ICMR-CDSCO/IVD/GD/PROTOCOLS/04/2024

Indian Council of Medical Research and Central Drugs Standard Control Organization
Department of Health Research and Drugs Controller General of India
Ministry of Health and Family Welfare
Government of India
Document No.: ICMR-CDSCO/IVD/GD/PROTOCOLS/04/2024

Subject: Inviting comments on standard IVD evaluation protocol drafted by ICMR and CDSCO

Licensure of In-Vitro Diagnostics (IVDs) under Medical Devices Rules 2017 requires a detailed evaluation protocol for the performance evaluation of IVDs to evaluate their quality and performance. To facilitate this process, the Indian Council of Medical Research (ICMR) and CDSCO have come together to draft standard evaluation protocols for use by IVD manufacturers testing labs in India. Currently, the HMPV real time PCR IVD evaluation protocol has been developed by ICMR and CDSCO.

The protocol is now being placed in the public domain for comments from relevant stakeholders. This window of opportunity will close on 15th March 2025, and, once finalized, there will be minimal scope for change in these documents. Therefore, all interested stakeholders are requested to provide their comments before 15th March 2025, at ivdevaluation@gmail.com as per the enclosed format. Once the public consultation period concludes, all comments will be reviewed and considered in finalizing the draft protocols before final clearance by ICMR and CDSCO.

Dated: 28th January 2025
Place: New Delhi

STANDARD IVD PERFORMANCE EVALUATION PROTOCOL

STAKEHOLDER FEEDBACK FORM

S.N.	Name of the Protocol	Document No.	Page No.	Line No.	Current Text	Proposed Text	Explanation/Reference

Name: _____

Designation and Affiliation: _____

STANDARD PERFORMANCE EVALUATION PROTOCOL

DRAFT FOR STAKEHOLDER COMMENTS

HUMAN METAPNEUMOVIRUS REAL-TIME PCR

ICMR-CDSCO/IVD/GD/PROTOCOLS/03/2024

JANUARY, 2025

New Delhi, India

Human Metapneumovirus Real Time PCR Performance Evaluation Protocol

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Performance evaluation protocol for Human Metapneumovirus real-time PCR kit

I. Background:

CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance evaluation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit (IVD) performance.

This recommendation focuses on the laboratory performance evaluation of Human Metapneumovirus (hMPV) virus real time PCR kit. All clinical samples tested in the study should be evaluated in accordance with the candidate test's instructions for use.

II. Purpose:

To evaluate the performance characteristics of hMPV real-time PCR kits in the diagnosis of hMPV infection/ disease.

III. Requirements:

1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If the kit to be evaluated works in a closed system format, the manufacturer needs to supply the required equipment.
2. Evaluation sites/laboratories (With required equipment)
3. Reference test kits
4. Characterised Evaluation panel
5. Laboratory supplies

IV. Ethical approvals:

Exempted from Ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory Validation Testing, 2024. A self-declaration form as provided in ICMR guidelines to be submitted by the investigators to the institutional authorities and ethics committee for information.

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using clinical/spiked samples
2. **Preparation of Evaluation sites/laboratories:**
   Identified IVD kit evaluation laboratories should be well-equipped and establish their proficiency through ALL of the following:

A. Accreditation from NABL for at least one of the Quality management systems for at least one respiratory viral pathogen molecular testing (NABL accreditation for testing Lab / calibration lab as per ISO/IES 17025, Medical Lab as per ISO 15189, PT provider as per ISO/IEC 17043), or CDSCO approved Reference laboratory.

B. Staff training: All the staff involved in hMPV virus IVD evaluation should undergo hands-on training and competency testing on following

- Preparation & characterization of reference sample panel (at least 2 staff)
- Handling of hMPV RT-PCR kits received for performance evaluation (Verification/Storage/Unpacking etc).
- Testing, interpreting, recording of results & reporting
- Data handling, data safety & confidentiality

3. Preparation of hMPV RNA evaluation panel

A well characterised panel of hMPV positive human samples is a critical requirement for evaluation of these RT-PCR IVD kits. A statistically significant number of clinical samples should be used for the evaluation.

The sample type for hMPV detection is nasopharyngeal/oropharyngeal swab. If a kit claims to detect hMPV across several sample types, attempt should be made to evaluate the assay across all the sample types. In case all the sample types mentioned in the IFU are not available with the lab, the performance evaluation report should clearly mention the sample type against which the kit is evaluated. There should be no ambiguity about the type of sample used for evaluation.

4. RNA extraction

RNA extraction should be performed using standard techniques. If the manufacturer of the index test recommends a specific RNA extraction kit, the same needs to be provided by the manufacturer if the evaluation lab is unable to procure the same.

5. Real-Time PCR System

PCR should be performed using IVD-approved machines. If any equipment(s) is specified in the IFU of the index test, it should be used for the evaluation, and it should be provided by the manufacturer if not available within the lab's IVD evaluation scope.

Real-time closed systems/devices awaiting evaluation should be provided by the manufacturer along with all necessary components, supplies and reagents.

6. Internal control/Extraction control

The index test must have an internal control (housekeeping gene), with or without an extraction control (RNA added before extraction to a sample).

7. Reference assay:

FDA approved real-time PCR assay/ ICMR-NIV Pune in-house Real Time PCR Assay should be used as the Reference Assay.

All positive samples should be confirmed positive by the reference assay.

All negative samples should be confirmed negative by the reference assay.

8. Sample size for performance evaluation: Sample size is calculated assuming 95% sensitivity and specificity of the index test, 95% confidence level, absolute precision of 5% and ≤5% invalid test rate. A minimum of 77 (rounded to 80) positive clinical samples and a minimum of 77 (rounded to 80) negative clinical samples are required for performance evaluation. However, for negative samples, a minimum of 115 specimens are suggested to account for a rigorous cross reactivity panel.

9. Sample panel composition:

A. Human samples

A.1 Positive samples (n=80): Clinical samples positive by the reference real-time PCR assay

A.1.1 Strong positive (Ct value <25) = 20 samples

A.1.2. Moderate positive (Ct value between 25-30) = 40 samples

A.1.3 Weak positive (Ct value >30-35) = 20 samples

Note:

If possible, attempt should be made to include all lineages of hMPV in the positive sample panel.

A.2 Negative samples (n=115): All negative samples should be negative by reference real-time PCR assay. Distribution of the negative samples should be as follows:

A.2.1 NP/OP swab from individuals with respiratory infection that are negative for hMPV RNA = 30 samples

A.2.2 NP/OP swab from apparently healthy individuals with no respiratory symptoms = 20 samples

A.2.3 Cross reactivity panel (Table 1): Samples negative for hMPV RNA but positive for other common respiratory viruses = 65 samples

127 *Table 1: Cross reactivity panel for performance evaluation of HMPV real time PCR kit*

S.N.	Pathogen	Minimum no. of positive samples needed (n=65)	Additional comments
i.	RSV A	5	In case adequate number of one RSV type is unavailable, supplement with the available RSV type
ii.	RSV B	5	
iii.	Measles	5	-
iv.	Mumps	5	Buccal swab is the preferred sample type for Mumps, and the same (or throat swab) should be used for evaluation
v.	Seasonal Influenza A (H1N1pdm09 **and** H3N2)	10 (5 of each)	-
vi.	Seasonal Influenza B (Victoria, with/without Yamagata)	5	-
vii.	SARS-CoV-2	5	-
viii.	Respiratory Adenovirus	5	Representation from all respiratory types is desirable
ix.	Human Respiroviruses 1 and 3, Human Rubulaviruses 2 and 4	5	Representation from all types is desirable
x.	Rhinovirus	5	In case samples available with the lab are not typed into Rhinovirus and non-Rhinovirus Enteroviruses, please use 10 such samples to represent these 2 pathogens
xi.	Enterovirus	5	

xii.	Seasonal coronaviruses	3	OC43 **AND** 229E
xiii.	Cytomegalovirus	2	Lower respiratory specimen positive for CMV is acceptable

If available, samples positive for relevant bacterial pathogens and other relevant viruses (with which majority of the population is likely to be infected), should also be included in the cross-reactivity panel.

10. Evaluation method:

The index test and the reference assay should be run simultaneously on the sample panel, and results should be recorded.

11. Test reproducibility

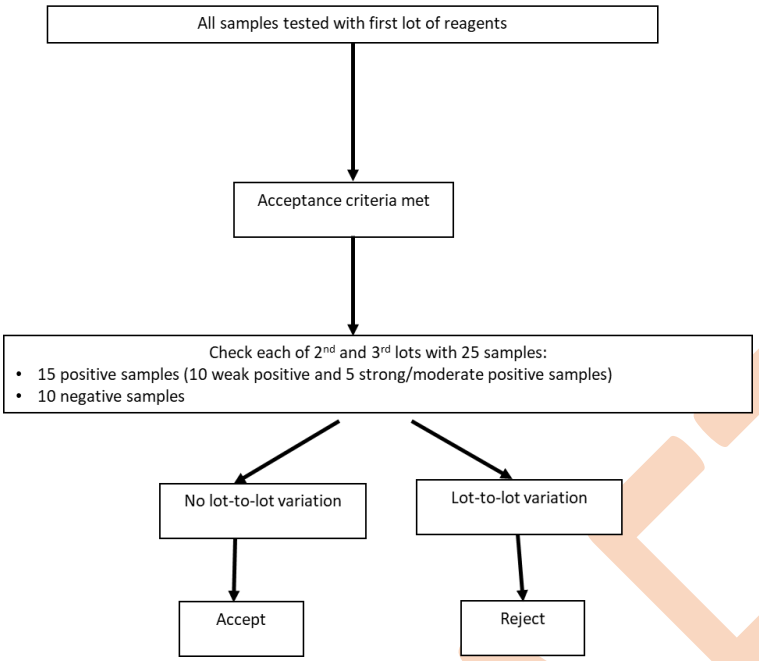
A. Sample size for lot-to-lot reproducibility

Three lots of an assay should be evaluated. Sample size for lot-to-lot reproducibility should be as follows:

- First lot of the assay: should be tested on statistically significant number of positive and negative samples as calculated in the protocol.
- Second lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- Third lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- There should be no lot-to-lot variation.

Refer the flowchart below (Fig. 1):

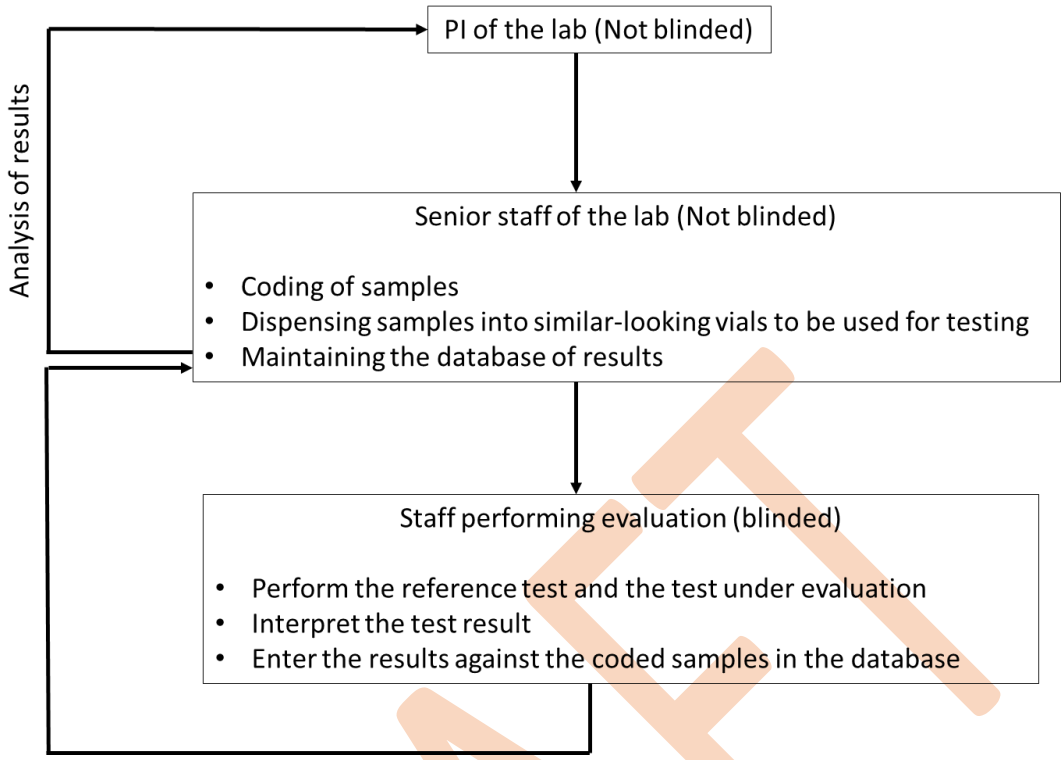
Fig.1: Sample size for Lot-to-lot reproducibility



12. Blinding of laboratory staff

To ensure rigor of the evaluation process, laboratory staff performing the evaluation should be blinded to the status of the clinical samples. The PI of the evaluation exercise should remain unblinded, i.e., privy to the status of the samples. Another senior laboratory staff selected by the PI may remain unblinded and carry out coding of samples and dispensing them into similar-looking vials to be used for testing, and maintaining the database of results. Staff performing the reference test and the test under evaluation, interpretation of the test result, and entering the results against the coded samples in the database, should remain blinded to the status of samples till the completion of evaluation. The data should be analyzed only by the PI of the evaluating lab. Refer to Fig. 2.

Fig.2: Blinding in evaluation exercise



13. Acceptance Criteria

Expected sensitivity: ≥95%

Expected specificity: ≥98%

Cross reactivity with other viruses as outlined in the negative sample panel: Nil

Invalid test rate: ≤5%

13. Publication Rights:

The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

After following due procedure as defined in this document, once any kit is found to be Not of Standard Quality, thereafter, no request for repeat testing of the same kit will be acceptable. Any request of re-validation from the same manufacturer for the same test type will only be entertained if valid proof of change in the kit composition is submitted.

VI. References:

1. U.S. Food and Drug Administration: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays - Class II Special Controls Guidance for Industry and FDA Staff. 2009. Available at: https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/testing-human-metapneumovirus-hmpv-using-nucleic-acid-assays-class-ii-special-controls-guidance#3 [Accessed on January 11, 2025]

2. Amarasinghe, G.K., Ayllón, M.A., Bào, Y. *et al.* Taxonomy of the order *Mononegavirales*: update 2019. *Arch Virol* 164, 1967–1980 (2019). https://doi.org/10.1007/s00705-019-04247-4

VII. Performance evaluation report format

PERFORMANCE EVALUATION REPORT FOR HUMAN METAPNEUMOVIRUS (HMPV) REAL-TIME PCR KITS

Name of the product (Brand /generic)		
Name and address of the legal manufacturer		
Name and address of the actual manufacturing site		
Name and address of the Importer		
Name of supplier: Manufacturer/Importer/Port office of CDSCO/State licensing Authority		
Lot No / Batch No.:		
Product Reference No/ Catalogue No		
Type of Assay		
Kit components		
Manufacturing Date		
Expiry Date		
Pack size (Number of tests per kit)		
Intended Use		
Number of Tests Received		
Regulatory Approval: Import license / Manufacturing license/ Test license License Number:Issue date: Valid Up to:		
Application No.		
Sample Panel	Positive samples (provide details: clinical/spiked, strong, moderate, weak)	
	Negative samples (provide details (clinical/spiked), including cross reactivity panel)	

Results

		Reference assay …… (name)		
		Positive	Negative	Total
Name of HMPV virus real-time PCR	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Details of cross reactivity with other viruses:
- **Conclusions:**

- Sensitivity, specificity
- Performance: **Satisfactory / Not satisfactory**

(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from the batch mentioned above using ….. sample. Results should not be extrapolated to other sample types.)

Disclaimers

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

Note:

This report is exclusively for Human Metapneumovirus…….. Kit (Lot No……) manufactured by …… (supplied by …….)

The kit has been validated against the pathogen (as a whole) with statistically significant sample size, and NOT against different lineages of the pathogen.

Evaluation Done on ……

Evaluation Done by …….

Signature of Director/ Director-In-charge …… Seal ……

**********End of the Report**********

Annexure-1: Information on Operational and Test Performance Characteristics Required from Manufacturers

The manufacturer should provide the following details about the IVD:

1. Instructions for Use
2. Scope of the IVD: to diagnose hMPV.
3. Intended Use Statement
4. Principle of the assay
5. Intended testing population (cases of ARI/ILI/SARI)
6. Intended user (laboratory professional and/or health care worker at point-of-care)
7. Lot/batch No.
8. Date of manufacture
9. Date of Expiry
10. Information on operational Characteristics

i. Configuration of the kit/device

ii. Requirement of any additional equipment, device

iii. Requirement of any additional reagents

iv. Operation conditions

v. Storage and stability before and after opening

vi. Internal control provided or not

vii. Quality control and batch testing data

viii. Biosafety aspects- waste disposal requirements

11. Information on Test Performance Characteristics

i. Type of sample-NP/OP swab, other respiratory specimen

ii. Volume of sample

iii. Any specific sample NOT to be tested

iv. Any additional sample processing required

v. Any additional device/consumable like sample transfer device, pipette, tube, etc required

vi. Name of analyte to be detected

vii. Pathogens targeted by the kit

viii. Time taken for testing

ix. Time for result reading and interpretation

x. Manual or automated(equipment)reading

xi. Limit of detection

xii. Diagnostic sensitivity

xiii. Diagnostic specificity

xiv. Stability and reproducibility

xv. Training required for testing

xvi. If yes, duration

xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test

xviii. Interpretation of invalid and indeterminate results to be provided

xix. It is recommended to provide data demonstrating the precision

*Please mention “Not applicable” against sections not pertaining to the kit.