Qnostics

SARS-CoV-2 Product Range

SCV2

Technical Note SCV2-01

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General Product Description

Qnostics' range of SARS-CoV-2 (SCV2) products listed in Table 1 are designed to support laboratories in the verification, validation, and performance monitoring of their SARS-CoV2 molecular assay. The product range contains whole inactivated SCV2 virus covering the entire viral genome in a transport medium containing background human cells. The SCV2 materials have been extensively characterised across a wide range of different molecular workflows / assays both commercial and in house currently in use. The SCV2 product range are external quality controls and are independent of manufacturers' kit controls. These reagents enable laboratories using them to monitor assay, equipment and operator performance on a day-to-day basis. The SCV2 product range fulfils the requirements of a reference material as described in ISO 15189.

Table 1: Qnostics SARS-CoV-2 associated products

Product Code	Product Description	Vials	Fill	Range Log_{10} Digital Copies / ml
SCV2AQP	Analytical Q Panel	8 positive 1 negative	0.5ml	6.0 to 1.7 Log_{10} dC/ml
SCV2MQP	Molecular Q Panel	3 positive 1 negative	0.5ml	4, 3 & 2 Log_{10} dC/ml
SCVQC	Q Control	5 positives	0.5ml	4.0 Log_{10} dC/ml
TMNQC	Negative Control	5 negatives	0.5ml	Negative for SCV2

Clinical range

A performance evaluation was carried out to assess the distribution of the positive samples in a typical cohort of asymptomatic and symptomatic population (n=1174). Figures 1a and 1b show the distribution of 262 positive patient samples identified over 60 consecutive days of testing.

Figure 1a – Performance of Qnostics SCV2 products relative to the distribution of SARS-CoV2 positive patient results within study

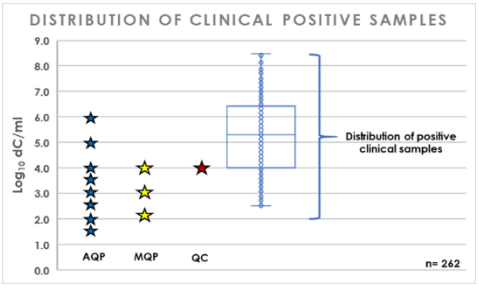


Figure 1a shows the distribution of clinical positives observed in the study plotted against the Qnostics product range. The AQP runs from above mean down to the LOD. The MQP focuses on the last quartile and the Q control provides a representative positive control.

Figure 1b – Distribution of SARS-CoV2 positive patient test results within clinical study

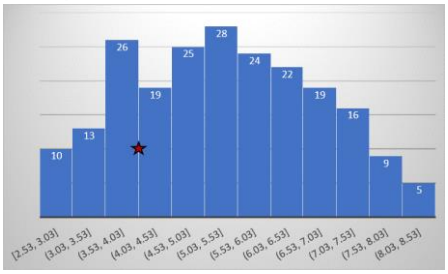


Figure 1b shows the distribution of positive samples observed in the study. For reference the position of the QC is indicated by a red star This demonstrates how the Qnostics SCV2 controls are representative of clinical samples observed during the study.

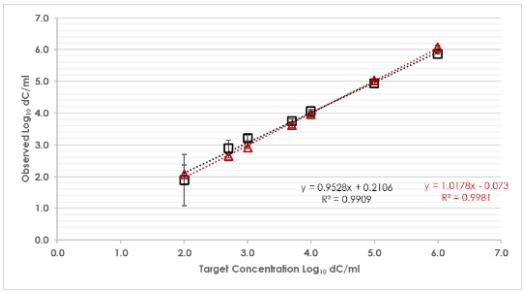
The SARS-CoV2 Product Range

1) Analytical Q Panel (AQP)

The Qnostics AQP is designed to be used to support laboratories in the assessment of assay linearity and the relative Limit of Detection (LOD) of their molecular workflows. It has been designed to cover the observed clinical range from the mean / median values down to the relative limit of detection.

The AQP has been calibrated using digital PCR and subsequently tested across a range of molecular workflows for the screening and detection of SCV2. Based upon a performance evaluation of over 30 different molecular workflows the LOD defined as the concentration of SCV2 that can be detected as positive with a 95% confidence interval, was between 2.0 Log_{10} dC/ml and 3.0 Log_{10} dC/ml. See Figure 2 for an example of AQP performance.

Figure 2 – Relative performance of the Qnostics AQP panel



Using an AQP to help identify Limit of Detection

When using molecular assays for screening purposes, it is important to understand the limit of detection (LOD) of the assay in terms of its analytical sensitivity and specificity, relative to its application in the clinical setting. The LOD is dependent on the analytical procedure, the nucleic acid extraction and molecular assay used.

In order to establish the qualitative LOD of a molecular workflow, standard practice would be to test a linearity panel of dilutions across the known dynamic range of the assay. This would typically be at log and/or 0.5 Log_{10} dilutions down to the point where the panel member within the dilution series is no longer detected. The last panel member in the series to be detected 'positive' would typically be tested multiple times in order to establish a detection rate.

In order to be able to compare relative LOD between workflows/assays and across laboratories, the molecular workflows need to be calibrated to a common reference material or standard. In the absence of available international standards and certified reference materials digital PCR provides a mechanism for supporting traceability and comparability of results and has become accepted as a molecular reference method within ISO17511.

2) Molecular Q Control (MQP)

The Molecular Q Panels (MQPs) are designed to support laboratories in monitoring the performance of their assays. They are useful when there is a requirement to verify assay performance periodically. For example when changing lots of reagents or after servicing and maintenance.
It can also be used to help train staff on the assay and for demonstrating assay performance in the field.

When assay performance has been established, rather than running the full AQP dilution series, a subset of the linearity panel can be used to verify performance. The MQP is a subset of the AQP product that covers the lower quarter of the observed clinical range, see Figure 1a and 1b. The MQP contains a high, medium, low and a negative sample to help verify that any changes to the assay have not significantly impacted on the overall performance.

3) Q Control (QC)

The Q controls are third party, positive controls developed to support laboratories with their routine Quality Control (QC) monitoring. It is essential that the laboratory conducts appropriate quality control (QC) & quality assurance (QA) measures. These measures are often driven by International and regional regulations and include a requirement for verifying and/or validating the performance specifications of the molecular assay as well as monitoring it's intended use and performance in the routine clinical setting over time.

The target concentration of the Q control has been designed to be within the dynamic range of most molecular assays and is representative of clinical specimens seen in the performance evaluation, see Figures 1a and 1b. The performance of the Q control was evaluated across 30 different molecular assay / workflows. The performance evaluation looked at quantitative and qualitative performance and the gene loci targeted by the assays used.

QC Performance

Using whole, inactivated SARS-CoV-2 provides the full viral genome ensuring compatibility with all molecular assays. This can be left up to five days after thawing in the fridge. Please see Figures 3 and 4 for the control performance across 11 commercial and inhouse workflows and the combinations of loci used to correctly identify SCV2.

Figure 3 Performance of the QC control across multiple workflows

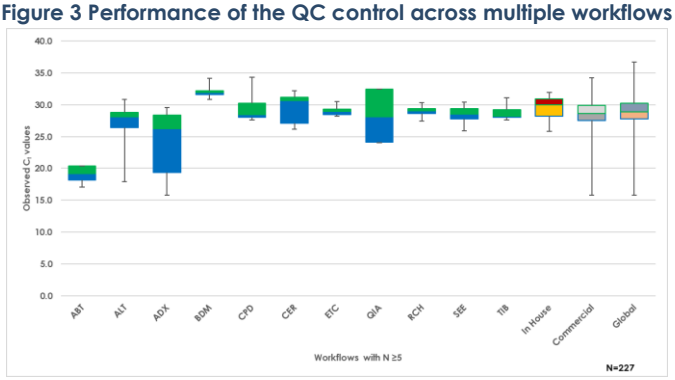


Figure 3 shows the performance for assays where there were ≥5 datasets returned with Cₜ values. In house covers all non-commercial assays, Commercial groups all of the commercially assays, Global covers all data.

Figures 4a and 4b - Combinations of loci used to detect SCV.

Figure 4a: Major loci and combinations used to detect SCV2.

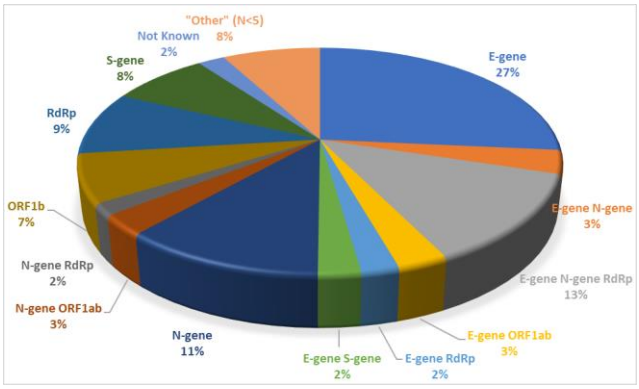


Figure 4a shows the loci targeted by the commercial and in house assays used in the performance evaluation where there were at least 5 datasets returned. All workflows shown, correctly identified the control as positive for SCV2. The group labelled "Other" is made up of the combinations used less frequently (N<5). The combination that makes up the "Other" group are shown in Figure 4b below.

Figure 4b: Less commonly used loci and combinations used to detect SCV2.

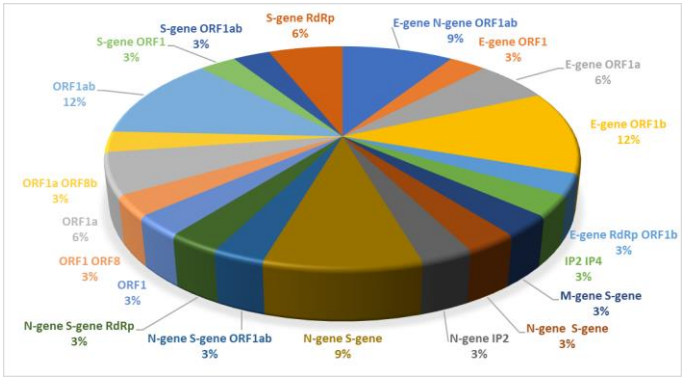


Figure 4b shows the loci targeted less frequently by the commercial and in house assays used in the evaluation. All workflows correctly identified the control as positive for SCV2.

4) Metrological traceability

Metrological traceability describes the process whereby a clinical test result can be traced back through an unbroken chain of comparisons to an appropriate International standard or higher order reference material with an estimated uncertainty of measurement at each step. This in turn supports the comparability of results between different molecular methods and laboratories over time and provides confidence in the laboratory's ability to deliver accurate and reproducible patient test results. The highest order standard is one which is traceable to the metric system/international system of units (SI).

WHO International standards are established through consensus studies. They are not traceable to an SI based value and do not have an uncertainty of measurement. Instead have an assigned arbitrary value in International Units (IU). In molecular virology and in particular the quantitation of viral nucleic acid WHO standards, where available, are recognised as one of the primary reference materials in support of assay and control standardisation.

Digital PCR (dPCR) provides a way of counting target viral nucleic acid molecules in order to establish an accurate and reproducible measure of copy number. Digital PCR is recognised in the latest version of ISO17511 as a molecular reference method which can be used in combination with WHO standards or on their own.

ISO17511*(In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples)*

Calibration to the 1st WHO International Standard for SARS-CoV2

The Qnostics SARS-CoV2 product range are fully traceable to the first WHO international standard (20/146) and have been characterised using digital PCR in accordance with ISO 17511. The product range can be used to support the initial validation and verification as well as ongoing assay performance monitoring in line with the requirements of ISO 17025 and ISO 15189.

The controls have also been shown to be at clinically appropriate levels (Figures 1a and 1b) and show consistent, repeatable and reproducible performance across all the molecular workflow / assays evaluated (Figure 3).

Table 2: SARS-CoV-2 product values in digital copies & international units

Product Code	Expected value Log_{10} dC/ml*	Expected value Log_{10} IU/ml*	MQP	QC	PC
SCV2AQP01-S01	6.0	6.88			
SCV2AQP01-S02	5.0	5.88			
SCV2AQP01-S03	4.0	4.88	☑	☑	
SCV2AQP01-S04	3.7	4.57			
SCV2AQP01-S05	3.0	3.88	☑		
SCV2AQP01-S06	2.7	3.57			☑
SCV2AQP01-S07	2.0	2.88	☑		
SCV2AQP01-S08	1.7	2.57			
SCV2AQP01-S09	Below LoD	Below LoD	☑		

***values on the Qnostics' in house N-gene assay. Values in Table 2 are molecular workflow specific.**

5) Negative Controls (TMNQC)

Negative controls support laboratories in the assessment and regular monitoring of specificity and potential contamination. Where a laboratory also needs a negative control for routine use or troubleshooting Qnostics provides the TMNQC. This is a transport medium control containing background human cells and negative for SCV2.

Additional information

Warnings and Precautions

The controls contain whole, inactivated virus but must only be handled by trained laboratory personnel and in accordance with Good Laboratory Practices, which must include the use of personal protective equipment (PPE). All residual materials must be treated as potentially hazardous and disposed of accordingly. This must be carried out according to the established procedures of the laboratory and in accordance with national and international regulations.

Hazard and Precautionary Statements:

H303, H333, P202, P270, P280, P314

IMPORTANT NOTE: The SCV2 range of product samples have **no assigned value.** The values provided are indicative and were established using digital PCR and the first international standard. These values are specific to the Qnostics' reference assays used. The actual values on different workflows may vary from those shown as they are dependent on the evaluation procedure, the nucleic acid extraction and molecular assay used, See Figure 3. It is the responsibility of the end user to establish their own target values for the controls using their laboratory's molecular procedures.

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