INDIAN COUNCIL OF MEDICAL RESEARCH
DEPARTMENT OF HEALTH RESEARCH

STANDARD PROTOCOL FOR VALIDATION OF COMMERCIAL RT-PCR BASED SARS-COV-2 DETECTION KIT

Objectives:

- To compare sensitivity and specificity of the new assay with that of the ICMR-**NIV assay**
- To rule out cross-reactivity with other respiratory viruses
- To assess analytical sensitivity of the assay (optional: if in-vitro transcribed RNA available for the viral target genes in the kit)

Sample Panel:

Clinical samples: Nasopharyngeal or oropharyngeal swabs in virus transport medium (VTM)

1. SARS-CoV-2 positive clinical samples (n =75) (equal representation of samples with low, medium and high Ct values as per ICMR-NIV RT-qPCR assay)

2. SARS-CoV-2 negative clinical samples (n=85)[1]
   a. [2]Clinical samples negative for SARS-CoV-2 but positive for other respiratory viruses (ORVs) such as Influenza A/ Influenza B virus, Human corona virus, Parainfluenza virus, Rhino virus, Respiratory Syncytial virusas per availability (n =10)
   b. SARS-CoV-2 negative clinical samples as per ICMR-NIV RT-qPCR assay (n=75)

Methodology:

Determination of sensitivity and specificity of the assay:

1) Extraction of viral RNA from the 160 clinical samples using any validated RNA extraction kit
2) Testing of RNA for detection of SARS-CoV-2 target genes and human internal control gene as per the manufacturer's protocol
3) Interpretation of results as per the cut off threshold cycle (Ct) values recommended by the manufacturer for-
   a. Detection of positive samples as positive
   b. Detection of negative samples as negative

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[1] Assuming pre-test probability of 5%, a specificity of 99.5% will yield a positive predictive value of 90%.
[2]Must be included in the sample panel when the kit is being validated for the first time

Determination of analytical sensitivity of the assay

Make 10-fold serial dilutions (~10^8 to 10 RNA copies/ µl) of in-vitro transcripts (RNA) of either E or ORF1ab or RdRp or N gene of SARS-CoV-2 and use these RNA dilutions to determine the lower limit of detection (LOD) of the RNA copies in the assay

Results

1) Compare performance of the assay under validation for the 75 positive and 85 negatives (10 SARS-CoV-2 negative but ORV positive +75 two SARS-CoV-2 negative) clinical samples with that of the **ICMR-NIV RT-qPCR assay**.
2) Calculate the Sensitivity and Specificity of the assay in comparison with the ICMR-NIV assay as the gold standard.
3) Calculate the LOD of the assay

The kit performance is *satisfactory if-

1. The **sensitivity** of the assay is ≥94.7% for detection of positive samples (i.e. atleast 71 samples out of 75 positive samples tested should come positive)
   AND
2. **Specificity** of the assay is ≥98.8% for detection of negative samples (i.e. atleast 84 samples out of 85 negative samples tested should come negative)

Comments/suggestions to be provided

1. Check whether the Cut-off Ct values are provided and protocol is very specific rather than general, and mention if corrections are needed in the kit insert.

Additional points to be mentioned in the kit validation report-

Requirement of any specific equipment, additional steps or adjustments in machine while interpreting final results.

REPORT FORMAT

NAME OF THE VALIDATION CENTRE

PERFORMANCE EVALUATION REPORT FOR RT-PCR DIAGNOSTIC KIT

- Name of the kit
- Name of the manufacturer
- Batch number
- Date of Expiry
- Application
- Kit components
- Sample Panel
  - Positive samples
  - Negative samples (provide details)
- Results

		RT PCR Results		
		Positive	Negative	Total
Name of RT PCR Kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Limit of detection
- Conclusions: Satisfactory or Not Satisfactory

(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from the batch mentioned above)

Disclaimers

1. ICMR's validation process does not approve / disapprove the kit design
2. ICMR's validation process does not certify user friendliness of the kit / assay

3. Validation of a kit by ICMR is not an assurance that the kit specifications would be included in the tendering process.

In addition to the above (1,2,3), the following disclaimer/Limitation needs to be included in all validation reports by all ICMR approved validation centres for SYBR green based real-time PCR assay

Interpretation of the SYBR green based test results requires expertise and experience which may not be available in many routine diagnostic laboratories involved in COVID-19 testing.

Note: This report is exclusively for RT-PCR Kit (Lot No……) manufactured by …… (supplied by …….)

The company shall not use or publish information or report for advertising or promotional purposes

Evaluation Done on ……

Evaluation Done by …….

Signature of Director/ Director-Incharge ……