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Guidance

Technical validation protocol for SARS-CoV-2 nucleic acid detection

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This publication is available at https://www.gov.uk/government/publications/assessment-and-procurement-of-coronavirus-covid-19-tests/technical-validation-protocol-for-sars-cov-2-nucleic-acid-detection

1. Assay description and intended purpose

1.1 What is the principle and method of the assay (description of the assay according to the manufacturer's instructions for use (IFU)?

1.2 What is the use for which the device is intended according to the data supplied by the manufacturer on the label, in the IFU, in promotional or sales materials or statements, or as specified by the manufacturer in the performance evaluation?

1.3 Is the test a stand-alone device/test or to be used in conjunction with other equipment and is biosafety containment is required?

2. Type of sample to be used in validation

2.1 Stipulate the sample type (for example whole non-extracted virus, extracted RNA, synthetic RNA, plasmid DNA containing assay target regions) and any sample matrices (for example saliva, plasma, nasopharyngeal/oronasal swab [dry or in VTM] and so on) in which the material is to be spiked.

2.2 Stipulate if the material is required to be extracted: that is volume received, volume extracted, volume eluted, elution buffer to be used in the assay.

2.3 If possible, stipulate if any interfering substances such as preservatives are likely to be present. Shelf life and number of freeze thaw events should also be stated, if known. Where dry swabs are to be used, samples will need to be collected prospectively; 2 swabs per participant, one for a new test and one to be tested using reference method; or one swab for a new test collected within 24 hours of a positive reference method swab.

3. Equipment and reagents

3.1 List all the equipment required that are not supplied by the manufacturer with calibration/service requirements and dates where applicable.

3.2 List all the reagents required that are not provided by the manufacturer with shelf-life expiry dates and storage conditions. Include positive and negative control materials.

4. Performance characteristics

4.1 Analytical sensitivity and linearity of SARS COV-2 targets

4.1.1 Dilution series: this should be calculated using a validated standard dilution series. If this is not possible (as standard material is not available), use 5 clinical positive replicates, with a 5 log10 dilution, plus 5 negatives, plus the use of inhibition controls. If feasible, repeat over several days, different users/machines (feasibility may be limited due to availability of positive material). Where dry swabs are to be used, known amounts of standard material should be added to the swab, and then tested as per IFU. Reference to be made on the reporting of results and for example CT values.

4.1.2 Linearity and efficiency: Ideally for linearity, the use of a standardised reference panel should be used to ensure effective benchmarking and assure that dilutions are accurate. If an alternative route for establishing linearity is undertaken, the method will need to be documented For LAMP assays the linearity will need to be standardised via dilutions in appropriate matrixes using untreated virus. Plot the data from 4.1.1 and calculate linearity and efficiency. Compare the data with that supplied by the manufacturer, if applicable.

4.1.3 Lowest Limits of Detection (LLOD): Where a validated standard dilution series was used LLOD should be calculated, using data from 4.1.2, in copies/ml (to align with the relevant MHRA).TPP). Where clinical positive material is used, copies/ml cannot be calculated; median CT value or dPCR should be given for the lowest dilution detected from the samples used in 4.1.1.

5. Precision and robustness

5.1 Intra-assay precision: Use the data for 5 replicate values from a single day from 4.1.1 to calculate Standard Deviation & Coefficient of Variation measurement, with the values for the latter to be <10%. To include the use of inhibition controls.

5.2 Inter-assay precision: Use the data for 5 replicate values from multiple days from 4.1.1 for Standard Deviation & Coefficient of Variation with the values for the latter to be <15%.

5.3 Repeatability: Spike 30 negative samples from different individuals with known amount of agent/positive material (suggested 3x the LLOD), all should be positive.

6. Analytical specificity (interferences and cross-reactions)

6.1 Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (such as other coronaviruses), syndromic diseases (such as other respiratory viruses and bacteria) and common diseases (such as HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

7. Diagnostic sensitivity and specificity (clinical validation with confirmed positives and negatives)

7.1 Samples selected for this validation will be appropriate to the assay. Low medium and high viral load samples will be be equally distributed to avoid increasing or lowering DSe and DSp.

7.2 Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~150 samples should be included to align with MHRA TPP. Clinical sensitivity (95% CI) and positive predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has sensitivity and specificity and a limit of detection within the specifications of the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays must be included in the validation report.

7.3 Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) should be used. Preferably, depending on the availability of samples, ~250 samples should be included to align with MHRA TPP. Clinical specificity (95% CI) and negative predictive value (PPV) should be calculated in comparison with a CE marked reference method that itself has sensitivity and specificity in line with the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays must be included in the validation report.

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