



ICMR-CDSCO/IVD/GD/PROTOCOLS/11/2025

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/11/2025

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 following IVD evaluation protocols have been developed by ICMR and CDSCO:

- 1. Performanceevaluation protocol for Influenza virus molecular detection and/or differentiation assay (single plex/multiplex format)
- 2. PerformanceevaluationprotocolforSARS-CoV-2moleculardetectionassay(single plex/multiplex format)
- 3. Performance evaluation protocol for Respiratory Syncytial Virus molecular detection assay (single plex/multiplex format)
- 4. Performance evaluation protocol for Influenza virus and SARS-CoV-2 molecular detection differentiation assay (multiplex format)
- 5. PerformanceevaluationprotocolforInfluenzavirus,SARS-CoV-2andRSVmoleculardetection and differentiation assay (multiplex format)
- 6. Performance evaluation protocol for Malaria rapid diagnostic test (RDT) for P falciparum and/or P vivax
- 7. Performance evaluation protocol for Malaria ELISA assay
- 8. Performance evaluation protocol for Malaria real time PCR assay
- 9. Field evaluation protocol for combo Malaria Rapid Diagnostic Test (RDT) kits (detecting P vivax and P falciparum)
- 10. Performance evaluation protocol for Nipah virus Real Time PCR
- 11. Performance evaluation protocol for Chandipura virus Real Time PCR
- 12. Performanceevaluationprotocolformultiplexrespiratoryvirus(expandedpanel)Real Time PCR
- 13. Performance evaluation protocol for Dengue IgG RDT
- 14. Performance evaluation protocol for Dengue IgM/IgG Combo RDT
- 15. Performance evaluation protocol for Dengue IgG ELISA

These protocols are now being placed in the public domain for comments from relevant stakeholders. This window of opportunity will close on 25th August 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 25th August 2025, at ivdevaluation@gmail.com as per the enclosed format. Once the public





ICMR-CDSCO/IVD/GD/PROTOCOLS/11/2025

Consultation period concludes, all comments will be reviewed and considered in finalizing the draft protocols before final clearance by ICMR and CDSCO.

Dated: 11thAugust2025

Place: New Delhi

STANDARDIVDPERFORMANCEEVALUATIONPROTOCOL

STAKEHOLDERFEEDBACKFORM

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 PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

Influenza virus, SARS-CoV-2, Respiratory Syncytial Virus
In-Vitro Diagnostics

ICMR-CDSCO/IVD/GD/PROTOCOLS/05/2025





> AUGUST, 2025 New Delhi, India

13 <u>Table of Contents</u>

S.N.	Торіс	Page Number
1.	General Guidelines	2
2.	Protocol A: Protocol for performance evaluation of influenza virus targets in single plex or multiplex molecular assay formats	6
3.	Protocol B: Protocol for performance evaluation of SARS-CoV-2 in single plex or multiplex molecular assay formats	14
4.	Protocol C: Protocol for performance evaluation of Respiratory Syncytial Virus (RSV) in single plex or multiplex molecular assay formats	21
5.	Protocol D: Protocol for performance evaluation of influenza viruses and SARS-CoV-2 in multiplex molecular assay format	28
6.	Protocol E: Protocol for performance evaluation of influenza viruses, SARS-CoV-2 and RSV in multiplex molecular assay format	31
7.	Performance Evaluation Report Format	34
8.	Annexure-1: Information on operational and test performance characteristics required from manufacturers	39

Page **1** of **41**

37 <u>GENERAL GUIDELINES</u>

38

39

40

41

42

43

44 45

46

47

48

49

69 70

<u>Protocols for performance evaluation of in vitro molecular diagnostic kits for detection and</u> differentiation of Influenza virus and/or SARS-CoV-2 and/or RSV

1. Introduction:

- This document provides a framework for evaluating the performance characteristics of *in vitro* diagnostic (IVD) kits used in identifying and distinguishing various strains of Influenza viruses and/or SARS-CoV-2 and/or RSV, aligning with international standards to ensure reliability and accuracy in diagnosis. The coronavirus diseases 2019 (COVID-19) pandemic, caused by the SARS-CoV-2 virus, has necessitated the rapid development and validation of in vitro molecular diagnostic kits. These kits are crucial for the timely detection and differentiation of major respiratory viruses (influenza/SARS-CoV-2/RSV) to control their spread. This protocol outlines a systematic approach for validating these diagnostic kits to ensure their accuracy, sensitivity,
- specificity, and reliability.
- 51 Although SARS-CoV -2 is no longer a public health emergency globally, it is prudent to
- 52 implement integrated surveillance for Influenza, SARS-CoV-2 and other respiratory viruses,
- making differential diagnosis for these viruses essential. Additionally, timely diagnosis of other
- 54 respiratory viruses, particularly Respiratory syncytial virus (RSV), is crucial for providing
- effective clinical management to pediatric cases.
- This document provides guidance for single plex or multiplex assays for the differential diagnosis
- of Influenza and/or SARS-CoV-2 and/or RSV. It outlines the evaluation of IVD devices/kits
- 58 intended for the detection and differentiation of influenza virus strains and/or detection of SARS-
- 59 CoV-2 and/or detection and differentiation of RSV using nucleic acid detection methods as
- outlined in the scope below. This includes IVD devices/kits that detect and differentiate between
- 61 influenza virus types (Influenza A or B), subtypes (A (H1N1) pdm09 or A (H3N2)), and/or
- multiple influenza virus types/subtypes; kits that identify only SARS-CoV-2, as well as kits that
- only detect and/or differentiate RSV. Additionally, this protocol may be used for multiplex IVD
- devices/kits designed to simultaneously detect Influenza A & B (with or without subtyping), and/or
- 65 SARS-CoV-2, and/or RSV. This document outlines the following aspects of performance
- evaluation of IVD devices/kits as per the scope outlined in the document:
- 1.1 The procedure for validating entities to determine operational parameters of IVD devices/kits that detect influenza virus gene segment(s).
 - **1.2** T procedure for validating entities to determine operational parameters of IVD devices/kits that detect SARS-CoV-2 gene segment(s).
- 1.3 The procedure for validating entities to determine operational parameters of IVD devices/kits that detect RSV gene segment(s).
- 1.4 The techniques for identifying influenza virus/SARS-CoV-2/RSV nucleic acid targets in single-plex or multiplex formats (using appropriate protocols listed in the document).

1.5 This document is not useful for performance evaluation of serological assays for detection of antigen and antibody for influenza viruses/SARS-CoV-2/RSV. The IVD device/kit to be validated is henceforth known as the "Kit under Evaluation."

2. Objective:

- 79 This document aims to offer a comprehensive set of instructions for evaluating the performance of
- 80 molecular IVD assays mentioned in the scope below for detecting Influenza A and Influenza B
- viruses with/without subtyping, and other common respiratory viruses such as SARS-CoV-2 and
- 82 RSV. This evaluation will focus on measuring the analytical sensitivity and specificity, cross-
- reactivity, repeatability, and reproducibility as compared against a reference assay using clinical
- sample panel.

78

91

- 85 In brief, the objectives are as follows:
- 2.1 To validate the performance characteristics of in vitro molecular diagnostic kits for
- detecting Influenza A & B (with/without subtyping)/ SARS-CoV-2/ RSV.
- 2.2 To ensure the kits under evaluation meet the necessary standards for sensitivity,
- specificity, repeatability, and reproducibility.
- 2.3 To evaluate the cross-reactivity of the kits with other respiratory viruses.

3. Scope:

- This guideline is solely for the evaluation and establishment of the performance characteristics of
- 93 IVD kits and devices designed for the detection and subtyping of commonly circulating seasonal
- 94 Influenza viruses (Influenza A(H1N1) pdm09, Influenza A(H3N2), Influenza B(Yamagata) and
- 95 Influenza B(Victoria) subtypes) and/or other common respiratory viruses such as SARS-CoV-2
- and RSV, using single or multiplex molecular assays (as outlined in the scope below) intended for
- 97 human clinical samples. This document is a guide to assess:
- 3.1 The analytical assay performance characteristics with clinical specimens for the detection
 and/or differentiation of influenza viruses. (Protocol A)
- 3.2 The analytical assay performance characteristics with clinical specimens for the detection of SARS-CoV-2 (Protocol B)
- 3.3 The analytical assay performance characteristics with clinical specimens for the detection of RSV (Protocol C)
- 3.4 The analytical performance characteristics of multiplex assay for detection of two or more
 of these viruses by combining Protocols A, B & C as per the kit format.
- 3.5 Analytical performance characteristics which should include sensitivity, specificity, crossreactivity, and lot-to-lot variation including functionality of devices that identify and/or
- reactivity, and lot-to-lot variation including functionality of devices that identify ar differentiate influenza viruses, SARS-CoV-2 and/or RSV depending on the kit format.
- 3.6 The performance of the kit, only if the kit includes an internal control (**preferably** endogenous, or exogenous).

111 112	3.7 This document may also apply to forthcoming influenza, SARS-CoV-2 and RSV molecular diagnostic devices that do not fit within these current classifications.
113 114	3.8 The document will serve as a reference for assessing kits based on Nucleic Acid Amplification Test (single plex or multiplex assays) as listed below:
115	
116 117 118 119 120 121	 3.8.1 Real-time Reverse Transcription Polymerase Chain Reaction format (rRT-PCR): including Real-time PCR probe-based assays or non-probe based assays 3.8.2 Other NAT testing platforms such as LAMP/RPA, and other closed system platforms such as TrueNat /cartridge-based assays Note: This protocol is not suitable for the kits where amplicons are handled outside the
122	amplification system.
123 124 125 126	 4. Requirements: 4.1 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 and consumables.
127	4.2 Evaluation sites/laboratories (With required equipment)
128	4.3 Reference test kits
129	4.4 Characterized samples for evaluation panel
130 131	4.5 Laboratory supplies
132	5. Ethical approvals:
133 134 135 136 137 138	Laboratory validation of IVDs using irreversibly de-identified samples is 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 (https://ethics.ncdirindia.org/asset/pdf/Guidance on Ethical Requirements for Laboratory Validation Testing.pdf)
139 140 141	6. Procedure:6.1 Study design/type: Diagnostic accuracy study using leftover irreversibly de-identified archived clinical samples.
142 143	6.2 Evaluation site/laboratory considerations: Identified IVD kit evaluation laboratories should establish their proficiency through
144 145 146	6.2.1 Accreditation for at least one of the Quality management systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO 15189), PT provider (ISO: 17043) or CDSCO approved Reference laboratory.

147 148 149 150	6.2.2 Have sufficient numbers of archived as well as contemporary clinical specimens positive for respiratory viruses targeted by the kit under evaluation (Influenza A(H1N1)pdm09, A(H3N2), B(Yamagata), B(Victoria), and/or SARS-CoV-2 and/or RSV A & B), with aliquots stored at -80 °C deep freezers or in lyophilized form.
151 152 153 154	6.2.3 Virus strains should be well-characterized by ICMR approved or US FDA/ ATAGI Australia/PMDA Japan approved/WHO Pre-Qualified reference assay and/or by influenza virus HA gene/segment or gene-specific sequencing (for SARS-CoV-2 and RSV) or Next-Generation Sequencing.
155 156 157	6.2.4 Have a minimum BSL-2 level facility with trained manpower and at least two different Real Time platforms to perform molecular diagnostic assays for Influenza virus and other respiratory viruses.
158 159 160 161	6.2.5 Have a good record of External Quality Assurance programs for influenza, SARS-CoV-2, and other respiratory viruses.6.2.6 Staff training: All the staff involved in IVD kit evaluation should undergo hands-on training and competency testing on the following:
162	6.2.6.1 Preparation & characterization of kit evaluation panel
163 164	6.2.6.2 Handling of respiratory virus PCR kits received for performance evaluation (Verification/Storage/Unpacking etc).
165	6.2.6.3 Testing, interpreting, recording of results & reporting
166	6.2.6.4 Data handling, data safety & confidentiality
167	
168 169 170 171 172 173 174 175 176 177	 6.3 Performance characteristics: To be assessed for all assay targets of influenza A/B, SARS-CoV-2 and RSV (single plex or multi-plex assays) 6.3.1 Analytical Sensitivity and specificity 6.3.2 Cross-reactivity 6.4.3 Repeatability 6.4.4 Reproducibility
179	

180	Protocol A				
181		aluation of performance characteristics of Molecular Kit detecting influenza A & B			
182	viruses, and subtyping into A (H1N1) pdm 09, A(H3N2), B(Yamagata) & B(Victoria) in				
183		single plex or multiplex format			
184	1.	Objective:			
185		1.1 To evaluate the performance of molecular IVD device /KIT for detection and			
186		differentiation of Influenza viruses as per the scope outlined in this document.			
187		1.2 To ensure the kits under evaluation meet the necessary standards for sensitivity,			
188		specificity, repeatability, and reproducibility.			
189		1.3 To evaluate the cross-reactivity of the kits with other respiratory viruses.			
190					
191	2.	Evaluation of performance characteristics should be done for the following			
192		parameters:			
193		2.1 Sensitivity and specificity			
194		2.2 Cross-reactivity			
195		2.3 Repeatability			
196		2.4 Reproducibility			
197					
198	3.	Panel development: Clinical sample (archived/contemporary) panel for testing:			
199		3.1 Contemporary leftover irreversibly de-identified clinical/archived respiratory samples			
200		(in VTM) for the panel should be irreversibly de-identified.			
201		3.2 Samples to be used for panel preparation shall be stored properly at -80 °C or			
202		lyophilized.			
203		3.3 Unless the manufacturer has specific requirement of nucleic acid extraction kit, the			
204		validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA			
205		Japan approved/ICMR validated total RNA / viral RNA extraction kits for the evaluation.			
206		3.4 Clinical samples for evaluation should be characterized by a reference kit /			
207		Sequencing/NGS.			
208 209		3.5 All positive samples should be confirmed positive for the target pathogens by the reference assay.			
209		reference assay.			
210		3.6 All negative samples should be confirmed negative for the target pathogens by the			
211		reference assay.			
212					
213					

4. Sample size and sample panel composition for evaluation of performance characteristics:

Sample sizes of positive and negative samples of the analyte/pathogen targeted by the kit against different values of sensitivity and specificity are provided in Table 1. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate ≤5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity. Sample sizes are calculated using the formulae:

$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

$$n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- n (se) is the number of positive samples.
- \cdot *n (sp) is the number of negative samples.*
- \cdot Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
- · Se is the predetermined sensitivity.
- · Sp is the predetermined specificity.
- *d is the predetermined marginal error (5%)*
- · IR is the invalid test rate

Sample sizes for positive samples and their composition for evaluating subtyping are provided in Table 2.

Table 1. Sample sizes per target pathogen for different values of sensitivity/ specificity claimed by the manufacturer.

Sensitivity/ Specificity	Sample size: Minimum number of positive samples [¥]	Composition of positive samples#	Sample size: Minimum number of negative samples (rounded) *	Minimum number of cross reactive* samples among the negative samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	20	5
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28	80	20
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55	150	38
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76	210	53
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91	260	65

^{*}Strong positive: (Ct value <25)

246

247

248

249

Moderate positive: (Ct value between 25-30) Weak positive: (Ct value >30 and and \le 34)

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Sample sizes for positive samples and their composition for evaluating subtyping

	Sample size* (per target pathogen)			
	Influenza A (H1N1) pdm09	Influenza A/H3N2	Influenza B	
Sensitivity	Minimum number of nasopharyngeal swabs/ oropharyngeal swabs (rounded figures)	Minimum number of nasopharyngeal swabs/ oropharyngeal swabs (rounded figures)	Minimum number of nasopharyngeal swabs/ oropharyngeal swabs (rounded figures)	Minimum total number of positive samples (rounded figures)
99%	20	20	20	60
95%	80	80	80	240

[¥] Equal distribution of positive nasopharyngeal and/or oropharyngeal swabs in virus transport medium (VTM) to be used

^{*} Samples positive for common respiratory viruses (such as SARS-CoV-2, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus (including its types and subtypes), common human coronaviruses), other than the ones targeted by the kit under evaluation. Equal distribution of cross-reactive viruses is desirable.

90%	150	150	150	450
85%	210	210	210	630
80%	260	260	260	780

*Combination of strong, moderate and weak positive samples should be considered as per the information provided in Table 1.

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

4.1 Repeatability testing will be performed on 3 positive (strong, moderate and weak positive) and 3 Negative samples (within the selected positive and negative samples) per target pathogen 5 times (replicates of 5).

5. Methodology:

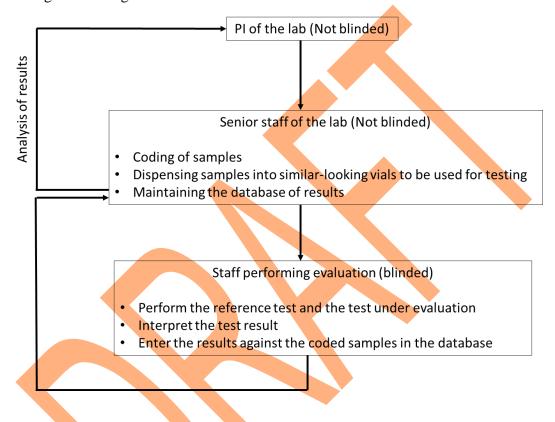
- **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference assay. The ICMR-NIV RT-qPCR assay for Influenza/SARS-CoV-2 or WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved kit will be considered as the reference assay for these parameters.
- **5.2** The validation laboratory can use the established total RNA / viral RNA extraction protocol for the evaluation.
- **5.3** The instruction for the assay setup and the interpretation of the results will be as per the protocol outlined by the manufacturer of the reference test and the kit under evaluation.
- **5.4** The results shall be compared with the reference assay for sensitivity and specificity calculations.
- **5.5** If there is a discrepancy observed in the results with the index test, this discrepancy should be taken as discordant. Repetition of the assay may introduce bias. If the reference kit itself has failed, then these samples with discrepancies should be discarded, and new well-characterized samples should be used instead.
- True positive samples: These are samples positive by both reference assay and index test.
- True negative samples: These are samples negative by both reference assay and index test.
- False positive samples: These are samples negative by reference assay and positive by index test.
- False negative samples: These are samples positive by reference assay and negative by index test.
- **5.6** The interpretation for internal control (**preferably** endogenous, or exogenous) will be as per manufacturer's instruction.
- **5.7** 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 283 manufacturer along with all necessary components, supplies and reagents. 284 The details on the Real-time Equipment used for validation should be recorded, including 285 calibration status. 286 287 288 6. Cross-reactivity Analysis: **6.1** Objective: 289 To assess the primer-probe set for true detection of influenza viruses and assess its cross-290 reactivity with other respiratory viruses. 291 **6.2 Methodology:** 292 **6.2.1** Potential cross-reactivity of the kit shall be ruled out by testing other 293 respiratory pathogen positive samples (N=30), with equal representation (n=5 each) 294 of samples positive for SARS-CoV-2, Parainfluenza viruses, Adenoviruses, 295 Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 296 **6.2.2** Cross-reactivity will be assessed by comparing the results of these samples 297 using kit under evaluation and reference kit. 298 **6.2.3** The kit targets should not show any amplification with other respiratory 299 viruses (ORVs). If amplification is observed for ORV then the kit will fail 300 validation and the same needs to be mentioned in the report. 301 302 * For multiplex assays targeting influenza, SARS-CoV-2, and RSV, samples positive for 303 these viruses may be suitably interchanged for assessing cross-reactivity, apart from the 304 305 ORV panel. (i.e. Influenza A positive samples may be used for detecting cross-reactivity against Influenza B) 306 307 7. Acceptance criteria for the kit: 308 Sensitivity for each pathogen/ type/ subtype: ≥95% 309 Specificity for each pathogen/ type/ subtype: ≥99% 310 Cross-reactivity: Nil 311 Invalid test rate: ≤5% 312 313 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80 positive samples and ≥ 20 negative samples should be tested for evaluation for each 314 315 pathogen/ type/ subtype. 8. Repeatability Assessment: 316

317	8.1 Objectives:
318	To assess the repeatability of the detection of Influenza virus and its subtypes using the
319	kit under evaluation
320	8.2 Sample size:
321	3 positive samples (strong, moderate and weak positive-as per the Ct values outlined in
322	the document) and 3 negative samples for each target pathogen should be tested 5 times.
323	
324	8.3 Result: Concordance should be 100% based on positive and negative test result
325	(qualitative).
326	
327	9. Precision (Reproducibility):
328	Lot to Lot Reproducibility
329	9.1 Objectives: To assess Precision (Reproducibility) among 3 different lots of the
330	kit under evaluation.
331	9.2 Sample size: Three lots of an assay shall be evaluated. Sample size for lot-to-lot
332	reproducibility should be as follows:
333	• First lot of the assay: should be tested on statistically significant number of positive
334	and negative samples as calculated in the protocol.
335	• Second lot of the assay: should be tested on 25 samples (15 positive samples
336	comprising 10 low positive AND 5 moderate/high positive samples, and 10 negative
337	samples).
338	• Third lot of the assay: should be tested on 25 samples (15 positive samples comprising
339	10 low positive AND 5 moderate/high positive samples, and 10 negative samples).
340	
341	9.3 Result: Concordance should be 100% based on positive and negative test result
342	(qualitative)
343	10. Internal Control Analysis:
344	10.1 Monitor the internal control (preferably RNaseP or other housekeeping gene) to
345	ensure consistent extraction and amplification efficiency across samples and runs.
346	10.2 Ct-values of internal controls should be within the manufacturer's prescribed limit.
347	10.3 Tests will be marked invalid if Ct-values are outside the prescribed limit.
348	Total Tests will be marked invalid if et values are outside the presented mint.
349	11. Blinding of Laboratory Staff:
350	To ensure rigor of the evaluation process, laboratory staff performing the evaluation should
351	be blinded to the status of the clinical samples. The PI of the evaluation exercise should
352	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. 1.

Fig.1: Blinding in evaluation exercise



12. Publication Rights:

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

13. Conclusion:

Based on the comprehensive evaluation conducted, the [Kit & Manufacturer's Name] Influenza Virus RT-PCR Assay has been found [Satisfactory/Not Satisfactory] for its intended *in vitro* diagnostic (IVD) use.

The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and performance characteristics compared to established reference IVD approved RT-PCR kits. 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information. Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%. 14. Performance evaluation report format The performance evaluation report format (page 34) is designed for multiplex assays with several targets. It should be modified and used accordingly for single plex assays/multiplex assays with fewer targets.

404	Protocol B
405 406	Evaluation of performance characteristics of Molecular Kit detecting SARS-CoV-2 in single plex or multiplex format
407	1. Objective:
408 409	1.1. To validate the performance characteristics of in vitro molecular diagnostic kits for detecting SARS-CoV-2 as per the scope outlined in this document.
410 411	1.2. To ensure the kits under evaluation meet the necessary standards for sensitivity, specificity, repeatability, and reproducibility.
412 413	1.3. To evaluate the cross-reactivity of the kits with other respiratory viruses.
414	2. Evaluation of Performance characteristic should be done for the following:
415	2.1 Sensitivity and specificity
416	2.2 Cross-reactivity
417	2.3 Repeatability
418	2.4 Reproducibility
419	3. Panel development: Clinical sample (archived/contemporary) panel for testing:
420 421	3.1 Contemporary leftover irreversibly de-identified clinical/archived respiratory samples in VTM for the panel should be irreversibly de-identified.
422 423	3.2 Samples to be used for panel preparation shall be stored properly at -80 °C or lyophilized.
424 425 426 427	3.3 Unless the manufacturer has specific requirement of nucleic acid extraction kit, the MDTLs/ validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved/ICMR validated an established total RNA / viral RNA extraction kits for the evaluation.
428 429	3.4 Clinical samples for evaluation should be characterized by a reference kit / Sequencing/NGS.
430 431	3.5 All positive samples should be confirmed positive for the target pathogens by the reference assay.
432 433	3.6 All negative samples should be confirmed negative for the target pathogens by the reference assay.
434	
435	4. Sample size and sample panel composition for evaluation of performance characteristics:

Sample sizes of positive and negative samples of SARS-CoV-2 against different values of sensitivity and specificity are provided in Table 3. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate ≤5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size that is outlined for 85% specificity. Sample sizes are calculated using the formulae:

448
$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

449
$$n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- \cdot n (se) is the number of positive samples.
- \cdot *n (sp) is the number of negative samples.*
- Z^2 is the critical value from the standard normal distribution corresponding 455 to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
 - Se is the predetermined sensitivity.
 - Sp is the predetermined specificity.
- 458 d is the predetermined marginal error (5%)
- 459 IR is the invalid test rate

Table 3. Sample sizes for different values of sensitivity/ specificity claimed by the manufacturer.

		Composition of positive		Minimum
		samples [#]	Sample size:	number of
	Cample size. Minimum		Minimum	cross
Sensitivity/	Sample size: Minimum		number of	reactive*
Specificity	number of positive		negative	samples
	samples [¥]		samples	among the
			(rounded) [¥]	negative
				samples

99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	20	5
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28	80	20
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55	150	38
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76	210	53
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91	260	65

^{*}Strong positive: (Ct value <25)

Moderate positive: (Ct value between 25-30) Weak positive: (Ct value >30 and \le 34)

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

4.1 Repeatability testing will be performed on 3 positive (strong, moderate and weak positive) and 3 negative samples (within the selected positive and negative samples) per target pathogen 5 times (replicates of 5).

- **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference assay. The ICMR-NIV RT-qPCR assay for Influenza/SARS-CoV-2 or WHO Pre-Qualified/ US FDA/ PMDA Japan/ ATAGI Australia approved kit will be considered as the reference assay for these parameters.
- **5.2** The validation laboratory can use established total RNA / viral RNA extraction protocol for the evaluation.
- **5.3** The instruction for the assay setup and the interpretation of the results will be as per the protocol outlined by the manufacturer of the reference test and the kit under evaluation. The results shall be compared with the reference assay for sensitivity and specificity calculations.
- **5.4** If there is a discrepancy observed in the results with the index test, this discrepancy should be taken as discordant. Repetition of the assay may introduce bias. If the reference kit itself has failed, then these samples with discrepancies should be discarded, and new well-characterized samples should be used instead.

True positive samples: These are samples positive by both reference assay and index test.

5. Methodology:

^{*}Nasopharyngeal/ oropharyngeal swabs in virus transport medium (VTM) to be used

^{*}Samples positive for common respiratory viruses (such as Influenza (including its types and subtypes), Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus (including its types and subtypes), common human coronaviruses), other than the ones targeted by the kit under evaluation. Equal distribution of cross-reactive viruses is desirable.

486 487 488	True negative samples: These are samples negative by both reference assay and index test. False positive samples: These are samples negative by reference assay and positive by index test.
489	False negative samples: These are samples positive by reference assay and negative by
490	index test.
491	
492	5.5 The interpretation for internal control (preferably endogenous, or exogenous) will be
493	as per manufacturer's instruction.
494	5.6 PCR should be performed using IVD-approved machines. If any equipment(s) is
495	specified in the IFU of the index test, it should be used for the evaluation, and it should
496	be provided by the manufacturer if not available within the lab's IVD evaluation
497	scope.
498	Real-time closed systems/devices awaiting evaluation should be provided by the
499	manufacturer along with all necessary components, supplies and reagents.
500	The details on the Real-time Equipment used for validation should be recorded,
501	including calibration status.
502	The details on the Real-time Equipment used for validation should be recorded
503	including calibration status.
504	
505 6.	Cross-reactivity Analysis:
506	6.1 Objective:
507	To assess the primer-probe set for true detection of SARS-CoV-2 and assess its cross-
507 508	To assess the primer-probe set for true detection of SARS-CoV-2 and assess its cross-reactivity with other respiratory viruses.
508	reactivity with other respiratory viruses.
508 509	reactivity with other respiratory viruses. 6.2 Methodology:
508 509 510	reactivity with other respiratory viruses. 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory
508 509 510 511	reactivity with other respiratory viruses. 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of
508 509 510 511 512	reactivity with other respiratory viruses. 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses,
508 509 510 511 512 513	reactivity with other respiratory viruses. 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).*
508 509 510 511 512 513 514	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using
508 509 510 511 512 513	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit.
508 509 510 511 512 513 514	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses
508 509 510 511 512 513 514 515 516 517	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation
508 509 510 511 512 513 514 515 516 517 518	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses
508 509 510 511 512 513 514 515 516 517 518 519	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation and the same needs to be mentioned in the report.
508 509 510 511 512 513 514 515 516 517 518 519 520 7.	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation
508 509 510 511 512 513 514 515 516 517 518 519 520 7.	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation and the same needs to be mentioned in the report. Acceptance criteria for the kit:
508 509 510 511 512 513 514 515 516 517 518 519 520 7.	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation and the same needs to be mentioned in the report. Acceptance criteria for the kit: Sensitivity: ≥95%
508 509 510 511 512 513 514 515 516 517 518 519 520 7.	 6.2 Methodology: 6.1.1 Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of samples positive for Influenza, Parainfluenza viruses, Adenoviruses, Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).* 6.1.2 Cross-reactivity will be assessed by comparing the results of these samples using kit under evaluation and reference kit. 6.1.3 The kit targets should not show any amplification with other respiratory viruses (ORVs). If amplification is observed for ORV then the kit will fail validation and the same needs to be mentioned in the report. Acceptance criteria for the kit:

525		Invalid test rate: ≤5%
526 527 528		To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80 positive samples and ≥ 20 negative samples should be tested for evaluation for each pathogen/ type/ subtype.
529	8.	Repeatability Assessment:
530 531 532 533 534 535		 8.1 Objectives: To assess the repeatability of the detection of SARS-CoV-2 using the kit under evaluation 8.2 Sample size: Five replicates of 3 positive samples (strong, moderate and weak positive-as per the Ct values outlined in the document), and five replicates of 3 negative samples for SARS-CoV-2 should be tested. For multiplex panels, these sample numbers shall
536		be used per target pathogen for repeatability assessment.
537 538 539 540		8.3 Result: Concordance should be 100% based on positive and negative test result (qualitative).
541 542 543 544	9.	Precision (Reproducibility): Lot to Lot Reproducibility
545 546		9.1 Objectives: To assess precision (reproducibility) among 3 different lots of the kit under evaluation.
547 548 549 550	•	 9.2 Sample size: Lot to lot variation testing: Three lots of an assay shall 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.
551 552 553 554		 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
555 556		10 low positive AND 5 moderate/high positive samples, and 10 negative samples).
557 558		9.3 Result: Concordance should be 100% based on positive and negative test result (qualitative).
559 560 561 562 563	10	 Internal Control Analysis: 10.1 Monitor the internal control (preferably RNaseP or other housekeeping gene) to ensure consistent extraction and amplification efficiency across samples and runs.

- 10.2 Ct-values of internal controls should be within the manufacturer's prescribed limit.
 - **10.3** Tests will be marked invalid if Ct-values are outside the prescribed limit.

11. 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. 1 in Protocol A.

12. Conclusion:

566567

568

569

570

571

572

573

574

575

576

577 578

579

585

586

588

- Based on the comprehensive evaluation conducted, the [Kit & Manufacturer's Name] SARS-CoV-
- 2 RT-PCR Assay has been found [Satisfactory/Not Satisfactory] for its intended in vitro
- 582 diagnostic (IVD) use.
- The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and performance
- characteristics compared to established reference IVD approved RT-PCR kits.

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
- 591 acceptable.
- Any request of re-validation from the same manufacturer for the same test type will only be
- entertained after a minimum of 3 months and only if a high-level technical summary of
- modifications or functional improvements to the kit design is submitted, without explicit
- 595 disclosure of proprietary information.
- 596 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different
- 597 well-characterized sample panel at a different laboratory may be considered only for kits
- which claim high performance characteristics (sensitivity and specificity 95% and above),
- 599 but which fail the performance evaluation by a margin of 5%.

14. Performance evaluation report format:

600

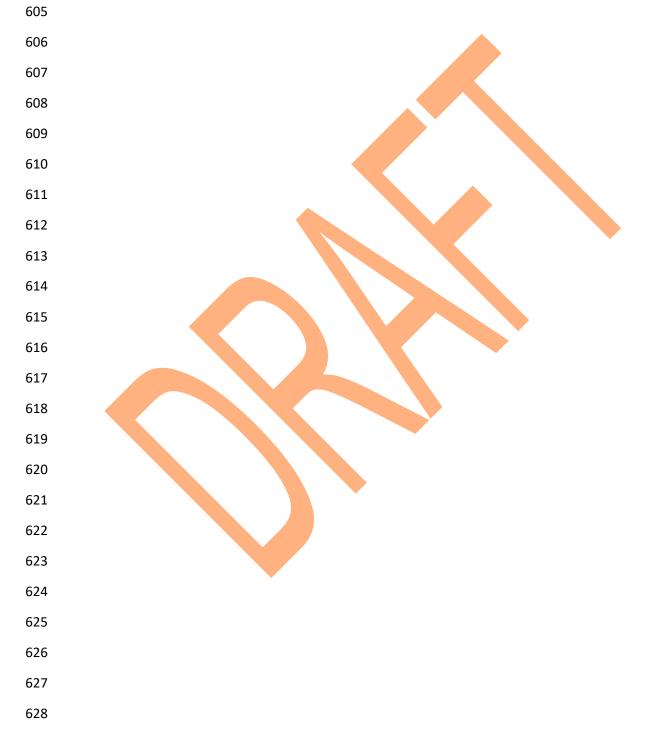
601

602

603

604

The performance evaluation report format (page 34) is designed for multiplex assays with several targets. It should be modified and used accordingly for single plex assays/multiplex assays with fewer targets.



629	Protocol C	
630 631	Evaluation of performance characteristics of Molecular Kit detecting Respirator Syncytial Virus (RSV) in single plex or multiplex format	<u>ry</u>
632	1. Objective:	
633 634	1.1. To validate the performance characteristics of in vitro molecular diagnostic detecting and/or differentiating RSV A/B as per the scope outlined in this document	
635 636	1.2. To ensure the kits under evaluation meet the necessary standards for sens specificity, repeatability, and reproducibility.	sitivity,
637	1.3. To evaluate the cross-reactivity of the kits with other respiratory viruses.	
638		
639	2. Evaluation of Performance characteristic should be done for the following:	
640	2.1 Sensitivity and specificity	
641	2.2 Cross-reactivity	
642	2.3 Repeatability	
643	2.4 Reproducibility	
644	3. Panel development: Clinical sample (archived/contemporary) panel for testing:	
645 646	3.1 Contemporary leftover irreversibly de-identified clinical/archived respiratory s in VTM for the panel should be irreversibly de-identified.	amples
647 648	3.2 Samples to be used for panel preparation shall be stored properly at -80 lyophilized.	°C or
649 650 651 652	3.3 Unless the manufacturer has specific requirement of nucleic acid extraction MDTLs/ validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Au PMDA Japan approved/ ICMR validated an established total RNA / viral RNA ext kits for the evaluation.	stralia/
653 654	3.4 Clinical samples for evaluation should be characterized by a reference Sequencing/NGS.	e kit /
655 656	3.5 All positive samples should be confirmed positive for the target pathogens reference assay.	by the
657 658	3.6 All negative samples should be confirmed negative for the target pathogens reference assay.	by the
659		
660	4. Sample size and sample panel composition for evaluation of performance character	ristics:

Sample sizes of positive and negative samples of the RSV A/B against different values of sensitivity and specificity are provided in Table 4. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate ≤5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity. Sample sizes for positive samples and their composition for evaluating subtyping (RSV A/B) are provided in Table 5. Sample sizes are calculated using the formulae:

675
$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

676
$$n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- \cdot *n (se) is the number of positive samples.*
- \cdot *n (sp) is the number of negative samples.*
- Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
 - · Se is the predetermined sensitivity.
 - · Sp is the predetermined specificity.
 - d is the predetermined marginal error (5%)
- 686 IR is the invalid test rate

Table 4. Sample sizes per target pathogen (RSV A/B) for different values of sensitivity/ specificity claimed by the manufacturer.

Sensitivity/ Specificity	Sample size: Minimum number of positive samples [¥]	Composition of positive samples [#]	Sample size: Minimum number of negative	Minimum number of cross reactive* samples
-----------------------------	--	---	--	---

			samples (rounded) [¥]	among the negative samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	20	5
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28	80	20
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55	150	38
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76	210	53
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91	260	65

^{*}Strong positive: (Ct value <25)

691 692

693

694

695

696

697

Moderate positive: (Ct value between 25-30) Weak positive: (Ct value >30 and and \le 34)

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 5. Sample sizes for positive samples and their composition for evaluating subtyping

		RSV A	RSV B	
Sensitivity	Sample size* (per target pathogen)	Minimum number of nasopharyngeal swabs/oropharyngeal swabs	Minimum number of nasopharyngeal swabs/oropharyngeal swabs	Minimum total positive samples
99%	20	20	20	40
95%	80	80	80	160
90%	150	150	150	300
85%	210	210	210	420
80%	260	260	260	520

*Combination of strong, moderate and weak positive samples should be considered as per the information provided in Table 4.

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

^{*}Nasopharyngeal/ oropharyngeal swabs in virus transport medium (VTM) to be used

^{*}Samples positive for common respiratory viruses (such as Influenza (including its types and subtypes), SARS-CoV-2, Parainfluenza viruses, Adenoviruses, Rhinoviruses, common human coronaviruses), other than the ones targeted by the kit under evaluation. Equal distribution of cross-reactive viruses is desirable.

4.1 Repeatability testing will be performed on 3 positive (strong, moderate and weak 698 699 positive) and 3 negative samples (within the selected positive and negative samples) per 700 target pathogen 5 times (replicates of 5). 5. Methodology: 701 **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference 702 assay. The ICMR-NIV RT-qPCR assay for RSV or WHO Pre-Qualified/ US FDA/ ATAGI 703 Australia/ PMDA Japan approved kit will be considered as the reference assay for these 704 parameters. 705 **5.2** The validation laboratory can use established total RNA / viral RNA extraction protocol 706 for the evaluation. 707 **5.3** The instruction for the assay setup and the interpretation of the results will be as per 708 the protocol outlined by the manufacturer of the Kit Under Evaluation. 709 **5.4** The results shall be compared with the reference assay for sensitivity and specificity 710 calculations. 711 **5.5** If there is a discrepancy observed in the results with the index test, this discrepancy 712 should be taken as discordant. Repetition of the assay may introduce bias. If the reference 713 kit itself has failed, then these samples with discrepancies should be discarded, and new 714 well-characterized samples should be used instead. 715 True positive samples: These are samples positive by both reference assay and index test. 716 True negative samples: These are samples negative by both reference assay and index test. 717 False positive samples: These are samples negative by reference assay and positive by 718 index test. 719 False negative samples: These are samples positive by reference assay and negative by 720 index test. 721 722 **5.6** The interpretation for internal control (**preferably** endogenous, or exogenous) will be 723 724 as per manufacturer's instruction. **5.7** PCR should be performed using IVD-approved machines. If any equipment(s) is 725 specified in the IFU of the index test, it should be used for the evaluation, and it should be 726 provided by the manufacturer if not available within the lab's IVD evaluation scope. 727 728 Real-time closed systems/devices awaiting evaluation should be provided by the manufacturer along with all necessary components, supplies and reagents. 729 The details on the Real-time Equipment used for validation should be recorded, including 730 calibration status. 731 The details on the Real-time Equipment used for validation should be recorded including 732 calibration status. 733

734

6. Cross-reactivity Analysis: 735 736 **6.1 Objective:** To assess the primer-probe set for true detection of RSV and assess its cross-reactivity with 737 other respiratory viruses. 738 **6.2 Methodology:** 739 740 **6.1.1** Potential cross-reactivity of the kit shall be ruled out by testing other respiratory pathogen positive samples (N=30), with equal representation (n=5 each) of 741 samples positive for Influenza, SARS-CoV-2, Parainfluenza viruses, 742 Adenoviruses, Rhinoviruses, common human coronaviruses.* 743 Cross-reactivity will be assessed by comparing the results of these samples 744 6.1.2 using kit under evaluation and reference kit. 745 746 **6.1.3** The kit targets should not show any amplification with other respiratory viruses 747 (ORVs). If amplification is observed for ORV then the kit will fail validation 748 and the same needs to be mentioned in the report. * For multiplex assays targeting influenza, SARS-CoV-2, and RSV detection, samples positive for 749 these viruses may be suitably interchanged for assessing cross-reactivity 750 751 752 7. Acceptance criteria for the kit: 753 754 Sensitivity for each pathogen/ type/ subtype: ≥95% 755 Specificity for each pathogen/ type/ subtype: ≥99% 756 Cross-reactivity: Nil 757 Invalid test rate: <5% 758 759 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80 positive samples and ≥ 20 negative samples should be tested for evaluation for each 760 pathogen/ type/ subtype. 761 762 8. Repeatability Assessment: 763 **8.1 Objectives:** To assess the repeatability of the detection of SARS-CoV-2 using the kit 764 under evaluation 765 766 **8.2 Sample size:** Five replicate of 3 positive samples per target pathogen (strong, 767 768 moderate and weak positive) and five replicates of 3 negative samples per target pathogen 769 should be tested.

8.3 Result: Concordance should be 100% based on positive and negative test result (qualitative).

9. Precision (Reproducibility):

770 771

772773

Lot to Lot Reproducibility

- **1.1 Objectives:** To assess precision (reproducibility) among 3 different lots of the kit under evaluation.
 - **9.2 Sample size:** Lot to lot variation testing: Three lots of an assay shall 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).
 - **9.3 Result:** Concordance should be 100% based on positive and negative test result (qualitative).

10. Internal Control Analysis:

- **10.1** Monitor the internal control (RNaseP or other endogenous housekeeping gene) to ensure consistent extraction and amplification efficiency across samples and runs.
- 10.2 Ct-values of internal controls should be within the manufacturer's prescribed limit.
- 10.3 Tests will be marked invalid if Ct-values are outside the prescribed limit.

11. 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. 1 in Protocol A.

12. Conclusion:

- Based on the comprehensive evaluation conducted, the [Kit & Manufacturer's Name] SARS-CoV-
- 2 RT-PCR Assay has been found [Satisfactory/Not Satisfactory] for its intended in vitro
- 810 diagnostic (IVD) use.

811 812	The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and performance characteristics compared to established reference IVD approved RT-PCR kits.
813	
814	13. Publication Rights:
815	The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).
816	
817 818 819	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.
820 821 822 823	Any request of re-validation from the same manufacturer for the same test type will only be entertained after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.
824 825 826 827	Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.
828	
829	14. Performance evaluation report format:
830 831 832 833	The performance evaluation report format (page 34) is designed for multiplex assays with several targets. It should be modified and used accordingly for single plex assays/multiplex assays with fewer targets.
834	
835	
836	
837	
838	
839	
840	
841	

842 **Protocol D** Evaluation of performance characteristics of Molecular Kit detecting Influenza virus and 843 844 **SARS-CoV-2** in multiplex format 845 To assess the performance of multiplex assays, Protocols A and B can be used as per kit format to 846 check the performance of each virus for its sensitivity and specificity assessment, including cross reactivity, repeatability, reproducibility and Lot to lot variation. 847 848 A comprehensive report can be generated which will include sensitivity and specificity for all targets. 849 Sample size for multiplex molecular assay (as per the scope outlined in the document) detecting 850 Influenza virus and SARS-CoV-2 in multiplex format is given below. All other 851 parameters/conditions outlined in the single plex protocols (Protocols A and B) are to be 852 essentially followed. 853 1. Sample size and sample panel composition for evaluation of performance 854 characteristics: 855 Sample sizes of positive and negative samples against different values of sensitivity and 856 specificity are provided in Table 6. Sample sizes have been calculated assuming 95% level of 857 significance, an absolute precision of 5%, and invalid test rate \leq 5%. Appropriate sample size 858 has to be chosen from the tables according to the values of sensitivity and specificity being 859 claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the 860 manufacturer needs to consider the sample size associated with the largest 861 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per 862 the next smaller value of the sensitivity/ specificity available in the table). For example, if a 863 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned 864 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the 865 sample size outlined for 85% specificity. Sample sizes are calculated using the formulae: 866 867 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$ 868 $n_{sp} \ge \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2 \times \left(1 - IR\right)}$ 869 870 871 n (se) is the number of positive samples. 872 873 n (sp) is the number of negative samples. Z^2 is the critical value from the standard normal distribution corresponding 874 to the desired confidence level (95% CI corresponds to $\mathbb{Z}^2 = 1.96$). 875

Se is the predetermined sensitivity.

876

877 · Sp is the predetermined specificity.

878 • d is the predetermined marginal error (5%)

879 · IR is the invalid test rate

880 881

Table 6. Sample sizes for different values of sensitivity/ specificity claimed by the manufacturer.

Sensitivit y/ Specificit y	Sample size for each of the 04 target pathogens ^a : Minimum number of positive samples [¥]	Composition of positive samples for ea <mark>ch pat</mark> hogen [#]	Total number of positive samples (includin g all 04 pathogen s)	Sample size: Minimum number of negative samples [¥]	Minimum number of cross reactive* samples among the negative samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	80	20	5
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28	320	80	20
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55	620	150	38
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76	860	210	53
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91	1040	260	65

^aInfluenza A: (H1N1) pdm09, Influenza A/H3N2, Influenza B, and SARS CoV-2

Moderate positive: (Ct value between 25-30) Weak positive: (Ct value >30 and and \le 34)

For multiplex assays targeting influenza and SARS-CoV-2, samples positive for these viruses may be suitably interchanged for assessing cross-reactivity

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

^{*}Strong positive: (Ct value <25)

[¥] Nasopharyngeal or oropharyngeal swabs in virus transport medium (VTM) to be used

^{*}Samples positive for common respiratory viruses (such as Parainfluenza viruses, Adenoviruses, Rhinoviruses, common human coronaviruses, RSV), other than the ones targeted by the kit under evaluation. Equal distribution of cross-reactive viruses is desirable.

2. Acceptance Criteria for the kit: 887 Sensitivity for each pathogen/ type/ subtype: ≥95% 888 Specificity for each pathogen/ type/ subtype: ≥99% 889 Cross-reactivity: Nil 890 Invalid test rate: ≤5% 891 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥80 892 positive samples and ≥20 negative samples should be tested for evaluation for each 893 894 pathogen/ type/ subtype. 895 After following due procedure as defined in this document, once any kit is found to be Not 896 897 of Standard Quality, thereafter, no request for repeat testing of the same kit will be acceptable. 898 Any request of re-validation from the same manufacturer for the same test type will only be 899 900 entertained after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit 901 902 disclosure of proprietary information. 903 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits 904 905 which claim high performance characteristics (sensitivity and specificity 95% and above), 906 but which fail the performance evaluation by a margin of 5%. 907 908 909 910 911 912 913 914 915 916

917

918 **Protocol E** Evaluation of performance characteristics of Molecular Kit detecting Influenza virus, 919 920 SARS-CoV-2 and Respiratory Syncytial Virus (RSV) in multiplex format 921 To assess the performance of multiplex assays, Protocols A, B or C can be used as per kit format 922 to check the performance of each virus for its sensitivity and specificity assessment, including cross reactivity, repeatability, reproducibility and Lot to lot variation. 923 A comprehensive report can be generated which will include sensitivity and specificity for all 924 925 targets. Sample size for multiplex molecular assay (as per the scope outlined in the document) detecting 926 Influenza virus, SARS-CoV-2 and Respiratory Syncytial Virus (RSV) in multiplex format is 927 given below. All other parameters/conditions outlined in the single plex protocols (Protocol A, B 928 and C) are to be essentially followed. 929 1. Sample size and sample panel composition for evaluation of performance 930 characteristics: 931 Sample sizes of positive and negative samples against different values of sensitivity and 932 specificity are provided in Table 7. Sample sizes have been calculated assuming 95% level of 933 significance, an absolute precision of 5%, and invalid test rate \leq 5%. Appropriate sample size 934 has to be chosen from the tables according to the values of sensitivity and specificity being 935 claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the 936 manufacturer needs to consider the sample size associated with the largest 937 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per 938 the next smaller value of the sensitivity/ specificity available in the table). For example, if a 939 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned 940 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the 941 sample size outlined for 85% specificity. Sample sizes are calculated using the formulae: 942 943 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$ 944 $n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$ 945 946 947 n (se) is the number of positive samples. 948 949 n (sp) is the number of negative samples. Z^2 is the critical value from the standard normal distribution corresponding 950 to the desired confidence level (95% CI corresponds to $\mathbb{Z}^2 = 1.96$). 951 *Se is the predetermined sensitivity.* 952

953 · Sp is the predetermined specificity.

954 · d is the predetermined marginal error (5%)

955 · IR is the invalid test rate

956957

958

959

Table 7. Sample sizes for different values of sensitivity/ specificity claimed by the manufacturer.

Sensitivit y/ Specificit y	Sample size for each of the 06 target pathogens ^a : Minimum number of positive samples [‡]	Composition of positive samples samples for each pathogen# Total number of positive samples (including all 06 pathogens)	Sample size: Minimum number of negative samples [¥]	Minimu m number of cross reactive * sample s among the negativ e sample s
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	20	5
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28 480	80	20
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55 930	150	38
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76 1290	210	53
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91 1560	260	65

^aInfluenza A: (H1N1) pdm09, Influenza A/H3N2, Influenza B, SARS CoV-2, RSV A, and RSV B

*Strong positive: (Ct value <25)

Moderate positive: (Ct value between 25-30) Weak positive: (Ct value >30 and and \le 34)

For multiplex assays targeting influenza, SARS-CoV-2, and RSV, samples positive for these viruses may be suitably interchanged for assessing cross-reactivity

[¥] Nasopharyngeal/ oropharyngeal swabs in virus transport medium (VTM) to be used

^{*} Samples positive for common respiratory viruses (such as Parainfluenza viruses, Adenoviruses, Rhinoviruses, common human coronaviruses), other than the ones targeted by the kit under evaluation. Equal distribution of cross-reactive viruses is desirable.

It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics. 2. Acceptance Criteria for the kit: Sensitivity for each pathogen/ type/ subtype: ≥95% Specificity for each pathogen/ type/ subtype: ≥99% Cross-reactivity: Nil Invalid test rate: ≤5% To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80 positive samples and ≥ 20 negative samples should be tested for evaluation for each pathogen/ type/ subtype. 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information. Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.

998 <u>Performance evaluation report for Respiratory Virus in-vitro molecular diagnostic kit</u> 999

<i>55</i>		
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	
CDSC	O/State licensing Authority	_
Lot No	o / Batch No.:	
Produc	et Reference No/ Catalogue No	
Type o	of Assay	
Kit con	mponents	
Manuf	acturing Date	
Expiry	Date	
Pack s	ize (Number of tests per kit)	
Intend	ed Use	
Numbe	er of Tests Received	
Impo	atory Approval: rt license / Manufacturing license/ Test license e Number:Issue date:	
Valid	Up to:	
Applic	ation No.	
Sample	Sample type	
Panel	Positive samples (provide details: strong, moderate, weak)	
	Negative samples (provide details, including cross reactivity panel)	•
00		

	1 /	
1000		
1001		
1002	i.	Analytes/Pathogens targeted by the kit under evaluation:
1003		
1004	ii.	
1005	iii.	
1006	iv.	
1007	v.	
1008	vi.	
1009	vii.	
1010		
1011		
1012		
1013		
1014		

1015 **RESULTS INTERPRETATION** 1016 1017 SENSITIVITY AND SPECIFICITY FOR INDIVIDUAL VIRUS TARGETS 1018 1. Sensitivity and specificity for Influenza A (H1N1) pdm09 1019 Name of the Kit Reference assay Under **Positive Negative** Total **Evaluation Positive** Negative **Total** 1020 Estimate (%) CI 95% Sensitivity **Specificity** 1021 2. Sensitivity and specificity for Influenza A (H3N2) 1022 Name of the Kit Reference assay Under **Positive Negative Total Evaluation Positive Negative Total** 1023 CI 95% Estimate (%) Sensitivity

1024
1025
3. Sensitivity and specificity for Influenza B (Victoria)

Name of the Kit		Reference assay		
Under		Positive	Negative	Total
Evaluation	Positive			
	Negative			
	Total			

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1027

1026

Specificity

1028	4.	Sensitivity	y and s	specificity	y for	Influenza	B	(Yamagata
------	----	-------------	---------	-------------	-------	-----------	---	-----------

Name of the Kit		Reference assay		
Under		Positive	Negative	Total
Evaluation	Positive			
	Negative			
	Total			

1029

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

10301031

5. Sensitivity and specificity for SARS-CoV-2

Name of the Kit		Reference assay	1	
Under		Positive	Negative	Total
Evaluation	Positive			
	Negative			
	Total			*

1032

		Est	timate	e (%)		CI 95%
Sensitivity						
Specificity	K					

10331034

6. Sensitivity and specificity for RSV A

Name of the Kit		Reference assay		
Under		Positive	Negative	Total
Evaluation	Positive			
	Negative			
	Total			

1035

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

10361037

7. Sensitivity and specificity for RSV B

Name of the Kit		Reference assay				
Under		Positive	Negative	Total		
Evaluation	Positive					
	Negative					

		Total				
			E.4°4. (0/)		CI 050/	
C.			Estimate (%)		CI 95%	
	ensitivity Decificity					
SI	pecificity					
a.	Cross-reactive	ity Analysis:				
b.	Repeatability	Assessment:				
c.	Precision (Re	producibility):			
	• Lo	t to Lot				
De	tails of lots tes	ted (3 lots to	be tested):			
	1. Lot No.:		ot No:		Tested By:	
	2. Lot No.:		ot No:		Tested By:	
	3. Lot No.:	Lo	ot No:		Tested By:	
	- Lot to lot	variation wa	s obse <mark>rved / not</mark> ol	bowwoo		
	- Lot-to-lot	variation wa	s observed / not or	<u>Jsei veu.</u>		
d.	Internal Con	trol <mark>Analysis</mark>	<u>:</u>			
	Conclusion: S	Satisfactory / I	Not satisfactory			
RE	COMMENDA	TIONS:				
Su	gges <mark>tion</mark> s for ii	mprove <mark>ment</mark> s	or modifications	(<mark>if applic</mark> ab	ole):	
	- ICMR-CI	SCO guideli	nes were followed	for kit nerf	formance evaluation.	
		beo guiaen	nes were ronowed	Tor the peri	tormunee evanauron.	
Th	is evaluation r	eport is excl	u <mark>sivel</mark> y for			_ In Vitro
Mo	lecular Diagn	ostic Kit <mark>man</mark>	ufactured by			·
Ser	ncitivity and ci	necificity hav	e heen assessed in	controlled	lab settings using the	kits of the
	t number:	seemeny nuv	e been absessed in	controlled	in settings using the	Mis of the
i						
ii						
iii	. Lot No		,			

1065 1066	Provided by the manufacturer, using samples. Results should not be extrapolated to other sample types.
1067	DISCLAIMER:
1068	1. This validation process does not approve/disapprove the Kit design.
1069	2. This validation process does not certify user friendliness of the Kit.
1070	3. Influenza and SARS-CoV-2 are continuously evolving viruses and therefore primer
1071	probe sequences of the assay may require periodic updates, which will amount to a changed
1072	version of the assay. Re-validation is required for changed version of the assay, and needs
1073	to be considered while issuing license
1074	
1075	
1076	Signature of the Lab Manager Signature of the Lab Director
1077	
1078	Signature of Head of the Institute
1079	
1080	Seal of Head of the Institute
1081	
1082	
1083	**************************************
1084	
1085	
1086	
1087	
1088	
1089	
1090	
1091	

1092	Annexure-1: Information on Operational and Test Performance Characteristics Required
1093	<u>from Manufacturers</u>
1094	The manufacturer should provide the following details about the IVD:
1095	1. Instructions for Use
1096	2. Scope of the IVD: to diagnose influenza and/or SARS-CoV-/RSV.
1097	3. Intended Use Statement
1098	4. Principle of the assay
1099	5. Intended testing population (cases of ARI/ILI/SARI)
1100	6. Intended user (laboratory professional and/or health care worker at point-of-care)
1101	7. Lot/batch No.
1102	8. Date of manufacture
1103	9. Date of Expiry
1104	10. Information on operational Characteristics
1105	i. Configuration of the kit/device
1106	ii. Requirement of any additional equipment, device
1107	iii. Requirement of any additional reagents
1108	iv. Operation conditions
1109	v. Storage and stability before and after opening
1110	vi. Internal control provided or not
1111	vii. Quality control and batch testing data
1112	viii. Biosafety aspects- waste disposal requirements
1113	11. Information on Test Performance Characteristics
1114	i. Type of sample-NP/OP swab, other respiratory specimen
1115	ii. Volume of sample
1116	iii. Any specific sample NOT to be tested
1117	iv. Any additional sample processing required
1118	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required

1119	vi. Name of analyte to be detected
1120	vii. Pathogens targeted by the kit
1121	viii. Time taken for testing
1122	ix. Time for result reading and interpretation
1123	x. Manual or automated(equipment)reading
1124	xi. Limit of detection
1125	xii. Diagnostic sensitivity
1126	xiii. Diagnostic specificity
1127	xiv. Stability and reproducibility
1128	xv. Training required for testing
1129	xvi. If yes, duration
1130	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
1131	xviii. Interpretation of invalid and indeterminate results to be provided
1132	xix. It is recommended to provide data demonstrating the precision
1133	xx. Limit of detection
1134	
1135	
1136	
1137	
1138	
1139	
1140	
1141	
1142	
1143	
1144	
1145	▼
1146	
1147	
1148	
1149	







2

3

4

5

1

STANDARD PERFORMANCE EVALUATION PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

6

7

8

MALARIA IN-VITRO DIAGNOSTICS

ICMR-CDSCO/IVD/GD/PROTOCOLS/06/2025

9 10



AUGUST, 2025 New Delhi, India

Table of Contents

S.N.	Topic	Page Number
1.	Performance evaluation protocol for Malaria Rapid diagnostic test (RDT) kits	2
2.	Performance evaluation protocol for Malaria ELISA kits	14
3.	Performance evaluation protocol for Malaria real-time PCR kits	25
4.	Field evaluation protocol for combo Malaria Rapid Diagnostic Test (RDT) kits (detecting <i>P vivax</i> and <i>P falciparum</i>)	38
5.	Information on operational and test performance characteristics required from manufacturers	49

Performance evaluation protocol for Malaria Rapid diagnostic test (RDT) kits

34 I. Background:

- 35 CDSCO/ICMR, New Delhi have aimed to facilitate the evaluation and supply of Quality-
- 36 Assured in vitro Diagnostics (IVD) kits suitable for use in India. Hence, the following
- 37 guidelines shall establish the uniformity during the performance evaluation of IVD kits The
- 38 objective of performance evaluation is to independently validate the manufacturer's claim
- regarding in-vitro diagnostic kit (IVD) performance.

40 **II. Purpose**:

- To evaluate the performance characteristics of rapid diagnostic test kit for the diagnosis of
- malaria parasite using irreversibly de-identified leftover archived/spiked clinical samples.

43 III. Requirements:

- a) Instructions for use (IFU)
- b) Supply of RDT kits under evaluation (with batch no.; lot no.; manufacturing and expiry date and other required details).
- c) Evaluation sites/laboratories (With required equipment)
- d) Reference test kits
- e) Characterised Evaluation panel
- 50 f) Laboratory supplies

51 IV. Ethical approvals:

- Performance evaluation activities using irreversibly de-identified leftover clinical samples
- are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for
- Laboratory Validation Testing, 2024.
- Investigators are required to submit a self-declaration form, as outlined in the ICMR
- 56 guidelines, to the institutional authorities and ethics committee for information.

57 V. Procedure:

61

62

63

64

65

66

67

68

- **1. Study design/type**: Diagnostic accuracy study using irreversibly de-identified leftover clinical/spiked samples.
- 2. Preparation of Evaluation sites/laboratories:
 - Identified IVD kit evaluation laboratories should establish their proficiency through:
 - a) Laboratory accreditation: Accreditation for at least one of the Quality management systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or CDSCO approved Reference laboratory.
 - b) It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in Quality Control exercises such as EQAP (External Quality Assurance Programme).

- c) **Staff training:** All the staff involved in IVD kit evaluation should undergo hands on training and competency testing on the following at referral level malaria labs before initiation of MDTL activity:
 - Preparation and characterization of evaluation panel for the respective IVD kit.
 - ➤ Management of RDT kits (specific for *Plasmodium falciparum / Plasmodium vivax*) received for performance evaluation (Verification/Storage/Unpacking etc.).
 - > Perform tests interpretation and documentation of results, and reporting.
 - > Data management and safety and confidentiality.

3. Preparation of QC panel members for Malaria RDT kit evaluation

To evaluate the performance of IVD kit, a well characterized species specific malaria antigen sample panel is required. Statistically significant number of blood samples as defined in this protocol should be collected from malaria confirmed cases in health facilities, (as mentioned in Table 1). The panel should comprise positive and negative samples as described in section 7.

The reference sample panel should be stored in appropriate storage conditions, and the quality of the panel should be checked periodically through appropriate testing.

4. Reference assay:

WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved RDT should be used as reference standard.

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

5. Sample size and sample panel composition for performance evaluation:

Sample sizes of positive and negative samples of each species targeted by the kit against different values of sensitivity and specificity are provided in Tables 1 and 2, with recommended composition. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate of 5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity.

Sample sizes are calculated using the formulae:

109

110
$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

111

 $n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$

112113

 \cdot *n (se) is the minimum number of positive samples.*

114 115

 \cdot n (sp) is the minimum number of negative samples.

116 117 \cdot Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).

118119

· Se is the predetermined sensitivity.

120

· Sp is the predetermined specificity.

121

 \cdot d is the predetermined marginal error (5%)

122

· IR is the invalid test rate

305 (rounded to 310 for better

distribution of samples)

123124

125

126

127

Table 1. Positive sample sizes (per species) and composition for different values of sensitivity claimed by the manufacturer for evaluation of Pf (single/combo RDT) or Pv (single/combo RDT)

Sensitivity	Sample size: Minimum number of positive samples #	Composition of positive samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91

128

75%

Strong positive = 92

Weak positive = 109

Moderate positive = 109

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Negative sample sizes and composition for different values of specificity claimed by the manufacturer for evaluation of Pf (single/combo RDT) or Pv (single/combo RDT)

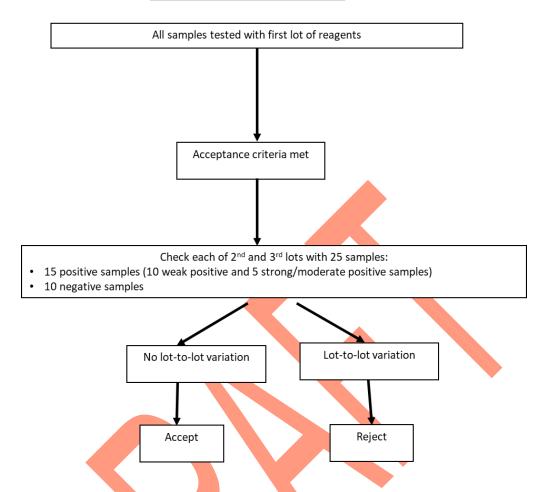
Specificity Specificity Specificity Minimum number of negative samples # Dengue NS1/IgM positive samples: 03 Chikungunya IgM positive samples: 03 Chikungunya IgM positive samples: 03 Serum reactive for RA factor – low positive and high positive: 02 Serum reactive for TPHA/other specific test for syphilis: 02 Healthy controls from endemic regions: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive samples: 10 Serum reactive for RA factor – low positive and high positive: 10 Serum reactive for TPHA/other specific test for syphilis: 10 Healthy controls from endemic regions: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 40 Chikungunya IgM positive s		Cample size:	Composition of reactive samples#
99% l6 (rounded to 20) Pongue NS1/IgM positive samples: 03 Chikungunya IgM positive and high positive: 02 Serum reactive for RA factor – low positive and high positive: 03 Healthy controls from endemic regions: 10 Pongue NS1/IgM positive samples: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive and high positive: 10 Serum reactive for RA factor – low positive and high positive: 10 Serum reactive for TPHA/other specific test for syphilis: 10 Healthy controls from endemic regions: 40 Pongue NS1/IgM positive samples: 18 Chikungunya IgM positive and high positive: 18 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Pongue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Pongue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungu			Composition of negative samples
99% 16 (rounded to 20) Serum reactive for RA factor – low positive and high positive: 02 Serum reactive for TPHA/other specific test for syphilis: 02 Healthy controls from endemic regions: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive samples: 10 Serum reactive for RA factor – low positive and high positive: 10 Serum reactive for TPHA/other specific test for syphilis: 10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphili	C :::::::::::::::::::::::::::::::::		
99% 16 (rounded to 20) Serum reactive for RA factor – low positive and high positive:02 Serum reactive for RA factor – low positive and high positive:02 Healthy controls from endemic regions: 10 Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive: 33 Serum reactive for RA factor – low positive and high positive: 10 Serum reactive for RA factor – low positive and high positive: 10 Serum reactive for TPHA/other specific test for syphilis: 10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive: 34 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive: 36 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Serum reactive for TPHA/other specific tes	Specificity	v	
Dengue NS1/IgM positive samples: 03 Chikungunya IgM positive samples: 03 Serum reactive for RA factor – low positive and high positive: 02 Healthy controls from endemic regions: 10 Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive: 10 Serum reactive for RA factor – low positive and high positive: 10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 40 Chikungunya		0	
Chikungunya IgM positive samples:03 Serum reactive for RA factor – low positive and high positive:02 Serum reactive for TPHA/other specific test for syphilis:02 Healthy controls from endemic regions: 10 Dengue NS1/IgM positive samples:10 Chikungunya IgM positive samples:10 Chikungunya IgM positive and high positive:10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 40 Chikungunya IgM positive samples: 40 Chikungunya IgM positive samp		samples #	
99% 20) Serum reactive for RA factor – low positive and high positive:02 Serum reactive for TPHA/other specific test for syphilis:02 Healthy controls from endemic regions: 10 Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive samples: 10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30			
95% 20) Serum reactive for TPHA/other specific test for syphilis:02 Healthy controls from endemic regions: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive and high positive:10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35			
Serum reactive for TPHA/other specific test for syphilis:02 Healthy controls from endemic regions: 10 Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive samples:10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35	99%		
Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive samples: 10 Chikungunya IgM positive samples: 10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30)) / 0	20)	
Chikungunya IgM positive samples:10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Healthy controls from endemic regions: 10
Chikungunya IgM positive samples:10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
95% 77 (rounded to 80) Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples:18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35			
90% Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples:18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Chikungunya IgM positive samples:10
Power Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40 Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30	050/	77 (rounded to	Serum reactive for RA factor – low positive and high positive:10
Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive and high positive: 26 Serum reactive for RA factor – low positive and high positive: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30	93%	80)	Serum reactive for TPHA/other specific test for syphilis:10
Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive: 18 Serum reactive for TPHA/other specific test for syphilis: 18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive and high positive: 26 Serum reactive for RA factor – low positive and high positive: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30			Healthy controls from endemic regions: 40
Chikungunya IgM positive samples:18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
Chikungunya IgM positive samples:18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Dengue NS1/IgM positive samples: 18
90% Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples:26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78 Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30	000/	146 (rounded	
Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30	90%	to 150)	Serum reactive for TPHA/other specific test for syphilis:18
Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples: 26 Chikungunya IgM positive samples: 26 Serum reactive for RA factor – low positive and high positive: 26 Serum reactive for TPHA/other specific test for syphilis: 26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30			Healthy controls from endemic regions: 78
Chikungunya IgM positive samples:26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples:35 Chikungunya IgM positive samples:35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Dengue NS1/IgM positive samples: 26
Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples:35 Chikungunya IgM positive samples:35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Chikungunya IgM positive samples:26
Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples:35 Chikungunya IgM positive samples:35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30	050/	207 (rounded	Serum reactive for RA factor – low positive and high positive:26
Healthy controls from endemic regions: 106 Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30	85%	to 210)	
Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30			
Chikungunya IgM positive samples:35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
Chikungunya IgM positive samples:35 Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			Dengue NS1/IgM positive samples: 35
80% 259 (rounded to 260) Serum reactive for RA factor – low positive and high positive:30 Serum reactive for TPHA/other specific test for syphilis:30			
to 260) Serum reactive for TPHA/other specific test for syphilis:30	900/	259 (rounded	
	80%	`	
			,

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Sample panel composition:

Positive samples: Malaria positive samples should be obtained from health facilities 141 (tertiary care centers and their linked hospitals, private clinics, field practice areas 142 etc.) and confirmed using PCR (Snounou protocol/FDA approved assay). 143 Malaria samples confirmed positive by PCR should be characterized for parasite load 144 on in-house calibrated equipment using blood smear microscopy and ELISA. Samples 145 with analyte values satisfying the range of acceptance criteria (as mentioned in this 146 document) should be included in the positive sample panel for the evaluation of malaria 147 RDT kits. 148 For the RDT kits which have other antigen/antibody as target analyte (for which limits 149 of detection have not been established), characterization of samples should be 150 performed on calibrated equipment, leading to their classification as low and high 151 parasitemic samples, which should then be used for performance evaluation of the 152 153 assay. 154 Range of Parasitemia: Panel members should have low (≤ 200 parasites per microliter) 155 to high (≥ 2000 parasites per microliter) range of Plasmodium falciparum, P. vivax 156 and/or other Plasmodium species, as obtained from ELISA results. Characterized 157 panels **must** contain equal number of samples of both low and high parasitemia. 158 Consistent ELISA quantification results should be obtained in ≥3 runs of ELISA 159 experiments performed for each of the three antigens (PfHRP2, LDH and aldolase) with 160 the results obtained at the 200 p/µL and the 2,000 p/µL being consistent with each other 161 as well (factor of roughly 10 between results). The limit of detection of Pfhrp2 is 5-10 162 ng/ μ L, and PvLDH is 15-45 ng/ μ L. 163 ** It should be noted that no such limit of detection is defined for aldolase. Where values/standard 164 165 reference assay not available, standard procedure on calibrated equipment will be followed for obtaining 166 results. 167 6. Test reproducibility: 168 169 A. Sample size for lot-to-lot reproducibility 170 171 Three lots of an assay shall be evaluated. The first lot shall be evaluated on the entire 172 panel of samples (statistically significant sample size). For the subsequent two lots, 25 173 samples should be used for evaluation (15 positive samples including 10 weak positive 174 samples and 5 moderate/strong positive samples, and 10 negative samples). 175 176 Refer the flowchart below (Fig. 1):

Fig.1: Lot-to-lot reproducibility



weak positive samples and 5 strong/moderate positive samples, and 10 negative samples) need to be tested by at least 2 trained personnel. Agreement should be 100%.

Note: Testing Methodology

Read the instructions for use (IFU) thoroughly. Take out the required number of RDTs kits from the recommended storage conditions. Bring RDTs to room temperature (20°C - 30°C) and thaw the required number of QC/sample panel aliquots for a minimum of 20 minutes to maximum 60 minutes before performing the test. Note that more than one aliquot may be needed for the testing of each sample. Record the results of the performance evaluation on the recommended report format (Annexure 1).

B. Reader-to-reader reproducibility: 25 samples (15 positive samples including 10

7. Evaluation method:

The reference assay and the index test should be run on the sample panel in parallel.

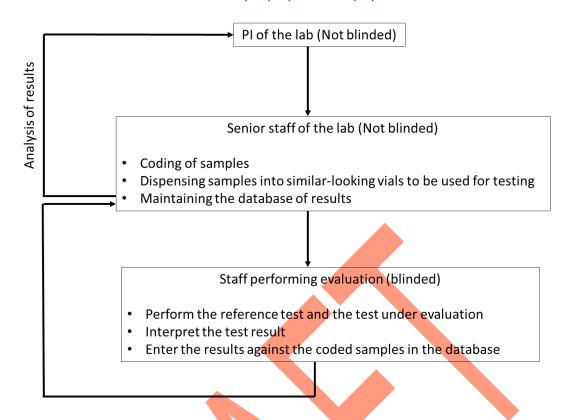
8. Interpretation of results:

Results should be interpreted as per the IFU of the reference assay and the index test.

198		
199	9.	Resolution of discrepant results:
200		True positive samples: These are samples positive by both reference assay and index
201		test.
202		True negative samples: These are samples negative by both reference assay and index
203		test.
204		False positive samples: These are samples negative by reference assay and positive by
205		index test.
206		False negative samples: These are samples positive by reference assay and negative by
207		index test.
208		
209	10.	Acceptance criteria ¹ :
210		Expected sensitivity: $\geq 75\%$ for P _. vivax and $\geq 95\%$ for P _. falciparum
211		Expected specificity: $\geq 90\%$ for P _. vivax and $\geq 95\%$ for P _. falciparum
212		Cross-reactivity: Nil
213		Invalid test rate: ≤5%
214		
215		To achieve at least the performance characteristics outlined in the acceptance criteria,
216		\geq 310 positive samples and \geq 150 negative samples should be tested for <i>P vivax</i> , and \geq 80
217		positive samples and ≥ 80 negative samples should be tested for <i>P falciparum</i> .
218		
219	11.	Blinding of laboratory staff
220		To ensure rigor of the evaluation process, laboratory staff performing the evaluation
221		should be blinded to the status of the clinical samples. The PI of the evaluation exercise
222		should remain unblinded, i.e., privy to the status of the samples. Another senior
223		laboratory staff selected by the PI may remain unblinded and carry out coding of
224		samples and dispensing them into similar-looking vials to be used for testing, and
225		maintaining the database of results. Staff performing the reference test and the test
226		under evaluation, interpretation of the test result, and entering the results against the
227		coded samples in the database, should remain blinded to the status of samples till the
228		completion of evaluation. The data should be analyzed only by the PI of the evaluating
229		lab. Refer to Fig. 2.

Fig.2: Blinding in evaluation exercise

229 230



12. Publication Rights

The PI(s) of the evaluating labs shall retain publication rights to 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.

- 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.
 - Clinical samples are precious, therefore, repeat evaluation of a kit using the same/different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.

VI. References:

- 1. Ministry of Health and Family Welfare. Guidelines for Bivalent RDT. Available at: guidelines-for-bivalent-rdt.pdf (mohfw.gov.in)
 - 2. World Health Organization. Malaria Rapid Diagnostic Test Performance Results of WHO product testing of malaria RDTs: round 8 (2016–2018): Available at: https://iris.who.int/bitstream/handle/10665/276190/9789241514965-eng.pdf?sequence=1
 - 3. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315–20.
 - 4. Krishna S, Bharti PK, Chandel HS, Ahmad A, Kumar R, Singh PP, et al. Detection of Mixed Infections with Plasmodium spp. by PCR, India, 2014. Emerg Infect Dis. 2015;21(10):1853-7.

VII. Performance evaluation report format



270	REPORT FORMA	<u>T</u>		
271	Name of the Laborat	cory		
272	Name of the Institute, (wit	h station)		
273	Certificate of Analy	sis		
274	File No.:			
Name of t	he 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 s	supplier: Manufacturer/Importer/Port office of			
CDSCO/S	State licensing Authority			
Lot No / I	Batch No.:			
Product R	eference No/ Catalogue No			
Type of A	ssay			
Kit compo	onents			
Manufact	uring Date			
Expiry Da	ate			
Pack size	(Number of tests per kit)			
Intended 1	Use			
Number o	f Tests Received			
Regulato	ry Approval:			
Import lic	mport license / Manufacturing license/ Test license			
License N	Tumber: Issue date:			
Valid Up	to:			
Application	on No.			
Sample	Sample type			
Panel	Positive samples (provide details: strong, moderate weak)			

275

276

277 Results:

278

		Reference a (name)	assay	
		Positive	Negative	Total
Name of index malaria RDT	Positive			
	Negative			
	Total			

279

280

	Estimate (%) 95% CI
Sensitivity	
Specificity	

281

289

- Details of cross reactivity with other agents:
- 283 Conclusions:
- 284 o Sensitivity, specificity
- 285 Performance: Satisfactory / Not Satisfactory
- 286 (Sensitivity and specificity have been assessed in controlled lab setting on serum samples only,
- using kits provided by the manufacturer from the batch mentioned above. Results should not
- 288 be extrapolated for any other sample type.)

Disclaimers

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

292 293	Note: This report is exclusively for Kit (Lot No) manufactured by
294	
295	Evaluation Done on
296	Evaluation Done by
297298299	Signature of Director/ Director-In-charge Seal
300	**************************************
301	
302	
303	
304	
305	
306	
307	
308	
309	
310	
311	
312	
313	
314	
315	
316	
317	
318	
319	
320	
321	
322	

Performance evaluation protocol for Malaria ELISA kits

CDSCO/ICMR, New Delhi, have aimed to facilitate the evaluation and supply of Quality-

Assured In Vitro Diagnostics kits suitable for use in India. Hence, the following guidelines

shall establish the uniformity during the performance evaluation of IVD kits. The objective

323

324

325

326

327

359

I.

Background:

328 329	of performance evaluation is to independently validate the manufacturer's claim regarding in-vitro diagnostic kit (IVD) performance.
330	II. Purpose:
331 332 333 334 335 336	To evaluate the performance characteristics of malaria ELISA kits for the diagnosis of malaria parasite infection using irreversibly de-identified leftover archived/spiked clinical samples. The malaria ELISA kits are designed to detect antigens (hrp2, LDH, aldolases) occurring in subjects infected with species specific (<i>P. falciparum</i> , <i>P. vivax</i>) and stage specific antibodies (MSP1, MSP3, CSP, EBA175 etc parasite markers for the purpose of sero-survey).
337	III. Requirements:
338	a) Instructions for use (IFU)
339 340 341	b) Supply of ELISA kits under evaluation (with batch no./lot no. expiry date & required details). In case the kit to be evaluated is designed to work in a closed system format, the manufacturer needs to supply the required equipment.
342	c) Evaluation sites/laboratories (With required equipment)
343	d) Reference test kits
344	e) Characterised Evaluation panel
345	f) Laboratory supplies
346	IV. Ethical approvals:
347 348 349	Performance evaluation activities using irreversibly de-identified leftover clinical samples are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory Validation Testing, 2024.
350 351	Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines, to the institutional authorities and ethics committee for information.
352	V. Procedure:
353 354 355 356 357	 Study design/type: Diagnostic accuracy study using irreversibly de-identified leftover clinical samples. Preparation of Evaluation sites/laboratories: Identified ELISA kit evaluation laboratories should establish their proficiency through
358	a) Laboratory accreditation: Accreditation for at least one of the Quality management

systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical

360

Lab (ISO:15189), PT provider ISO: 17043 or CDSCO approved Reference

361			laboratory.
362 363 364		b)	It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in Quality Control exercises such as EQAP (External Quality Assurance Programme).
365 366 367		c)	Staff training: All the staff involved in ELISA kit evaluation should undergo hands on training and competency testing on the following at referral level malaria labs before initiation of MDTL activity:
368 369			Preparation and characterization of evaluation panel for the respective ELISA kit.
370 371			Management of malaria ELISA kits received for performance evaluation (Verification/Storage/Unpacking etc).
372			Perform tests, interpretation and documentation of results and reporting.
373			> Data management and safety and confidentiality
374	3.	Ref	Perence sample panel:
375 376 377 378 379		anti abs mal	evaluate the performance of ELISA kit a well characterised malaria stage specific gens/species specific antibody ELISA evaluation sample panel is required. In the ence of WHO Pre-Qualified/US FDA/ ATAGI Australia/ PMDA Japan approved aria ELISA assay, it is recommended that performance evaluation of ELISA assays performed on a rigorously well characterized panel of positive and negative samples.
380 381 382		con	statistically significant number of sera samples should be collected from malaria firmed cases from health facilities. All samples should be further confirmed by PCR ay (Snounou protocol/FDA approved assay).
383 384 385 386 387 388 389 390 391 392	A.	on the the For dete sho	laria samples confirmed positive by PCR should be characterized for parasite load in-house calibrated equipment using ELISA. Samples with analyte values satisfying range of acceptance criteria (as mentioned in this document) should be included in positive sample panel for the evaluation of malaria RDT kits. those kits which have other antigen/antibody as target analyte (for which limits of ection have not been established), characterization of samples for that analyte uld be performed on calibrated equipment, leading to their classification as low and h parasitemic samples, which will then be used for performance evaluation of the ay.
393 394 395 396		to 1	nge of Parasitemia: Panel members should have low (≤200 parasites per microliter) nigh (≥2000 parasites per microliter) range of Plasmodium falciparum, P. vivax /or other Plasmodium species, as obtained from ELISA results. Characterized els must contain equal number of samples of both low and high parasitemia.
397 398 399		exp	nsistent ELISA quantification results should be obtained in ≥ 3 runs of ELISA periments performed for each of the three antigens (PfHRP2, LDH and aldolase – ombinantly expessed proteins) with the results obtained at the 200 p/ μ L and the

2,000 p/µL being consistent with each other as well (factor of roughly 10 between 400 results). The limit of detection of Pfhrp2 is 5-10 ng/ μ L, and Pvldh is 15-45 ng/ μ L. 401 ** It should be noted that no such limit of detection is defined for aldolase. Where values/standard 402 403 reference assay not available, standard procedure on calibrated equipment will be followed for obtaining 404 The above-mentioned activities should not be performed with spiked/contrived samples. 405 Equal representation of samples positive for Plasmodium (P.falciparum /P.vivax) species 406 preferred. 407 408 **B.** Negative panel should constitute malaria negative samples (confirmed by PCR) as described in point 6B. 409 The reference sample panel should be stored in appropriate storage conditions, and the quality 410 of the panel should be checked periodically with appropriate tests (including parasite culture) 411 as needed. 412 Malaria positive samples should be obtained from health facilities, including tertiary care 413 centers and their linked hospitals, private clinics, field practice areas etc. 414 Wherever any WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved 415 assay is available, it should be used as reference standard. 416 417 Sample size and sample panel composition for performance evaluation: Sample sizes 418 of positive and negative samples of each species targeted by the kit against different values 419 of sensitivity and specificity are provided in Table 1 and Table 2, with recommended 420 composition. Sample sizes have been calculated assuming 95% level of significance and 421 422 an absolute precision of 5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If 423 a claimed sensitivity/specificity is not present in the table, the manufacturer needs to 424 consider the sample size associated with the largest sensitivity/specificity provided in the 425 table that is smaller to the claimed value (that is, as per the next smaller value of the 426 sensitivity/ specificity available in the table). For example, if a manufacturer claims a 427 sensitivity of 93%, they are required to use a sample size mentioned against 90% 428 sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size 429 outlined for 85% specificity. Sample sizes are calculated using the formulae: 430 431 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2}$ 432 $n_{sp} \ge \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2}$ 433 434

16

n (se) is the minimum number of positive samples.

n (sp) is the minimum number of negative samples.

435

436

438 439 440	Z^2 is the critical value from the standard normal distribution esponding to the desired confidence level (95% CI corresponds to Z^2 96).
441	Se is the predetermined sensitivity.
442	Sp is the predetermined specificity.
443	d is the predetermined marginal error (5%)

Table 1. Positive sample sizes (per species) and composition for different values of sensitivity claimed by the manufacturer for evaluation of Pf (single/combo) or Pv (single/combo) ELISA

Sensitivity	Sample size: Minimum number of positive samples#	Composition of positive samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07
95%	73 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28
90%	139 (rounded to 140 for better distribution of samples)	Strong positive = 42 Moderate positive = 49 Weak positive = 49
85%	196 (rounded to 200 for better distribution of samples)	Strong positive = 60 Moderate positive = 70 Weak positive = 70
80%	246 (rounded to 255 for better distribution of samples)	Strong positive = 75 Moderate positive = 90 Weak positive = 90
75%	289 (rounded to 295 for better distribution of samples)	Strong positive = 87 Moderate positive = 104 Weak positive = 104

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Negative sample sizes and composition for different values of specificity claimed by the manufacturer for evaluation of Pf (single/combo) or Pv (single/combo) ELISA

	Sample size: Minimum	Composition of negative samples
Specificity	number of	
	negative	
	samples #	
		Dengue NS1/IgM positive samples: 03
		Chikungunya IgM positive samples:03
99%	16 (rounded to	Serum reactive for RA factor – low positive and high positive:02
) //0	20)	Serum reactive for TPHA/other specific test for syphilis:02
		Healthy controls from endemic regions: 10
050/	73 (rounded to	Dengue NS1/IgM positive samples: 10
95%	80)	Chikungunya IgM positive samples:10

		Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40
90%	139 (rounded to 140)	Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples: 18 Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 68
85%	196 (rounded to 200)	Dengue NS1/IgM positive samples: 25 Chikungunya IgM positive samples: 25 Serum reactive for RA factor – low positive and high positive: 25 Serum reactive for TPHA/other specific test for syphilis: 25 Healthy controls from endemic regions: 100
80%	246 (rounded to 250)	Dengue NS1/IgM positive samples: 30 Chikungunya IgM positive samples: 30 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Healthy controls from endemic regions: 130

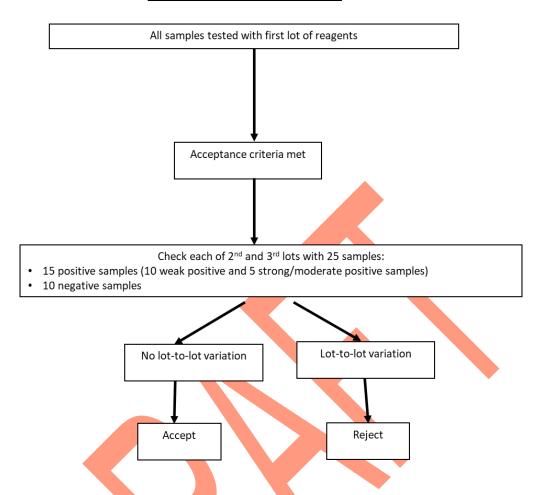
#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

4. Sample size for lot-to-lot reproducibility

Three lots of an assay shall be evaluated. The first lot shall be evaluated on the entire panel of samples (statistically significant sample size). For the subsequent two lots, 25 samples should be used for evaluation (15 positive samples including 10 weak positive samples and 5 moderate/strong positive samples, and 10 negative samples).

Refer the flowchart below (Fig. 1):

Fig.1: Lot-to-lot reproducibility



5. Evaluation Methodology:

 The index test should be tested on a rigorously well-characterized panel of samples from confirmed malaria positive and negative cases, which are further tested for the presence of malaria parasite using the Snounou protocol.

6. Interpretation of results:

Results should be interpreted as per the IFU of the reference assay.

7. Resolution of discrepant results:

True positive samples: These are well-characterized samples from confirmed malaria positive cases, which are also positive by the index test.

True negative samples: These are well-characterized samples from confirmed malaria negative cases, which are also negative by the index test.

False positive samples: These are well-characterized samples from confirmed malaria negative cases, which are positive by the index test.

False negative samples: These are well-characterized samples from confirmed malaria positive cases, which are negative by the index test.

8. Acceptance Criteria:

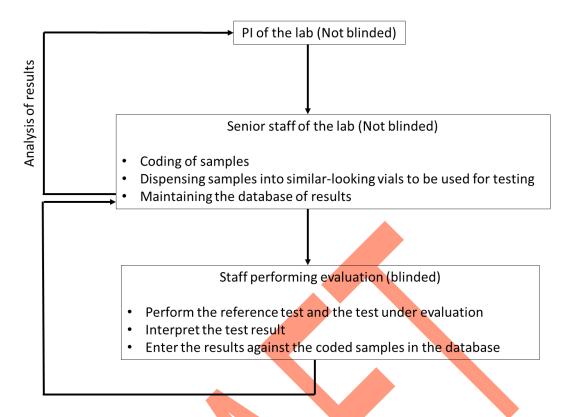
Type of assay	Acceptance criteria	Minimum no. of samples needed to achieve at least the performance characteristics outlined in the acceptance criteria
Malaria antibody ELISA	Sensitivity: ≥90% Specificity: ≥95%	Minimum no. of Positive samples = 140
		Minimum no. of Negative samples = 80
Pv ELISA	Sensitivity: ≥75% Specificity: ≥95%	Minimum no. of Positive samples = 295
		Minimum no. of Negative samples = 80
Pf ELISA	Sensitivity: ≥90% Specificity: ≥95%	Minimum no. of Positive samples = 140
		Minimum no. of Negative samples = 80

Cross-reactivity: Nil

9. 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



10. 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.

Clinical samples are precious, therefore, repeat evaluation of a kit using the same/different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.

VI. References:

1. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315–20.

VII. Performance evaluation report format

527	REPORT FORMAT
528	Name of the Laboratory
529	Name of the Institute, (with station)
530	Certificate of Analysis
531	File No.:
Name of	the product (Brand /generic)
Name and	l address of the legal manufacturer
Name and	l address of the actual manufacturing site
Name and	l address of the Importer
Name of	supplier: Manufacturer/Importer/Port office of
CDSCO/S	State licensing Authority
Lot No / l	Batch No.:
Product R	Reference No/ Catalogue No
Type of A	assay
Kit comp	onents
Manufact	uring Date
Expiry Date	
Pack size	(Number of tests per kit)
Intended	Use
Number o	of Tests Received
Regulato	ry Approval:
Import lic	eense / Manufacturing license/ Test license
License Number: Issue date:	
Valid Up	to:
Application	on No.
Sample	Sample type
Panel	Positive samples (provide details: strong, moderate, weak)

Negative samples (provide details, including cross reactivity panel)

532

533

534 **Results:**

535

		· •	n confirmed of frmed by Snow (assay)	
		Positive	Negative	Total
Name of malaria ELISA kit	Positive			
	Negative			
	Total			

536

537

	Estimate (%)	95% CI
Sensitivity		
Specificity		

538

- Details of cross reactivity with other agents:
- Conclusions:
- 541 o Sensitivity, specificity
- 542 o Performance: Satisfactory / Not Satisfactory
- 543 (Sensitivity and specificity have been assessed in controlled lab setting on samples only,
- 544 using kits provided by the manufacturer from the batch mentioned above. Results should not
- 545 be extrapolated for any other sample type.)
 - **Disclaimers**
- 1. This validation process does not approve / disapprove the kit design
- 548 2. This validation process does not certify user friendliness of the kit / assay

549 550	Note: This report is exclusively for Kit (Lot No) manufactured by (Supplied by)
551	
552	Evaluation Done on
553	Evaluation Done by
554	
555 556	Signature of Director/ Director-In-charge Seal
557	**************************************
558	
559	
560	
561	
562	
563	
564	
565	
566	
567	
568	
569	
570	
571	
572	
573	
574	
575	
576	
577	
578	
579	

Performance evaluation protocol for Malaria real-time PCR kits

580

616

617

581		I.	Background:			
582 583 584 585 586	CDSCO/ICMR, New Delhi, have aimed to facilitate the evaluation and supply 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 IVD kit performance.					
587		II.	Purpose:			
588 589		evaluate the performance characteristics of Malaria real-time PCR (RT-PCR) kits using reversibly de-identified leftover archived/spiked clinical samples.				
590		Ш	. Requirements:			
591		1.	Instructions for use (IFU)			
592		2.	Supply of kits under evaluation (with batch no. and lot no.; Manufacturing and			
593		2.	Expiry and other required details). If the kit to be evaluated works in a closed			
594			system format, the manufacturer needs to supply the required equipment.			
595		3.	Evaluation sites/laboratories (With required equipment)			
596		4.	Reference test kits			
597		5.	Characterised Evaluation panel			
598		6.	Laboratory supplies			
599						
600		IV	. Ethical approvals:			
601		Performance evaluation activities using irreversibly de-identified leftover clinical samples				
602		are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for				
603		Laboratory Validation Testing, 2024.				
604		Inv	vestigators are required to submit a self-declaration form, as outlined in the ICMR			
605		guidelines, to the institutional authorities and ethics committee for information.				
606						
607		v.	Procedure:			
608		1.	Study design/type: Diagnostic accuracy study using irreversibly de-identified leftover			
609		_,	clinical/spiked samples.			
610		2.	Preparation of Evaluation sites/laboratories:			
611			Identified IVD kit evaluation laboratories should establish their proficiency			
612			through			
613	a)	Lal	boratory accreditation: Accreditation for at least one of the Quality management systems			
614			creditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189),			
615		PT	provider (ISO: 17043) or CDSCO approved Reference laboratory.			

b) It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in

Quality Control exercises such as EQAP (External Quality Assurance Programme).

- 618 c) **Staff training:** All the staff involved in IVD kit evaluation should undergo hands-on training and competency testing on the following at referral level malaria labs before initiation of MDTL activity:
- Preparation and characterization of evaluation panel for the respective IVD kit.
- Management of RDT kits (specific for *Plasmodium falciparum / Plasmodium vivax*) received for performance evaluation (Verification/Storage/Unpacking etc.).
- Perform tests interpretation and documentation of results, and reporting.
- Data management and safety and confidentiality. ▶

1. Preparation of evaluation sample panel for Malaria

- To evaluate the performance of malaria RT-PCR IVD kit, a well characterized species specific
- malaria whole genome panel is required. Hence, statistically significant number of whole blood
- samples should be collected from malaria confirmed cases. The panel should comprise positive
- 630 and negative samples as described in section 8.
- 631 The reference sample panel should be stored in appropriate storage conditions, and the quality
- of the panel should be checked periodically with appropriate tests (including parasite culture)
- 633 as needed.

626

636

637

643

649

- 634 Malaria positive samples should be obtained from health facilities, including tertiary care
- 635 centers and their linked hospitals, private clinics, field practice areas etc.

2. DNA extraction

- DNA extraction should be performed using a standard protocol/kit as recommended by the
- manufacturer, or fully automated DNA extractor may be used (as per manufacturer's
- 640 instruction and compatible reagent kits).
- Note: If the manufacturer of the index test recommends a specific DNA extraction kit, it needs
- to be provided by the manufacturer, if the evaluation lab is unable to procure the same.

3. 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.

4. Internal Control/Extraction Control:

- The index test must have an internal control (housekeeping gene), with or without an extraction
- 651 control.

5. Reference assay:

- Two WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan-approved malaria RT-653
- PCR assays (or one FDA-approved assay and the Snounou protocol) should be used as 654
- reference assays for the characterization of samples, with 100% agreement between their 655
- results. 656
- All positive samples should be confirmed positive by the reference assay(s). 657
- All negative samples should be confirmed negative by the reference assay(s). 658

659

660

661

662

663

664

665

666

667

668

669

670

671

672

6. Sample size and sample panel composition for performance evaluation:

Sample sizes of positive and negative samples of each species targeted by the kit against different values of sensitivity and specificity are provided in Table 1 and Table 2, with recommended composition. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate of 5%. Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity. Sample sizes are calculated using the formulae:

 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$

673 674

$$n_{sp} \ge \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2 \times \left(1 - IR\right)}$$

- 678
- 679

- 680
- 681 682
- 683
- 684
- 685
- 687
- 686
- 688

- n (se) is the minimum number of positive samples.
- *n (sp) is the minimum number of negative samples.*
- Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to \mathbb{Z}^2 =1.96).
- *Se is the predetermined sensitivity.*
- *Sp is the predetermined specificity.*
- *d* is the predetermined marginal error (5%)
- IR is the invalid test rate

Table 1. Positive sample sizes (per species) and composition for different values of sensitivity claimed by the manufacturer for evaluation of Pf (single/combo RDT) or Pv (single/combo RDT)

Sensitivity	Sample size: Minimum number of positive samples#	Composition of positive samples
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07
95%	77 (rounded to 80 for better distribution of samples)	Strong positive = 24 Moderate positive = 28 Weak positive = 28
90%	146 (rounded to 155 for better distribution of samples)	Strong positive = 45 Moderate positive = 55 Weak positive = 55
85%	207 (rounded to 215 for better distribution of samples)	Strong positive = 63 Moderate positive = 76 Weak positive = 76
80%	259 (rounded to 260 for better distribution of samples)	Strong positive = 78 Moderate positive = 91 Weak positive = 91
75%	304 (rounded to 310 for better distribution of samples)	Strong positive = 92 Moderate positive = 109 Weak positive = 109

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Negative sample sizes and composition for different values of specificity claimed by the manufacturer for evaluation of Pf (single/combo RDT) or Pv (single/combo RDT)

Specificity	Sample size: Minimum number of negative samples#	Composition of negative samples
99%	16 (rounded to 20)	Dengue NS1/IgM positive samples: 03 Chikungunya IgM positive samples:03 Serum reactive for RA factor – low positive and high positive:02 Serum reactive for TPHA/other specific test for syphilis:02 Healthy controls from endemic regions: 10
95%	77 (rounded to 80)	Dengue NS1/IgM positive samples: 10 Chikungunya IgM positive samples: 10 Serum reactive for RA factor – low positive and high positive:10 Serum reactive for TPHA/other specific test for syphilis:10 Healthy controls from endemic regions: 40
90%	146 (rounded to 150)	Dengue NS1/IgM positive samples: 18 Chikungunya IgM positive samples:18

		Serum reactive for RA factor – low positive and high positive:18 Serum reactive for TPHA/other specific test for syphilis:18 Healthy controls from endemic regions: 78
85%	207 (rounded to 210)	Dengue NS1/IgM positive samples: 26 Chikungunya IgM positive samples:26 Serum reactive for RA factor – low positive and high positive:26 Serum reactive for TPHA/other specific test for syphilis:26 Healthy controls from endemic regions: 106
80%	259 (rounded to 260)	Dengue NS1/IgM positive samples: 35 Chikungunya IgM positive samples: 35 Serum reactive for RA factor – low positive and high positive: 30 Serum reactive for TPHA/other specific test for syphilis: 30 Healthy controls from endemic regions: 130

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Sample panel composition:

A. *Positive samples:* Malaria positive samples should be obtained from health facilities and confirmed using two FDA approved PCR Kits (including Snounou protocol). Once the positive samples are well-characterized with these two PCR assays (100% agreement between results), they should be classified as per their parasite load using ELISA on *in-house calibrated equipment*. Samples with analyte values satisfying the range of acceptance criteria (as mentioned in this document) should be included in the positive sample panel for the evaluation of malaria RT-PCR kits.

Additional analytes (whose cutoff values have not yet been established) may be used for further sample characterization by ELISA. However, this characterization of samples should also be performed on calibrated equipment, leading to their classification as low and high parasitemia samples, which should then be used for performance evaluation of the assay.

Range of Parasitemia: Panel members should have a low (≤ 200 parasites per microliter) to high (≥ 2000 parasites per microliter) range of Plasmodium falciparum, P. vivax, as obtained from ELISA results. Characterized panels **must** contain equal number of samples of both low and high parasitemia.

Consistent ELISA quantification results should be obtained in ≥ 3 runs of ELISA experiments performed for each of the three antigens (PfHRP2, LDH and aldolase), with the results obtained at the 200 p/ μ L and the 2,000 p/ μ L being consistent with each other as well (factor of roughly 10 between results). The limit of detection of Pfhrp2 is 5-10 ng/ μ L, and Pvldh is 15-45 ng/ μ L.

** It should be noted that no such limit of detection is defined for aldolase. Where values/standard reference assay not available, standard procedure on calibrated equipment will be followed for obtaining results.

The above mentioned activities should not be performed with spiked/contrived samples.

Equal representation of samples positive for all Plasmodium (P.falciparum /P.vivax) species preferred.

7. Test reproducibility

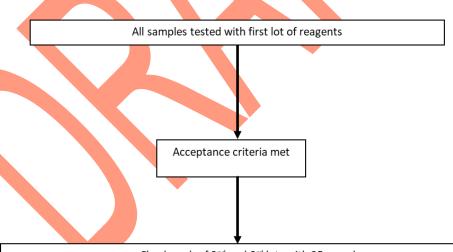
A. Lot-to-lot reproducibility:

• Sample size for lot-to-lot reproducibility

Three lots of an assay shall be evaluated. The first lot shall be evaluated on the entire panel of samples (statistically significant sample size). For the subsequent two lots, 25 samples should be used for evaluation (15 positive samples including 10 weak positive samples and 5 moderate/strong positive samples, and 10 negative samples).

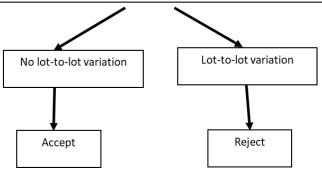
Refer the flowchart below (Fig. 1):

Fig.1: Lot-to-lot reproducibility



Check each of 2^{nd} and 3^{rd} lots with 25 samples:

- 15 positive samples (10 weak positive and 5 strong/moderate positive samples)
- 10 negative samples



748 749 750

751 752

753 754

756 757

755

758

760 761

759

762 763 764

765 766

767 768 769

770

771 772 **B. Reader-to-reader reproducibility:** 25 samples (15 positive samples including 10 weak positive samples and 5 strong/moderate positive samples, and 10 negative samples) need to be tested by at least 2 trained personnel. Agreement should be 100%.

C. Machine-to-machine reproducibility: 25 samples (15 positive samples including 10 weak positive samples and 5 strong/moderate positive samples, and 10 negative samples) to be tested on two different platforms (e.g.: ABI 7500 and BioRad CFX96). Agreement should be 100%.

8. Testing Methodology:

The reference assay and the index test should be run on the sample panel in parallel.

9. Interpretation of results:

Results should be interpreted as per the IFU of the reference assay and the index test.

10. Resolution of discrepant results:

True positive samples: These are samples positive by both reference assay and index test.

True negative samples: These are samples negative by both reference assay and index

False positive samples: These are samples negative by reference assay and positive by

False negative samples: These are samples positive by reference assay and negative by index test.

11. Acceptance Criteria:

Target Plasmodium species	Acceptance criteria	Minimum no. of samples needed to achieve at least the performance characteristics outlined in the acceptance criteria
Pf PCR	Sensitivity ≥98% Specificity ≥98% Limit of detection: 1	Minimum no. of Positive samples = 80
	parasite/µl Invalid test rate: ≤5%	Minimum no. of Negative samples = 80
Pv PCR	Sensitivity ≥95% Specificity ≥98% Limit of detection: 1-2	Minimum no. of Positive samples = 80
	parasites/μ1 Invalid test rate: ≤5%	Minimum no. of Negative samples = 80
Multiplex PCR - Pf & Pv	For Pf: • Sensitivity: ≥98% • Specificity: ≥98%	For Pf: Minimum no. of Positive samples = 80

 Absolute precision 5% 95% CI Invalid test rate ≤5% Limit of detection: 1 	Minimum no. of Negative samples = 80
parasite/μl For Pv: • Sensitivity: ≥95% • Sensitivity: >089/	For Pv: Minimum no. of Positive samples = 80
 Specificity: ≥98% Absolute precision 5% 95% CI Invalid test rate ≤5% Limit of detection: 1-2 	Minimum no. of Negative samples = 80
parasites/µl	

773

774 775

776

777 778

779 780

781

787 788 789

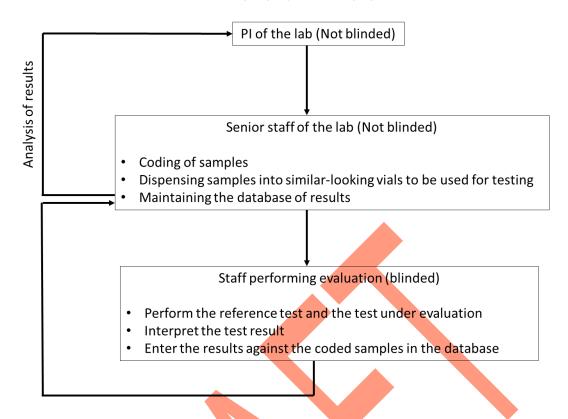
790

12. Blinding of laboratory staff

Cross-reactivity: nil Invalid test rate: ≤5%

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 similarlooking 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



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

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

Any request of re-validation from the same manufacturer for the same test type will only

be entertained after a minimum of 3 months and only if a high-level technical summary

of modifications or functional improvements to the kit design is submitted, without

Clinical samples are precious, therefore, repeat evaluation of a kit using the same/

different well-characterized sample panel at a different laboratory may be considered

only for kits which claim high performance characteristics (sensitivity and specificity

95% and above), but which fail the performance evaluation by a margin of 5%.

791

792

793

794 795

796

797

798

799

800 801

802 803 804

805 806

807 808 809

810

811 812

813

814

VI. **References:**

explicit disclosure of proprietary information.

13. Publication Rights

author(s).

acceptable.

1.

Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested

- polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315-815 20. 816
 - Ramírez AM, Tang THT, Suárez ML, Fernández AÁ, García CM, Hisam S, Rubio 2. JM. Assessment of Commercial Real-Time PCR Assays for Detection of Malaria Infection in a Non-Endemic Setting. Am J Trop Med Hyg. 2021 Oct 12;105(6):1732-1737. doi: 10.4269/ajtmh.21-0406. PMID: 34662870; PMCID: PMC8641344.
 - 3. Bouzayene, A., Zaffaroullah, R., Bailly, J. et al. Evaluation of two commercial kits and two laboratory-developed qPCR assays compared to LAMP for molecular diagnosis of malaria. Malar J 21, 204 (2022). https://doi.org/10.1186/s12936-022-04219-1
 - Aschar M, Sanchez MCA, Costa-Nascimento MJ, Farinas MLRN, Hristov AD, 4. Lima GFMC, Inoue J, Levi JE, Di Santi SM, Ultrasensitive molecular tests for *Plasmodium* detection: applicability in control and elimination programs and reference laboratories. Rev Panam Salud Publica. 2022 Mar 28;46:e11. doi: 10.26633/RPSP.2022.11. PMID: 35355692; PMCID: PMC8959250.

VII. Performance evaluation report format

817

818

819

820

821

822

823

824

825 826

827

828 829

830

851

852



853	REPORT FORMAT	_	
854	Name of the Laboratory		
855	Name of the Institute, (with station)		
856	Certificate of Analysi	is	
857	File No.:		
Name of t	the product (Brand /generic)		
Name and	d address of the legal manufacturer		
Name and	d address of the actual manufacturing site		
Name and	d address of the Importer		
Name of s	supplier: Manufacturer/Importer/Port office of		
CDSCO/S	State licensing Authority		
Lot No / I	Batch No.:		
Product R	Reference No/ Catalogue No		
Type of A	Assay		
Kit comp	onents		
Manufact	turing Date		
Expiry Da	ate		
Pack size	(Number of tests per kit)		
Intended	Use		
Number o	of Tests Received		
Regulato	ory Approval:		
Import lic	cense / Manufacturing license/ Test license		
License N	Number: Issue date:		
Valid Up	to:		
Application	on No.		
Sample	Sample type		
Panel	Positive samples (provide details: strong, moderate, weak)		

reactivity panel)	Negative samples (p
• •	vity panel)

858

859

860 **Results:**

861

		Reference a (name)	issay	•••••
		Positive	Negative	Total
Name of malaria real time PCR kit	Positive			
	Negative			
	Total			

862

863

	Estimate (%)	95% CI
Sensitivity		
Specificity		

864

872

- Details of cross reactivity with other agents:
- 866 Conclusions:
- o Sensitivity, specificity
- 868 o Performance: Satisfactory / Not Satisfactory

869 (Sensitivity and specificity have been assessed in controlled lab setting on samples only, 870 using kits provided by the manufacturer from the batch mentioned above. Results should not

- 871 *be extrapolated for any other sample type.)*
 - **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

875 876	Note: This report is exclusively for Kit (Lot No) manufactured by (Supplied by)
877	
878	Evaluation Done on
879	Evaluation Done by
880	
881	Signature of Director/ Director-In-charge Seal
882	
883	**************************************
884	
885	
886	
887	
888	
889	
890	
891	
892	
893	
894	
895	
896	
897	
898	
899	
900	
901	
902	
903	
904	
905	

906 907	(detecting P vivax and P falciparum)
908	I. Background:
909 910 911 912	CDSCO/ICMR, New Delhi, have aimed to facilitate 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 IVD kit performance.
913	II. Purpose:
914 915 916	To evaluate the performance characteristics of Malaria RDT kits (detecting <i>P. vivax</i> and/or <i>P. falciparum</i>) in the diagnosis of Malaria parasite infection in individuals with unknown disease status.
917	III. Requirements:
918 919 920	1. Supply of kits under evaluation (with batch no. and lot no. Manufacturing and Expiry dates other required details). If the kit to be evaluated works in a closed system format, the manufacturer needs to supply the required equipment.
921	2. Evaluation sites/laboratories (With required equipment)
922	3. Reference test kits
923	4. Laboratory supplies
924	IV. <u>Ethical approval:</u>
925	The study will be initiated after approval from the institutional human ethics committee.
926 927 928 929 930 931 932	 V. Procedure: 1. Study design/type: Cross-sectional study 2. Preparation of Evaluation sites/laboratories: Identified IVD kit evaluation laboratories should establish their proficiency through A. Laboratory accreditation: Accreditation for at least one of the Quality management systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or CDSCO approved Reference laboratory.
933 934	It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in Quality Control exercises such as EQAP (External Quality Assurance Programme).
935	
936 937 938	BStaff training: All the staff involved in IVD kit evaluation should undergo hands on training and competency testing on the following at referral level malaria labs before initiation of MDTL activity:
939	> Preparation and characterization of evaluation panel for the respective IVD kit.
940 941	➤ Management of RDT kits (specific for <i>Plasmodium falciparum / Plasmodium vivax</i>) received for performance evaluation (Verification/Storage/Unpacking etc.).

- > Perform tests interpretation and documentation of results, and reporting.
- Data management and safety and confidentiality.

3. Sample size for performance evaluation:

Sample sizes of positive and negative samples against different values of sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate 5%. It is further assumed that at least 5% of the individuals attending the health care facilities for acute febrile illness and suspected for Malaria will be positive for Malaria (*P. vivax* and *P. falciparum*). Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity. Sample sizes are calculated using the following formulae and assumption of 5% for prevalence of the disease:

- $n_{se} \ge \frac{Z^2 \times S_e (1 S_e)}{d^2 \times (1 IR) \times P}$
- $n_{sp} \ge \frac{Z^2 \times S_p (1 S_p)}{d^2 \times (1 IR) \times P}$
- \cdot n (se) is the minimum number of individuals to be enrolled to obtain the requisite number of positive samples.
- \cdot *n (sp) is the minimum number of individuals to be enrolled to obtain the requisite number of negative samples.*
- \cdot Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
- Se is the predetermined sensitivity.
- · Sp is the predetermined specificity.
- d is the predetermined marginal error (5%)
- · IR is the invalid test rate
- *P is prevalence of the disease*

Sample size has to be calculated based on both the sensitivity and the specificity. The final sample size will be the maximum of the two. For example, at 95% sensitivity and

95% specificity, the sample size required will be 1600 (maximum of 1600 and 84). Please note that since the prevalence is low, the final sample size is generally expected to be governed by the assumed sensitivity.

983

984

980

981

982

Table 1. Sample sizes for different values of species-specific sensitivity being claimed

Sensitivity	Minimum no. of positive samples required (rounded figure) #	Minimum number of individuals to be enrolled in the study to obtain requisite number of positive samples
99%	20	400
95%	80	1600
90%	150	3000
85%	210	4200
80%	260	5200
75%	305	6100

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Samples will be collected from individuals attending the health care facilities (tertiary care centers and their linked hospitals, private clinics, field practice areas etc.) for acute febrile illness in highly endemic areas.

The disease status of these cases will be unknown.

985

986

Table 2. Sample sizes for different values of species-specific specificity being claimed

Specificity	No. of negative samples required (rounded figure)	Minimum number of individuals to be enrolled to obtain requisite number of negative samples
99%	20	21
95%	80	84
90%	150	158
85%	210	221
80%	260	274
75%	305	321

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Samples will be collected from individuals attending the health care facilities (tertiary care centers and their linked hospitals, private clinics, field practice areas etc.) for acute febrile illness in highly endemic areas.

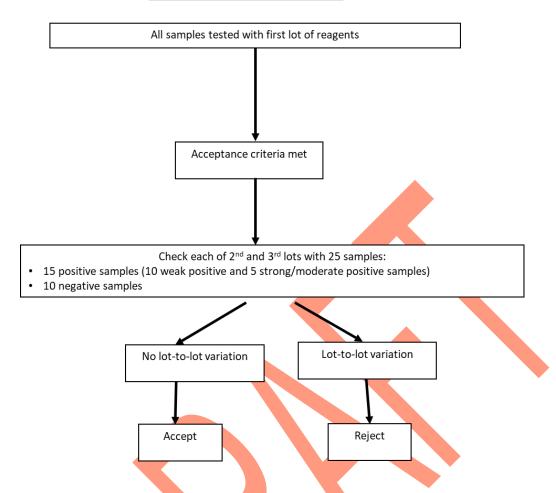
Since a large number of febrile cases have to be enrolled to obtain the requisite number of

988

989 990	malaria positive samples, enrolling the number of cases mentioned in Table 1 will be sufficient to obtain the requisite number of negative samples.
991	4. Inclusion criteria:
992	Individuals with the following clinical features may be enrolled in the study
993	Fever and any 2 of the following:
994	o Chills, sweating, headache, tiredness, nausea and vomiting, jaundice, splenomegaly
995	5. Exclusion criteria
996 997	 Individuals not satisfying inclusion criteria Individuals with already known positive history for other pathogens
998	6. Reference assay:
999 1000	WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved Malaria PCR assay/ Snounou protocol should be used as reference assay.
1001	7. Study implementation:
1002 1003 1004	The patients displaying Malaria like illness will be recruited into the study and five ml of whole blood will be collected in EDTA tubes. The whole blood sample will be subjected to the reference and the index test.
1005	The disease status of the enrolled cases will be unknown.
1006 1007 1008 1009 1010	8. Evaluation method: The index test and the reference tests should be run simultaneously on the sample panel, and results should be recorded.9. Interpretation of results:
1011	Reference test and index test results will be interpreted as per kit IFU.
1012	10. Positive samples:
1013	Samples positive by the reference assay will be considered as true positive samples.
1014	11. Negative samples:
1015	Samples negative by the reference assay will be considered as true negative samples.
1016 1017 1018 1019	False positive samples: These are samples negative by reference assay and positive by index test. False negative samples: These are samples positive by reference assay and negative by index test.
1020	
1021	A. Cross reactivity:

1022 1023	The RDT kit should have been evaluated against the following cross reactivity panel during the analytical performance evaluation:
1024	
1025	• Dengue NS1 positive samples (n=10 samples)
1026	 Chikungunya PCR positive samples (n=10 samples)
1027	 Healthy controls from endemic regions (n= 40 samples)
1028 1029	 Serum reactive for RA factor – low positive and high positive (n=15 samples)
1030	• Serum reactive for TPHA/other specific test for syphilis $(n=10 \text{ samples})$
1031	12. Statistical analysis:
1032	Sensitivity and specificity will be calculated.
1033 1034 1035	Interim analysis of data shall be conducted on completing evaluation of 25%, 50% and 75% of samples. If, at any point, the performance of the assay is found to be not satisfactory, the assay shall not be evaluated further. Evaluation fee shall be charged accordingly.
1036 1037	13. Test reproducibility A. Sample size for lot-to-lot reproducibility
1038	Three lots of an assay shall be evaluated. The first lot shall be evaluated on the entire panel
1039	of samples (statistically significant sample size). For the subsequent two lots, 25 samples
1040	should be used for evaluation (15 positive samples including 10 weak positive samples and
1041	5 moderate/strong positive samples, and 10 negative samples).
1042	Refer the flowchart below (Fig. 1):

Fig.1: Lot-to-lot reproducibility



B. Reader-to-reader reproducibility: 25 samples (15 positive samples including 10 weak positive samples and 5 strong/moderate positive samples, and 10 negative samples) need to be tested by at least 2 trained personnel. Agreement should be 100%.

14. Resolution of discrepant results:

True positive samples: These are samples positive by both reference assay and index test.

True negative samples: These are samples negative by both reference assay and index test.

False positive samples: These are samples negative by reference assay and positive by index test.

False negative samples: These are samples positive by reference assay and negative by index test.

15. Blinding of laboratory staff

To ensure rigor of the evaluation process, laboratory staff performing the evaluation should be blinded to the results of the reference assay. The PI of the evaluation exercise should remain unblinded, i.e., privy to the results of the reference test. Another senior laboratory staff selected by the PI may remain unblinded for overseeing the activity and

maintaining the database of results.. The data should be analyzed only by the PI of the evaluating lab.

1066

16. Acceptance criteria:

Expected sensitivity: $\geq 75\%$ for $P_{\underline{.}}$ vivax and $\geq 95\%$ for $P_{\underline{.}}$ falciparum Expected specificity: $\geq 90\%$ for $P_{\underline{.}}$ vivax and $\geq 95\%$ for $P_{\underline{.}}$ falciparum

1069 Cross-reactivity: Nil 1070 Invalid test rate: ≤5%

10711072

1073

1074

1075 1076

10771078

1067

1068

To achieve at least the performance characteristics outlined in the acceptance criteria for P vivax, ≥ 6100 individuals satisfying the case definition need to be enrolled to obtain the requisite number of positive samples. This sample size is sufficient for requisite number of negative samples.

To achieve at least the performance characteristics outlined in the acceptance criteria for P falciparum, ≥ 1600 individuals satisfying the case definition need to be enrolled to obtain the requisite number of positive samples. This sample size is sufficient for requisite number of negative samples.

1079 1080 1081

Recruitment should be terminated once the desired number of positive cases is enrolled and tested.

1082 1083 1084

17. 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.
- 1094 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/
 1095 different well-characterized sample panel at a different laboratory may be considered
 1096 only for kits which claim high performance characteristics (sensitivity and specificity
 1097 95% and above), but which fail the performance evaluation by a margin of 5%.

1098

1099

1100

1101

1106

1107

VI. References:

- 1. Ministry of Health and Family Welfare. Guidelines for Bivalent RDT. Available at: guidelines-for-bivalent-rdt.pdf (mohfw.gov.in)
- World Health Organization. Malaria Rapid Diagnostic Test Performance Results of WHO product testing of malaria RDTs: round 8 (2016–2018): Available at: https://iris.who.int/bitstream/handle/10665/276190/9789241514965- eng.pdf?sequence=1
 - 3. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested

1108 1109		polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315–20.
1110	4.	Integrated Disease Surveillance Project Training Manual For State & District
1111		Surveillance Officers - Case Definitions Of Diseases & Syndromes Under
1112		Surveillance (Module-5). Available at:
1113		https://idsp.mohfw.gov.in/WriteReadData/OldSite/2WkDSOSept08/Resources_fil
1114		es/DistrictSurvMan/Module5.pdf [Accessed on 25th June 2024]
1115	5.	CDC. National Notifiable Diseases Surveillance System (NNDSS). Malaria
1116		(Plasmodium spp.) 2014 Case Definition. Available at:
1117		https://ndc.services.cdc.gov/case-definitions/malaria-2014/ [Accessed on 28 th June,
1118		2024]
1119	6.	Kannambath R, Rajkumari N, Sivaradjy M. Prevalence of malaria: A 7-year trend
1120		analysis from a tertiary care center, Puducherry. Trop Parasitol. 2023 Jan-
1121		Jun;13(1):28-33. doi: 10.4103/tp.tp_41_22. Epub 2023 May 19. PMID: 37415756;
1122		PMCID: PMC10321582.
1123	VII.	Performance evaluation report format
1124		
1125		
1126		
1127		
1128		
1129		
1130		
1131		
11321133		
1134		
1135		
1136		
1137		
1138		
1139		
1140		
1141		
1142		
1143		

1144	REPORT FORMAT			
1145	Name of the Laboratory			
1146	Name of the Institute, (with station)			
1147	Certificate of Analysis			
1148 _	File No.:			
Name of	of the product (Brand /generic)			
Name an	and address of the legal manufacturer			
Name an	and address of the actual manufacturing site			
Name an	and address of the Importer			
Name of	of supplier: Manufacturer/Importer/Port office of			
CDSCO/	O/State licensing Authority			
Lot No /	o / Batch No.:			
Product I	t Reference No/ Catalogue No			
Type of A	f Assay			
Kit comp	mponents			
Manufac	acturing Date			
Expiry D	Date			
Pack size	ze (Number of tests per kit)			
Intended	ed Use			
Number	er of Tests Received			
Regulato	tory Approval:			
Import li	license / Manufacturing license/ Test license			
License l	e Number: Issue date:			
Valid Up	Jp to:			
Applicati	ation No.			
Sample	e Sample type			
Panel	Positive samples (provide details: strong, moderate, weak)			

Negative samples (provide details, including cross reactivity panel)

1149

1152

1151

Results:

		Reference assay		
		(name)		
		Positive	Negative	Total
Name of index malaria RDT	Positive			
	Negative			
	Total			

1153

1154

	Estimate (%)	95% CI
Sensitivity		
Specificity		

1155

- Details of cross reactivity with other agents:
- 1157 Conclusions:
- o Sensitivity, specificity
- o Performance: Satisfactory / Not Satisfactory
- (Sensitivity and specificity have been assessed in field/controlled lab setting on...... samples
- only, using kits provided by the manufacturer from the batch mentioned above. Results
- should not be extrapolated for any other sample type.)
- 1163 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

1166 1167	Note: This report is exclusively for
1168	
1169	Evaluation Done on
1170	Evaluation Done by
1171	
1172	Signature of Director/ Director-In-charge Seal
1173	
1174	**************************************
1175	
1176	
1177	
1178	
1179	
1180	
1181	
1182	
1183	
1184	
1185	
1186	
1187	
1188	
1189	
1190	
1191	
1192	
1193	
1194	
1195	
1106	

1197 1198	<u>Information on Operational and Test Performance Characteristics Required from</u> <u>Manufacturers for Malaria IVD</u>
1199	The manufacturer should provide the following details about the IVD:
1200	1. Instructions for Use
1201	2. Scope of the IVD: to diagnose Malaria (Pf and/or Pv)
1202	3. Intended Use Statement
1203	4. Principle of the assay
1204	5. Intended testing population (cases of acute febrile illness/suspected cases of Malaria)
1205	6. Intended user(laboratory professional and/or health care worker at point-of-care)
1206	7. Detailed test protocol
1207	8. Lot/batch No.
1208	9. Date of manufacture
1209	10. Date of Expiry
1210	11. Information on operational Characteristics
1211	i. Configuration of the kit/device
1212	ii. Requirement of any additional equipment, device
1213	iii. Requirement of any additional reagents
1214	iv. Operation conditions
1215	v. Storage and stability before and after opening
1216	vi. Internal control provided or not
1217	vii. Quality control and batch testing data
1218	viii. Biosafety aspects- waste disposal requirements
1219	10. Information on Test Performance Characteristics
1220	i. Type of sample-serum/plasma/whole blood/other specimen (specify)
1221	ii. Volume of sample
1222	iii. Sample rejection criteria (if any)
1223	iv. Any additional sample processing required
1224 1225	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required
1226	vi. Name of analyte to be detected
1227	vii. Pathogens targeted by the kit

1228	viii. Time taken for testing
1229	ix. Time for result reading and interpretation
1230	x. Manual or automated(equipment)reading
1231	xi. Limit of detection
1232	xii. Diagnostic sensitivity
1233	xiii. Diagnostic specificity
1234	xiv. Stability and reproducibility (including data)
1235	xv. Training required for testing (if any)
1236	xvi. If yes, duration
1237	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
1238	xviii. Details of cross reactivity, if any
1239	xix. Interpretation of invalid and indeterminate results to be provided
1240	xx. It is recommended to provide data demonstrating the precision
1241	xxi. Limit of detection
1242	
1243	*Please mention "Not applicable" against sections not pertaining to the kit.
1244	
1245	
1246	**************************************
1247	
1248	







STANDARD PERFORMANCE

DRAFT FOR STAKEHOLDER COMMENTS

EVALUATION PROTOCOL

NIPAH VIRUS REAL TIME PCR KIT

ICMR-CDSCO/IVD/GD/PROTOCOLS/08/2025



AUGUST, 2025 New Delhi, India

1

5

3

4

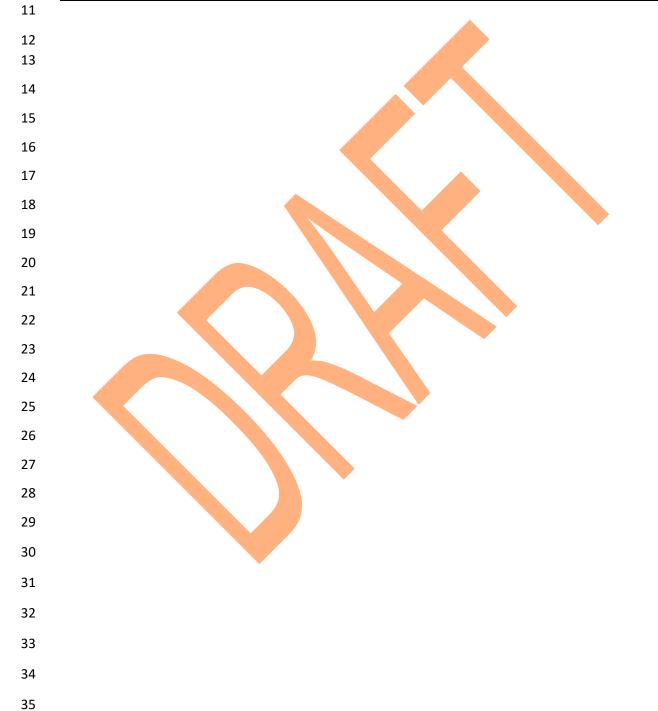
7

6

8

10 <u>Table of Contents</u>

S.N.	Topic	Page Number
1.	Performance evaluation protocol for Nipah virus real-time PCR kits	2
2.	Performance Evaluation Report Format	11
	Information on Operational and Test Performance Characteristics Required from Manufacturers	13



Performance evaluation protocol for Nipah virus real-time PCR kit

37 I. Background:

36

- 38 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured
- 39 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the
- 40 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
- 42 (IVD) performance.
- This recommendation focuses on the laboratory performance evaluation of Nipah virus real time
- 44 PCR kit. All clinical samples tested in the study should be evaluated in accordance with the
- 45 candidate test's instructions for use.

46 II. Purpose:

- To evaluate the performance characteristics of Nipah virus real-time PCR kits in the diagnosis of
- 48 Nipah virus infection/ disease using irreversibly de-identified leftover archived/ spiked clinical
- 49 samples.

50 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
- 53 the required equipment.
- 54 2. Evaluation sites/laboratories (With required equipment)
- 55 3. Reference test kits
- 56 4. Characterised Evaluation panel
- 5. Laboratory supplies

58 IV. Ethical approvals:

- Performance evaluation activities using irreversibly de-identified leftover clinical samples are
- exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory
- Validation Testing, 2024.
- Investigators are required to submit a self-declaration form, as outlined in the ICMR
- guidelines, to the institutional authorities and ethics committee for information.

64 V. <u>Procedure:</u>

- 1. Study design/type: Diagnostic accuracy study using spiked/clinical samples (human specimens).
- 2. Preparation of Evaluation sites/laboratories:

Identified IVD kit evaluation laboratories should be well-equipped and establish their proficiency through ALL of the following:

- 71 A. Availability of BSL-4 facility for handling of Nipah virus positive specimens
- B. Accreditation for at least one Quality management system for at least one respiratory viral pathogen molecular testing (accreditation for Testing Lab / Calibration Lab as per ISO/IES
- 74 17025, Medical Lab as per ISO 15189, PT provider as per ISO/IEC 17043), or CDSCO
- approved Reference laboratory.
- C. Staff training: All the staff involved in Nipah virus IVD evaluation should undergo hands on training and competency testing on following
- 78 ➤ BSL-4 practices

70

89

93

- 79 Nipah virus culture and handling
- Preparation & characterization of reference sample panel
- Handling of Nipah virus 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 Nipah virus RNA evaluation panel
- This is a zoonotic disease, and well characterised Nipah virus positive human samples is a critical
- 87 requirement for evaluation of RT-PCR IVD kits. A statistically significant number of clinical
- samples should be used for the evaluation.
 - 4. RNA extraction
- 90 RNA extraction should be performed as per manufacturer's instruction for reference assay as well
- as the assay under evaluation. If any extraction system is specified -in the IFU, that shall be used
- 92 for the test and shall be provided by the manufacturer.
 - 5. Real-Time PCR System
- 94 PCR shall be performed using IVD-approved machines. If any equipment(s) is specified in the
- 95 IFU, that shall be used for the test and shall be provided by the manufacturer.
- 96 Real-time closed systems/devices awaiting evaluation should be provided by the manufacturer
- 97 along with all necessary components, supplies and reagents.
- 98 6. Internal control/Extraction control
- 99 Assays must have an internal control (housekeeping gene), with or without an extraction control
- 100 (RNA added before extraction to a sample).

7. Reference assay:

- The Nipah virus Real Time PCR Assay developed by ICMR-NIV Pune, or a WHO Pre-Qualified/
- 103 US FDA/ ATAGI Australia/ PMDA Japan approved real time PCR assay should be used as the
- 104 Reference Standard.

101

107

113

116

117

122

126 127

128129

130

131

132

133

- 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. Sample sizes are calculated using the formulae:

114
$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

115
$$n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- 118 \cdot n (se) is the minimum number of positive samples.
- 119 n (sp) is the minimum number of negative samples.
- 120 Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
 - · Se is the predetermined sensitivity.
- Sp is the predetermined specificity.
- 124 · d is the predetermined marginal error (5%)
- 125 · IR is the invalid test rate

Nipah virus is detectable from throat swab, urine, CSF. The assay should be validated with positive clinical/spiked samples, and negative samples for all the formats claimed by the manufacturer. However, if a particular sample matrix is used to evaluate the assay (as opposed to all the sample types claimed by the manufacturer), the performance evaluation report should clearly mention the performance characteristics of the assay against the sample type used for validation. There should be no ambiguity about the sample type used for assay validation.

134 135 136 9. Sample panel composition: A. Human samples 137 **A.1 Positive samples (Minimum n=80 for each sample type):** Clinical/ Spiked samples 138 139 positive by the reference real-time PCR assay A.1.1 Strong positive (Ct value <25) = 24 samples 140 A.1.2. Moderate positive (Ct value between 25-30) = 28 samples 141 A.1.3 Weak positive (Ct value >30 to 34) = 28 samples 142 The sample type should be as per the index test IFU. If an assay claims to detect Nipah 143 virus RNA in several sample types, attempt should be made to use 80 positive samples 144 across each sample type, or at least the sample types available with the evaluating lab. This 145 relaxation is provided since clinical samples are scarce and obtained only during outbreaks 146 occurring every few years in India, which necessitates using spiked clinical samples. The 147 latter is difficult since Nipah virus is a BSL-4 level pathogen and its handling requires 148 sophisticated laboratory setup and trained manpower. 149 In case the requisite number of specimens for a particular sample type are not available and 150 a smaller number of samples are used for performance evaluation (i.e., sample size 151 calculated assuming higher performance characteristics), it is necessary to ensure that the 152 study has adequate power for acceptance of the evaluation results in case the assay falls 153 short of the assumed performance characteristics. 154 155 Note: If clinical samples positive for Nipah virus are not available, tissue culture fluid (Heat-inactivated) from reference 156 laboratories can be used, spiked in serum/urine/Throat swab samples to obtain the panel with Ct value <25, 25-30 and 157 158 >35 and tested by the reference assay, and the positive samples can be used for evaluation. 159 Confirmed negative samples would be used for spiking with Nipah virus.isolate. 160 161 A.2 Negative samples (number of samples will depend on sample type): All negative samples should be negative by reference real-time PCR assay. Distribution of the negative 162 163 samples should be as follows

Categories of		Sample type		
samples as per the sample type	NP/TS (Minimum n= 80)	Serum (Minimum n= 80)	Urine n=80)	(Minimum

A.2.1 Samples	Samples from individuals	Samples from cases of AES	5 positive clinical/
from cases having	presenting with ARI/ILI/SARI	(n=35):	spiked samples from
similar illness/	(n=45):	` ,	each of the following
spiked samples	(- 10)	5 positive clinical/ spiked	diseases, presenting
which are RT-	5 positive clinical/ spiked samples	samples from each of the	with respiratory
		_	1
PCR positive for	from each of the following	following diseases:	and/or encephalitis
common	diseases:		symptoms (n=20):
pathogens but		1. Japanese Encephalitis	
negative for	1. Influenza A virus @	@	1. Measles
Nipah virus	2. Influenza B virus @	2. Dengue @	Rubella
•	3. SARS-CoV-2 @	3. HSV @	3. Mumps
	4. RSV A/B @	4. VZV @	4. SARS-CoV-
	5. HPIV @	5. West Nile Virus *	2
	\cup		2
	\smile		
	7. Adenovirus @	7. Rabies virus *	
	8. Seasonal Coronaviruses *		
	9. Rhinovirus/Enterovirus*	Cross reactivity panel is	
		arranged in descending order of	
	Cross reactivity panel is arranged	priority.	
	in descending order of priority.	The pathogens marked @ are	
	The pathogens marked @ are	essentially to be tested.	
	essentially to be tested.	It is recommended to test for all	
	•	*	
	It is recommended to test for all	pathogens listed in the cross	
	pathogens listed in the cross	reactivity panel. However, if	
	reactivity panel. However, if there	there is an acute shortfall or	
	is an acute shortfall or non-	non-availability of clinical	
	availability of clinical samples,	samples, one may consider	
	one may consider reducing only	reducing only the pathogens of	
	the pathogens of lower priority	lower priority marked by *,	
	marked by *, while ensuring that	while ensuring that the actual	
	the actual numbers of cross	numbers of cross reactive	
		3	
	reactive sample panel remain the	sample panel remain the same	
	same by compensating with the	by compensating with the	
	available "essentially to be tested"	available "essentially to be	
	samples.	tested" samples.	
A.2.2 Samples	25	35	40
from cases with			
acute respiratory			
disease/ acute			
encephalitis/			
acute febrile			
illness and RT-	~		
PCR negative for			
the above-			
mentioned			
pathogens and			
Nipah virus			
-			

A.2.3 Healthy/ 10	10	20
symptomatic		
cases from		
endemic regions negative for		
negative for Nipah virus		
	collected from the same case may be used	for evaluation.
10. Evaluation method:		
The index test and the ref	ference tests should be run simultaneou	sly on the sample panel,
and results should be reco		J 1 1 /
11. Interpretation of results	:	
Reference test and index t	test results will be interpreted as per kit	IFU.
12. Resolution of discrepant		
True positive samples: Th	nese are samples positive by reference as	ssay and index test.
True negative samples: Tl	hese are samples negative by reference a	assay and index test.
False positive samples: T	These are samples negative by reference	ce assay and positive by
index test.		. 1
False negative samples:	These are samples positive by reference	e assay and negative by
index test.		
mach test.		
13. Test reproducibility		
A. Sample size for lot-to-lot	reproducibility	
Three lots of an assay shall b	e evaluated. Sample size for lot-to-lot r	eproducibility should be
as follows:	e evaluated. Sample Size for for to for f	eproductionity should be
	should be tested on statistically signifi	icant number of positive
	as calculated in the protocol.	cant number of positive
	ssay: should be tested on 25 sample	es (15 positive samples
	ositive AND 5 moderate/high positive s	

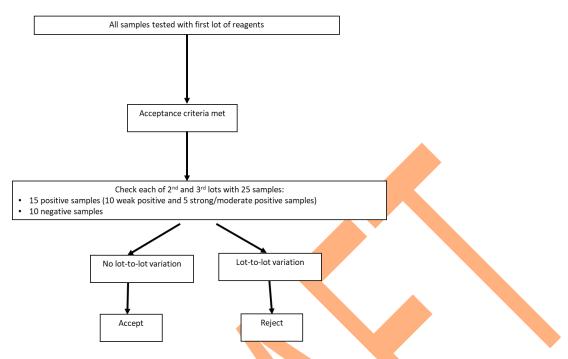
• Third lot of the assay: should be tested on 25 samples (15 positive samples comprising

10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

192 Refer the flowchart below (Fig. 1):

samples).

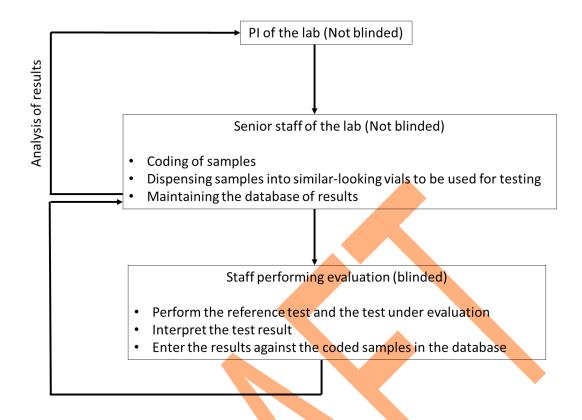
Fig.1: Sample size for Lot-to-lot reproducibility



14. 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



15. Acceptance Criteria

- 210 Expected sensitivity: ≥95%
- 211 Expected specificity: ≥98%
- 212 Cross reactivity with other viruses as outlined in the negative sample panel: Nil
- 213 Invalid test rate: ≤5%

207

208

209

214

215

217

16. Publication Rights:

- The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).
- 218 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
- 220 acceptable.
- Any request of re-validation from the same manufacturer for the same test type will only be
- entertained after a minimum of 3 months and only if a high-level technical summary of
- 223 modifications or functional improvements to the kit design is submitted, without explicit
- 224 disclosure of proprietary information.

Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different well-characterized sample panel at a different laboratory may be considered only for kits which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%.

VI. References:

- Yadav PD, Majumdar T, Gupta N, Kumar MA, Shete A, Pardeshi P, Sultana S, Sahay RR, Manoj MN, Patil S, Floura S, Gangakhedkar R, Mourya DT. Standardization & validation of Truenat[™] point-of-care test for rapid diagnosis of Nipah. Indian J Med Res. 2021 Apr;154(4):645-649. doi: 10.4103/ijmr.IJMR_4717_20. PMID: 34854433; PMCID: PMC9205002.
- 2. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification Diagnostic Assessment TGS-3. 2017. Available at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf:sequence=1

VII. **Performance evaluation report format**

257 <u>PERFORMANCE EVALUATION REPORT FOR NIPAH VIRUS REAL-TIME PCR</u> 258 <u>KITS</u>

Name o	f the product (Brand /generic)	
Name a	nd address of the legal manufacturer	
Name a	nd address of the actual manufacturing site	
Name a	nd address of the Importer	
Name o	f supplier: Manufacturer/Importer/Port office of	
CDSCC	/State licensing Authority	
Lot No	/ Batch No.:	
Product	Reference No/ Catalogue No	
Type of	Assay	
Kit com	ponents	
Manufa	cturing Date	
Expiry l	Date	
Pack siz	te (Number of tests per kit)	
Intended	i Use	
Number	of Tests Received	
Import	tory Approval: license / Manufacturing license/ Test license Number:Issue date: p to:	
	tion No.	
Sample	Sample type	
Panel	Positive samples (provide details: clinical/spiked, strong, moderate,	
	weak)	
	Negative samples (provide details (clinical/spiked,), including cross	
	reactivity panel)	
9		

260 Results

261

262

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

Estimate (%) 95% CI
Sensitivity
Specificity

• Details of cross reactivity with other Paramyxoviruses:

• Conclusions:

Page **11** of **15**

265 266 267 268	 Sensitivity, specificity Cross reactivity Invalid test rate Performance: Satisfactory / Not satisfactory
269 270	(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.)
271	<u>Disclaimers</u>
272 273	 This validation process does not approve / disapprove the kit design This validation process does not certify user friendliness of the kit / assay
274 275	Note: This report is exclusively for Nipah virus Kit (Lot No) manufactured by
276	Evaluation Done on
277	Evaluation Done by
278	Signature of Director/ Director-In-charge
279	**************************************
280	
281	
282	
283	
284	
285	
286	
287	
288	
289	
290	
291	
292	
293	
294	
295	

296	Annexure-1: Information on Operational and Test Performance Characteristics Required			
297	<u>from Manufacturers</u>			
298	The manufacturer should provide the following details about the IVD:			
299	1. Instructions for Use			
300	2. Scope of the IVD: to diagnose Nipah virus			
301	3. Intended Use Statement			
302	4. Principle of the assay			
303	5. Intended testing population (cases of AES/ARI/SARI)			
304	6. Intended user (laboratory professional and/or health care worker at point-of-care)			
305	7. Lot/batch No.			
306	8. Date of manufacture			
307	9. Date of Expiry			
308	10. Information on operational Characteristics			
309	i. Configuration of the kit/device			
310	ii. Requirement of any additional equipment, device			
311	iii. Requirement of any additional reagents			
312	iv. Operation conditions			
313	v. Storage and stability before and after opening			
314	vi. Internal control provided or not			
315	vii. Quality control and batch testing data			
316	viii. Biosafety aspects- waste disposal requirements			
317	11. Information on Test Performance Characteristics			
318	i. Type of sample- Nasopharyngeal swab/Throat swab/ CSF/Serum / Other specimen			
319	ii. Volume of sample			
320	iii. Any specific sample NOT to be tested			
321	iv. Any additional sample processing required			
322	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required			

323	vi. Name of analyte to be detected
324	vii. Pathogen(s) targeted by the kit
325	viii. Time taken for testing
326	ix. Time for result reading and interpretation
327	x. Manual or automated(equipment)reading
328	xi. Limit of detection
329	xii. Diagnostic sensitivity
330	xiii. Diagnostic specificity
331	xiv. Stability and reproducibility
332	xv. Training required for testing
333	xvi. If yes, duration
334	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
335	xviii. Interpretation of invalid and indeterminate results to be provided
336	xix. It is recommended to provide data demonstrating the precision
337	
338	*Please mention "Not applicable" against sections not pertaining to the kit.
339	
340	
341	**************************************



1

4

5

6

7

8





STANDARD PERFORMANCE
EVALUATION PROTOCOL

DRAFT FOR STAKEHOLDER COMMENTS

CHANDIPURA VIRUS REAL TIME PCR KIT

ICMR-CDSCO/IVD/GD/PROTOCOLS/07/2025



9 10

> AUGUST, 2025 New Delhi, India

11 <u>Table of Contents</u>

S.N.	Topic	Page Number
1.	Performance evaluation protocol for Chandipura virus real-time PCR kits	2
2.	Performance Evaluation Report Format	11
3.	Information on Operational and Test Performance Characteristics Required from Manufacturers	13

Performance evaluation protocol for Chandipura virus real-time PCR kits

38 <u>I. Background</u>

37

50

51

63

64

- 39 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured
- 40 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the
- 41 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
- 43 (IVD) performance.
- This recommendation focuses on the laboratory performance evaluation of Chandipura virus
- 45 (CHPV) 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.

47 **II. Purpose:**

- To evaluate the performance characteristics of CHPV real-time PCR kits in the diagnosis of CHPV
- 49 infection/ disease using irreversibly de-identified leftover archived/ spiked clinical samples.

III. Requirements:

- 1. **Kits Under Evaluation**: Include detailed information such as batch number, lot number, expiry
- date, and other relevant specifications. For kits designed to operate within a closed system,
- 54 manufacturers must provide the necessary equipment and consumables for testing.
- 2. Evaluation Sites/Laboratories: Identify laboratories equipped with the required instruments
- and infrastructure to conduct the evaluation.
- 3. **Reference Test Kits:** Use reference kits or in-house kits developed by the reference laboratory,
- 58 which have been validated to demonstrate satisfactory performance.
- 59 4. **Evaluation Panel**: Prepare a panel of well-characterised clinical samples from confirmed cases
- or spiked samples for a comprehensive evaluation.
- 5. **Laboratory Supplies**: Ensure all necessary laboratory materials and supplies are available for
- 62 the evaluation process.

IV. Ethical Approvals:

- Performance evaluation activities using irreversibly de-identified clinical samples are exempt from
- ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory Validation
- 67 Testing, 2024.
- 68 Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
- 69 to the institutional authorities and ethics committee for information.

70	
71	V. Procedure:
72 73	1. Study design/type: Diagnostic accuracy study using irreversibly de-identified archived clinical/spiked samples
74	2. Preparation of Evaluation sites/laboratories:
75	Identified IVD kit evaluation laboratories should establish their proficiency through the following:
76	A) Accreditation for at least one of the Quality management systems, such as
77	 Testing Laboratory or Calibration Laboratory (ISO/IEC 17025)
78	Medical Laboratory (ISO 15189)
79	 Proficiency Testing Provider (ISO/IEC 17043)
80	OR
81	CDSCO-approved reference laboratory
82 83	B) Staff training: All staff involved in IVD kit evaluation process should undergo hands on training and competency assessment in the following areas:
84 85 86 87 88	 Preparation and characterization of kit evaluation panel Handling of Chandipura real-time PCR kits received for performance evaluation (verification/storage/unpacking etc.). Testing procedures, interpretation and recording of results, and reporting Data handling, data safety & confidentiality
89	3. Preparation of Chandipura RNA evaluation panel:
90 91 92	A well characterised panel of CHPV positive clinical 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.
93 94 95 96 97 98	The sample type for CHPV detection is Cerebrospinal fluid (CSF) and serum. If a kit claims to detect CHPV in both sample types, attempt should be made to evaluate the assay across both serum and CSF using statistically significant sample size for each sample type. 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, ensuring statistical rigor. There should be no ambiguity about the type of sample used for evaluation.
99	4. RNA extraction:
100	RNA extraction should be performed as per manufacturer's instruction for reference assay as well

as the assay under evaluation. If the manufacturer of the index test recommends a specific RNA

extraction kit, it needs to be provided by the manufacturer if the evaluation lab is unable to procure 102 103 the same. 5. Real-time PCR system: 104 PCR should be performed using IVD-approved machines. If any equipment(s) is specified in the 105 IFU of the index test, it should be used for the evaluation, and it should be provided by the 106 manufacturer if not available within the lab's IVD evaluation scope. 107 Real-time closed systems/devices awaiting evaluation should be provided by the manufacturer 108 along with all necessary components, supplies and reagents. 109 6. Internal Control/Extraction Control: 110 The index test must have an internal control (housekeeping gene), with or without an extraction 111 control (RNA added before extraction to a sample). 112 7. Reference assay: 113 A WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved real time CHPV PCR 114 assay/ ICMR-National Institute of Virology, Pune developed protocol for detection of Chandipura 115 virus RNA will serve as the reference assay. 116 All positive samples should be confirmed positive by the reference assay. 117 All negative samples should be confirmed negative by the reference assay and CHPV IgM. 118 8. Sample size for performance evaluation: 119 1. Sample size is calculated assuming 95% sensitivity and specificity of the index test, 95% 120 121 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 for 122 each sample type are required for performance evaluation. Sample sizes are calculated using the 123 formulae: 124 125 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$ 126 $n_{sp} \geq \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2 \times \left(1 - IR\right)}$ 127 128 129 130 *n (se) is the minimum number of positive samples.* n (sp) is the minimum number of negative samples. 131 Z^2 is the critical value from the standard normal distribution corresponding 132

to the desired confidence level (95% CI corresponds to $\mathbb{Z}^2 = 1.96$).

Se is the predetermined sensitivity.
Sp is the predetermined specificity.
d is the predetermined marginal error (5%)
IR is the invalid test rate

139 9. Sample panel composition:

A) <u>Positive samples (Minimum n=80 for each sample type)</u>: These samples should be clinical/spiked samples positive by reference real-time PCR assay and preferably represent all genetic variants. The distribution of samples should be as follows:

Characteristic of positive	Minimum no, of serum	Minimum no. of CSF
sample	samples needed (for kits	samples needed (for kits
	detecting CHPV in serum)	detecting CHPV in CSF)
A.1 Strong positive [Ct value	24	24
≤ 25]		
A.2 Moderate positive [Ct	28	28
value between >25 and ≤ 31]		•
A.3 Weak positive [Ct value	28	28
$>31 \text{ and } \le 37$		

For kits detecting CHPV in both serum and CSF, 80 positive serum samples and 80 positive CSF samples should be used for performance evaluation. One sample type should not be substituted by the other to reach the desired sample size in case there is paucity of samples.

Note: Since such large number of positive clinical samples may NOT be available for Chandipura virus, pre-titrated and inactivated virus obtained from tissue culture fluid prepared in the laboratory will be used to spike serum and CSF samples [dilution factor: 1:10 to 1:1000 to generate samples with different intensities of positivity]. These spiked samples will be stored at -80°C, after being tested by the reference assay.

B) <u>Negative samples (n=80 for each sample type)</u>: All negative samples should be negative by reference assay and CHPV IgM. Distribution of the negative samples should be as follows:

Categories of samples as per the sample type	Sample type	
The management of the	Serum/plasma (Minimum n=80, (B.1 + B.2))	CSF (Minimum n=80, (B.1+B.2))

Page **5** of **15**

B.1 Samples from cases of AES/ spiked samples which are RT-PCR positive for known pathogens but negative for CHPV (CHPV RNA and serology)	5 positive clinical/ spiked samples from each of the following diseases (confirmed by PCR): 1. Dengue virus @	1. Seven (07) positive clinical/ spiked samples from each of the following diseases: a) Japanese Encephalitis @
	 2. Japanese Encephalitis @ 3. HSV 1/2 * 4. West Nile Virus* 5. VSV * 	 b) Dengue virus @ c) HSV 1/2 * d) West Nile Virus * 2. Rabies virus (n=4)* 3. VSV (n=3)*
B.2 Samples from cases with acute encephalitis and RT-PCR negative for the above-mentioned pathogens and CHPV (CHPV RNA and serology)	50	45
B.3 Healthy/ asymptomatic cases from endemic regions negative for CHPV (CHPV RNA and serology)	5 (desirable, not mandatory)	20 (desirable, not mandatory)

Serum/plasma and CSF samples collected from the same case may be used for evaluation.

Cross reactivity panel is arranged in descending order of priority.

The pathogens marked @ are essentially to be tested.

It is recommended to test for all pathogens listed in the cross-reactivity panel. However, if there is an acute shortfall or non-availability of clinical samples, one may consider reducing only the pathogens of lower priority marked by *, while ensuring that the actual numbers of cross-reactive sample panel remain the same by compensating with the available "essentially to be tested" samples.

Testing for Rabies and VSV is recommended since both the viruses belong to the same family as Chandipura virus (Rhabdoviridae). Spiked specimens/synthetic transcripts may be used for these viruses.

10. Evaluation method:

156

157

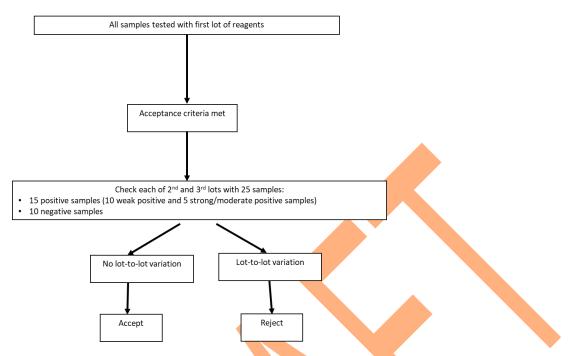
161

- 158 The index test and reference tests should be conducted simultaneously on the sample panel to
- minimize the risk of false-negative results from the index test due to freeze-thaw cycles or sample
- degradation from prolonged storage.

11. Interpretation of results:

Reference test and index test results will be interpreted as per kit IFU. 162 12. Resolution of discrepant results: 163 164 True positive samples: These are samples positive by both the reference assay and index test. True negative samples: These are samples negative by both the reference assay and index test. 165 False positive samples: These are samples negative by reference assay and positive by index test. 166 False negative samples: These are samples positive by reference assay and negative by index test. 167 168 169 13. Test reproducibility: 170 A) Sample size for lot-to-lot reproducibility: Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be as 171 follows: 172 First lot of the assay: should be tested on statistically significant number of positive and 173 negative samples as calculated in the protocol above 174 • Second lot of the assay: should be tested on 25 samples (15 positive samples comprising 175 10 low positives and 5 moderate/high positives, and 10 negative samples) 176 Third lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 177 low positives and 5 moderate/high positives, and 10 negative samples) 178 If there is no lot-to-lot variation, accept the assay. 179 180 Refer the flowchart below (Fig. 1):

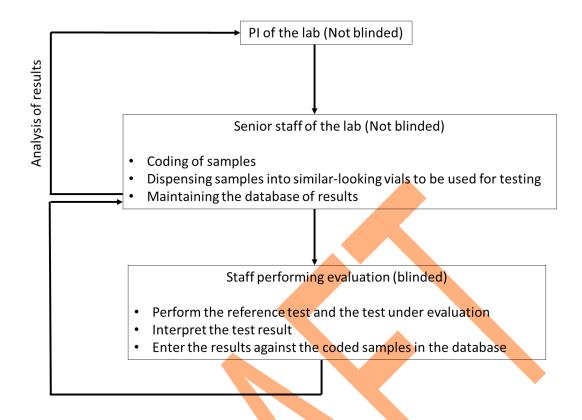
Fig.1: Sample size for Lot-to-lot reproducibility



14. 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



15. Acceptance criteria:

- 197 Expected sensitivity: $\geq 95\%$
- 198 Expected specificity: $\geq 98\%$
- 199 Cross-reactivity with other rhabdoviruses: Nil
- 200 Invalid test rate ≤5%

194

195

196

201

202

205

206

207

208

16. Publication Rights:

- The PI(s) of the evaluating labs shall retain publication rights of the field 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 after a minimum of 3 months and only if a high-level technical summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.

Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different 213 214 well-characterized sample panel at a different laboratory may be considered only for kits 215 which claim high performance characteristics (sensitivity and specificity 95% and above), but which fail the performance evaluation by a margin of 5%. 216 217 218 VI. References 1. Sudeep AB, Gurav YK, Bondre VP. Changing clinical scenario in Chandipura virus 219 infection. Indian J Med Res. 2016;143(6):712-721. doi:10.4103/0971-5916.191929. 220 2. Sapkal GN, Sawant PM, Mourya DT. Chandipura Viral Encephalitis: A Brief Review. Open 221 Virol J. 2018 Aug 31;12:44-51. doi: 10.2174/1874357901812010044. PMID: 30288194; PMCID: 222 PMC6142667. 223 3. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification-224 Diagnostic TGS-3. 2017. Available 225 assessment at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-226 eng.pdf;sequence=1 227 228 VII. Performance Evaluation Report Format 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243

244 <u>PERFORMANCE EVALUATION REPORT FOR CHANDIPURA VIRUS REAL-TIME</u> 245 <u>PCR KITS</u>

Name of	the product (Brand /generic)
Name an	d address of the legal manufacturer
Name an	d address of the actual manufacturing site
Name an	d address of the Importer
Name of	supplier: Manufacturer/Importer/Port office of
CDSCO	State licensing Authority
Lot No /	Batch No.:
Product 1	Reference No/ Catalogue No
Type of	Assay
Kit comp	ponents
Manufac	eturing Date
Expiry D	Date
Pack size	e (Number of tests per kit)
Intended	Use
Number of Tests Received	
Import	ory Approval: license / Manufacturing license/ Test license Number:Issue date:
Valid Up to:	
Applicat	
Sample	Positive samples (provide details: type, strong, moderate, weak)
Panel	Negative samples (provide details, type,including cross reactivity panel)
-6	

246247

Results

		Reference assay		name)
		Positive	Negative	Total
Name of	Positive			
Chandipura				
real-time PCR				
kits				
	Negative			
	Total			

248

	Estimate (%)	95% CI
Sensitivity		
Specificity		

249250

251

Conclusions:

Cross reactivity with related viruses:

o Invalid test rate:

253 o Performance: Satisfactory / Not satisfactory

254 255 256	(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.)
257	
258	<u>Disclaimers</u>
259 260	 This validation process does not approve / disapprove the kit design This validation process does not certify user friendliness of the kit / assay
261 262	Note: This report is exclusively for Chandipura Kit (Lot No) manufactured by (supplied by)
263	Evaluation Done on
264	Evaluation Done by
265	Signature of Director/ Director-In-charge Seal
266	**************************************
267	
268	
269	
270	
271	
272	
273	
274	
275	
276	
277	
278	
279	
280	
281	
282	
283	
284	

285	Annexure-1: Information on Operational and Test Performance Characteristics Required
286	<u>from Manufacturers</u>
287	The manufacturer should provide the following details about the IVD:
288	1. Instructions for Use
289	2. Scope of the IVD: to diagnose Chandipura virus
290	3. Intended Use Statement
291	4. Principle of the assay
292	5. Intended testing population (cases of Acute Febrile Illness/ AES)
293	6. Intended user (laboratory professional and/or health care worker at point-of-care)
294	7. Lot/batch No.
295	8. Date of manufacture
296	9. Date of Expiry
297	10. Information on operational Characteristics
298	i. Configuration of the kit/device
299	ii. Requirement of any additional equipment, device
300	iii. Requirement of any additional reagents
301	iv. Operation conditions
302	v. Storage and stability before and after opening
303	vi. Internal control provided or not
304	vii. Quality control and batch testing data
305	viii. Biosafety aspects- waste disposal requirements
306	11. Information on Test Performance Characteristics
307	i. Type of sample-CSF/Serum/Other specimen
308	ii. Volume of sample
309	iii. Any specific sample NOT to be tested
310	iv. Any additional sample processing required
311	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required

312	vi. Name of analyte to be detected
313	vii. Pathogen(s) targeted by the kit
314	viii. Time taken for testing
315	ix. Time for result reading and interpretation
316	x. Manual or automated (equipment) reading
317	xi. Limit of detection
318	xii. Diagnostic sensitivity
319	xiii. Diagnostic specificity
320	xiv. Stability and reproducibility
321	xv. Training required for testing
322	xvi. If yes, duration
323	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
324	xviii. Interpretation of invalid and indeterminate results to be provided
325	xix. It is recommended to provide data demonstrating the precision
326	xx. Limit of detection
327	
328	*Please mention "Not applicable" against sections not pertaining to the kit.
329	
330	**************************************







1

2

3

4

STANDARD PERFORMANCE EVALUATION PROTOCOL

DRAFT FOR STAKEHOLDER COMMENTS

5

6

7

8

9

MULTIPLEX RESPIRATORY VIRUS
REAL TIME PCR

ICMR-CDSCO/IVD/GD/PROTOCOLS/09/2025



10 11

> AUGUST, 2025 New Delhi, India

12 <u>Table of Contents</u>

S.N.	Topic	Page Number
1.	Performance evaluation protocol for Multiplex Respiratory Virus real-time PCR kits	2
2.	Performance Evaluation Report Format	13
3.	Information on Operational and Test Performance Characteristics Required from Manufacturers	15

Performance evaluation protocol for multiplex respiratory virus real-time PCR kit

I. <u>Background:</u>

38

39

47

50

- 40 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured
- 41 diagnostic kits appropriate for use in India. Hence the following guidelines shall establish the
- 42 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance
- evaluation is to independently verify the manufacturer's claim regarding IVD performance.
- This recommendation focuses on the laboratory performance evaluation of multiplex respiratory
- 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 multiplex respiratory virus real-time PCR kits using
- 49 irreversibly de-identified leftover archived clinical/spiked samples.

III. Scope of the document:

- 51 This document outlines performance evaluation protocol for multiplex real time PCR assays
- 52 detecting the following respiratory viruses of utmost importance in human clinical specimens
- 53 (Table 1), as determined by ICMR appointed working group and expert group of physicians and
- 54 clinical microbiologists following extensive literature review and real-life experience. This
- pathogen list has been developed as part of the National One Health Mission.
- 56 Table 1: List of respiratory viruses within the scope of this performance evaluation protocol
 - 1. Influenza virus A
 - 2. Influenza virus B
 - 3. SARS Coronavirus-2
 - 4. Respiratory syncytial virus
 - 5. Adenovirus
 - 6. Human Respiroviruses 1 and 3 and Human Rubulaviruses 2 and 4 (erstwhile Human Parainfluenzaviruses 1-4)
 - 7 11
 - 7. Human metapneumovirus
 - 8. Measles virus
 - 9. Rhinovirus
 - 10. Human Bocavirus
 - 11. Enterovirus
 - 12. Cytomegalovirus

57

58

IV. 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.
- 62 2. Evaluation sites/laboratories (With required equipment)
- 63 3. Reference test kits
- 4. Characterised Evaluation panel
- 5. Laboratory supplies

66 V. Ethical approvals:

- Performance evaluation activities using irreversibly de-identified leftover clinical samples are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for
- Laboratory Validation Testing, 2024.
- Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines, to the institutional authorities and ethics committee for information.

72 VI. Procedure:

- Study design/type: Diagnostic accuracy study using irreversibly de-identified archived/
 spiked clinical samples
- 75 **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 at least one of the Quality management systems for at least one respiratory viral pathogen molecular testing (accreditation for Testing Lab / Calibration Lab as per ISO 17025,
- Medical Lab as per ISO 15189, PT provider as per ISO/IEC 17043), or CDSCO approved
- 81 Reference laboratory.
- B. Staff training: All the staff involved in IVD evaluation should undergo hands-on training and competency testing on the following:
- Preparation & characterization of reference sample panel
- Handling of multiplex respiratory virus RT-PCR kits received for performance evaluation (Verification/Storage/Unpacking etc).
- 87 ➤ Testing
- Data handling, data safety & confidentiality
- 3. Preparation of multiplex respiratory virus evaluation panel
- 90 A well characterised panel of positive and negative clinical samples is a critical requirement for
- evaluation of these RT-PCR IVD kits. Also, a statistically significant number of clinical samples
- should be used for the evaluation.

- 93 The sample type for respiratory virus detection is usually nasopharyngeal/oropharyngeal swab. If
- a kit claims to detect these viruses across several sample types, attempt should be made to evaluate
- 95 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
- 98 evaluation.

99

105

111

4. Nucleic acid extraction

- Nucleic acid extraction should be performed using standard techniques. If the manufacturer of the
- index test recommends a specific nucleic acid extraction kit, it needs to be provided by the
- manufacturer if the evaluation lab is unable to procure the same.
- *Caution is advised in the selection of a nucleic acid extraction kit since the target pathogens comprise
- 104 both RNA and DNA viruses.

5. Real-Time PCR System

- PCR should be performed using IVD-approved machines. If any equipment(s) is specified in the
- 107 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 test under evaluation (index test) must have an internal control (housekeeping gene), with or
- without an extraction control (nucleic acid added before extraction to a sample).

7. Reference assay:

- The following points are to be noted:
- i.A WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved single plex (for a
- particular target pathogen) or multiplex real-time PCR assay/ ICMR-NIV Pune in-house single
- plex (for a particular target pathogen) or multiplex Real Time PCR Assay should be used as the
- reference assay.
- ii. Since the list of target pathogens is extensive, a combination of single plex and/or multiplex
- assays may be used as the reference assay(s), as long as these reference assays satisfy the criteria
- outlined in point 7(i).
- All samples positive for a particular pathogen should be confirmed positive by the reference assay.
- All samples negative for a particular pathogen should be confirmed negative by the reference
- assay.

8. Sample size for performance evaluation: The 2009 FDA guidance document "Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class II Special Controls Guidance for Industry and FDA Staff", recommends including a sufficient number of prospectively collected samples for each specimen type to generate a result with at least 90% sensitivity with a lower bound of the two-sided 95% confidence interval (CI) greater than 80, and demonstrate specificity with a lower bound of the two-sided 95% CI greater than 90%. In accordance with these guidelines and for feasibility of evaluation of these extensive multiplex panels, sample size for each pathogen is calculated assuming ≥90% sensitivity and specificity of the index test, 95% confidence level, absolute precision of 7.5%, and ≤5% invalid test rate. A minimum of 65 positive clinical samples (rounded to 70) and a minimum of 65 negative clinical samples for each target pathogen are required for performance evaluation of the assay. However, 120 negative samples are recommended per pathogen to account for an extensive cross reactivity panel. Sample sizes are calculated using the formulae:

$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

$$n_{sp} \ge \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- \cdot n (se) is the minimum number of positive samples.
 - · n (sp) is the minimum number of negative samples.
 - Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
 - · Se is the predetermined sensitivity.
 - · Sp is the predetermined specificity.
 - d is the predetermined marginal error (5%)
 - · IR is the invalid test rate
- The details of sample requirement are outlined in Table 2.

Table 2: No. of samples required for performance evaluation:

Pathogen	Minimum no. of positive samples needed per pathogen	Minimum no. of negative samples recommended per pathogen
1. Influenza virus A*	70	120
2. Influenza virus B*	70	120
3. SARS Coronavirus-2	70	120

4. Respiratory syncytial virus*	70	120
5. Adenovirus*	70	120
6. Human Respirovirus 1 and Human Respirovirus 3 and Human Rubulavirus 2 and Human Rubulavirus 4*	70	120
7. Human metapneumovirus *	70	120
8. Measles virus	70	120
9. Rhinovirus**	70	120
10. Human Bocavirus	70	120
11. Enterovirus**	70	120
12. Cytomegalovirus	70	120

*If a kit claims to differentiate between virus types/subtypes, please use minimum 70 positive samples and minimum 120 negative samples for each virus type/subtype. If such type/subtype specific samples are not available (only for predicate device) or if the kit does not claim to differentiate between pathogen types/subtypes, and the kit is evaluated against the pathogen as a whole, the reports should be issued with a disclaimer that performance characteristics against pathogen types/subtypes have not been evaluated separately. However, in such a scenario, the evaluating centre should try to include all types/subtypes of the pathogen in the evaluation panel (even if the numbers are not statistically significant for each pathogen type).

**If clinical samples positive separately for Rhinovirus/Enterovirus are not available (only for predicate device), or if the kit does not differentiate between Enteroviruses and Rhinoviruses, please use minimum 70 samples positive for Rhinovirus/Enterovirus in the positive sample panel and issue the reports with a disclaimer that performance characteristics against Rhinovirus/Enterovirus have not been evaluated separately.

Influenza virus, SARS Coronavirus 2, Respiratory Syncytial Virus and Human Metapneumovirus positive samples used for evaluation should have been collected within the past 1 year.

Notes for Table 2:

157

158

159

160

161

162

163164

165

166

167

168

169

- 1. Samples positive for currently circulating virus strains should be used in the positive sample panel, with representation from all virus types/subtypes.
- 2. Sample positive for a particular virus type and negative for the target pathogen being considered may be used in the negative sample panel for the target pathogen, e.g.: a sample positive for SARS-CoV-2 may be used as a negative sample for RSV.

9. Sample panel composition:

A. Human samples

A.1 Positive samples for each pathogen/ type or subtype of pathogen (Minimum n=70): Clinical samples positive by the reference real-time PCR assay should be included, as per the following criteria

170	A.1.1 Strong positive (Ct value <25) = 20 samples
171	A.1.2. Moderate positive (Ct value between $25-30$) = 25 samples
172	A.1.3 Weak positive (Ct value $>30-36$) = 25 samples
173 174 175 176	A.2 Negative samples for each pathogen/ type or subtype of pathogen (Minimum n=120): All negative samples should be negative for the target pathogen/ its type or subtype by the reference real-time PCR assay. Distribution of the negative samples should be as follows:
177 178	A.2.1 NP/OP swab from individuals with respiratory infection that are negative for the target pathogen/its type or subtype = 35 samples **
179 180	A.2.2 NP/OP swab from apparently healthy individuals with no respiratory symptoms = 23_samples **
181 182	A.2.3 Cross reactivity panel (Table 3): Samples negative for the target pathogen but positive for other common respiratory viruses = 62 samples ***
183 184	Archived frozen sample aliquots if used for the evaluation, should not be thawed more than once.
185 186 187	** If samples are available with the evaluating lab that satisfy these criteria and are negative for all the pathogens targeted by the kit, the same samples may be included in the negative sample panel for all target pathogens to prevent wastage of resources.
188 189 190 191 192	*** Same positive samples may be included in the cross-reactivity panel of several target pathogens to prevent wastage of resources e.g.: the same Influenza A virus positive sample may be included in the cross-reactivity panel for RSV, Human Metapneumovirus, SARS-CoV-2 etc. Table 3: Cross reactivity panel for performance evaluation of multiplex respiratory virus
193	real time PCR kit

				Vi	rus-wis	e no. of sa	mples neede	d for	cross rea	ctivity a	analysis				Total
Target Pathogen	Influe nza virus A *	Influe nza virus B*	SARS Coronav irus-2 *	Respira tory syncyti al virus	Adenov irus @	Human Respirovi ruses 1 and 3, Human Rubulavir uses 2 and 4#	Human metapneumov irus@	Meas les virus *	Rhinovir us @ \$	Huma n Bocav irus	Enterov irus \$	Cytomegalo virus.	Seasonal coronavir uses*	Rube lla	no. of cross reacti ve sampl es per patho gen
1. Influenza virus A	0	5	5	5	5	5	5	5	5	5	5	5	5	2	62
2. Influenza virus B	5	0	5	5	5	5	5	5	5	5	5	5	5	2	62
3. SARS Coronavirus-2	5	5	0	5	5	5	5	5	5	5	5	5	5	2	62
4. Respiratory syncytial virus	5	5	5	0	5	5	5	5	5	5	5	5	5	2	62
5. Adenovirus	5	5	5	5	0	5	5	5	5	5	5	5	5	2	62
6. Human Respi roviruses 1 and 3, Human Rubulaviruses 2 and 4	5	5	5	5	5	0	5	5	5	5	5	5	5	2	62
7. Human metapneumovirus	5	5	5	5	5	5	0	5	5	5	5	5	5	2	62
8. Measles virus	5	5	5	5	5	5	5	0	5	5	5	5	5	2	62
9. Rhinovirus	5	5	5	5	5	5	5	5	0	5	5	5	5	2	62
10. Human Bocavirus	5	5	5	5	5	5	5	5	5	0	5	5	5	2	62
11. Enterovirus	5	5	5	5	5	5	5	5	5	5	0	5	5	2	62
12. Cytomegalovirus	5	5	5	5	5	5	5	5	5	5	5	0	5	2	62

^{*}Include all currently circulating strains/types/subtypes

[@]It is desirable to have representation from all types of the pathogen, since even approved assays may not always differentiate between pathogen types.

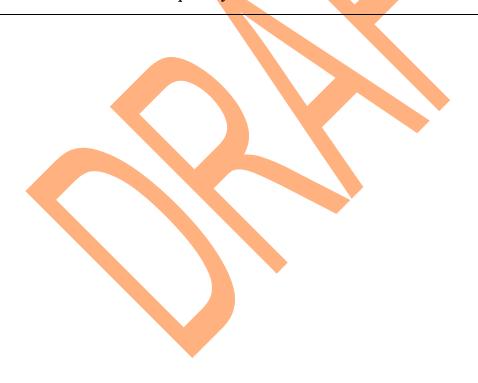
[#] Include at least 1 of each

- \$ If clinical samples positive separately for Rhinovirus/Enterovirus are not available, please use total 10 samples positive for Rhinovirus/Enterovirus in the cross-reactivity panel for remaining pathogens.
- Can use lower respiratory tract specimen

If a kit claims to differentiate between virus types/subtypes, please use 5 positive samples for each virus type in the cross reactivity panel for other target pathogens. If such type specific samples are not available and the kit is evaluated against the pathogen as a whole, it should be clearly mentioned in the report.

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.

Influenza virus, SARS Coronavirus 2, Respiratory Syncytial Virus and Human Metapneumovirus positive samples used for evaluation should have been collected within the past 1 year.



B. Contrived samples:

194

195

196

197

198 199

200201

202

203

204

205

206

207208

209210

211

212

213

219

220

221

222

223

224

225

226

227

228

229

230

Contrived positive and negative samples may be used for evaluation in case of paucity/unavailability of human clinical samples. Positive contrived samples should be positive and negative contrived samples should be negative for the target pathogen/type/subtype using the reference assay. The number and distribution of positive and negative samples, including the cross reactivity panel, should remain the same.

Contrived positive samples (as part of positive sample panel/ cross-reactivity panel) should be prepared by spiking a sample matrix negative for the pathogen with a pathogen-infected cell line, genomic DNA plasmids or RNA transcripts.

It is recommended to demonstrate equivalence between contrived and clinical specimens. Serial dilutions of clinical sample and serial dilutions of contrived sample with targeted levels of analyte should be compared for demonstrating equivalence.

10. Evaluation method:

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

11. Interpretation of results:

Reference test and index test results will be interpreted as per kit IFU.

12. Resolution of discrepant results:

- True positive samples: These are samples positive by reference assay and index test.
- True negative samples: These are samples negative by reference assay and index test.
- False positive samples: These are samples negative by reference assay and positive by index test.
- False negative samples: These are samples positive by reference assay and negative by index test.

13. 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 for each pathogen/type of pathogen as calculated in the protocol.
- Second lot of the assay: should be tested on 25 samples for each pathogen/type of pathogen (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 for each pathogen/type of pathogen (15 positive samples comprising 10 low positive AND 5 moderate/high positive samples, and 10 negative samples).
- There should be no lot-to-lot variability.

14. Blinding of laboratory staff

233

234235

236

237238

239

240

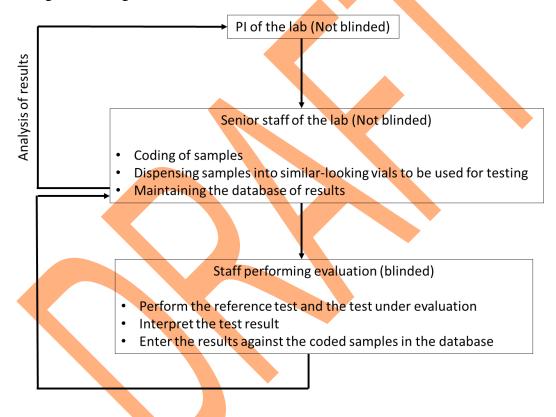
241

242243

244

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



15. Acceptance Criteria

- Expected sensitivity for each pathogen/type/subtype: ≥90%
- Expected specificity for each pathogen/type/subtype: ≥95%
- 251 Cross reactivity with other viruses as outlined in the negative sample panel: Nil
- 252 Invalid test rate: ≤5%

253

245246247

16. Publication Rights: 254 255 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s). 256 After following due procedure as defined in this document, once any kit is found to be Not 257 of Standard Quality, thereafter, no request for repeat testing of the same kit will be 258 acceptable. 259 260 Any request of re-validation from the same manufacturer for the same test type will only be entertained after a minimum of 3 months and only if a high-level technical summary of 261 modifications or functional improvements to the kit design is submitted, without explicit 262 disclosure of proprietary information. 263 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different 264 well-characterized sample panel at a different laboratory may be considered only for kits 265 which claim high performance characteristics (sensitivity and specificity 95% and above), 266 but which fail the performance evaluation by a margin of 5%. 267 268 VII. 269 **References:** 1. Food and Drug Administration. Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class 270 Special Controls Guidance for Industry and FDA Staff. Available 271 https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-272 emitting-products/respiratory-viral-panel-multiplex-nucleic-acid-assay-class-ii-special-273 controls-guidance-industry-and [Accessed on 22nd January, 2025]. 274 Food and Drug Administration. 510(k) Substantial Equivalence Determination Decision 275 2. Summary, Biofire Diagnostics LLC, FilmArray Pneumonia Panel. Available at: 276 https://www.accessdata.fda.gov/cdrh docs/reviews/K180966.pdf [Accessed on 19th January 277 2025] 278 279 Food and Drug Administration: Testing for Human Metapneumovirus (hMPV) Using Nucleic 3. 280 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-281 emitting-products/testing-human-metapneumovirus-hmpv-using-nucleic-acid-assays-class-ii-282 283 special-controls-guidance#3 [Accessed on January 11, 2025] 284 285 VIII. Performance evaluation report format 286 287 288 289

290 <u>PERFORMANCE EVALUATION REPORT FOR MULTIPLEX RESPIRATORY VIRUS</u> 291 <u>REAL-TIME PCR KITS</u>

Name of	f the product (Brand /generic)	
Name an	nd address of the legal manufacturer	
Name a	nd address of the actual manufacturing site	
Name an	nd address of the Importer	
Name of	f supplier: Manufacturer/Importer/Port office of	
CDSCO)/State licensing Authority	
Lot No	/ Batch No.:	
Product	Reference No/ Catalogue No	
Type of	Assay	
Kit com	ponents	
Manufa	cturing Date	
Expiry I	Date	
Pack siz	te (Number of tests per kit)	
Pathoge	ens detected by the assay	
Intended	d Use	
Number	of Tests Received	
Import	tory Approval: license / Manufacturing license/ Test license Number:Issue date:	
Valid U	p to:	
Applica	tion No.	
Sample	Sample type	
Panel	Positive samples (provide details: clinical/spiked, strong, moderate,	
	weak)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel)	
292		•

293 <u>Results</u>: Tables 1 and 2 should be made for each pathogen/type of pathogen targeted by the kit under evaluation

Table 1: 2x2 table for sensitivity and specificity calculation (prepare 1 table for each target pathogen/type/subtype)

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

295

296297

300 301	Table 2: Sens	itivity and specifi	city		
301			Estimate (%)	95% CI	7
		Sensitivity	Estimate (70)	7570 C1	-
		Specificity]
302	D 11 0				
303 304	Details of croConclusions:	•	other viruses in th	e cross-reactivity panel:	
305		ity, specificity			
306	Cross re				
307	 Invalid t 	•			
308	 Perform 	ance: Satisfactor	y / Not satisfactor	cy.	
309				setting using kits provided b	
310	the batch mentioned abo	ove using samp	ile. Results should no	ot be extrapolated to other san	nple types.)
311	<u>Disclaimers</u>				
312 313	 This validation p This validation p 	-		e the kit design ness of the kit / assay	
314	Note:				
315 316	This report is excluse (supplied		Metapneumovirus	Kit (Lot No) manufactured by
317	Evaluation Done on .				
318	Evaluation Done by .				
319				Seal	
320	*************	******	*End of the Repor	*** *************	*****
321					
322					
323					
324					
325					
326					
327					
328					
329					
330					
331					

332	Annexure-1: Information on Operational and Test Performance Characteristics Require
333	<u>from Manufacturers</u>
334	1. The manufacturer should provide the following details about the IVD:
335	2. Instructions for Use
336	3. Scope of the IVD:
337	4. Pathogens/type/subtype of pathogens targeted by the kit
338	5. Intended Use Statement
339	6. Principle of the assay
340	7. Intended testing population (cases of ARI/ILI/SARI)
341	8. Intended user (laboratory professional and/or health care worker at point-of-care)
342	9. Lot/batch No.
343	10. Date of manufacture
344	11. Date of Expiry
345	12. Information on operational Characteristics
346	i. Configuration of the kit/device
347	ii. Requirement of any additional equipment, device
348	iii. Requirement of any additional reagents
349	iv. Operation conditions
350	v. Storage and stability before and after opening
351	vi. Internal control provided or not
352	vii. Quality control and batch testing data
353	viii. Biosafety aspects- waste disposal requirements
354	11. Information on Test Performance Characteristics
355	i. Type of sample-NP/OP swab, other respiratory specimen
356	ii. Volume of sample
357	iii. Any specific sample NOT to be tested
358	iv. Any additional sample processing required
359	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required
360	vi. Name of analyte to be detected

361	vii. Pathogens targeted by the kit
362	viii. Time taken for testing
363	ix. Time for result reading and interpretation
364	x. Manual or automated(equipment)reading
365	xi. Limit of detection
366	xii. Diagnostic sensitivity
367	xiii. Diagnostic specificity
368	xiv. Stability and reproducibility
369	xv. Training required for testing
370	xvi. If yes, duration
371	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
372	xviii. Interpretation of invalid and indeterminate results to be provided
373	xix. It is recommended to provide data demonstrating the precision
374	xx. Limit of detection
375	*Please mention "Not applicable" against sections not pertaining to the kit.
376	
377	
378	**************************************







1

STANDARD PERFORMANCE EVALUATION PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

DENGUE IgG BASED ASSAYS

ICMR-CDSCO/IVD/GD/PROTOCOLS/10/2025

4

2

3



AUGUST, 2025 New Delhi, India

Dengue IgG Based Assays Performance Evaluation Protocols ICMR-CDSCO/IVD/GD/PROTOCOLS/10/2025

Table of Contents

S.No.	Content	Page Number
1.	Performance evaluation protocol for Dengue IgG RDT kits	2
2.	Performance evaluation protocol for Dengue IgM and IgG RDT combo kits	13
3.	Performance evaluation protocol for Dengue IgG ELISA kits	26
4.	Information on Operational and Test Performance Characteristics Required from Manufacturers	37



Dengue IgG Based Assays Performance Evaluation Protocols ICMR-CDSCO/IVD/GD/PROTOCOLS/10/2025

Performance evaluation protocol for Dengue IgG RDT kits

32 I. Background:

31

- 33 CDSCO/ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured
- 34 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the
- 35 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
- 37 (IVD) performance.

38 II. Purpose:

- To evaluate the performance characteristics of Dengue IgG RDT kits in the diagnosis of primary
- 40 and secondary dengue infections using irreversibly de-identified leftover archived clinical
- 41 samples.

42 III. Requirements:

- a) 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
- 45 the required equipment.
- b) Evaluation sites/laboratories (With required equipment)
- 47 c) Reference test kits
- d) Characterised Evaluation panel
- e) Laboratory supplies

50 IV. Ethical approvals:

- Performance evaluation activities using irreversibly de-identified leftover clinical samples are
- 52 exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory
- Validation Testing, 2024.
- Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
- 55 to the institutional authorities and ethics committee for information.

56 V. Procedure:

57

58

- **1. Study design/type:** Diagnostic accuracy study using irreversibly de-identified archived/spiked leftover clinical samples
- 2. Preparation of Evaluation sites/laboratories:
- Identified IVD kit evaluation laboratories should establish their proficiency through ALL of the following:
- A. Accreditation for at least one of the Quality management systems (accreditation for Testing
- Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or
- 64 CDSCO approved Reference laboratory.

- B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training and competency testing on following
- 67 ➤ Preparation & characterization of kit evaluation panel
- Handling of Dengue IgG Rapid IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).
- 70 Esting, interpreting, recording of results & reporting
- 71 Data handling, data safety & confidentiality

3. Preparation of Dengue IgG Rapid IVD kit evaluation panel

- Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance evaluation of IVD kits. Hence statistically significant number of sera samples should be collected from Dengue NS1/PCR/IgM confirmed cases. Further characterised for Dengue IgG positivity by using approved reference kits having high sensitivity and specificity.
- Dengue IgG performance evaluation panel need to be tested again by the reference assays at the time of evaluating a particular index test to confirm the positive and negative status of the samples.

4. Reference assay:

72

73

81

82

83

84

85

86 87

88

89

90

91

92 93 94

95

96

97

98

99

- Positive and negative samples should be characterized using composite reference standard of Dengue IgG AND one additional marker of Dengue (NS1 or IgM or PCR). The following kits should be used for characterization of the sample panel:
- Panbio Dengue IgG capture ELISA kit
 - WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue IgM ELISA kit
 - NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved NS1 ELISA kit
 - Serotype status to be assessed using a combination of CDC/NIV real-time PCR serotyping protocols.

5. Sample size for performance evaluation:

Sample sizes of positive and negative samples and sample panel composition against different values of sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance, an absolute precision of 5%, and invalid test rate \leq 5% using the following formulae:

$$n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

100	$n_{sp} \ge \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2 \times \left(1 - IR\right)}$
101	
102 103	\cdot n (se) is the minimum number of positive samples.
104	\cdot n (sp) is the minimum number of negative samples.
105 106	\cdot Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
107	· Se is the predetermined sensitivity.
108	· Sp is the predetermined specificity.
109	· d is the predetermined marginal error (5%)
110	· IR is the invalid test rate

Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity.

Positive samples: The panel of positive samples should include samples positive for IgG by the reference assay. The samples should also be positive for either dengue NS1 antigen or dengue IgM antibodies.

<u>Negative samples:</u> Samples which are negative by reference dengue IgG test should form the negative sample panel.

Table 1. Sample sizes and panel composition of positive dengue IgG samples for different values of sensitivity claimed by the manufacturer.

	Calculated	Minimum no. of	Sample Panel Composition
	sample size	Positive Samples	
Sensitivity		required	
		[Sample size rounded	
		off] #	
			Strong Positive: 6
99%	16	20	Moderate Positive: 7
			Weak Positive: 7

95%	77	80	Strong Positive: 24 Moderate Positive: 28 Weak Positive: 28
90%	145	150	Strong Positive: 44 Moderate Positive: 53 Weak Positive: 53
85%	206	210	Strong Positive: 62 Moderate Positive: 74 Weak Positive: 74
80%	258	260	Strong Positive: 78 Moderate Positive: 91 Weak Positive: 91

The samples need to be classified as strong, moderate and weak positives based on ELISA units of the reference assay.

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

128

129

130

131

Table 2. Sample sizes and panel composition of negative dengue IgG samples for different values of specificity claimed by the manufacturer.

	Calculated sample size	Minimu <mark>m n</mark> o. of Nega <mark>tive</mark> Samples	Sample Panel Composition
Specificity		required [Sample size rounded off] #	
99%#	16	20	 1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 7 2.Acute febrile illness cases: 8 Chikungunya positive samples:2 Dengue (NS1 & IgM & IgG & PCR) negative samples:6 3.Samples from other flavivirus disease cases (cross-reactive panel): 3 Japanese Encephalitis IgM/IgG positive: 1 @ West Nile Virus IgM/IgG positive: 1 * Zika Virus IgM/IgG positive: 1 * 4. ^aHealthy subjects from endemic regions: 2
95%	77	80	1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 27 2.Acute febrile illness cases: 32

	1		
			 Chikungunya positive samples:8 Dengue (NS1 & IgM & IgG & PCR) negative samples:24 3.Samples from other flavivirus disease cases(cross-reactive panel): 9 Japanese Encephalitis IgM/IgG positive: 3 @ West Nile Virus IgM/IgG positive: 3 * Zika Virus IgM/IgG positive: 3 * 4. aHealthy subjects from endemic regions: 12
90%	145	150	1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 50 2.Acute febrile illness cases: 60 • Chikungunya positive samples:15 • Dengue (NS1 & IgM & IgG & PCR) negative samples:45 3.Samples from other flavivirus disease cases(cross-reactive panel): 15 • Japanese Encephalitis IgM/IgG positive: 5 @ • West Nile Virus IgM/IgG positive: 5 * • Zika Virus IgM/IgG positive: 5 * 4. **AHEalthy subjects from endemic regions: 25
85%	206	210	 1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 70 2.Acute febrile illness cases: 84 Chikungunya positive samples:21 Dengue (NS1 & IgM & IgG & PCR) negative samples:63 3.Samples from other flavivirus disease cases(cross-reactive panel): 21 Japanese Encephalitis IgM/IgG positive: 7 @ West Nile Virus IgM/IgG positive: 7 * Zika Virus IgM/IgG positive: 7 * 4. ^aHealthy subjects from endemic regions: 35
80%	258	260	1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 85 2.Acute febrile illness cases: 104 • Chikungunya positive samples:26 • Dengue (NS1 & IgM & IgG & PCR) negative samples:78 3.Samples from other flavivirus disease cases(cross-reactive panel): 27 • Japanese Encephalitis IgM/IgG positive: 9 @ • West Nile Virus IgM/IgG positive: 9 * • Zika Virus IgM/IgG positive: 9 * 4. aHealthy subjects from endemic regions: 44

^a Samples from healthy subjects from endemic regions negative for all dengue markers (NS1, IgM, IgG, RNA)

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Cross reactivity panel is arranged in descending order of priority.

The pathogens marked @ are essentially to be tested.

It is recommended to test for all pathogens listed in the cross-reactivity panel. However, if there is an acute shortfall or non-availability of clinical samples, one may consider reducing only the pathogens of lower priority marked by *, while ensuring that the actual numbers of cross-reactive sample panel remain the same by compensating with the available "essentially to be tested" samples.

Note: If IgM/IgG positive samples for cross reactive flaviviruses are not available, commercially available IgM/IgG sera panel for different viruses can be procured and used to test cross reactivity.

132133

134

135

6. Evaluation method:

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

136137138

139

140

141

142

143

144

145

146

147

7. Interpretation of results:

Reference test and index test results will be interpreted as per kit IFU.

8. Resolution of discrepant results:

True positive samples: These are samples positive by reference assay and index test.

True negative samples: These are samples negative by reference assay and index test.

False positive samples: These are samples negative by reference assay and positive by index test.

False negative samples: These are samples positive by reference assay and negative by index test.

148

149

150

151

152153

154

9. Test reproducibility

A. Sample size for lot-to-lot reproducibility

Three lots of an assay shall 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.

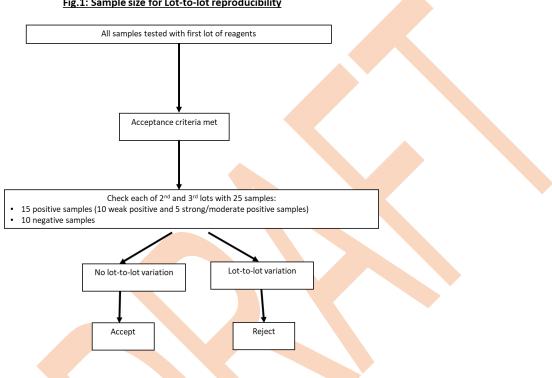
- 155 156 157
- Second lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 low positive AND 5 moderate/high positive samples, and 10 negative samples).
- 158 159
- Third lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

160

161

Refer the flowchart below (Fig. 1):

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



162 163

164

165

166

B. Sample size for reader-to-reader reproducibility

For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10 low positive AND 5 moderate/high positive samples, and 10 negative samples).

167 168 169

Two operators will be reading the test results independently as per manufacturer's instruction. Agreement should be 100% between the operators.

170 171

172

10. Blinding of laboratory staff

173 174

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

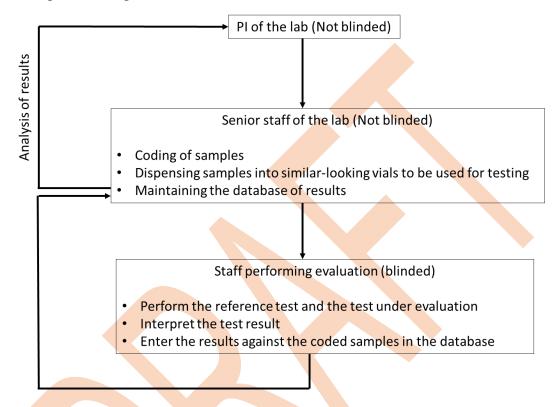
results. Staff performing the reference test and the test under evaluation, interpretation of the test result, and entering the results against the coded samples in the database, should remain blinded to the status of samples till the completion of evaluation. The data should be analyzed only by the PI of the evaluating lab. Refer to Fig. 2.

180 181 182

177

178179

Fig.2: Blinding in evaluation exercise



183

184 185

186

11. Acceptance Criteria

187 Expected sensitivity: ≥80%

188 Expected specificity: ≥90%

189 Cross reactivity: Nil

190 Invalid test rate: $\leq 5\%$

To achieve at least the performance characteristics outlined in the acceptance criteria, ≥260

positive samples and \geq 150 negative samples should be used for evaluation.

12. Publication Rights:

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

195

191

193

194

- 196 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
- 198 acceptable.
- Any request of re-validation from the same manufacturer for the same test type will only be
- 200 entertained after a minimum of 3 months and only if a high-level technical summary of
- 201 modifications or functional improvements to the kit design is submitted, without explicit
- 202 disclosure of proprietary information.
- 203 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different
- well-characterized sample panel at a different laboratory may be considered only for kits
- which claim high performance characteristics (sensitivity and specificity 95% and above),
- but which fail the performance evaluation by a margin of 5%.

208 VI. References:

207

- 2091. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G capture
- enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections. J Clin Virol. 2007
- 211 Jul;39(3):194-8. doi: 10.1016/j.jcv.2007.04.003...
- 212 2. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics
- evaluation series, 3). ISBN 978 92 4 159775 3.
- 214 3. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro Diagnostic
- 215 Medical Devices. 2018. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-
- 216 documents/medical device/guidanceperformanceivd.pdf
- 217 4. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently
- 218 Asked Questions. 2022. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-
- 219 documents/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf
- 220 5. U.S. Food and Drug Administration. Dengue Virus Serological Reagents Class II Special Controls
- 221 Guideline for Industry and Food and Drug Administration Staff. 2014. Available at:
- 222 https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-
- products/Dengue-virus-serological-reagents-class-ii-special-controls-guideline-industry-and-food-and-
- 224 drug
- 225 6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification Diagnostic
- 226 Assessment TGS-3. 2017. Available at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-
- 227 RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1
- *The validation protocols need to be revisited after introduction of Dengue vaccines and the
- 230 acceptance criteria needs revisiting every year so as to enable the availability of best
- 231 diagnostic kits.

233 VII. Performance evaluation report format

234

232

228

235

236 PERFORMANCE EVALUATION REPORT FOR DENGUE IGG RDT KIT

Name of	f the product (Brand /generic)	
Name ar	nd address of the legal manufacturer	
Name ar	nd address of the actual manufacturing site	
Name ar	nd address of the Importer	
Name of	f supplier: Manufacturer/Importer/Port office of	
CDSCO	/State licensing Authority	
Lot No /	/ Batch No.:	
Product	Reference No/ Catalogue No	
Type of	Assay	
Kit com	ponents	
Manufac	cturing Date	
Expiry I	Date	
Pack siz	te (Number of tests per kit)	
Intended	i Use	
Number	of Tests Received	
	tory Approval: icense / Manufacturing license/ Test license	
License	Number:Issue date:	
Valid U	p to:	
Applicat	tion No.	
Sample	Sample type	
Panel	Positive samples (provide details: strong, moderate, weak)	
	Negative samples (provide details: clinical/spiked, including cross	•
	reactivity panel)	

237

238 Results:

		Reference assay (name)		(name)
		Positive	Negative	Total
Name of	Positive			
Dengue IgG	Negative			
antibody -				
based RDT kit				
	Total			

239

	Estimate (%)	95% CI
Sensitivity		
Specificity		

240 Conclusions:

241 o Sensitivity, specificity

242 o Cross-reactivity:

243 o Invalid test rate:

244

245 o 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 (Supplied by) Evaluation Done on Evaluation Done by Signature of Director/ Director-In-charge Seal

276 <u>Performance evaluation protocol for Dengue IgM and IgG RDT combo kits</u>

277 I. Background:

- 278 CDSCO/ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured
- 279 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the
- 280 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
- 282 (IVD) performance.

283 II. Purpose:

- To evaluate the performance characteristics of Dengue IgM and IgG RDT combo kits in the
- 285 diagnosis of dengue and discriminating primary and secondary dengue infections using
- 286 irreversibly de-identified leftover archived clinical samples.

287 III. Requirements:

- 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.
- g) Evaluation sites/laboratories (With required equipment)
- 292 h) Reference test kits
- 293 i) Characterised Evaluation panel
- j) Laboratory supplies

295 IV. Ethical approvals:

- 296 Performance evaluation activities using irreversibly de-identified leftover clinical samples are
- 297 exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory
- 298 Validation Testing, 2024.
- Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
- to the institutional authorities and ethics committee for information.

301 V. Procedure:

302

303

304

- 1. Study design/type: Diagnostic accuracy study using irreversibly de-identified archived/spiked leftover clinical samples
- 2. Preparation of Evaluation sites/laboratories:
- Identified IVD kit evaluation laboratories should establish their proficiency through
 ALL of the following:
- A.Accreditation for at least one of the Quality management systems (accreditation for Testing
- Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or
- 309 CDSCO approved Reference laboratory.

B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training 310 311 and competency testing on following > Preparation & characterization of kit evaluation panel 312 > Handling of Dengue IgM and IgG Rapid IVD kits received for performance evaluation 313 (Verification/Storage/Unpacking etc). 314 > Testing, interpreting, recording of results & reporting 315 > Data handling, data safety & confidentiality 316 317 3. Preparation of Dengue IgM and IgG Rapid IVD kit evaluation panel 318 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance 319 evaluation of IVD kits. Hence statistically significant number of sera samples should be 320 collected from Dengue NS1/PCR/IgM confirmed cases, Further characterised for Dengue IgG 321 positivity by using approved reference kits having high sensitivity and specificity. 322 Dengue IgG performance evaluation panel need to be tested again by the reference assays at 323 the time of evaluating a particular index test to confirm the positive and negative status of the 324 samples. 325 4. Reference assay: 326 Positive and negative samples should be characterized using reference standard for Dengue 327 IgG (and one additional marker of Dengue - NS1 or IgM or PCR) AND IgM. The following 328 kits should be used for characterization of the sample panel: 329 • Panbio Dengue IgG capture ELISA kit 330 • WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue 331 IgM ELISA kit 332 • NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI 333 Australia/ PMDA Japan approved NS1 ELISA kit 334 Serotype status to be assessed using a combination of CDC/NIV real-time PCR 335 serotyping protocols. 336 Sample size and sample panel composition: Sample sizes of positive and negative samples 337 of Dengue against different values of sensitivity and specificity are provided in Tables 1 and 338 2. Sample sizes have been calculated assuming 95% level of significance, an absolute precision 339 of 5%, and invalid test rate \leq 5% using the following formulae: 340 341 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$ 342 $n_{sp} \geq \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2 \times \left(1 - IR\right)}$ 343

344

345

346

347

348 349

350

351

352

353 354

355 356 357

358 359 360

361 362

363 364

> 365 366

367 368

369

370 371 n (se) is the minimum number of positive samples.

n (sp) is the minimum number of negative samples.

 Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $\mathbb{Z}^2 = 1.96$).

- *Se is the predetermined sensitivity.*
- *Sp is the predetermined specificity.*
- d is the predetermined marginal error (5%)
- IR is the invalid test rate

Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity.

Positive samples: The samples should be positive for dengue IgM antibodies. The panel of positive samples should include 50% of samples positive for IgG by the reference assay. Samples should be representative of varying degrees of positivity:

Negative samples: These should include samples negative by all the reference assays (True negatives).

Table 1. Sample sizes and panel composition of positive Dengue samples for different values of sensitivity claimed by the manufacturer.

	Calculated	Minimum no. of	Sample Panel Composition
	sample	Positive Samples	
Consitivity	size	required	
Sensitivity		[Sample size rounded	
		off for balanced	
		allocation] #	
			1. 10 samples positive for Dengue
99%	16	20	IgM
	16	20	• Strong positive:3
			• Moderate positive: 3

			 Weak positive: 4 2. 10 samples positive for both Dengue IgM and IgG Strong positive IgG:3 Moderate positive IgG: 3 Weak positive IgG: 4
95%	77	80	40 samples positive for Dengue IgM Strong positive:12 Moderate positive: 14 Weak positive: 14 40 samples positive for both Dengue IgM and IgG Strong positive IgG:12 Moderate positive IgG: 14 Weak positive IgG: 14
90%	145	150	75 samples positive for Dengue IgM Strong positive: 23 Moderate positive: 26 Weak positive: 26 75 samples positive for both Dengue IgM and IgG Strong positive IgG: 23 Moderate positive IgG: 26 Weak positive IgG: 26
85%	206	210	105 samples positive for Dengue IgM • Strong positive:31 • Moderate positive: 37 • Weak positive: 37 105 samples positive for both Dengue IgM and IgG • Strong positive IgG: 31 • Moderate positive IgG: 37 • Weak positive IgG: 37
80%	258	260	130 samples positive for Dengue IgM • Strong positive:38 • Moderate positive: 46 • Weak positive: 46

130 sample	s positive for both
Dengue IgN	I and IgG
• Stro	ng positive IgG: 38
• Mod	lerate positive IgG: 46
• Wea	k positive IgG: 46

372

373

374

375

376

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Sample sizes and panel composition of negative Dengue samples for different values of specificity claimed by the manufacturer.

	Calculated	Minimum no.	Sample Panel Composition
	sample	of Negative	Sample I unei Composition
	•		
	size	Samples	
Specificity		required	
		[Sample size	
		rounded off	
		for <mark>bal</mark> anced	
		allocation] #	
			1. ^a Samples from acute febrile illness cases
			negative for dengue: 9
			• Samples positive for chikungunya: 2
			Other Acute febrile cases negative
			for Dengue (NS1 & IgM & IgG &
			PCR):7
			1 311)11
99%	16	20	2.Samples from other flavivirus disease
			cases (cross-reactive panel): 3
			Japanese Encephalitis IgM/IgG
			positive: 1@
			West Nile Virus IgM/IgG positive:1*
			• Zika Virus IgM/IgG positive: 1 *
			3. ^b Healthy subjects from endemic regions: 8
			1. ^a Samples from acute febrile illness cases
			negative for dengue: 44
			• Samples positive for chikungunya: 8
0.504	77	0.0	Other Acute febrile cases negative
95%	77	80	for Dengue (NS1 & IgM & IgG &
			PCR):36
			1 010,100
			2.Samples from other flavivirus disease
			cases (cross-reactive panel): 6
			cases (cross reactive paner). V

			 Japanese Encephalitis IgM/IgG positive: 2@ West Nile Virus IgM/IgG positive: 2* Zika Virus IgM/IgG positive: 2 * 3. bHealthy subjects from endemic regions: 30
90%	145	150	 1. Samples from acute febrile illness cases negative for dengue: 80 Samples positive for chikungunya: 15 Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):65 2. Samples from other flavivirus disease cases (cross-reactive panel): 15 Japanese Encephalitis IgM/IgG positive: 5 @ West Nile Virus IgM/IgG positive: 5* Zika Virus IgM/IgG positive: 5*
			3. bHealthy subjects from endemic regions: 55 1.aSamples from acute febrile illness cases negative for dengue: 110
85%	206	210	 Samples positive for chikungunya: Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):89 2.Samples from other flavivirus disease cases (cross-reactive panel): 24 Japanese Encephalitis IgM/IgG
			positive: 8 @ • West Nile Virus IgM/IgG positive: 8* • Zika Virus IgM/IgG positive: 8* 3. bHealthy subjects from endemic regions: 76
80%	258	260	1. Samples from acute febrile illness cases negative for dengue: 138

	 Samples positive for chikungunya: 26 Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):112
	 2.Samples from other flavivirus disease cases (cross-reactive panel): 27 Japanese Encephalitis IgM/IgG positive: 9 @ West Nile Virus IgM/IgG positive: 9* Zika Virus IgM/IgG positive: 9* 3. bHealthy subjects from endemic regions: 95

^a Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR)

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Cross reactivity panel is arranged in descending order of priority.

The pathogens marked @ are essentially to be tested.

It is recommended to test for all pathogens listed in the cross reactivity panel. However, if there is an acute shortfall or non-availability of clinical samples, one may consider reducing only the pathogens of lower priority marked by *, while ensuring that the actual numbers of cross reactive sample panel remain the same by compensating with the available "essentially to be tested" samples.

Note: If IgM/IgG positive samples for cross reactive flaviviruses are not available, commercially available IgM/IgG sera panel for different viruses can be procured and used to test cross reactivity.

5. Evaluation method:

377378379

380

381 382

383

384

385

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

6. Resolution of discrepant results:

True positive samples: These are samples positive by reference assay and index test. True negative samples: These are samples negative by reference assay and index test.

^b Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA)

False positive samples: These are samples negative by reference assay and positive by index test.

False negative samples: These are samples positive by reference assay and negative by index test.

7. Test reproducibility

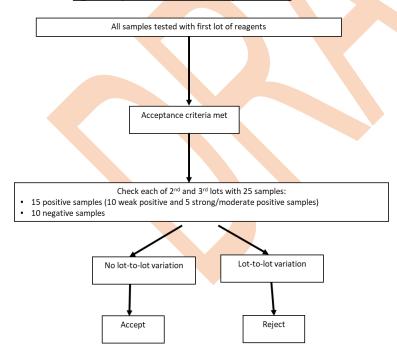
C. Sample size for lot-to-lot reproducibility

Three lots of an assay shall 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).

Refer the flowchart below (Fig. 1):

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



D. Sample size for reader-to-reader reproducibility

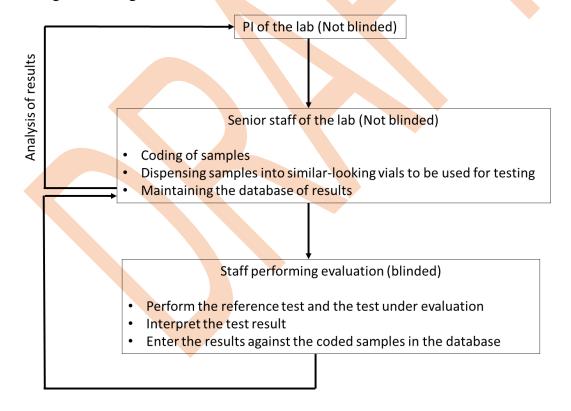
For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

Two operators will be reading the test results independently as per manufacturer's instruction. Agreement should be 100% between the operators.

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

8. Blinding of laboratory staff



9. Acceptance Criteria

Expected sensitivity for each analyte: ≥80%

429 Expected specificity for each analyte: ≥90%

- Cross-reactivity: Nil 430
- Invalid test rate: <5% 431
- To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 260 432
- positive samples and ≥ 150 negative samples should be used for evaluation. 433
- 10. Publication Rights: 434
- The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s). 435
- After following due procedure as defined in this document, once any kit is found to be Not 437
- 438 of Standard Quality, thereafter, no request for repeat testing of the same kit will be
- acceptable. 439

436

448

- Any request of re-validation from the same manufacturer for the same test type will only be 440
- entertained after a minimum of 3 months and only if a high-level technical summary of 441
- modifications or functional improvements to the kit design is submitted, without explicit 442
- disclosure of proprietary information. 443
- Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different 444
- well-characterized sample panel at a different laboratory may be considered only for kits 445
- which claim high performance characteristics (sensitivity and specificity 95% and above), 446
- but which fail the performance evaluation by a margin of 5%. 447

449 VI. References:

- 1. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G 450 capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections. J Clin Virol. 451 452 2007 Jul;39(3):194-8. doi: 10.1016/j.jcv.2007.04.003...
- 453 2. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3. 454
- 455 3. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro 456 Diagnostic Medical Devices. 2018. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO WEB/Pdf-documents/medical 457
- 458 device/guidanceperformanceivd.pdf
- 4. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently 459 460 Asked Questions. 2022. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdfdocuments/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf 461
- 462 5. U.S. Food and Drug Administration. Dengue Virus Serological Reagents - Class II Special Controls Guideline for Industry and Food and Drug Administration Staff. 2014. Available at:
- 463 https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-
- 464
- products/Dengue-virus-serological-reagents-class-ii-special-controls-guideline-industry-and-food-465
- 466 and-drug

6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment TGS-3. 2017. Available at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1

*The validation protocols need to be revisited after introduction of Dengue vaccines and the acceptance criteria needs revisiting every year so as to enable the availability of best diagnostic kits.

VII. Performance evaluation report format





PERFORMANCE EVALUATION REPORT FOR DENGUE IgM and IgG COMBO RDT 497 **KIT** 498

499

Name o	of the product (Brand /generic)	
Name a	and address of the legal manufacturer	
Name a	and address of the actual manufacturing site	
Name a	and address of the Importer	
Name o	of supplier: Manufacturer/Importer/Port office of	
CDSC	D/State licensing Authority	
Lot No	/ Batch No.:	
Produc	t Reference No/ Catalogue No	
Type of	f Assay	
	nponents	
Manufa	acturing Date	
Expiry	Date	
Pack si	ze (Number of tests per kit)	
Intende	d Use	
Numbe	r of Tests Received	
Regula	tory Approval:	Y .
Import	license / Manufacturing license/ Test license	
T:	Number:Issue date:	
License	e Number: Issue date:	
Valid U	Jp to:	
Applica	ation No.	
	Sample type	
Panel	Positive samples (provide details: strong, moderate, weak)	
	Negative samples (provide details:,clinical/spiked, including	
	cross reactivity panel)	
00		•

500

Results for IgM: 501

		Reference assay	•••••	. (name)
		Positive	Negative	Total
Name of Dengue antibody combo RDT kit	Positive			
	Negative			
	Total			

502

Results for IgG: 503

	Reference assay		. (name)
	Positive	Negative	Total

	ne of Dengue antibody abo RDT kit	Positive					
		Negative					_
		Total					_
504		1					_
505							
506	Details of cross res	activity with other	r flavivirus antibo	odies:			
507 508	• Invalid test rate:						
509	• Conclusions:						
510		pecificity for den					
511 512		pecificity for den	gue IgG:				
513		: Satisfactory / No	t satisfactory fo	r Dengue IgM			
514		Satisfactory / No					
515							
516 517	(Sensitivity and specificity the batch mentioned above				-		er from
518	<u>Disclaimers</u>						
519 520 521	1. This validation process 2. This validation process						
522	Note: This report is exclusive	ely for	Kit (Lot No) manufactured	by	(Supplied by)
523	Evaluation Done on						
524	Evaluation Done by						
525	Signature of Director/ Director/	ctor-In-charge		Seal			
526	*********	*********End	of the Report***	******	*****	*	
527							
528			·				
529							
530							
531							
532							
533							
534							
535							

536		Performance evaluation protocol for Dengue IgG ELISA kits			
537	I. <u>B</u> a	nckground:			
538 539 540 541 542	Diagn unifor evalua	CO/ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured ostics kits appropriate for use in India. Hence the following guidelines shall establish the mity in performance evaluation of in-vitro diagnostic kits (IVD). The performance ation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit performance.			
543	II. <u>P</u> u	irpose:			
544 545		aluate the performance characteristics of Dengue IgG ELISA kits in the diagnosis of Dengue on using irreversibly de-identified leftover archived clinical samples.			
546	III. <u>R</u> e	equirements:			
547 548 549	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.			
550	2.	Evaluation sites/laboratories (With required equipment)			
551	3.	Reference test kits			
552	4.	Characterised Evaluation panel			
553	5.	Laboratory supplies			
554	IV. Et	hical approval:			
555 556 557	exemp	mance evaluation activities using irreversibly de-identified leftover clinical samples are of from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory ation Testing, 2024.			
558 559		igators are required to submit a self-declaration form, as outlined in the ICMR guidelines, institutional authorities and ethics committee for information.			
560	V. <u>Pr</u>	rocedure:			
561 562 563		Study design/type: Diagnostic accuracy study using irreversibly de-identified archived/spiked leftover clinical samples Preparation of Evaluation sites/laboratories: Identified IVD kit evaluation leberatories should establish their preficiency through			
564 565		Identified IVD kit evaluation laboratories should establish their proficiency through ALL of the following:			
566 567		Accreditation for at least one of the Quality management systems (accreditation for Testing ab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or			
568	CDSCO approved Reference laboratory.				

B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training 569 570 and competency testing on following > Preparation & characterization of kit evaluation panel 571 > Handling of Dengue IgG ELISA IVD kits received for performance evaluation 572 (Verification/Storage/Unpacking etc). 573 > Testing, interpreting, recording of results & reporting 574 > Data handling, data safety & confidentiality 575 3. Preparation of Dengue IgG ELISA IVD kit evaluation panel 576 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance 577 evaluation of IVD kits. Hence statistically significant number of sera samples should be 578 collected from Dengue NS1/PCR/IgG confirmed cases. Further characterised for Dengue IgM 579 positivity by using approved reference kits having high sensitivity and specificity. 580 Dengue IgG performance evaluation panel need to be tested again by the reference assays at 581 the time of evaluating a particular index test to confirm the positive and negative status of the 582 samples. 583 4. Reference assay: 584 Positive and negative samples should be characterized using composite reference standard 585 of Dengue IgG AND one additional marker of Dengue (NS1 or IgM or PCR). The 586 following kits should be used for characterization of the sample panel: 587 588 • Panbio Dengue IgG capture ELISA kit • WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue 589 IgM ELISA kit 590 • NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI 591 Australia/ PMDA Japan approved NS1 ELISA kit 592 Serotype status to be assessed using a combination of CDC/NIV real-time PCR 593 serotyping protocols. 594 595 5. Sample size for performance evaluation: 596 Sample sizes of positive and negative samples and sample panel composition against different 597 values of sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been 598 calculated assuming 95% level of significance, and an absolute precision of 5% using the 599

601 $n_{se} \ge \frac{Z^2 \times S_e (1 - S_e)}{d^2}$

following formulae:

600

$$n_{sp} \geq \frac{Z^2 \times S_p \left(1 - S_p\right)}{d^2}$$

- \cdot *n (se) is the minimum number of positive samples.*
- \cdot \cdot n (sp) is the minimum number of negative samples.
 - Z^2 is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
 - · Se is the predetermined sensitivity.
- 611 Sp is the predetermined specificity.
- \cdot d is the predetermined marginal error (5%)

Appropriate sample size has to be chosen from the tables according to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the sample size outlined for 85% specificity.

<u>Positive samples:</u> The panel of positive samples should include samples positive for IgG by the reference assay. The samples should also be positive for either dengue NS1 antigen or dengue IgM antibodies. Samples should be representative of varying degrees of positivity:

<u>Negative samples:</u> These should include samples negative by the reference assays for dengue IgG.

Table 1. Sample sizes and panel composition of positive Dengue samples for different values of sensitivity claimed by the manufacturer.

	Calculated	Minimum no. of	Sample Panel Composition
	sample size	Positive Samples	
Sensitivity		required	
		[Sample size rounded	
		off] #	
			Strong Positive: 6
99%	15	20	Moderate Positive: 7
			Weak Positive: 7
050/	72	90	Strong Positive: 24
95%	73	80	Moderate Positive: 28

			Weak Positive: 28
			Strong Positive: 42
90%	138	140	Moderate Positive: 49
			Weak Positive: 49
			Strong Positive: 60
85%	196	200	Moderate Positive: 70
			Weak Positive: 70
			Strong Positive: 75
80%	246	250	Moderate Positive: 87
			Weak Positive: 88

631

632

633 634

635

636

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Table 2. Sample sizes and panel composition of negative Dengue samples for different values of specificity claimed by the manufacturer.

	Calculated	Minimum	Sample Panel Composition
			Sample I unei Composition
	sample 	no. of	
	size	Negative	
~		Samples	
Specificity		required	
		[Sample	
		size	
		rou <mark>nde</mark> d	
		off]#	
			1.Samples positive for dengue IgM/NS1/RNA
			but negative for IgG: 7
			2.Acute febrile illness cases: 8
			Chikungunya positive samples:2
			Dengue (NS1 & IgM & IgG & PCR)
	15		negative samples:6
99%		20	3. Samples from other flavivirus disease cases
7770			(cross-reactive panel): 3
			Japanese Encephalitis IgM/IgG
			positive: 1 @
			West Nile Virus IgM/IgG positive: 1 *
			• Zika Virus IgM/IgG positive: 1 *
			4. ^a Healthy subjects from endemic regions: 2
			1.Samples positive for dengue IgM/NS1/RNA
			but negative for IgG: 27
050/	73	80	2.Acute febrile illness cases: 32
95%	13	80	Chikungunya positive samples:8
			Dengue (NS1 & IgM & IgG & PCR)
			negative samples:24

			2 0 1 6 1 - 0 - 1 - 1 -
			3. Samples from other flavivirus disease
			cases(cross-reactive panel): 9
			Japanese Encephalitis IgM/IgG
			positive: 3 @
			 West Nile Virus IgM/IgG positive: 3 *
			• Zika Virus IgM/IgG positive: 3 *
			4. ^a Healthy subjects from endemic regions: 12
			1.Samples positive for dengue IgM/NS1/RNA
			but negative for IgG: 45
			2. Acute febrile illness cases: 60
			Chikungunya positive samples:15
			Dengue (NS1 & IgM & IgG & PCR)
			negative samples:45
90%	138	140	3. Samples from other flavivirus disease
7070	136	140	cases(cross-reactive panel): 15
		•	Japanese Encephalitis IgM/IgG
			positive: 5 @
			• West Nile Virus IgM/IgG positive: 5 *
			• Zika Virus IgM/IgG positive: 5 *
_			4. aHealthy subjects from endemic regions: 20
			1.Samples positive for dengue IgM/NS1/RNA
			but negative for IgG: 65
			2.Acute febrile illness cases: 84
			Chikungunya positive samples:21
			• Dengue (NS1 & IgM & IgG & PCR)
			negative samples:63
85%	196	200	3.Samples from other flavivirus disease
			cases(cross-reactive panel): 21
			 Japanese Encephalitis IgM/IgG
			positive: 7 @
			• West Nile Virus IgM/IgG positive: 7 *
			• Zika Virus IgM/IgG positive: 7 *
			4. ^a Healthy subjects from endemic regions: 30
			1.Samples positive for dengue IgM/NS1/RNA
			but negative for IgG: 80
			2. Acute febrile illness cases: 104
			Chikungunya positive samples:26
			Dengue (NS1 & IgM & IgG & PCR)
			negative samples:78
80%	246	250	3.Samples from other flavivirus disease
			_
			cases(cross-reactive panel): 27
			Japanese Encephalitis IgM/IgG Japanese Encephalitis IgM/IgG
			positive: 9 @
			West Nile Virus IgM/IgG positive: 9 *
			Zika Virus IgM/IgG positive: 9 *

4. ^aHealthy subjects from endemic regions: **39**

#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.

Cross reactivity panel is arranged in descending order of priority.

The pathogens marked @ are essentially to be tested.

It is recommended to test for all pathogens listed in the cross reactivity panel. However, if there is an acute shortfall or non-availability of clinical samples, one may consider reducing only the pathogens of lower priority marked by *, while ensuring that the actual numbers of cross reactive sample panel remain the same by compensating with the available "essentially to be tested" samples.

Note: If IgM/IgG positive samples for cross reactive flaviviruses are not available, commercially available IgM/IgG sera panel for different viruses can be procured and used to test cross reactivity.

637

638 639

640

641

6. Evaluation method:

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

642643644

645

646

647

648

649

650

651

652

7. Interpretation of results:

Reference test and index test results will be interpreted as per kit IFU.

8. Resolution of discrepant results:

True positive samples: These are samples positive by reference assay and index test.

True negative samples: These are samples negative by reference assay and index test.

False positive samples: These are samples negative by reference assay and positive by index test.

False negative samples: These are samples positive by reference assay and negative by index test.

653

654

655

656

657

9. Test reproducibility

A. Sample size for lot-to-lot reproducibility

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

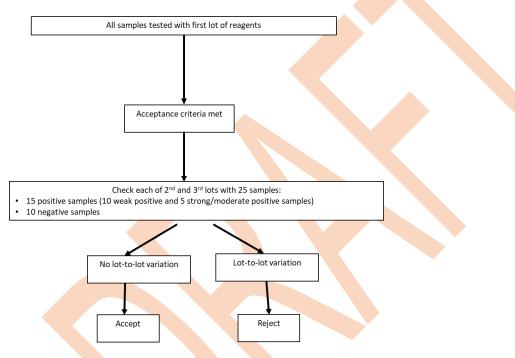
^a Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR)

^b Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA)

- 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).

Refer the flowchart below (Fig. 1):

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

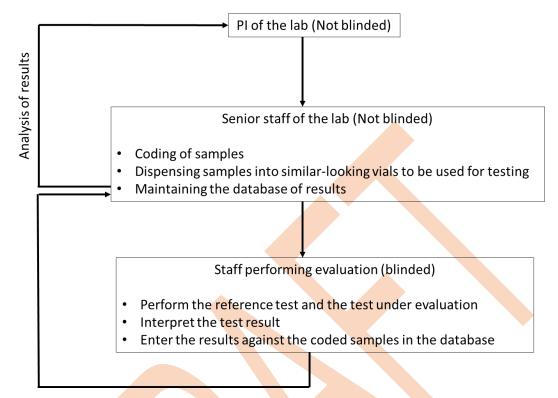


10. Blinding of laboratory staff

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.

681 682

Fig.2: Blinding in evaluation exercise



683

684

685

687

690

691

692

693 694

686 11. Acceptance criteria

Expected sensitivity: ≥90%

688 Expected specificity: ≥95%

689 Cross-reactivity: Nil

To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 140 positive samples and ≥ 80 negative samples should be used for evaluation.

12. Publication Rights:

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

695

696

697 698 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
- 700 entertained after a minimum of 3 months and only if a high-level technical summary of
- 701 modifications or functional improvements to the kit design is submitted, without explicit
- 702 disclosure of proprietary information.
- 703 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different
- well-characterized sample panel at a different laboratory may be considered only for kits
- which claim high performance characteristics (sensitivity and specificity 95% and above),
- but which fail the performance evaluation by a margin of 5%.

VI. References:

- 1. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections. J Clin Virol. 2007 Jul;39(3):194-8. doi: 10.1016/j.jcv.2007.04.003..
- 2. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.
- 3. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro Diagnostic Medical Devices. 2018. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical-device/guidanceperformanceivd.pdf
- 4. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently Asked Questions. 2022. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO WEB/Pdf-documents/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf
- U.S. Food and Drug Administration. Dengue Virus Serological Reagents Class II Special Controls Guideline for Industry and Food and Drug Administration Staff. 2014. Available at: https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/Dengue-virus-serological-reagents-class-ii-special-controls-guideline-industry-and-food-and-drug
- 6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification Diagnostic Assessment TGS-3. 2017. Available at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf; sequence=1

*The validation protocols need to be revisited after introduction of Dengue vaccines and the acceptance criteria needs revisiting every year so as to enable the availability of best diagnostic kits.

VII. Performance evaluation report format

739 PERFORMANCE EVALUATION REPORT FOR DENGUE IgG ELISA KIT

Mana	file and deat (Dear down down down down	
	f the product (Brand /generic)	
Name a	nd address of the legal manufacturer	
Name a	nd address of the actual manufacturing site	
Name a	nd address of the Importer	
Name o	f supplier: Manufacturer/Importer/Port office of	
CDSCC	D/State licensing Authority	
Lot No	/ Batch No.:	
Product	Reference No/ Catalogue No	
Type of	Assay	
Kit com	nponents	
Manufa	cturing Date	
Expiry	Date	
Pack siz	ze (Number of tests per kit)	
Intende	d Use	
Number	r of Tests Received	
	tory Approval: license / Manufacturing license/ Test license	
License	Number:Issue date:	
Valid U	Ip to:	
Applica	tion No.	
Sample	Sample type	
Panel	Positive samples (provide details: strong, moderate, weak)	
	Negative samples (provide details: clinical/spiked, including cross	
	reactivity panel)	
10		

740

741 Results:

		Reference assay (name)			
		Positive		Negative	Total
Name of Dengue IgG	Positive				
antibody -based	Negative				
ELISA kit					
	Total				

742

	Estimate (%)	95% CI
Sensitivity		
Specificity		

743 Conclusions:

744 o Sensitivity, specificity

745 o Cross-reactivity:

746 o Invalid test rate:

747 o 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.)

750	<u>Disclaimers</u>
751 752	 This validation process does not approve / disapprove the kit design This validation process does not certify user friendliness of the kit / assay
753 754	Note: This report is exclusively for
755	Evaluation Done on
756	Evaluation Done by
757	Signature of Director/ Director-In-charge
758	
759	**************************************
760	
761 762	
763	
764	
765	
766	
767	
768	
769	
770	
771	
772	
773	
774	
775	
776	
777	
778	
779	
780	

781 782	Information on Operational and Test Performance Characteristics Required from Manufacturers for Dengue IgG Based Assays
783	The manufacturer should provide the following details about the IVD:
784	1. Instructions for Use
785	2. Scope of the IVD: to diagnose Dengue
786	3. Intended Use Statement
787	4. Principle of the assay
788	5. Intended testing population(cases of acute febrile illness/suspected cases of Dengue)
789	6. Intended user(laboratory professional and/or health care worker at point-of-care)
790	7. Detailed test protocol
791	8. Lot/batch No.
792	9. Date of manufacture
793	10. Date of Expiry
794	11. Information on operational Characteristics
795	i. Configuration of the kit/device
796	ii. Requirement of any additional equipment, device
797	iii. Requirement of any additional reagents
798	iv. Operation conditions
799	v. Storage and stability before and after opening
800	vi. Internal control provided or not
801	vii. Quality control and batch testing data
802	viii. Biosafety aspects- waste disposal requirements
803	10. Information on Test Performance Characteristics
804	i. Type of sample-serum/plasma/whole blood/other specimen (specify)
805	ii. Volume of sample
806	iii. Sample rejection criteria (if any)
807	iv. Any additional sample processing required
808	v. Any additional device/consumable like sample transfer device, pipette, tube, etc required
809	vi. Name of analyte to be detected
810	vii. Pathogens targeted by the kit

811	viii. Time taken for testing
812	ix. Time for result reading and interpretation
813	x. Manual or automated(equipment)reading
814	xi. Limit of detection
815	xii. Diagnostic sensitivity
816	xiii. Diagnostic specificity
817	xiv. Stability and reproducibility (including data)
818	xv. Training required for testing (if any)
819	xvi. If yes, duration
820	xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
821	xviii. Details of cross reactivity, if any
822	xix. Interpretation of invalid and indeterminate results to be provided
823	xx. It is recommended to provide data demonstrating the precision
824	
825	*Please mention "Not applicable" against sections not pertaining to the kit.
826	
827	
828	**************************************