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# Robust detection of SARS-CoV-2 in a multi-centre evaluation of clinical performance using the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay

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## INTRODUCTION

QuantuMDx set out to design, develop and manufacture a SARS-CoV-2 detection assay which is sensitive, specific and user friendly in an attempt to help combat the ongoing pandemic. The result is an extremely accurate test which has many benefits over other developed assays, both from a biological and end-user stand-point.

## AIM

The main goals of this work were as follows and were targeted to solve problems often seen in commercial assay design:

- Design and develop a highly sensitive and specific SARS-CoV-2 assay
- Future-proof the assay performance against predictable mutations
- Produce an assay suitable for use on a range of commercial machines
- No indeterminate results are called if controls are valid
- Provide a long shelf-life for assay without requiring a freezer
- Ensure assay is rapid and easy to use

## METHOD

Various techniques were employed to deliver an assay which overcomes the issues set out in the Aims section.

Primers and probes for 3 SARS-CoV-2 targets (N Gene, S Gene and ORF1 region) were used to provide high sensitivity as well as redundancy and improved detection against any arising strain variants. Chosen sequences were stringently selected against deposited sequences from NCBI and GISAID, to negate the risk of off-target amplification. In silico and wet analysis were performed against commensal and respiratory disease causing organisms, including those specified by the FDA

The likelihood of mutations within the designed primer/probe regions was heavily reduced by using intelligent bioinformatic design. All SARS-CoV-2 sequences available at the time (from NCBI and GISAID) were used alongside SARS and MERS sequences to predict likely areas of variation in the future. These areas were avoided.

Primers and probes for RNase P detection were designed to give a specimen process control (SPC) to allow for identification of correct sampling. The assay is designed to return concise SARS-CoV-2 status without 'indeterminate' results when this SPC is positive

Fluorophores (FAM and HEX) were chosen to ensure assay compatibility with many commercially available Real-Time thermal cyclers

Reagents were chosen in order to provide an assay which is rapid, sensitive, convenient and stable. The test is a single lyophilised mix in a vial.

## RESULTS

### Ease-of-Use

The QuantuMDx SARS-CoV-2 Detection Assay was built from the ground up with the end-user in mind. Many aspects were tailored towards ease-of-use and convenience.

Firstly, the mix is provided as a single, lyophilised vial, guaranteeing reagent stability for up to 18-months in the refrigerator. A freezer is not required and therefore, reagents do not need thawing prior to use and only need rehydrating with water before being ready to use. No need for addition of further reagents. Figure 1 demonstrates the minimal workflow required for use of the assay.

The assay is validated for use with the following commonly used RNA extraction methodologies; Qiagen QIAamp© Viral RNA Mini Kit (automated QIAcube), Promega Maxwell® RSC Viral TNA Purification Kit (automated Maxwell RSC 48), PerkinElmer chemagic™ Prime Viral DNA/RNA and the Roche MagNA Pure 96 DNA and Viral RNA SV Kit (automated MagNA Pure 96).

The assay is also validated for use on the following, commonly used thermal cyclers; Qiagen Rotor-Gene Q, Bio-Rad CFX96™ Dx , Bio-Rad CFX96™ Deepwell, Thermo Fisher QuantStudio™ 7 384 well, Roche LightCycler® 480 II, Thermo Fisher ABI™ 7500 Fast Dx. The chemistry chosen allows the PCR reaction to be performed in ~75 minutes.

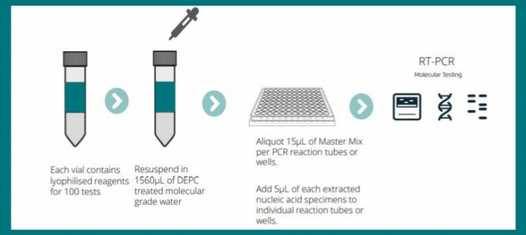


**Figure 1 – User workflow for the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay**

### Clinical Performance

Table 1 demonstrates the outstanding clinical performance of the QuantuMDx SARS-CoV-2 RT-PCR Detection Assay, with a Positive Percent Agreement of **99.0%** and a Negative Percent Agreement of **100%**. These validation studies were performed independently at 5 external clinical laboratories against other validated comparator assays. In total, 496 clinical samples were tested. The QuantuMDx assay correctly called 201/203 positive and 293/293 negative. External sites used a wide range of extraction methodologies as well as multiple thermal cycler models.

| | | Comparator Assay | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| QMDx SARS-CoV-2 RT-PCR Detection Assay | Positive | 201 | 0 | 201 |
| | Negative | 2 | 293 | 295 |
| | Total | 203 | 293 | 496 |
| Positive Percent Agreement | | 99.0% (201/203) | | |
| Negative Percent Agreement | | 100% (293/293) | | |

**Table 1 - Positive and Negative Percent Agreement between QuantuMDx SARS-CoV-2 detection assay and validated comparator assays.**
The testing was performed at 5 external sites clinical laboratories:

- Manchester Centre for Genomic Medicine (MCGM) Clinical Laboratory - Comparator assay used from IDT (N-Gene assay)
- NHS Hampshire Clinical Laboratory - Comparator assay used from PrimerDesign
- St George University of London – Comparator assay used from Altona
- Foundation for Innovative New Diagnostics (FIND) – University Hospitals of Geneva – Comparator assay used from TIL MOLBIOL
- MNDA Life Sciences Laboratories – Comparator assay used from PrimerDesign

### Additional Performance Characteristics

- Analytical sensitivity – 10 copies per reaction
- Analytical Specificity – 100% (in silico and wet testing)
- Reproducibility and repeatability – 100% (day-to-day, technician-to-technician, instrument-to-instrument, site-to-site, lot-to-lot)
- Interfering organisms – No interference when commensals and relevant respiratory disease causing organisms were tested at physiologically relevant concentrations

### Bioinformatics

Bioinformatic decisions made at the time of assay conception mean the QuantuMDx SARS-CoV-2 detection assay has not been significantly impacted by the emergence of new strains and variants to date. We assessed the mutation behaviour of previous disease-causing (MERS, SARS) coronaviruses to attempt to predict likely areas of genomic mutation and coupled this with assessment of the SARS-CoV-2 sequences available at the time.

Bi-weekly sequence analysis shows the assay detects 98-99% of the variants of concern (VOC) or variants under investigation (VUI) with at least 2 of the 3 loci targeted.

Additionally, primers and probes for RNase P allow detection of an endogenous specimen process control (SPC). A positive signal for this ensures sampling has taken place correctly and tests with a positive SPC signal will have a definitive result. No indeterminate results are reported when using this assay.

## CONCLUSIONS

The QuantuMDx SARS-CoV-2 RT-PCR Detection Assay has demonstrated excellent clinical performance across 5 external validation sites with a Positive and Negative Percent Agreement of 99.0 % and 100% respectively. Use of various extraction chemistries and thermal cycler models during this evaluation further verifies the robustness and accessibility of this assay.

The assay is now CE-IVD marked and has also been validated by NHS Test and Trace.

## REFERENCES

www.gov.uk/dhsc [Internet]. Department of Health and Social Care. Nov 2020. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/ uploads/attachment_data/file/944909/QuantumDX_TVG_Report-V0.1-201208.pdf

## CONTACT INFORMATION

For more information, please email **info@quantumdx.com** or go to **www.quantumdx.com**

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