

COVID-19 (SARS-2) diagnostics at Hampshire Hospitals NHS Foundation Trust

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Introduction

In response to increasing numbers of requests for COVID19 tests and to pre-empt the inevitable call for NHS labs to support the reference labs we decided to explore setting up in-house testing as soon as possible.

At the beginning of February we procured two new assays prior to public release and proceeded to set-up a parallel testing service to compare results of these assays with the PHE reference lab service.

Methods

Assays were challenged with inactivated respiratory viruses from ZeptoMetrix (NATrol Resp verification panel 2) and known respiratory virus positive patient samples before testing ?COVID19 samples.

Throughout February and early March we tested in parallel 57 patient combined nose and throat swabs that were sent to PHE Colindale and Southampton for ?COVID19 testing.

We received positive control material from PHE Colindale to complete the validation (Lot 07/02/2020).

Extraction - PerkinElmer chemagic Prepito-D using the NA Body Fluid kit; plasma protocol.

Diagnostic platforms - Biomolecular Systems Magnetic Induction Cyclers (MIC)

Diagnostic assays (20 µl total volume): both these assays are now CE marked and both use Taqman chemistry.

- Primer Design Genesig COVID-19 assay – direct from company - <http://www.primerdesign.co.uk/home>
- VIASURE 2019-nCoV by CerTest BIOTEC – via Pro-Lab Diagnostics - <https://www.pro-lab.co.uk/product-category/viasure/viasure-real-time-pcr-detection-kits/>

Initial screen - Primer Design Genesig COVID-19 assay – RdRP gene target.

4 µl of IAC RNA spiked pre-extraction.

Assay 1 – COVID19 and IAC

1x

10 µl Mastermix
 1 µl COVID Primer/Probe (FAM)
 1 µl IAC Primer/Probe (VIC)
3 µl Water
 15 µl

Assay 2 – Endogenous human control
 (sample quality indicator <30Ct)

1x

10 µl Mastermix
 1 µl Endog Primer/Probe (FAM)
4 µl Water
 15 µl

Both assays run on the same cycling conditions, and require the same volumes.

- 15 µl Mastermix
- 5 µl Sample/POS/NEG

| Cycles | Step | Time | Tm |
|--------|-----------------------|--------|------|
| 1 | Reverse transcription | 10 min | 55°C |
| 1 | Initial denaturation | 2 min | 95°C |
| 45 | Denaturation | 10 sec | 65°C |
| | Annealing/Extension | 60 sec | 60°C |

Positive confirmation - VIASURE 2019-nCoV – S gene target.

- S gene FAM

- IAC VIC

- 15 µl Mastermix (all in one buffer, enzyme, P/P FAM & VIC)
- 5 µl Sample/POS/NEG

| Cycles | Step | Time | Tm |
|--------|-----------------------|--------|------|
| 1 | Reverse transcription | 15 min | 45°C |
| 1 | Initial denaturation | 2 min | 95°C |
| 45 | Denaturation | 10 sec | 95°C |
| | Annealing/Extension | 50 sec | 60°C |

Results

Inactivated virus control material

| | |
|-----------------------------|---------------------------|
| Adenovirus 1 | Nucleic acid NOT detected |
| Adenovirus 3 | Nucleic acid NOT detected |
| Coronavirus 229E | Nucleic acid NOT detected |
| Coronavirus HKU-1 | Nucleic acid NOT detected |
| Coronavirus NL63 | Nucleic acid NOT detected |
| Coronavirus OC43 | Nucleic acid NOT detected |
| Flu A H1N1 _{pdm09} | Nucleic acid NOT detected |
| Flu A H3/brisbane/10/07 | Nucleic acid NOT detected |
| Flu A H1 | Nucleic acid NOT detected |
| Flu B/florida/02/06 | Nucleic acid NOT detected |
| RSV A2 | Nucleic acid NOT detected |
| Human Metapneumovirus | Nucleic acid NOT detected |
| Parainfluenza 1 | Nucleic acid NOT detected |
| Parainfluenza 2 | Nucleic acid NOT detected |
| Parainfluenza 3 | Nucleic acid NOT detected |
| Parainfluenza 4 | Nucleic acid NOT detected |
| Rhinovirus | Nucleic acid NOT detected |

Comparison to PHE COVID-19 assay

| Target | Count | True Pos | False Pos | True Neg | False Neg | Sn | Sp |
|------------------------------|-------|----------|-----------|----------|-----------|-------------|---------------|
| PD COVID19 | 57 | 4 | 0 | 53 | 0 | 4/4 100% | 53/53 100% |
| VIASURE 2019-nCoV | 29 | n/a | 0 | 29 | 0 | n/a | 29/29 100% |

14 of these patients were also tested on the BioFire Filmarray Resp 2+ panel (see spreadsheet)

Challenge with PHE positive control material

| Assay | Mean CT (n=10) | PHE mean CT - RdRP |
|-----------------------------------|----------------|--------------------|
| PD COVID19 - RdRP | 28.33 | 28.95 |
| VIASURE 2019-nCoV - S gene | 28.59 | |