical Microbiology laboratories (61.2%). 15.7% of participants were based within Clinical Virology laboratories

- Virus detection methods employed by participating labs varied across the board (Figure 2). Cepheid: GeneXpert was used by 36 laboratories and was the most commonly used method. 100% of participants who reported using this method returned the intended results across all specimens
- Altona: RealStar was the second most commonly used method, with 10 labs using this. 100% of participants that used this method, returned the intended results across all specimens

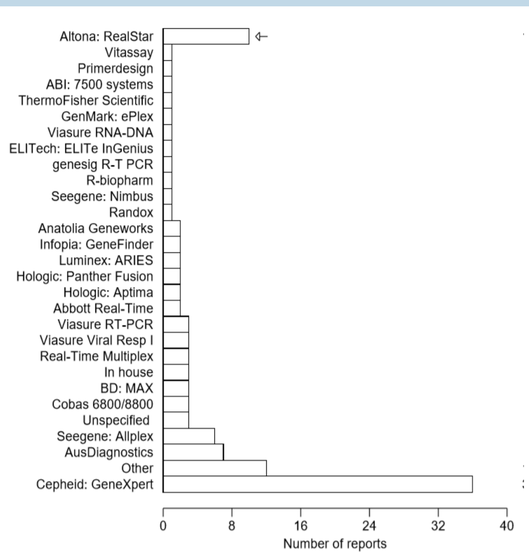


**Figure 2: Virus Detection Methods Used By Participating Labs**

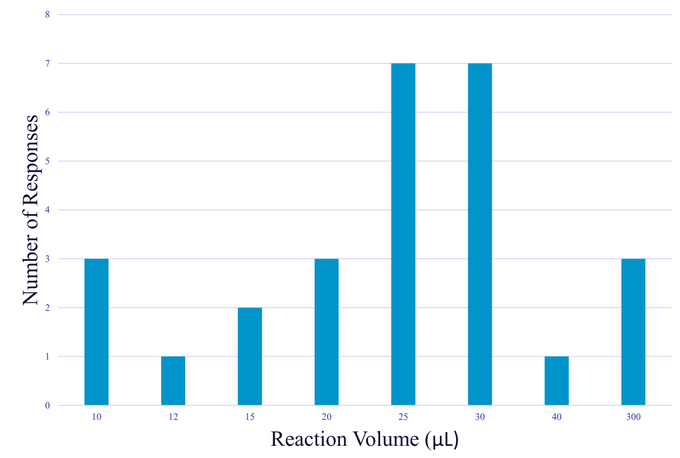


**Figure 3: Reaction volumes used, as reported by participants**

- Thirty eight participants provided the volume of material used for nucleic acid extraction. These volumes ranged from 50 µL to 1300 µL, with 36.9% responders reported to have used 200 µL
- Twenty seven responses were received on the volumes of nucleic acid template used for amplification. 40.7% of responders used 10 µl of nucleic acid template in their amplification
- Reaction volume data (Figure 3) reported, ranged from 10 µL to 300 µL). Reaction volumes of 25 µL and 30 µL were used by approximately 7 participants (26%) each

## DISCUSSION

As the number of cases continue to increase and the burden on the NHS continues to rise, there is demand to deliver accurate and reliable COVID-19 diagnostics. Although most service providers are from clinical microbiology/virology laboratories, there has been participation from a range of laboratories to help diagnose SARS-CoV-2 infection including non microbiology laboratories which account for 23.1% of our participants. Despite heavy workloads, 78.2% of participants returned their results within a week of receiving our samples. The reaction volumes differed amongst participants. The majority of laboratories reported that the reaction volumes were between 25-30µl. There are varied diagnostics platforms being used in clinical and non-clinical laboratories. This introduces varied levels of sensitivity and limits of detection. Pressure on supply chains resulted in numerous COVID-19 kits being adopted by laboratories. Our EQA scheme helps to ensure these kits are fit for purpose.

## CONCLUSIONS

- The results show an excellent return rate.
- Varying levels of sensitivity in diagnostic platform emphasizes the need of an EQA scheme to monitor the results of these platforms.
- Diagnostic laboratories have different processes in place for COVID-19 testing.
- 38.3% of participants received specimens that were thawed, however this did not effect the quality of the material prepared by NIBSC. This was established by the results submitted by participants.
- Our EQA scheme effectively compares these results in order to help us identify trends and patterns in the use of different diagnostic platforms.
- In rare cases, the results from our participants can highlight any issues or concerns with manufacturer kits. It can be used as an educational tool to train and improve testing services.
- We are dedicated to providing high-quality inactivated specimens that are representative of real clinical samples in order to help evaluate and monitor the accuracy of diagnostic kits.

## ACKNOWLEDGEMENTS

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