

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. *Performance evaluation protocol for Influenza virus molecular detection and/or differentiation assay (singleplex/multiplex format)*
2. *Performance evaluation protocol for SARS-CoV-2 molecular detection assay (singleplex/multiplex format)*
3. *Performance evaluation protocol for Respiratory Syncytial Virus molecular detection assay (singleplex/multiplex format)*
4. *Performance evaluation protocol for Influenza virus and SARS-CoV-2 molecular detection differentiation assay (multiplex format)*
5. *Performance evaluation protocol for Influenza virus, SARS-CoV-2 and RSV molecular detection 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. *Performance evaluation protocol for multiplex respiratory virus (expanded panel) 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

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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: _____



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STANDARD PERFORMANCE EVALUATION PROTOCOLS

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DRAFT FOR STAKEHOLDER COMMENTS

3

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

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

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AUGUST, 2025
New Delhi, India

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GENERAL GUIDELINES

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

41 1. Introduction:

42 This document provides a framework for evaluating the performance characteristics of *in vitro*
43 diagnostic (IVD) kits used in identifying and distinguishing various strains of Influenza viruses
44 and/or SARS-CoV-2 and/or RSV, aligning with international standards to ensure reliability and
45 accuracy in diagnosis. The coronavirus diseases 2019 (COVID-19) pandemic, caused by the
46 SARS-CoV-2 virus, has necessitated the rapid development and validation of in vitro molecular
47 diagnostic kits. These kits are crucial for the timely detection and differentiation of major
48 respiratory viruses (influenza/SARS-CoV-2/RSV) to control their spread. This protocol outlines a
49 systematic approach for validating these diagnostic kits to ensure their accuracy, sensitivity,
50 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,
53 making differential diagnosis for these viruses essential. Additionally, timely diagnosis of other
54 respiratory viruses, particularly Respiratory syncytial virus (RSV), is crucial for providing
55 effective clinical management to pediatric cases.

56 This document provides guidance for singleplex or multiplex assays for the differential diagnosis
57 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
60 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
62 multiple influenza virus types/subtypes; kits that identify only SARS-CoV-2, as well as kits that
63 only detect and/or differentiate RSV. Additionally, this protocol may be used for multiplex IVD
64 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
66 evaluation of IVD devices/kits as per the scope outlined in the document:

- 67 **1.1** The procedure for validating entities to determine operational parameters of IVD
68 devices/kits that detect influenza virus gene segment(s).
- 69 **1.2** The procedure for validating entities to determine operational parameters of IVD devices/kits
70 that detect SARS-CoV-2 gene segment(s).
- 71 **1.3** The procedure for validating entities to determine operational parameters of IVD
72 devices/kits that detect RSV gene segment(s).
- 73 **1.4** The techniques for identifying influenza virus/SARS-CoV-2/RSV nucleic acid targets in
74 single-plex or multiplex formats (using appropriate protocols listed in the document).

75 **1.5** This document is not useful for performance evaluation of serological assays for detection
76 of antigen and antibody for influenza viruses/SARS-CoV-2/RSV. The IVD device/kit to be
77 validated is henceforth known as the “Kit under Evaluation.”

78 **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
81 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-
83 reactivity, repeatability, and reproducibility as compared against a reference assay using clinical
84 sample panel.

85 In brief, the objectives are as follows:

- 86 **2.1** To validate the performance characteristics of in vitro molecular diagnostic kits for
87 detecting Influenza A & B (with/without subtyping)/ SARS-CoV-2/ RSV.
- 88 **2.2** To ensure the kits under evaluation meet the necessary standards for sensitivity,
89 specificity, repeatability, and reproducibility.
- 90 **2.3** To evaluate the cross-reactivity of the kits with other respiratory viruses.

91 **3. Scope:**

92 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
96 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:

- 98 **3.1** The analytical assay performance characteristics with clinical specimens for the detection
99 and/or differentiation of influenza viruses. (Protocol A)
- 100 **3.2** The analytical assay performance characteristics with clinical specimens for the detection
101 of SARS-CoV-2 (Protocol B)
- 102 **3.3** The analytical assay performance characteristics with clinical specimens for the detection
103 of RSV (Protocol C)
- 104 **3.4** The analytical performance characteristics of multiplex assay for detection of two or more
105 of these viruses by combining Protocols A, B & C as per the kit format.
- 106 **3.5** Analytical performance characteristics which should include sensitivity, specificity, cross-
107 reactivity, and lot-to-lot variation including functionality of devices that identify and/or
108 differentiate influenza viruses, SARS-CoV-2 and/or RSV depending on the kit format.
- 109 **3.6** The performance of the kit, only if the kit includes an internal control (**preferably**
110 endogenous, or exogenous).

111 **3.7** This document may also apply to forthcoming influenza, SARS-CoV-2 and RSV molecular
112 diagnostic devices that do not fit within these current classifications.

113 **3.8** The document will serve as a reference for assessing kits based on Nucleic Acid
114 Amplification Test (single plex or multiplex assays) as listed below:

115

116 **3.8.1** Real-time Reverse Transcription Polymerase Chain Reaction format (rRT-PCR):
117 including Real-time PCR probe-based assays or non-probe based assays

118 **3.8.2** Other NAT testing platforms such as LAMP/RPA, and other closed system platforms
119 such as TrueNat /cartridge-based assays

120

121 ***Note:** This protocol is not suitable for the kits where amplicons are handled outside the*
122 *amplification system.*

123 **4. Requirements:**

124 **4.1** Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If
125 the kit to be evaluated works in a closed system format, the manufacturer needs to supply
126 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 **4.5** Laboratory supplies

131 **5. Ethical approvals:**

133 Laboratory validation of IVDs using irreversibly de-identified samples is exempted from ethics
134 approval as per ICMR's Guidance on Ethical Requirements for Laboratory Validation Testing,
135 2024. A self-declaration form as provided in ICMR guidelines to be submitted by the
136 investigators to the institutional authorities and ethics committee for information
137 ([https://ethics.ncdirindia.org/asset/pdf/Guidance on Ethical Requirements for Laboratory Validation Testing.pdf](https://ethics.ncdirindia.org/asset/pdf/Guidance%20on%20Ethical%20Requirements%20for%20Laboratory%20Validation%20Testing.pdf))

139 **6. Procedure:**

140 **6.1 Study design/type:** Diagnostic accuracy study using leftover irreversibly de-identified
141 archived clinical samples.

142 **6.2 Evaluation site/laboratory considerations:** Identified IVD kit evaluation laboratories should
143 establish their proficiency through

144 **6.2.1** Accreditation for at least one of the Quality management systems (accreditation for
145 Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO 15189), PT provider (ISO:
146 17043) or CDSCO approved Reference laboratory.

147 **6.2.2** Have sufficient numbers of archived as well as contemporary clinical specimens positive
148 for respiratory viruses targeted by the kit under evaluation (Influenza A(H1N1)pdm09,
149 A(H3N2), B(Yamagata), B(Victoria), and/or SARS-CoV-2 and/or RSV A & B), with aliquots
150 stored at -80 °C deep freezers or in lyophilized form.

151 **6.2.3** Virus strains should be well-characterized by ICMR approved or US FDA/ ATAGI
152 Australia/PMDA Japan approved/WHO Pre-Qualified reference assay and/or by influenza
153 virus HA gene/segment or gene-specific sequencing (for SARS-CoV-2 and RSV) or Next-
154 Generation Sequencing.

155 **6.2.4** Have a minimum BSL-2 level facility with trained manpower and at least two different
156 Real Time platforms to perform molecular diagnostic assays for Influenza virus and other
157 respiratory viruses.

158 **6.2.5** Have a good record of External Quality Assurance programs for influenza, SARS-CoV-
159 2, and other respiratory viruses.

160 **6.2.6** Staff training: All the staff involved in IVD kit evaluation should undergo hands-on
161 training and competency testing on the following:

162 **6.2.6.1** Preparation & characterization of kit evaluation panel

163 **6.2.6.2** Handling of respiratory virus PCR kits received for performance evaluation
164 (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

168 **6.3 Performance characteristics:** To be assessed for all assay targets of influenza A/B,
169 SARS-CoV-2 and RSV (single plex or multi-plex assays)

170 **6.3.1** Analytical Sensitivity and specificity

171 **6.3.2** Cross-reactivity

172 **6.4.3** Repeatability

173 **6.4.4** Reproducibility

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Protocol A

Evaluation of performance characteristics of Molecular Kit detecting influenza A & B viruses, and subtyping into A (H1N1) pdm 09, A(H3N2), B(Yamagata) & B(Victoria) in single plex or multiplex format

1. Objective:

- 1.1** To evaluate the performance of molecular IVD device /KIT for detection and differentiation of Influenza viruses as per the scope outlined in this document.
- 1.2** To ensure the kits under evaluation meet the necessary standards for sensitivity, specificity, repeatability, and reproducibility.
- 1.3** To evaluate the cross-reactivity of the kits with other respiratory viruses.

190

2. Evaluation of performance characteristics should be done for the following parameters:

- 2.1** Sensitivity and specificity
- 2.2** Cross-reactivity
- 2.3** Repeatability
- 2.4** Reproducibility

195

3. Panel development: Clinical sample (archived/contemporary) panel for testing:

- 3.1** Contemporary leftover irreversibly de-identified clinical/archived respiratory samples (in VTM) for the panel should be irreversibly de-identified.
- 3.2** Samples to be used for panel preparation shall be stored properly at – 80 °C or lyophilized.
- 3.3** Unless the manufacturer has specific requirement of nucleic acid extraction kit, the validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved/ICMR validated total RNA / viral RNA extraction kits for the evaluation.
- 3.4** Clinical samples for evaluation should be characterized by a reference kit / Sequencing/NGS.
- 3.5** All positive samples should be confirmed positive for the target pathogens by the reference assay.
- 3.6** All negative samples should be confirmed negative for the target pathogens by the reference assay.

212

213

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

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

228

$$229 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

230

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



232

- 233 · *n (se) is the number of positive samples.*

234 · *n (sp) is the number of negative samples.*

235 · *Z² is the critical value from the standard normal distribution corresponding*
236 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*

237 · *Se is the predetermined sensitivity.*

238 · *Sp is the predetermined specificity.*

239 · *d is the predetermined marginal error (5%)*

240 · *IR is the invalid test rate*

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

243

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

<i>Sensitivity/ Specificity</i>	<i>Sample size: Minimum number of positive samples[¥]</i>	<i>Composition of positive samples[#]</i>	<i>Sample size: Minimum number of negative samples (rounded)[¥]</i>	<i>Minimum number of cross reactive* samples among the negative samples</i>
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 and \leq 34)

[¥] 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.

246 *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.*

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

	Sample size* (per target pathogen)			Minimum total number of positive samples (rounded figures)
	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

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.

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

253 **4.1** Repeatability testing will be performed on 3 positive (strong, moderate and weak
254 positive) and 3 Negative samples (within the selected positive and negative samples) per
255 target pathogen 5 times (replicates of 5).

256

257 **5. Methodology:**

258 **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference
259 assay. The ICMR-NIV RT-qPCR assay for Influenza/SARS-CoV-2 or WHO Pre-
260 Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved kit will be considered as
261 the reference assay for these parameters.

262 **5.2** The validation laboratory can use the established total RNA / viral RNA extraction
263 protocol for the evaluation.

264 **5.3** The instruction for the assay setup and the interpretation of the results will be as per
265 the protocol outlined by the manufacturer of the reference test and the kit under evaluation.

266 **5.4** The results shall be compared with the reference assay for sensitivity and specificity
267 calculations.

268 **5.5** If there is a discrepancy observed in the results with the index test, this discrepancy
269 should be taken as discordant. Repetition of the assay may introduce bias. If the reference
270 kit itself has failed, then these samples with discrepancies should be discarded, and new
271 well-characterized samples should be used instead.

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

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

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

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

278 **5.6** The interpretation for internal control (**preferably** endogenous, or exogenous) will be
279 as per manufacturer's instruction.

280 **5.7** PCR should be performed using IVD-approved machines. If any equipment(s) is
281 specified in the IFU of the index test, it should be used for the evaluation, and it should be
282 provided by the manufacturer if not available within the lab's IVD evaluation scope.

283 Real-time closed systems/devices awaiting evaluation should be provided by the
284 manufacturer along with all necessary components, supplies and reagents.
285 The details on the Real-time Equipment used for validation should be recorded, including
286 calibration status.
287

288 **6. Cross-reactivity Analysis:**

289 **6.1 Objective:**

290 To assess the primer-probe set for true detection of influenza viruses and assess its cross-
291 reactivity with other respiratory viruses.

292 **6.2 Methodology:**

293 **6.2.1** Potential cross-reactivity of the kit shall be ruled out by testing other
294 respiratory pathogen positive samples (N=30), with equal representation (n=5 each)
295 of samples positive for SARS-CoV-2, Parainfluenza viruses, Adenoviruses,
296 Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).*

297 **6.2.2** Cross-reactivity will be assessed by comparing the results of these samples
298 using kit under evaluation and reference kit.

299 **6.2.3** The kit targets should not show any amplification with other respiratory
300 viruses (ORVs). If amplification is observed for ORV then the kit will fail
301 validation and the same needs to be mentioned in the report.

302
303 * For multiplex assays targeting influenza, SARS-CoV-2, and RSV, samples positive for
304 these viruses may be suitably interchanged for assessing cross-reactivity, apart from the
305 ORV panel. (i.e. Influenza A positive samples may be used for detecting cross-reactivity
306 against Influenza B)

308 **7. Acceptance criteria for the kit:**

309 Sensitivity for each pathogen/ type/ subtype: $\geq 95\%$

310 Specificity for each pathogen/ type/ subtype: $\geq 99\%$

311 Cross-reactivity: Nil

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

313 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80
314 positive samples and ≥ 20 negative samples should be tested for evaluation for each
315 pathogen/ type/ subtype.

316 **8. Repeatability Assessment:**

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).

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).

341 **9.3 Result:** Concordance should be 100% based on positive and negative test result
342 (qualitative)

343 **10. Internal Control Analysis:**

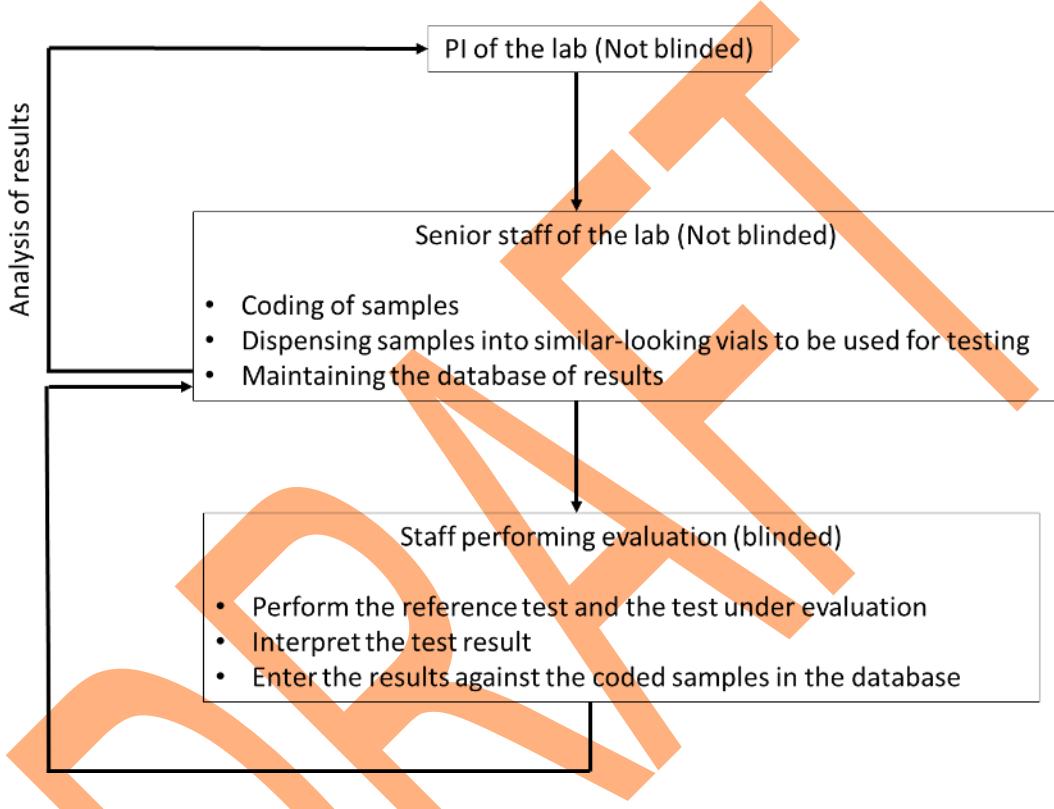
344 **10.1** Monitor the internal control (preferably RNaseP or other housekeeping gene) to
345 ensure consistent extraction and amplification efficiency across samples and runs.
346 **10.2** Ct-values of internal controls should be within the manufacturer's prescribed limit.
347 **10.3** Tests will be marked invalid if Ct-values are outside the prescribed limit.

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



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362
363

12. Publication Rights:

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

367 13. Conclusion:

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

371 The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and
372 performance characteristics compared to established reference IVD approved RT-PCR kits.

373

374 **After following due procedure as defined in this document, once any kit is found to be Not**
375 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
376 **acceptable.**

377 **Any request of re-validation from the same manufacturer for the same test type will only be**
378 **entertained after a minimum of 3 months and only if a high-level technical summary of**
379 **modifications or functional improvements to the kit design is submitted, without explicit**
380 **disclosure of proprietary information.**

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

385

14. Performance evaluation report format

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

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Protocol B

Evaluation of performance characteristics of Molecular Kit detecting SARS-CoV-2 in single plex or multiplex format

407 1. Objective:

408 **1.1.** To validate the performance characteristics of in vitro molecular diagnostic kits for
409 detecting SARS-CoV-2 as per the scope outlined in this document.

410 **1.2.** To ensure the kits under evaluation meet the necessary standards for sensitivity,
411 specificity, repeatability, and reproducibility.

412 **1.3.** To evaluate the cross-reactivity of the kits with other respiratory viruses.

413

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 **3.1** Contemporary leftover irreversibly de-identified clinical/archived respiratory samples
421 in VTM for the panel should be irreversibly de-identified.

422 **3.2** Samples to be used for panel preparation shall be stored properly at – 80 °C or
423 lyophilized.

424 **3.3** Unless the manufacturer has specific requirement of nucleic acid extraction kit, the
425 MDTLs/ validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Australia/
426 PMDA Japan approved/ICMR validated an established total RNA / viral RNA extraction
427 kits for the evaluation.

428 **3.4** Clinical samples for evaluation should be characterized by a reference kit /
429 Sequencing/NGS.

430 **3.5** All positive samples should be confirmed positive for the target pathogens by the
431 reference assay.

432 **3.6** All negative samples should be confirmed negative for the target pathogens by the
433 reference assay.

434

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

436 Sample sizes of positive and negative samples of SARS-CoV-2 against different values of
437 sensitivity and specificity are provided in Table 3. Sample sizes have been calculated assuming
438 95% level of significance, an absolute precision of 5%, and invalid test rate $\leq 5\%$. Appropriate
439 sample size has to be chosen from the tables according to the values of sensitivity and
440 specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present
441 in the table, the manufacturer needs to consider the sample size associated with the largest
442 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per
443 the next smaller value of the sensitivity/ specificity available in the table). For example, if a
444 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned
445 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the
446 sample size that is outlined for 85% specificity. Sample sizes are calculated using the formulae:
447

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

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

450

451

- 452 · *n (se) is the number of positive samples.*
- 453 · *n (sp) is the number of negative samples.*
- 454 · *Z² is the critical value from the standard normal distribution corresponding*
455 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*
- 456 · *Se is the predetermined sensitivity.*
- 457 · *Sp is the predetermined specificity.*
- 458 · *d is the predetermined marginal error (5%)*
- 459 · *IR is the invalid test rate*

460

461

462 *Table 3. Sample sizes 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)
 Moderate positive: (Ct value between 25-30)
 Weak positive: (Ct value >30 and \leq 34)

[¥] 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.

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

466 **4.1** Repeatability testing will be performed on 3 positive (strong, moderate and weak
 467 positive) and 3 negative samples (within the selected positive and negative samples) per
 468 target pathogen 5 times (replicates of 5).

469

470 5. Methodology:

471 **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference
 472 assay. The ICMR-NIV RT-qPCR assay for Influenza/SARS-CoV-2 or WHO Pre-
 473 Qualified/ US FDA/ PMDA Japan/ ATAGI Australia approved kit will be considered
 474 as the reference assay for these parameters.

475 **5.2** The validation laboratory can use established total RNA / viral RNA extraction
 476 protocol for the evaluation.

477 **5.3** The instruction for the assay setup and the interpretation of the results will be as per
 478 the protocol outlined by the manufacturer of the reference test and the kit under
 479 evaluation. The results shall be compared with the reference assay for sensitivity and
 480 specificity calculations.

481 **5.4** If there is a discrepancy observed in the results with the index test, this discrepancy
 482 should be taken as discordant. Repetition of the assay may introduce bias. If the
 483 reference kit itself has failed, then these samples with discrepancies should be
 484 discarded, and new well-characterized samples should be used instead.

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

486 *True negative samples: These are samples negative by both reference assay and index test.*
487 *False positive samples: These are samples negative by reference assay and positive by*
488 *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-
508 reactivity with other respiratory viruses.

509 **6.2 Methodology:**
510 **6.1.1** Potential cross-reactivity of the kit shall be ruled out by testing other respiratory
511 pathogen positive samples (N=30), with equal representation (n=5 each) of
512 samples positive for Influenza, Parainfluenza viruses, Adenoviruses,
513 Rhinoviruses, Respiratory Syncytial Virus, common human coronaviruses).*
514 **6.1.2** Cross-reactivity will be assessed by comparing the results of these samples using
515 kit under evaluation and reference kit.
516 **6.1.3** The kit targets should not show any amplification with other respiratory viruses
517 (ORVs). If amplification is observed for ORV then the kit will fail validation
518 and the same needs to be mentioned in the report.

519
520 **7. Acceptance criteria for the kit:**
521
522 Sensitivity: $\geq 95\%$
523 Specificity: $\geq 99\%$
524 Cross-reactivity: Nil

525 Invalid test rate: $\leq 5\%$
526 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80
527 positive samples and ≥ 20 negative samples should be tested for evaluation for each
528 pathogen/ type/ subtype.

529 **8. Repeatability Assessment:**

530 **8.1 Objectives:** To assess the repeatability of the detection of SARS-CoV-2 using the kit
531 under evaluation

533 **8.2 Sample size:** Five replicates of 3 positive samples (strong, moderate and weak positive-
534 as per the Ct values outlined in the document), and five replicates of 3 negative samples
535 for SARS-CoV-2 should be tested. For multiplex panels, these sample numbers shall
536 be used per target pathogen for repeatability assessment.

538 **8.3 Result: Concordance should be 100% based on positive and negative test result
539 (qualitative).**

542 **9. Precision (Reproducibility):**

543 **Lot to Lot Reproducibility**

545 **9.1 Objectives:** To assess precision (reproducibility) among 3 different lots of the kit
546 under evaluation.

547 **9.2 Sample size:** Lot to lot variation testing: Three lots of an assay shall be evaluated.
548 Sample size for lot-to-lot reproducibility should be as follows:

- 549 • First lot of the assay: should be tested on statistically significant number of positive
550 and negative samples as calculated in the protocol.
- 551 • Second lot of the assay: should be tested on 25 samples (15 positive samples
552 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative
553 samples).
- 554 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising
555 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

557 **9.3 Result: Concordance should be 100% based on positive and negative test result
558 (qualitative).**

559 **10. Internal Control Analysis:**

561 **10.1** Monitor the internal control (preferably RNaseP or other housekeeping gene) to
562 ensure consistent extraction and amplification efficiency across samples and runs.

564 **10.2** Ct-values of internal controls should be within the manufacturer's prescribed limit.

565

566 **10.3** Tests will be marked invalid if Ct-values are outside the prescribed limit.

567

568 **11. Blinding of Laboratory Staff:**

569 To ensure rigor of the evaluation process, laboratory staff performing the evaluation should
570 be blinded to the status of the clinical samples. The PI of the evaluation exercise should
571 remain unblinded, i.e., privy to the status of the samples. Another senior laboratory staff
572 selected by the PI may remain unblinded and carry out coding of samples and dispensing
573 them into similar-looking vials to be used for testing, and maintaining the database of
574 results. Staff performing the reference test and the test under evaluation, interpretation of
575 the test result, and entering the results against the coded samples in the database, should
576 remain blinded to the status of samples till the completion of evaluation. The data should
577 be analyzed only by the PI of the evaluating lab. Refer to Fig. 1 in Protocol A.

578

579 **12. Conclusion:**

580 Based on the comprehensive evaluation conducted, the [Kit & Manufacturer's Name] SARS-CoV-
581 2 RT-PCR Assay has been found **[Satisfactory/Not Satisfactory]** for its intended *in vitro*
582 diagnostic (IVD) use.

583 The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and performance
584 characteristics compared to established reference IVD approved RT-PCR kits.

585

586 **13. Publication Rights:**

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

588

589 **After following due procedure as defined in this document, once any kit is found to be Not**
590 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
591 **acceptable.**

592 **Any request of re-validation from the same manufacturer for the same test type will only be**
593 **entertained after a minimum of 3 months and only if a high-level technical summary of**
594 **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**
598 **which claim high performance characteristics (sensitivity and specificity 95% and above),**
599 **but which fail the performance evaluation by a margin of 5%.**

600

601 **14. Performance evaluation report format:**

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

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A large, stylized orange watermark reading "DRAFT" is positioned in the center of the page. The letters are bold and slightly slanted, with a thick orange outline. The watermark covers the majority of the page content area.

629 **Protocol C**

630 **Evaluation of performance characteristics of Molecular Kit detecting Respiratory**
631 **Syncytial Virus (RSV) in single plex or multiplex format**

632 **1. Objective:**

633 **1.1.** To validate the performance characteristics of in vitro molecular diagnostic kits for
634 detecting and/or differentiating RSV A/B as per the scope outlined in this document.

635 **1.2.** To ensure the kits under evaluation meet the necessary standards for sensitivity,
636 specificity, repeatability, and reproducibility.

637 **1.3.** To evaluate the cross-reactivity of the kits with other respiratory viruses.

638 **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 **3.1** Contemporary leftover irreversibly de-identified clinical/archived respiratory samples
646 in VTM for the panel should be irreversibly de-identified.

647 **3.2** Samples to be used for panel preparation shall be stored properly at – 80 °C or
648 lyophilized.

649 **3.3** Unless the manufacturer has specific requirement of nucleic acid extraction kit, the
650 MDTLs/ validation laboratory can use WHO Pre-Qualified/ US FDA/ ATAGI Australia/
651 PMDA Japan approved/ ICMR validated an established total RNA / viral RNA extraction
652 kits for the evaluation.

653 **3.4** Clinical samples for evaluation should be characterized by a reference kit /
654 Sequencing/NGS.

655 **3.5** All positive samples should be confirmed positive for the target pathogens by the
656 reference assay.

657 **3.6** All negative samples should be confirmed negative for the target pathogens by the
658 reference assay.

659

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

661 Sample sizes of positive and negative samples of the RSV A/B against different values of
662 sensitivity and specificity are provided in Table 4. Sample sizes have been calculated assuming
663 95% level of significance, an absolute precision of 5%, and invalid test rate $\leq 5\%$. Appropriate
664 sample size has to be chosen from the tables according to the values of sensitivity and
665 specificity being claimed by the manufacturer. If a claimed sensitivity/specificity is not present
666 in the table, the manufacturer needs to consider the sample size associated with the largest
667 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per
668 the next smaller value of the sensitivity/ specificity available in the table). For example, if a
669 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned
670 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the
671 sample size outlined for 85% specificity. Sample sizes for positive samples and their
672 composition for evaluating subtyping (RSV A/B) are provided in Table 5. Sample sizes are
673 calculated using the formulae:

674

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

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

677

678

- 679 · n_{se} is the number of positive samples.
- 680 · n_{sp} is the number of negative samples.
- 681 · Z^2 is the critical value from the standard normal distribution corresponding
682 to the desired confidence level (95% CI corresponds to $Z^2 = 1.96$).
- 683 · S_e is the predetermined sensitivity.
- 684 · S_p is the predetermined specificity.
- 685 · d is the predetermined marginal error (5%)
- 686 · IR is the invalid test rate

687

688

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

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

			<i>samples (rounded)[‡]</i>	<i>among the negative samples</i>
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 and \leq 34)

[‡] 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.

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

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

Sensitivity	Sample size* (per target pathogen)	RSV A	RSV B	Minimum total positive samples
		Minimum number of nasopharyngeal swabs/ oropharyngeal swabs	Minimum number of nasopharyngeal swabs/ oropharyngeal swabs	
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.

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

698 **4.1** Repeatability testing will be performed on 3 positive (strong, moderate and weak
699 positive) and 3 negative samples (within the selected positive and negative samples) per
700 target pathogen 5 times (replicates of 5).

701 **5. Methodology:**

702 **5.1** Samples should be tested in parallel with the Kit Under Evaluation and the reference
703 assay. The ICMR-NIV RT-qPCR assay for RSV or WHO Pre-Qualified/ US FDA/ ATAGI
704 Australia/ PMDA Japan approved kit will be considered as the reference assay for these
705 parameters.

706 **5.2** The validation laboratory can use established total RNA / viral RNA extraction protocol
707 for the evaluation.

708 **5.3** The instruction for the assay setup and the interpretation of the results will be as per
709 the protocol outlined by the manufacturer of the Kit Under Evaluation.

710 **5.4** The results shall be compared with the reference assay for sensitivity and specificity
711 calculations.

712 **5.5** If there is a discrepancy observed in the results with the index test, this discrepancy
713 should be taken as discordant. Repetition of the assay may introduce bias. If the reference
714 kit itself has failed, then these samples with discrepancies should be discarded, and new
715 well-characterized samples should be used instead.

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

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

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

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

722

723 **5.6** The interpretation for internal control (**preferably** endogenous, or exogenous) will be
724 as per manufacturer's instruction.

725 **5.7** PCR should be performed using IVD-approved machines. If any equipment(s) is
726 specified in the IFU of the index test, it should be used for the evaluation, and it should be
727 provided by the manufacturer if not available within the lab's IVD evaluation scope.

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

730 The details on the Real-time Equipment used for validation should be recorded, including
731 calibration status.

732 The details on the Real-time Equipment used for validation should be recorded including
733 calibration status.

734

735 **6. Cross-reactivity Analysis:**

736 **6.1 Objective:**

737 To assess the primer-probe set for true detection of RSV and assess its cross-reactivity with
738 other respiratory viruses.

739 **6.2 Methodology:**

740 **6.1.1** Potential cross-reactivity of the kit shall be ruled out by testing other respiratory
741 pathogen positive samples (N=30), with equal representation (n=5 each) of
742 samples positive for Influenza, SARS-CoV-2, Parainfluenza viruses,
743 Adenoviruses, Rhinoviruses, common human coronaviruses.*

744 **6.1.2** Cross-reactivity will be assessed by comparing the results of these samples
745 using kit under evaluation and reference kit.

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.

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

751 **7. Acceptance criteria for the kit:**

752 Sensitivity for each pathogen/ type/ subtype: $\geq 95\%$

753 Specificity for each pathogen/ type/ subtype: $\geq 99\%$

754 Cross-reactivity: Nil

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

756 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80
757 positive samples and ≥ 20 negative samples should be tested for evaluation for each
758 pathogen/ type/ subtype.

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 **8.2 Sample size:** Five replicate of 3 positive samples per target pathogen (strong,
766 moderate and weak positive) and five replicates of 3 negative samples per target pathogen
767 should be tested.

768 **8.3 Result:** Concordance should be 100% based on positive and negative test result (qualitative).

769 **9. Precision (Reproducibility):**

774 **Lot to Lot Reproducibility**

775
776 **1.1 Objectives:** To assess precision (reproducibility) among 3 different lots of the kit
777 under evaluation.

778 **9.2 Sample size:** Lot to lot variation testing: Three lots of an assay shall be evaluated.
779 Sample size for lot-to-lot reproducibility should be as follows:

- 780 • First lot of the assay: should be tested on statistically significant number of positive
781 and negative samples as calculated in the protocol.
- 782 • Second lot of the assay: should be tested on 25 samples (15 positive samples
783 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative
784 samples).
- 785 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising
786 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

788 **9.3 Result:** Concordance should be 100% based on positive and negative test result
789 (qualitative).

790
791 **10. Internal Control Analysis:**

- 792 **10.1** Monitor the internal control (RNaseP or other endogenous housekeeping gene) to ensure
793 consistent extraction and amplification efficiency across samples and runs.
- 794 **10.2** Ct-values of internal controls should be within the manufacturer's prescribed limit.
- 795 **10.3** Tests will be marked invalid if Ct-values are outside the prescribed limit.

796
797 **11. Blinding of Laboratory Staff:**

798 To ensure rigor of the evaluation process, laboratory staff performing the evaluation should
799 be blinded to the status of the clinical samples. The PI of the evaluation exercise should
800 remain unblinded, i.e., privy to the status of the samples. Another senior laboratory staff
801 selected by the PI may remain unblinded and carry out coding of samples and dispensing
802 them into similar-looking vials to be used for testing, and maintaining the database of
803 results. Staff performing the reference test and the test under evaluation, interpretation of
804 the test result, and entering the results against the coded samples in the database, should
805 remain blinded to the status of samples till the completion of evaluation. The data should
806 be analyzed only by the PI of the evaluating lab. Refer to Fig. 1 in Protocol A.

807 **12. Conclusion:**

808 Based on the comprehensive evaluation conducted, the [Kit & Manufacturer's Name] SARS-CoV-
809 2 RT-PCR Assay has been found **[Satisfactory/Not Satisfactory]** for its intended *in vitro*
810 diagnostic (IVD) use.

811 The assay demonstrates [Strengths/Concerns] in terms of sensitivity, specificity, and performance
812 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 **After following due procedure as defined in this document, once any kit is found to be Not**
818 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
819 **acceptable.**

820 **Any request of re-validation from the same manufacturer for the same test type will only be**
821 **entertained after a minimum of 3 months and only if a high-level technical summary of**
822 **modifications or functional improvements to the kit design is submitted, without explicit**
823 **disclosure of proprietary information.**

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

828

829 14. Performance evaluation report format:

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

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Protocol D

Evaluation of performance characteristics of Molecular Kit detecting Influenza virus and 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
847 reactivity, repeatability, reproducibility and Lot to lot variation.

848 A comprehensive report can be generated which will include sensitivity and specificity for all
849 targets.

850 Sample size for multiplex molecular assay (as per the scope outlined in the document) detecting
851 Influenza virus and SARS-CoV-2 in multiplex format is given below. All other
852 parameters/conditions outlined in the single plex protocols (Protocols A and B) are to be
853 essentially followed.

854 1. Sample size and sample panel composition for evaluation of performance 855 characteristics:

856 Sample sizes of positive and negative samples against different values of sensitivity and
857 specificity are provided in Table 6. Sample sizes have been calculated assuming 95% level of
858 significance, an absolute precision of 5%, and invalid test rate $\leq 5\%$. Appropriate sample size
859 has to be chosen from the tables according to the values of sensitivity and specificity being
860 claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the
861 manufacturer needs to consider the sample size associated with the largest
862 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per
863 the next smaller value of the sensitivity/ specificity available in the table). For example, if a
864 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned
865 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the
866 sample size outlined for 85% specificity. Sample sizes are calculated using the formulae:

$$868 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

$$869 n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

870

871

- 872 · *n (se) is the number of positive samples.*
- 873 · *n (sp) is the number of negative samples.*
- 874 · *Z² is the critical value from the standard normal distribution corresponding*
875 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*
- 876 · *Se is the predetermined sensitivity.*

877 · *Sp* is the predetermined specificity.
 878 · *d* is the predetermined marginal error (5%)
 879 · *IR* is the invalid test rate

880
 881
 882 Table 6. Sample sizes for different values of sensitivity/ specificity claimed by the
 883 manufacturer.

Sensitivity/ Specificity	Sample size for each of the 04 target pathogens ^a : Minimum number of positive samples [¥]	Composition of positive samples for each pathogen [#]	Total number of positive samples (including all 04 pathogens)	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
[#]Strong positive: (Ct value <25)
 Moderate positive: (Ct value between 25-30)
 Weak positive: (Ct value >30 and and \leq 34)
[¥] 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.

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

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

887 **2. Acceptance Criteria for the kit:**

888 Sensitivity for each pathogen/ type/ subtype: $\geq 95\%$

889 Specificity for each pathogen/ type/ subtype: $\geq 99\%$

890 Cross-reactivity: Nil

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

892 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80
893 positive samples and ≥ 20 negative samples should be tested for evaluation for each
894 pathogen/ type/ subtype.

895

896 **After following due procedure as defined in this document, once any kit is found to be Not**
897 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
898 **acceptable.**

899 **Any request of re-validation from the same manufacturer for the same test type will only be**
900 **entertained after a minimum of 3 months and only if a high-level technical summary of**
901 **modifications or functional improvements to the kit design is submitted, without explicit**
902 **disclosure of proprietary information.**

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

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Protocol E

Evaluation of performance characteristics of Molecular Kit detecting Influenza virus, 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
923 cross reactivity, repeatability, reproducibility and Lot to lot variation.

924 A comprehensive report can be generated which will include sensitivity and specificity for all
925 targets.

926 Sample size for multiplex molecular assay (as per the scope outlined in the document) detecting
927 Influenza virus, SARS-CoV-2 and Respiratory Syncytial Virus (RSV) in multiplex format is
928 given below. All other parameters/conditions outlined in the single plex protocols (Protocol A, B
929 and C) are to be essentially followed.

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

932 Sample sizes of positive and negative samples against different values of sensitivity and
933 specificity are provided in Table 7. Sample sizes have been calculated assuming 95% level of
934 significance, an absolute precision of 5%, and invalid test rate $\leq 5\%$. Appropriate sample size
935 has to be chosen from the tables according to the values of sensitivity and specificity being
936 claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the table, the
937 manufacturer needs to consider the sample size associated with the largest
938 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as per
939 the next smaller value of the sensitivity/ specificity available in the table). For example, if a
940 manufacturer claims a sensitivity of 93%, they are required to use a sample size mentioned
941 against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the
942 sample size outlined for 85% specificity. Sample sizes are calculated using the formulae:

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

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

946

947

948 · *n (se) is the number of positive samples.*

949 · *n (sp) is the number of negative samples.*

950 · *Z² is the critical value from the standard normal distribution corresponding*
951 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*

952 · *Se is the predetermined sensitivity.*

953 · *Sp* is the predetermined specificity.
 954 · *d* is the predetermined marginal error (5%)
 955 · *IR* is the invalid test rate

956
 957
 958 Table 7. Sample sizes for different values of sensitivity/ specificity claimed by the
 959 manufacturer.

<i>Sensitivity/ Specificity</i>	<i>Sample size for each of the 06 target pathogens^a: Minimum number of positive samples[¥]</i>	<i>Composition of positive samples for each pathogen[#]</i>	<i>Total number of positive samples (including all 06 pathogens)</i>	<i>Sample size: Minimum number of negative samples[¥]</i>	<i>Minimum number of cross reactive * sample s among the negative samples[¥]</i>
99%	16 (rounded to 20 for better distribution of samples)	Strong positive = 06 Moderate positive = 07 Weak positive = 07	120	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 \leq 34)

[¥] 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.

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

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

963

964 **2. Acceptance Criteria for the kit:**

965 Sensitivity for each pathogen/ type/ subtype: $\geq 95\%$

966 Specificity for each pathogen/ type/ subtype: $\geq 99\%$

967 Cross-reactivity: Nil

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

969 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 80
970 positive samples and ≥ 20 negative samples should be tested for evaluation for each
971 pathogen/ type/ subtype.

972

973 After following due procedure as defined in this document, once any kit is found to be Not
974 of Standard Quality, thereafter, no request for repeat testing of the same kit will be
975 acceptable.

976 Any request of re-validation from the same manufacturer for the same test type will only be
977 entertained after a minimum of 3 months and only if a high-level technical summary of
978 modifications or functional improvements to the kit design is submitted, without explicit
979 disclosure of proprietary information.

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

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998 **Performance evaluation report for Respiratory Virus in-vitro molecular diagnostic kit**

999

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

1000

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1002

i. **Analytes/Pathogens targeted by the kit under evaluation:**

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v.

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1015 **RESULTS INTERPRETATION**

1016

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1018 **SENSITIVITY AND SPECIFICITY FOR INDIVIDUAL VIRUS TARGETS**

1019 **1. Sensitivity and specificity for Influenza A (H1N1) pdm09**

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

1020

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1021

1022 **2. Sensitivity and specificity for Influenza A (H3N2)**

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

1023

	Estimate (%)	CI 95 %
Sensitivity		
Specificity		

1024

1025

3. Sensitivity and specificity for Influenza B (Victoria)

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

1026

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1027

1028

4. Sensitivity and specificity for Influenza B (Yamagata)

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

1029

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1030

1031

5. Sensitivity and specificity for SARS-CoV-2

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

1032

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1033

1034

6. Sensitivity and specificity for RSV A

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

1035

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1036

1037

7. Sensitivity and specificity for RSV B

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

	Total			
--	--------------	--	--	--

1038

	Estimate (%)	CI 95%
Sensitivity		
Specificity		

1039

1040 a. **Cross-reactivity Analysis:**
1041 b. **Repeatability Assessment:**
1042 c. **Precision (Reproducibility):**
1043 • **Lot to Lot**

1044

Details of lots tested (3 lots to be tested):

1046

1. Lot No.: Lot No:
1047 2. Lot No.: Lot No:
1048 3. Lot No.: Lot No:

Tested By:
Tested By:
Tested By:

1049

1050 ▪ **Lot-to-lot variation was observed / not observed.**

1051

d. Internal Control Analysis:

1052

Conclusion: Satisfactory / Not satisfactory

1053

RECOMMENDATIONS:

1055

Suggestions for improvements or modifications (if applicable):

1056

1057

▪ **ICMR-CDSCO guidelines were followed for kit performance evaluation.**

1058

This evaluation report is exclusively for _____ In Vitro

1059 Molecular Diagnostic Kit manufactured by _____.

1060 **Sensitivity and specificity have been assessed in controlled lab settings using the kits of the**
1061 **Lot number:**

1062

i. **Lot No._____,**

1063

ii. **Lot No._____,**

1064

iii. **Lot No._____,**

1065 **Provided by the manufacturer, using samples. Results should not be extrapolated to**
1066 **other sample types.**

1067 **DISCLAIMER:**

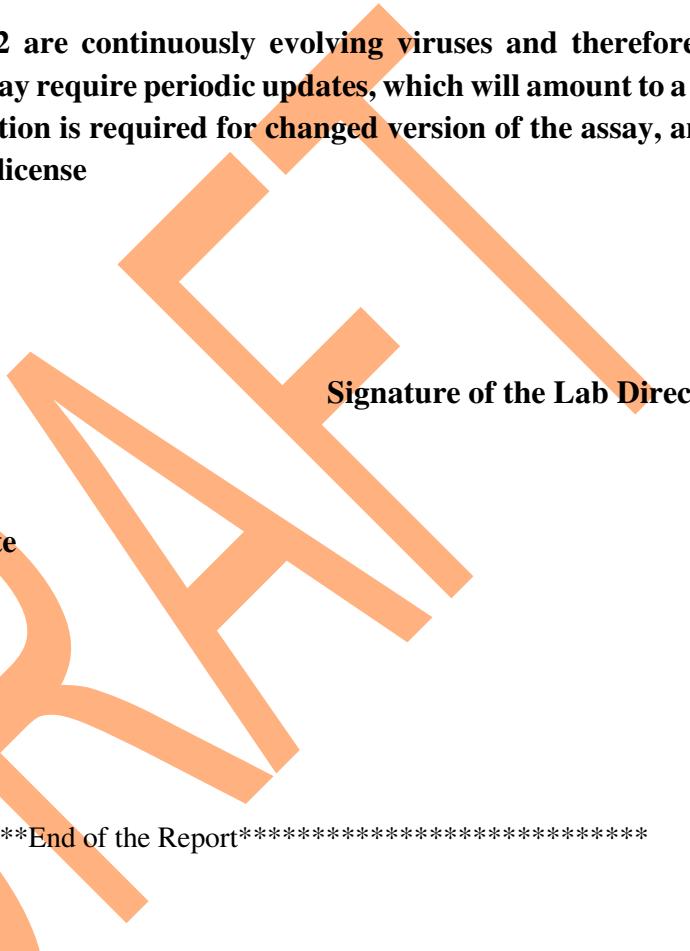
1068 **1. This validation process does not approve/disapprove the Kit design.**

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**

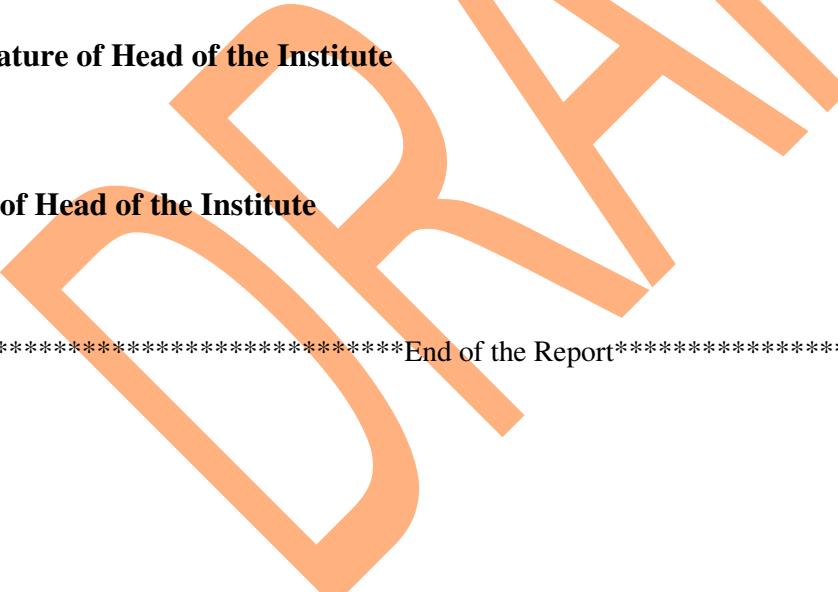
1074

1075

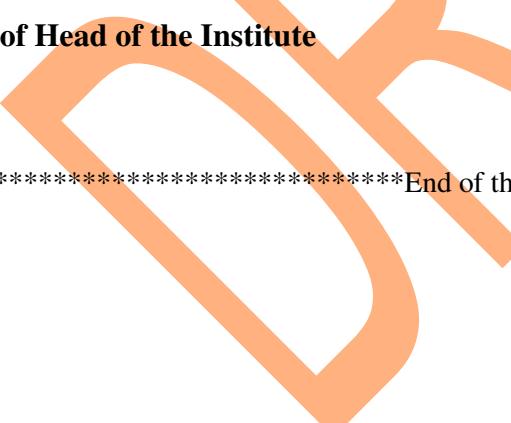
1076 **Signature of the Lab Manager** 

Signature of the Lab Director

1077

1078 **Signature of Head of the Institute** 

1079

1080 **Seal of Head of the Institute** 

1081

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1083 *****End of the Report*****

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1092 **Annexure-1: Information on Operational and Test Performance Characteristics Required**
1093 **from Manufacturers**

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

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2 STANDARD PERFORMANCE 3 EVALUATION PROTOCOLS

4 DRAFT FOR STAKEHOLDER 5 COMMENTS

6 MALARIA IN-VITRO DIAGNOSTICS

7
8 ICMR-CDSCO/IVD/GD/PROTOCOLS/06/2025

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AUGUST, 2025
New Delhi, India

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33 **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
39 regarding in-vitro diagnostic kit (IVD) performance.

40 **II. Purpose:**

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

43 **III. Requirements:**

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

51 **IV. Ethical approvals:**

52 Performance evaluation activities using irreversibly de-identified leftover clinical samples
53 are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for
54 Laboratory Validation Testing, 2024.

55 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:**

58 1. **Study design/type:** Diagnostic accuracy study using irreversibly de-identified leftover
59 clinical/spiked samples.

60 2. **Preparation of Evaluation sites/laboratories:**

61 **Identified IVD kit evaluation laboratories should establish their proficiency
62 through:**

- 63 a) Laboratory accreditation: Accreditation for at least one of the Quality management
64 systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical
65 Lab (ISO: 15189), PT provider (ISO: 17043) or CDSCO approved Reference
66 laboratory).
- 67 b) It is recommended that malaria Medical Device Testing Labs (MDTLs) participate
68 in Quality Control exercises such as EQAP (External Quality Assurance
69 Programme).

70 c) **Staff training:** All the staff involved in IVD kit evaluation should undergo hands
71 on training and competency testing on the following at referral level malaria labs
72 before initiation of MDTL activity:

73 ➤ Preparation and characterization of evaluation panel for the respective IVD
74 kit.

75 ➤ Management of RDT kits (specific for *Plasmodium falciparum* / *Plasmodium*
76 *vivax*) received for performance evaluation (Verification/Storage/Unpacking
77 etc.).

78 ➤ Perform tests interpretation and documentation of results, and reporting.

79 ➤ Data management and safety and confidentiality.

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

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

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

88 **4. Reference assay:**

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

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

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

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

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

108 Sample sizes are calculated using the formulae:

109

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

111

$$112 \quad n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

113

- 114 · *n (se) is the minimum number of positive samples.*
- 115 · *n (sp) is the minimum number of negative samples.*
- 116 · *Z² is the critical value from the standard normal distribution*
117 *corresponding to the desired confidence level (95% CI corresponds to Z²*
118 *=1.96).*
- 119 · *Se is the predetermined sensitivity.*
- 120 · *Sp is the predetermined specificity.*
- 121 · *d is the predetermined marginal error (5%)*
- 122 · *IR is the invalid test rate*

123

124

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

<i>Sensitivity</i>	<i>Sample size: Minimum number of positive samples #</i>	<i>Composition of positive samples</i>
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%	305 (rounded to 310 for better distribution of samples)	Strong positive = 92 Moderate positive = 109 Weak positive = 109

128

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

132
 133 *Table 2. Negative sample sizes and composition for different values of specificity*
 134 *claimed by the manufacturer for evaluation of Pf (single/combo RDT) or Pv*
 135 *(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

136
 137 #It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and
 138 specificity; however, a higher sample size is suggested to ensure adequate power of the study
 139 in case the kit falls short of claimed performance characteristics.

140 **Sample panel composition:**

141 **Positive samples:** Malaria positive samples should be obtained from health facilities
142 (*tertiary care centers and their linked hospitals, private clinics, field practice areas*
143 *etc.*) and confirmed using PCR (Snounou protocol/FDA approved assay).

144 Malaria samples confirmed positive by PCR should be characterized for parasite load
145 on in-house calibrated equipment using blood smear microscopy and ELISA. Samples
146 with analyte values satisfying the range of acceptance criteria (as mentioned in this
147 document) should be included in the positive sample panel for the evaluation of malaria
148 RDT kits.

149 For the RDT kits which have other antigen/antibody as target analyte (for which limits
150 of detection have not been established), characterization of samples should be
151 performed on calibrated equipment, leading to their classification as low and high
152 parasitemic samples, which should then be used for performance evaluation of the
153 assay.

154
155 **Range of Parasitemia:** Panel members should have low (≤ 200 parasites per microliter)
156 to high (≥ 2000 parasites per microliter) range of *Plasmodium falciparum*, *P. vivax*
157 and/or other *Plasmodium* species, as obtained from ELISA results. Characterized
158 panels **must** contain equal number of samples of both low and high parasitemia.

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

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

167
168 **6. Test reproducibility:**

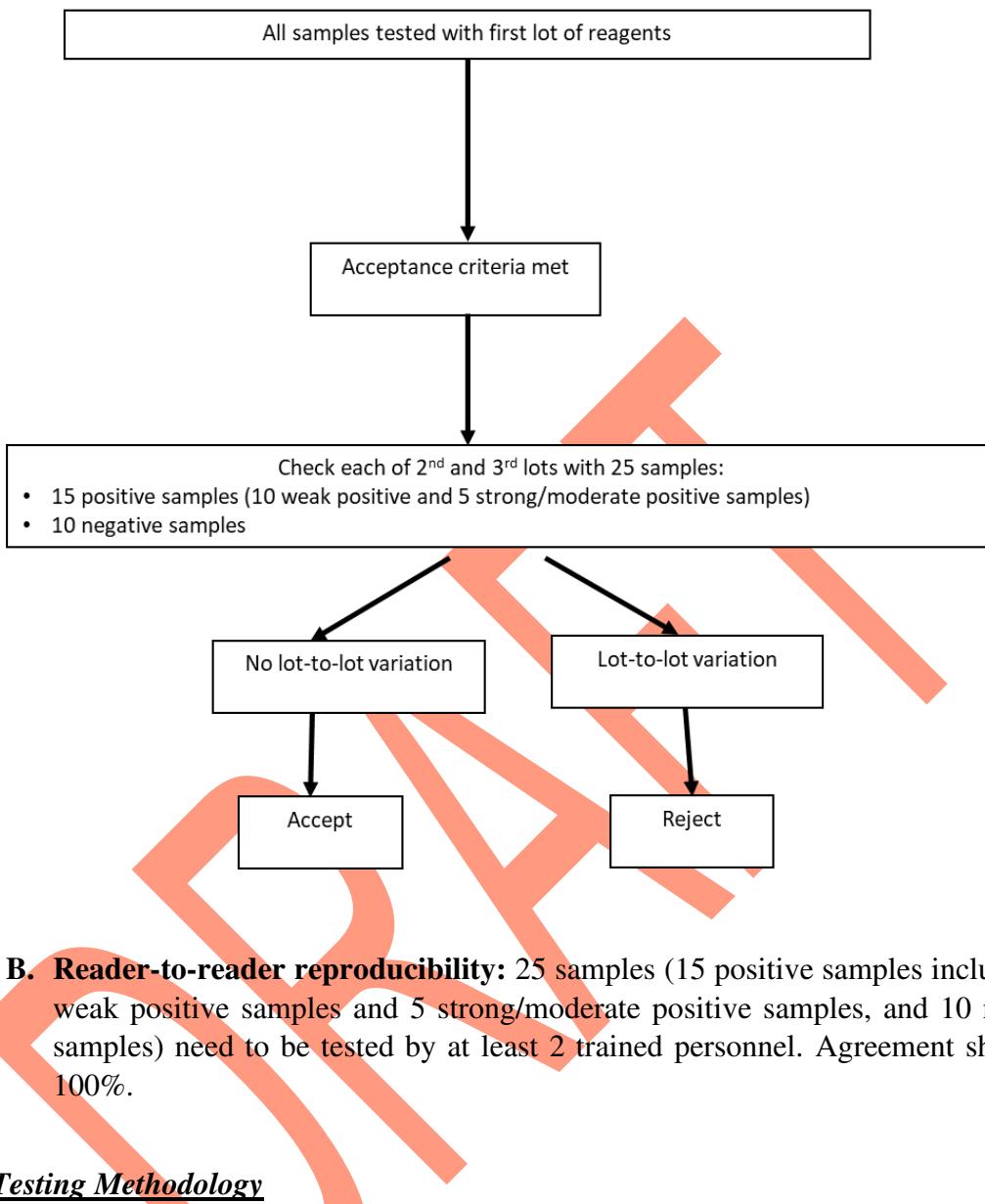
169
170 **A. Sample size for lot-to-lot reproducibility**

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):

177

Fig.1: Lot-to-lot reproducibility



178
179
180
181 **B. Reader-to-reader reproducibility:** 25 samples (15 positive samples including 10
182 weak positive samples and 5 strong/moderate positive samples, and 10 negative
183 samples) need to be tested by at least 2 trained personnel. Agreement should be
184 100%.
185

186 **Note: Testing Methodology**

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

193 **7. Evaluation method:**

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

195
196 **8. Interpretation of results:**

197 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 criteria¹:**

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: $\leq 5\%$

214

215 To achieve at least the performance characteristics outlined in the acceptance criteria,
216 ≥ 310 positive samples and ≥ 150 negative samples should be tested for *P. vivax*, and ≥ 80
217 positive samples and ≥ 80 negative samples should be tested for *P. falciparum*.

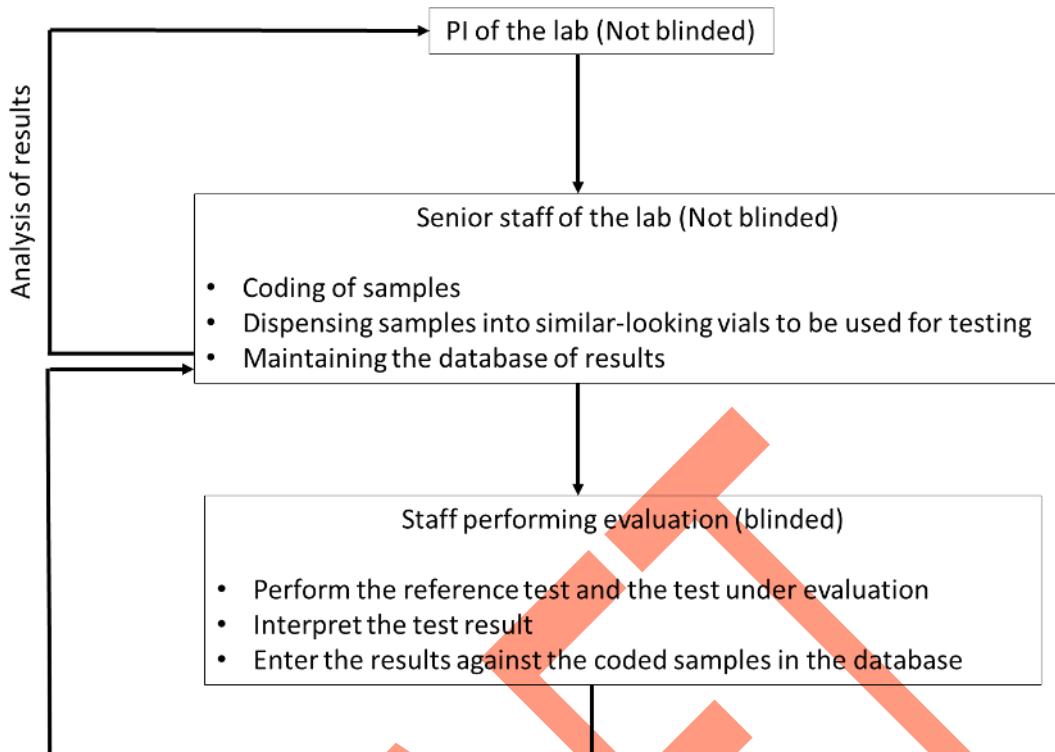
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219 **11. Blinding of laboratory staff**

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.

230

231 Fig.2: Blinding in evaluation exercise



232

233

234

12. Publication Rights

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

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

241

242 **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.**

243 **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.**

244 **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%.**

245

246

VI. References:

255 1. Ministry of Health and Family Welfare. Guidelines for Bivalent RDT. Available at:
256 [guidelines-for-bivalent-rdt.pdf \(mohfw.gov.in\)](http://guidelines-for-bivalent-rdt.pdf (mohfw.gov.in))

257 2. World Health Organization. Malaria Rapid Diagnostic Test Performance - Results of
258 WHO product testing of malaria RDTs: round 8 (2016–2018): Available at:
259 [https://iris.who.int/bitstream/handle/10665/276190/9789241514965-
eng.pdf?sequence=1](https://iris.who.int/bitstream/handle/10665/276190/9789241514965-eng.pdf?sequence=1)

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

263 4. Krishna S, Bharti PK, Chandel HS, Ahmad A, Kumar R, Singh PP, et al. Detection of
264 Mixed Infections with Plasmodium spp. by PCR, India, 2014. Emerg Infect Dis.
265 2015;21(10):1853–7.

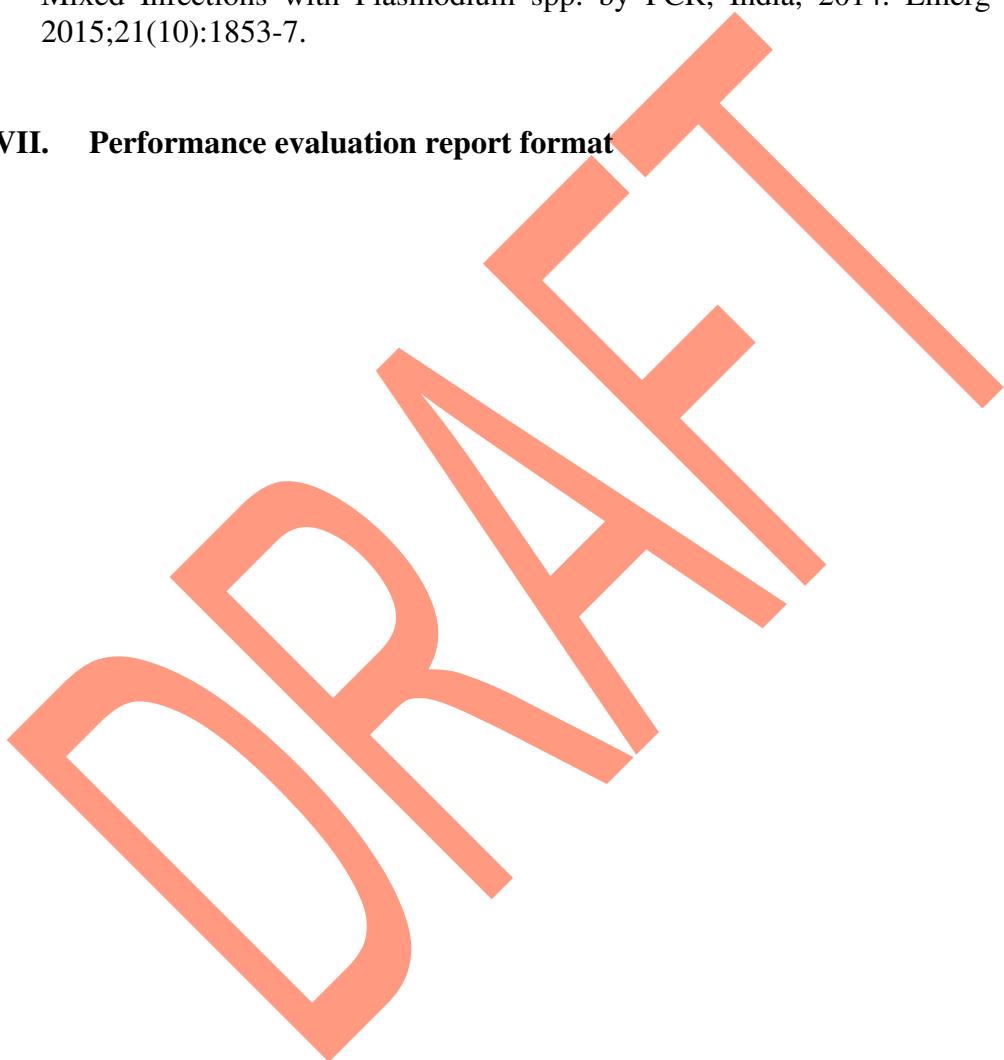
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VII. Performance evaluation report format



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REPORT FORMAT

271

Name of the Laboratory

272

Name of the Institute, (with station)

273

Certificate of Analysis

274

File No.: _____

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

	Negative samples (provide details, including cross reactivity panel)	
--	----------------------------------------------------------------------	--

275

276

277 **Results:**

278

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

279

280

	Estimate (%)	95% CI
Sensitivity		
Specificity		

281

282 • Details of cross reactivity with other agents:

283 • **Conclusions:**

284 ○ 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 be extrapolated for any other sample type.*)

289 **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 Note: This report is exclusively for Kit (Lot No.....) manufactured by
293 (Supplied by

294

295 Evaluation Done on

296 Evaluation Done by

297

298 Signature of Director/ Director-In-charge Seal
299

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

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DBAF

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Performance evaluation protocol for Malaria ELISA kits

I. Background:

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 of performance evaluation is to independently validate the manufacturer's claim regarding in-vitro diagnostic kit (IVD) performance.

II. Purpose:

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 (*P. falciparum*, *P. vivax*) and stage specific antibodies (MSP1, MSP3, CSP, EBA175 etc.- parasite markers for the purpose of sero-survey).

III. Requirements:

- a) Instructions for use (IFU)
- 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.
- c) Evaluation sites/laboratories (With required equipment)
- d) Reference test kits
- e) Characterised Evaluation panel
- f) Laboratory supplies

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.

V. Procedure:

- 1. Study design/type:** Diagnostic accuracy study using irreversibly de-identified leftover clinical samples.
- 2. Preparation of Evaluation sites/laboratories:**
Identified ELISA 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

360 Lab (ISO:15189), PT provider ISO: 17043 or CDSCO approved Reference
361 laboratory.

362 b) It is recommended that malaria Medical Device Testing Labs (MDTLs) participate
363 in Quality Control exercises such as EQAP (External Quality Assurance
364 Programme).

365 c) **Staff training:** All the staff involved in ELISA kit evaluation should undergo
366 hands on training and competency testing on the following at referral level malaria
367 labs before initiation of MDTL activity:

- 368 ➤ Preparation and characterization of evaluation panel for the respective
369 ELISA kit.
- 370 ➤ Management of malaria ELISA kits received for performance evaluation
371 (Verification/Storage/Unpacking etc).
- 372 ➤ Perform tests , interpretation and documentation of results and reporting.
- 373 ➤ Data management and safety and confidentiality

374 3. Reference sample panel:

375 To evaluate the performance of ELISA kit a well characterised malaria stage specific
376 antigens/species specific antibody ELISA evaluation sample panel is required. In the
377 absence of WHO Pre-Qualified/US FDA/ ATAGI Australia/ PMDA Japan approved
378 malaria ELISA assay, it is recommended that performance evaluation of ELISA assays
379 be performed on a rigorously well characterized panel of positive and negative samples.

380 A statistically significant number of sera samples should be collected from malaria
381 confirmed cases from health facilities. *All samples should be further confirmed by PCR
382 assay (Snounou protocol/FDA approved assay).*

383 A. ***Malaria samples confirmed positive by PCR should be characterized for parasite load
384 on in-house calibrated equipment using ELISA. Samples with analyte values satisfying
385 the range of acceptance criteria (as mentioned in this document) should be included in
386 the positive sample panel for the evaluation of malaria RDT kits.***

387 *For those kits which have other antigen/antibody as target analyte (for which limits of
388 detection have not been established), characterization of samples for that analyte
389 should be performed on calibrated equipment, leading to their classification as low and
390 high parasitemic samples, which will then be used for performance evaluation of the
391 assay.*

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

396 Consistent ELISA quantification results should be obtained in ≥ 3 runs of ELISA
397 experiments performed for each of the three antigens (PfHRP2, LDH and aldolase –
398 recombinantly expressed proteins) with the results obtained at the 200 p/ μ L and the

400 2,000 p/µL being consistent with each other as well (factor of roughly 10 between
401 results). The limit of detection of Pfhrp2 is 5-10 ng/ µL, and Pvldh is 15-45 ng/ µL.

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

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

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

408 **B. Negative panel should constitute malaria negative samples (confirmed by PCR) as**
409 **described in point 6B.**

410 *The reference sample panel should be stored in appropriate storage conditions, and the quality*
411 *of the panel should be checked periodically with appropriate tests (including parasite culture)*
412 *as needed.*

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

415 **Wherever any WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved**
416 **assay is available, it should be used as reference standard.**

417

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

431

$$432 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2}$$

$$433 n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2}$$

434

435

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

437

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

438 · Z^2 is the critical value from the standard normal distribution
439 corresponding to the desired confidence level (95% CI corresponds to Z^2
440 =1.96).

441 · Se is the predetermined sensitivity.

442 · Sp is the predetermined specificity.

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

444

445 **Table 1.** Positive sample sizes (per species) and composition for different values of sensitivity
446 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

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

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

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%	73 (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%	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

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

455

456

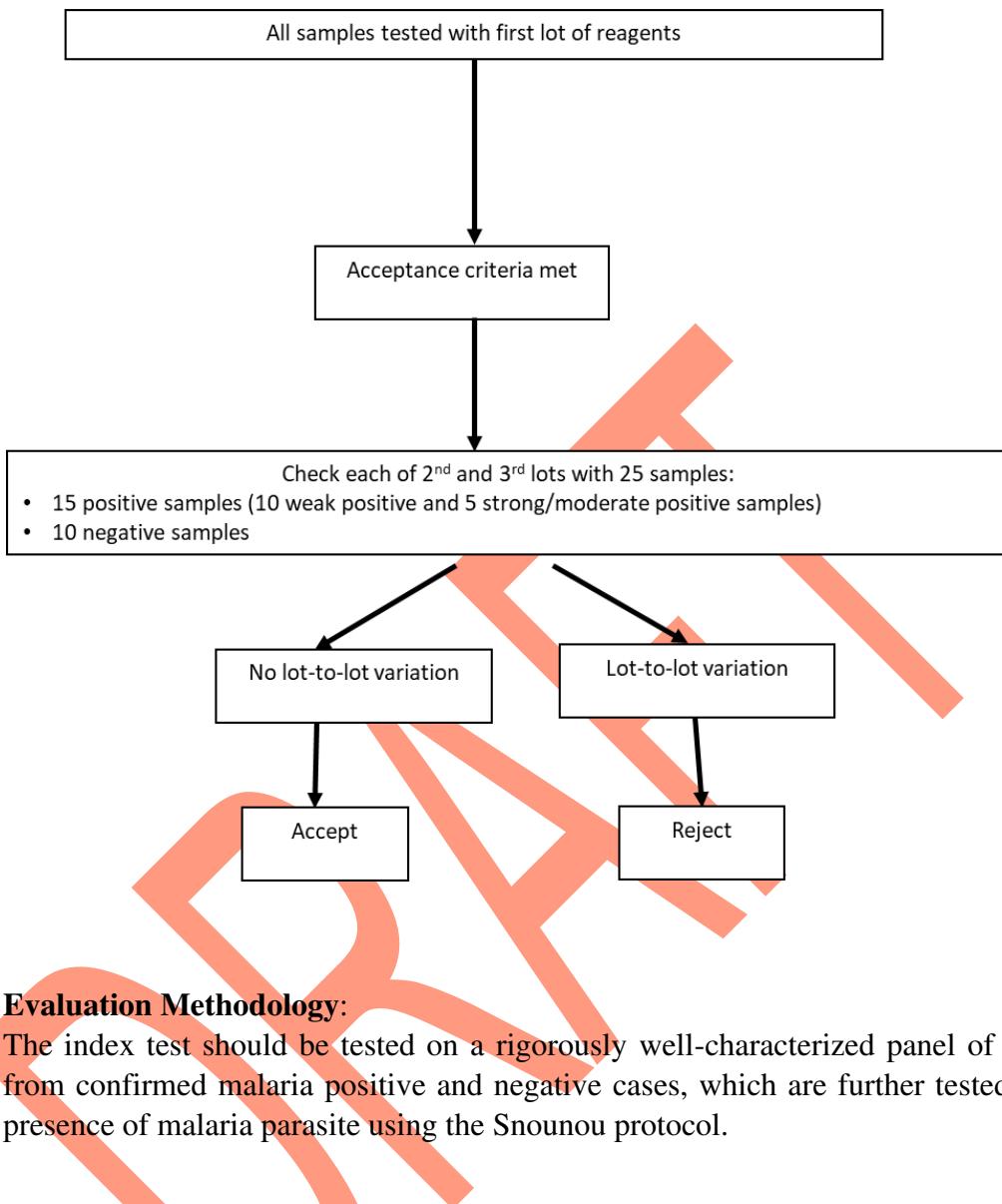
457 **4. Sample size for lot-to-lot reproducibility**

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

462

Refer the flowchart below (Fig. 1):

Fig.1: Lot-to-lot reproducibility



463
464
465
466 **5. Evaluation Methodology:**

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

470
471 **6. Interpretation of results:**

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

473
474 **7. Resolution of discrepant results:**

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

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

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

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

483

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485
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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: $\geq 90\%$ Specificity: $\geq 95\%$	Minimum no. of Positive samples = 140 Minimum no. of Negative samples = 80
Pv ELISA	Sensitivity: $\geq 75\%$ Specificity: $\geq 95\%$	Minimum no. of Positive samples = 295 Minimum no. of Negative samples = 80
Pf ELISA	Sensitivity: $\geq 90\%$ Specificity: $\geq 95\%$	Minimum no. of Positive samples = 140 Minimum no. of Negative samples = 80

487

Cross-reactivity: Nil

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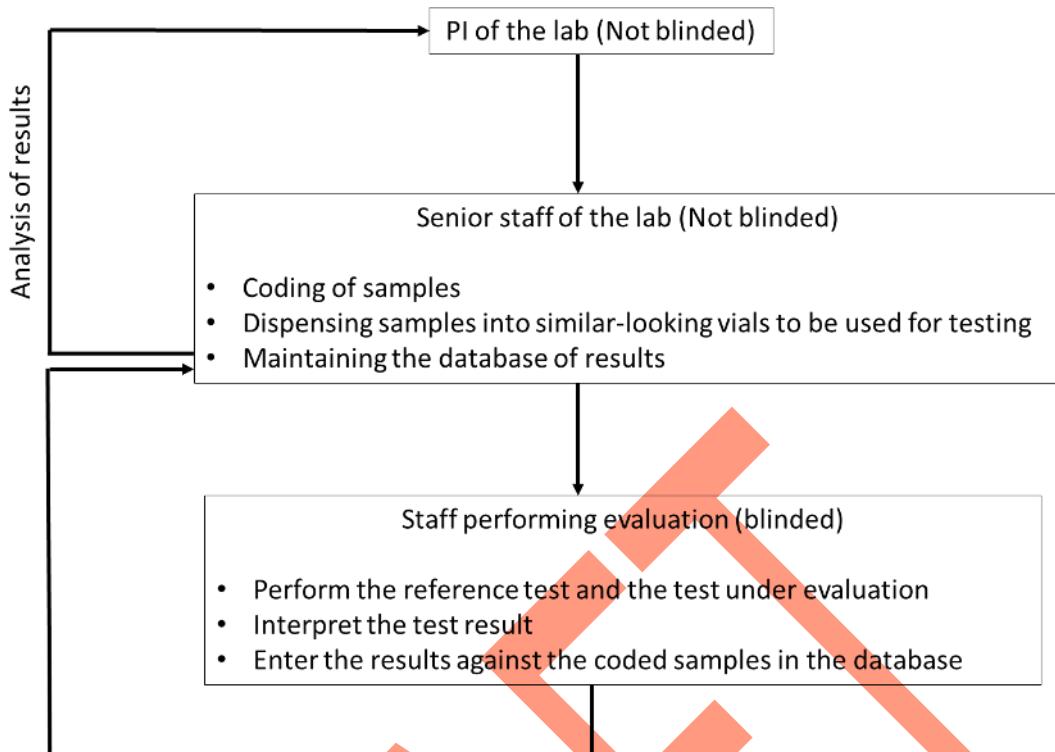
9. Blinding of laboratory staff

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.

501

502

Fig.2: Blinding in evaluation exercise



503

504

505 10. Publication Rights

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

508 **After following due procedure as defined in this document, once any kit is found to be
509 Not of Standard Quality, thereafter, no request for repeat testing of the same kit will be
510 acceptable.**

511 **Any request of re-validation from the same manufacturer for the same test type will only
512 be entertained after a minimum of 3 months and only if a high-level technical summary
513 of modifications or functional improvements to the kit design is submitted, without
514 explicit disclosure of proprietary information.**

515 **Clinical samples are precious, therefore, repeat evaluation of a kit using the same/
516 different well-characterized sample panel at a different laboratory may be considered
517 only for kits which claim high performance characteristics (sensitivity and specificity
518 95% and above), but which fail the performance evaluation by a margin of 5%.**

519

520

521 **VI. References:**

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

525

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

	Negative samples (provide details, including cross reactivity panel)	
--	----------------------------------------------------------------------	--

532

533

534 **Results:**

535

		Samples with confirmed disease status (Further confirmed by Snounou protocol/ FDA approved assay)		
		Positive	Negative	Total
Name of malaria ELISA kit	Positive			
	Negative			
	Total			

536

537

	Estimate (%)	95% CI
Sensitivity		
Specificity		

538

539 • Details of cross reactivity with other agents:

540 • **Conclusions:**

- 541 ○ Sensitivity, specificity
- 542 ○ 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.)*

546 **Disclaimers**

- 547 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 Note: This report is exclusively for Kit (Lot No.....) manufactured by
550 (Supplied by

551

552 Evaluation Done on

553 Evaluation Done by

554

555 Signature of Director/ Director-In-charge Seal
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557 *****End of the Report*****

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580 **Performance evaluation protocol for Malaria real-time PCR kits**

581 **I. Background:**

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

587 **II. Purpose:**

588 To evaluate the performance characteristics of Malaria real-time PCR (RT-PCR) kits using
589 irreversibly de-identified leftover archived/ spiked clinical samples.

590 **III. Requirements:**

- 591 1. Instructions for use (IFU)
- 592 2. Supply of kits under evaluation (with batch no. and lot no. ; Manufacturing and
593 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 **IV. Ethical approvals:**

600 Performance evaluation activities using irreversibly de-identified leftover clinical samples
601 are exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for
602 Laboratory Validation Testing, 2024.

603 Investigators are required to submit a self-declaration form, as outlined in the ICMR
604 guidelines, to the institutional authorities and ethics committee for information.

606 **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) Laboratory accreditation: Accreditation for at least one of the Quality management systems
614 (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189),
615 PT provider (ISO: 17043) or CDSCO approved Reference laboratory.
 - 616 b) It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in
617 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
619 training and competency testing on the following at referral level malaria labs before
620 initiation of MDTL activity:

- 621 ➤ Preparation and characterization of evaluation panel for the respective IVD kit.
- 622 ➤ Management of RDT kits (specific for *Plasmodium falciparum* / *Plasmodium vivax*)
623 received for performance evaluation (Verification/Storage/Unpacking etc.).
- 624 ➤ Perform tests interpretation and documentation of results, and reporting.
- 625 ➤ Data management and safety and confidentiality.

1. Preparation of evaluation sample panel for Malaria

627 To evaluate the performance of malaria RT-PCR IVD kit, a well characterized species specific
628 malaria whole genome panel is required. Hence, statistically significant number of whole blood
629 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*
632 *of the panel should be checked periodically with appropriate tests (including parasite culture)*
633 *as needed.*

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.*

636

2. DNA extraction

638 DNA extraction should be performed using a standard protocol/kit as recommended by the
639 manufacturer, or fully automated DNA extractor may be used (as per manufacturer's
640 instruction and compatible reagent kits).

641 Note: If the manufacturer of the index test recommends a specific DNA extraction kit, it needs
642 to be provided by the manufacturer, if the evaluation lab is unable to procure the same.

3. Real-time PCR system:

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

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

4. Internal Control/Extraction Control:

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

5. Reference assay:

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

657 All positive samples should be confirmed positive by the reference assay(s).

658 All negative samples should be confirmed negative by the reference assay(s).

659

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:

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

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

- $n(se)$ 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

689

690

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

<i>Sensitivity</i>	<i>Sample size: Minimum number of positive samples#</i>	<i>Composition of positive samples</i>
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

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

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

<i>Specificity</i>	<i>Sample size: Minimum number of negative samples#</i>	<i>Composition of negative samples</i>
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

699

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

703

704 **Sample panel composition:**

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

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

717

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

722

723 Consistent ELISA quantification results should be obtained in ≥ 3 runs of ELISA
724 experiments performed for each of the three antigens (PfHRP2, LDH and aldolase),
725 with the results obtained at the 200 p/ μ L and the 2,000 p/ μ L being consistent with each
726 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.

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

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

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

734

735 **7. Test reproducibility**

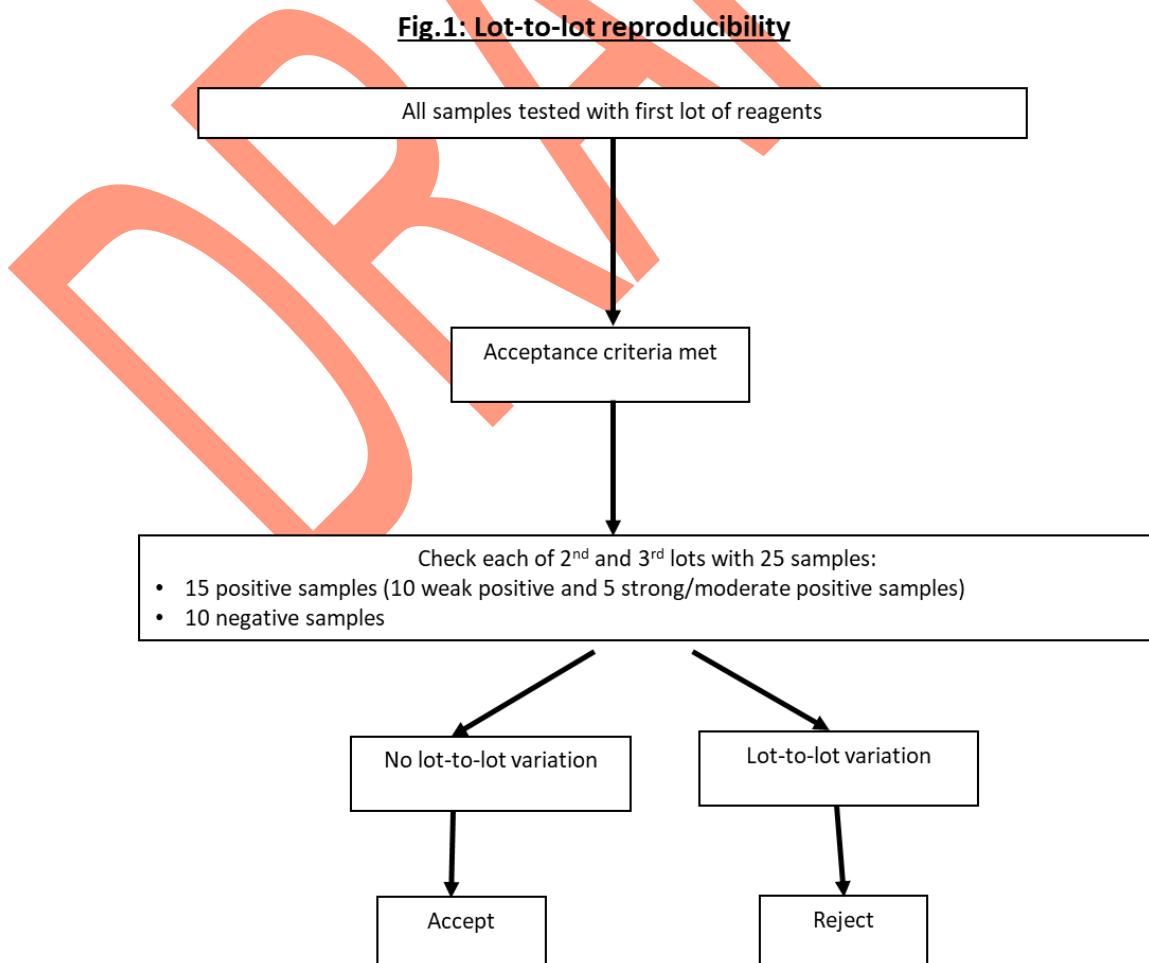
736 **A. Lot-to-lot reproducibility:**

- 737 **• Sample size for lot-to-lot reproducibility**

738 Three lots of an assay shall be evaluated. The first lot shall be evaluated on the
739 entire panel of samples (statistically significant sample size). For the subsequent
740 two lots, 25 samples should be used for evaluation (15 positive samples including
741 10 weak positive samples and 5 moderate/strong positive samples, and 10 negative
742 samples).

743 Refer the flowchart below (Fig. 1):

744



745

746

747

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

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

755

756 **8. Testing Methodology:**

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

758

759 **9. Interpretation of results:**

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

761

762 **10. Resolution of discrepant results:**

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

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

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

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

771

772

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 $\geq 98\%$ Specificity $\geq 98\%$ Limit of detection: 1 parasite/ μ l Invalid test rate: $\leq 5\%$	Minimum no. of Positive samples = 80 Minimum no. of Negative samples = 80
Pv PCR	Sensitivity $\geq 95\%$ Specificity $\geq 98\%$ Limit of detection: 1-2 parasites/ μ l Invalid test rate: $\leq 5\%$	Minimum no. of Positive samples = 80 Minimum no. of Negative samples = 80
Multiplex PCR - Pf & Pv	For Pf: • Sensitivity: $\geq 98\%$ • Specificity: $\geq 98\%$	For Pf: Minimum no. of Positive samples = 80

	<ul style="list-style-type: none"> • Absolute precision 5% • 95% CI • Invalid test rate $\leq 5\%$ • Limit of detection: 1 parasite/μl <p>For Pv:</p> <ul style="list-style-type: none"> • Sensitivity: $\geq 95\%$ • Specificity: $\geq 98\%$ • Absolute precision 5% • 95% CI • Invalid test rate $\leq 5\%$ • Limit of detection: 1-2 parasites/μl 	<p>Minimum no. of Negative samples = 80</p> <p>For Pv:</p> <p>Minimum no. of Positive samples = 80</p> <p>Minimum no. of Negative samples = 80</p>
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774 Cross-reactivity: nil
 775 Invalid test rate: $\leq 5\%$

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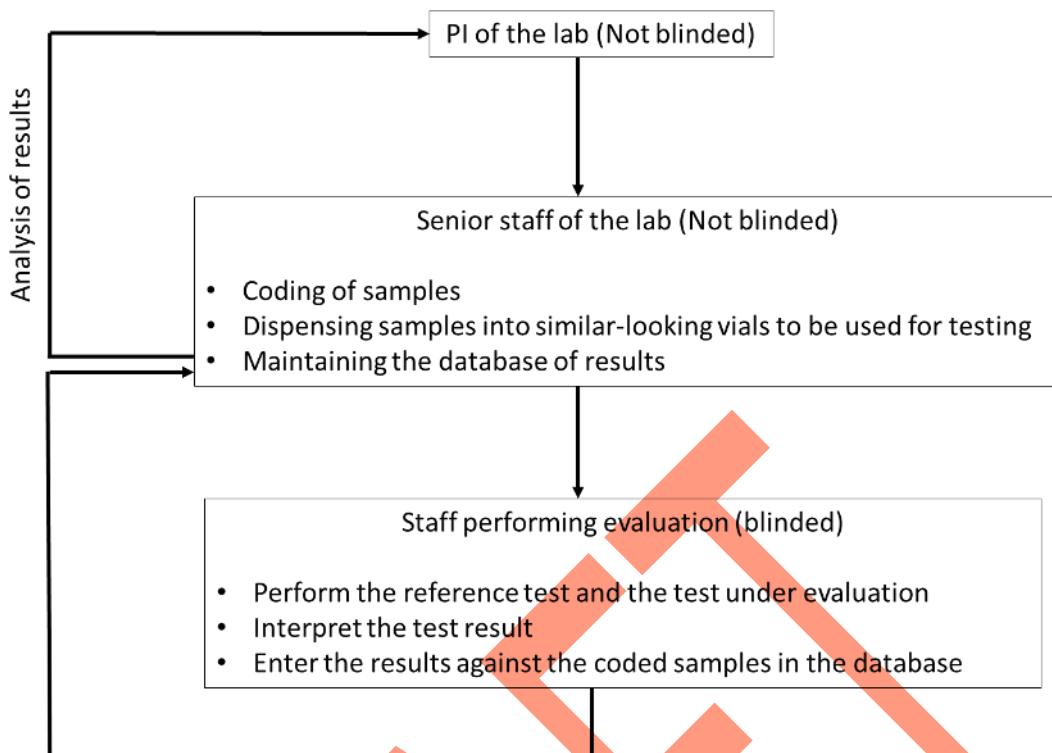
779

12. Blinding of laboratory staff

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

789

790 Fig.2: Blinding in evaluation exercise



791

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794

795 **13. Publication Rights**

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

797 author(s).

798

799 **After following due procedure as defined in this document, once any kit is found to be**

800 **Not of Standard Quality, thereafter, no request for repeat testing of the same kit will be**

801 **acceptable.**

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

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

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

805 **explicit disclosure of proprietary information.**

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

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

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

809 **95% and above), but which fail the performance evaluation by a margin of 5%.**

810

811

812 **VI. References:**

813 1. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, Do Rosario VE, et al.

814 High sensitivity of detection of human malaria parasites by the use of nested

815 polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315–
816 20.

817 2. Ramírez AM, Tang THT, Suárez ML, Fernández AA, García CM, Hisam S, Rubio
818 JM. Assessment of Commercial Real-Time PCR Assays for Detection of Malaria
819 Infection in a Non-Endemic Setting. Am J Trop Med Hyg. 2021 Oct
820 12;105(6):1732-1737. doi: 10.4269/ajtmh.21-0406. PMID: 34662870; PMCID:
821 PMC8641344.

822 3. Bouzayene, A., Zaffarullah, R., Bailly, J. *et al.* Evaluation of two commercial kits
823 and two laboratory-developed qPCR assays compared to LAMP for molecular
824 diagnosis of malaria. *Malar J* 21, 204 (2022). <https://doi.org/10.1186/s12936-022-04219-1>

825

826 4. Aschar M, Sanchez MCA, Costa-Nascimento MJ, Farinas MLRN, Hristov AD,
827 Lima GFMC, Inoue J, Levi JE, Di Santi SM. Ultrasensitive molecular tests
828 for *Plasmodium* detection: applicability in control and elimination programs and
829 reference laboratories. Rev Panam Salud Publica. 2022 Mar 28;46:e11. doi:
830 10.26633/RPSP.2022.11. PMID: 35355692; PMCID: PMC8959250.

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833 **VII. Performance evaluation report format**

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REPORT FORMAT

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Name of the Laboratory

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Name of the Institute, (with station)

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Certificate of Analysis

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File No.: _____

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

	Negative samples (provide details, including cross reactivity panel)	
--	----------------------------------------------------------------------	--

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860 **Results:**

861

		Reference assay (name)		
		Positive	Negative	Total
Name of malaria real time PCR kit	Positive			
	Negative			
Total				

862

863

	Estimate (%)	95% CI
Sensitivity		
Specificity		

864

865 • Details of cross reactivity with other agents:

866 • **Conclusions:**

867 ○ Sensitivity, specificity

868 ○ Performance: **Satisfactory / Not Satisfactory**

869 (*Sensitivity and specificity have been assessed in 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.*)

872 **Disclaimers**

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

875 Note: This report is exclusively for Kit (Lot No.....) manufactured by
876 (Supplied by

877

878 Evaluation Done on

879 Evaluation Done by

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881 Signature of Director/ Director-In-charge Seal
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883 *****End of the Report*****

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906 **Field evaluation protocol for combo Malaria Rapid Diagnostic Test (RDT) kits**
907 **(detecting *P. vivax* and *P. falciparum*)**

908 **I. Background:**

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

913 **II. Purpose:**

914 To evaluate the performance characteristics of Malaria RDT kits (detecting *P. vivax* and/or *P. falciparum*) in the diagnosis of Malaria parasite infection in individuals with unknown disease
915 status.

917 **III. Requirements:**

- 918 1. Supply of kits under evaluation (with batch no. and lot no. Manufacturing and Expiry
919 dates other required details). If the kit to be evaluated works in a closed system format,
920 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. Ethical approval:**

925 The study will be initiated after approval from the institutional human ethics committee.

926 **V. Procedure:**

927 **1. Study design/type:** Cross-sectional study

928 **2. Preparation of Evaluation sites/laboratories:**

929 Identified IVD kit evaluation laboratories should establish their proficiency through
930 A. Laboratory accreditation: Accreditation for at least one of the Quality management
931 systems (accreditation for Testing Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO:
932 15189), PT provider (ISO: 17043) or CDSCO approved Reference laboratory.

933 It is recommended that malaria Medical Device Testing Labs (MDTLs) participate in
934 Quality Control exercises such as EQAP (External Quality Assurance Programme).

936 **B. Staff training:** All the staff involved in IVD kit evaluation should undergo hands on
937 training and competency testing on the following at referral level malaria labs before
938 initiation of MDTL activity:

- 939 ➤ Preparation and characterization of evaluation panel for the respective IVD kit.
- 940 ➤ Management of RDT kits (specific for *Plasmodium falciparum* / *Plasmodium vivax*)
941 received for performance evaluation (Verification/Storage/Unpacking etc.).

942 ➤ Perform tests interpretation and documentation of results, and reporting.

943 ➤ Data management and safety and confidentiality.

944 3. Sample size for performance evaluation:

945 Sample sizes of positive and negative samples against different values of sensitivity and
946 specificity are provided in Tables 1 and 2. Sample sizes have been calculated assuming
947 95% level of significance, an absolute precision of 5%, and invalid test rate 5%. It is further
948 assumed that at least 5% of the individuals attending the health care facilities for acute
949 febrile illness and suspected for Malaria will be positive for Malaria (*P. vivax* and *P.
950 falciparum*). Appropriate sample size has to be chosen from the tables according to the
951 values of sensitivity and specificity being claimed by the manufacturer. If a claimed
952 sensitivity/specificity is not present in the table, the manufacturer needs to consider the
953 sample size associated with the largest sensitivity/specificity provided in the table that is
954 smaller to the claimed value (that is, as per the next smaller value of the sensitivity/
955 specificity available in the table). For example, if a manufacturer claims a sensitivity of
956 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly,
957 a claim of 87% specificity would require usage of the sample size outlined for 85%
958 specificity. Sample sizes are calculated using the following formulae and assumption of
959 5% for prevalence of the disease:

$$960$$

$$961 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR) \times P}$$

$$962 n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR) \times P}$$

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- *n (se)* is the minimum number of individuals to be enrolled to obtain the requisite number of positive samples.
- *n (sp)* is the minimum number of individuals to be enrolled to obtain the requisite number of negative samples.
- *Z²* is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to *Z²* = 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

980 95% specificity, the sample size required will be 1600 (maximum of 1600 and 84).
981 Please note that since the prevalence is low, the final sample size is generally expected
982 to be governed by the assumed sensitivity.

983

984 *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.

987

988 Since a large number of febrile cases have to be enrolled to obtain the requisite number of
989 malaria positive samples, enrolling the number of cases mentioned in Table 1 will be sufficient
990 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 ○ Chills, sweating, headache, tiredness, nausea and vomiting, jaundice, splenomegaly

995 **5. Exclusion criteria**

996 ● Individuals not satisfying inclusion criteria
997 ● Individuals with already known positive history for other pathogens

998 **6. Reference assay:**

999 WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved Malaria PCR assay/
1000 Snounou protocol should be used as reference assay.

1001 **7. Study implementation:**

1002 The patients displaying Malaria like illness will be recruited into the study and five ml of whole
1003 blood will be collected in EDTA tubes. The whole blood sample will be subjected to the
1004 reference and the index test.

1005 The disease status of the enrolled cases will be unknown.

1006 **8. Evaluation method:**

1007 The index test and the reference tests should be run simultaneously on the sample panel, and
1008 results should be recorded.

1009

1010 **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 *False positive samples: These are samples negative by reference assay and positive by index test.*

1018 *False negative samples: These are samples positive by reference assay and negative by index test.*

1020

1021 **A. Cross reactivity:**

1022 The RDT kit should have been evaluated against the following cross reactivity panel during
1023 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 • *Serum reactive for RA factor – low positive and high positive (n=15 samples)*
- 1029 • *Serum reactive for TPHA/other specific test for syphilis (n= 10 samples)*

12. Statistical analysis:

1031 Sensitivity and specificity will be calculated.

1032 Interim analysis of data shall be conducted on completing evaluation of 25%, 50% and 75% of
1033 samples. If, at any point, the performance of the assay is found to be not satisfactory, the assay
1034 shall not be evaluated further. Evaluation fee shall be charged accordingly.

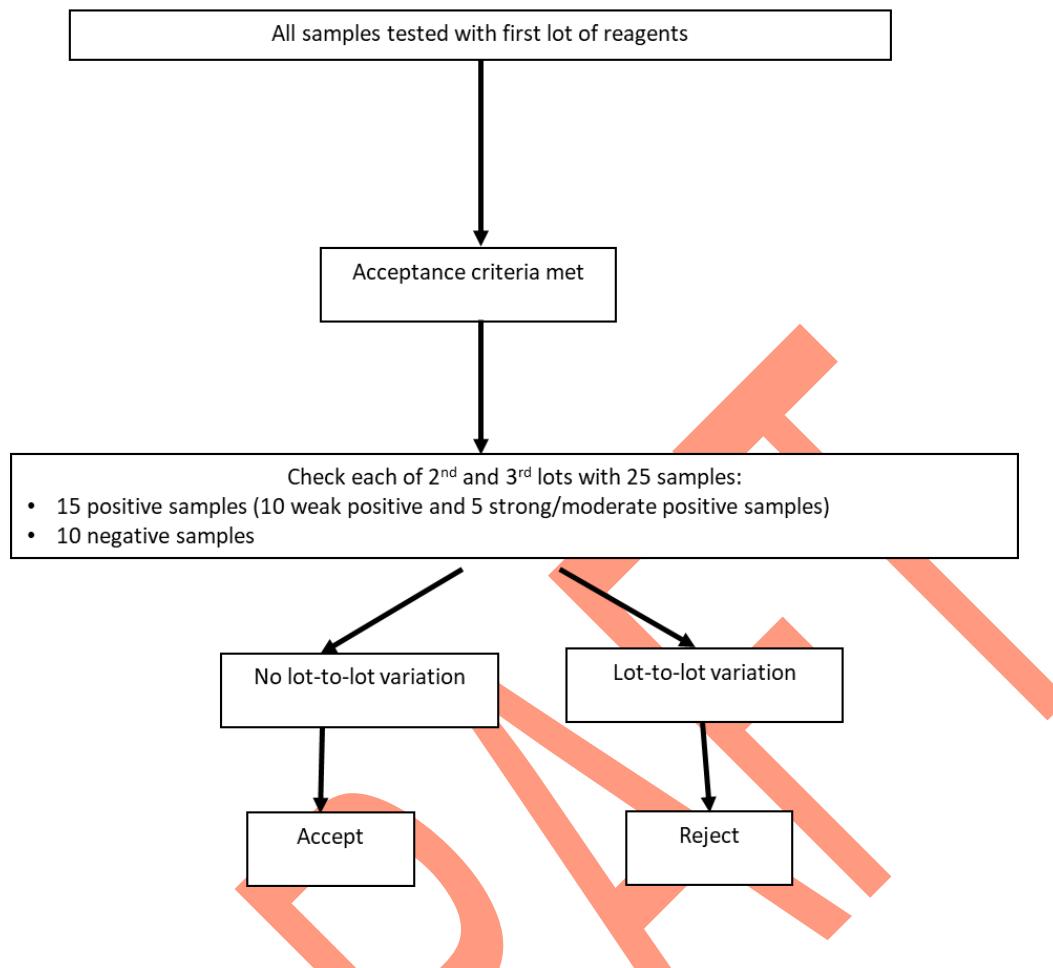
13. Test reproducibility

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

1064 maintaining the database of results.. The data should be analyzed only by the PI of the
1065 evaluating lab.

1066 **16. Acceptance criteria:**

1067 Expected sensitivity: $\geq 75\%$ for *P. vivax* and $\geq 95\%$ for *P. falciparum*

1068 Expected specificity: $\geq 90\%$ for *P. vivax* and $\geq 95\%$ for *P. falciparum*

1069 Cross-reactivity: Nil

1070 Invalid test rate: $\leq 5\%$

1072 To achieve at least the performance characteristics outlined in the acceptance criteria
1073 for *P. vivax*, ≥ 6100 individuals satisfying the case definition need to be enrolled to
1074 obtain the requisite number of positive samples. This sample size is sufficient for
1075 requisite number of negative samples.

1076 To achieve at least the performance characteristics outlined in the acceptance criteria
1077 for *P. falciparum*, ≥ 1600 individuals satisfying the case definition need to be enrolled
1078 to obtain the requisite number of positive samples. This sample size is sufficient for
1079 requisite number of negative samples.

1081 Recruitment should be terminated once the desired number of positive cases is enrolled
1082 and tested.

1084 **17. Publication Rights**

1085 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead
1086 author(s).

1087 **After following due procedure as defined in this document, once any kit is found to be
1088 Not of Standard Quality, thereafter, no request for repeat testing of the same kit will be
1089 acceptable.**

1090 **Any request of re-validation from the same manufacturer for the same test type will only
1091 be entertained after a minimum of 3 months and only if a high-level technical summary
1092 of modifications or functional improvements to the kit design is submitted, without
1093 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%.**

1099 **VI. References:**

1. Ministry of Health and Family Welfare. Guidelines for Bivalent RDT. Available at: [guidelines-for-bivalent-rdt.pdf \(mohfw.gov.in\)](http://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](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 polymerase chain reaction. Molecular and Biochemical Parasitology. 1993;61:315–
1109 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_files/DistrictSurvMan/Module5.pdf [Accessed on 25th June 2024]

1114 5. CDC. National Notifiable Diseases Surveillance System (NNDSS). Malaria
1115 (Plasmodium spp.) 2014 Case Definition. Available at:
1116 <https://ndc.services.cdc.gov/case-definitions/malaria-2014/> [Accessed on 28th June,
1117 2024]

1118 6. Kannambath R, Rajkumari N, Sivaradjy M. Prevalence of malaria: A 7-year trend
1119 analysis from a tertiary care center, Puducherry. Trop Parasitol. 2023 Jan-
1120 Jun;13(1):28-33. doi: 10.4103/tp.tp_41_22. Epub 2023 May 19. PMID: 37415756;
1121 PMCID: PMC10321582.

1123 **VII. Performance evaluation report format**

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REPORT FORMAT

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Name of the Laboratory

1146

Name of the Institute, (with station)

1147

Certificate of Analysis

1148

File No.: _____

Name of the product (Brand /generic)					
Name and address of the legal manufacturer					
Name and address of the actual manufacturing site					
Name and address of the Importer					
Name of supplier: Manufacturer/Importer/Port office of CDSCO/State licensing Authority					
Lot No / Batch No.:					
Product Reference No/ Catalogue No					
Type of Assay					
Kit components					
Manufacturing Date					
Expiry Date					
Pack size (Number of tests per kit)					
Intended Use					
Number of Tests Received					
Regulatory Approval:					
Import license / Manufacturing license/ Test license					
License Number: Issue date:					
Valid Up to:					
Application No.					
Sample Panel	<table border="1" style="width: 100%;"> <tr> <td style="width: 15%;">Sample type</td> <td></td> </tr> <tr> <td>Positive samples (provide details: strong, moderate, weak)</td> <td></td> </tr> </table>	Sample type		Positive samples (provide details: strong, moderate, weak)	
Sample type					
Positive samples (provide details: strong, moderate, weak)					

	Negative samples (provide details, including cross reactivity panel)	
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1151 Results:

1152

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

1153

1154

	Estimate (%)	95% CI
Sensitivity		
Specificity		

1155

1156 • Details of cross reactivity with other agents:

1157 • Conclusions:

1158 ○ Sensitivity, specificity

1159 ○ Performance: Satisfactory / Not Satisfactory

1160 (*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

1164 1. This validation process does not approve / disapprove the kit design

1165 2. This validation process does not certify user friendliness of the kit / assay

1166 Note: This report is exclusively for Kit (Lot No.....) manufactured by
1167 (Supplied by

1168

1169 Evaluation Done on

1170 Evaluation Done by

1171

1172 Signature of Director/ Director-In-charge Seal
1173

1174 *****End of the Report*****

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1197 **Information on Operational and Test Performance Characteristics Required from**
1198 **Manufacturers for Malaria IVD**

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 v. Any additional device/consumable like sample transfer device, pipette, tube, etc
1225 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 *****End of the Document*****

1247

1248

1



2 **STANDARD PERFORMANCE
EVALUATION PROTOCOL**
3
4 **DRAFT FOR STAKEHOLDER COMMENTS**

5
6 **NIPAH VIRUS REAL TIME PCR KIT**
7

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

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9



AUGUST, 2025
New Delhi, India

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DRAFT

36 **Performance evaluation protocol for Nipah virus real-time PCR kit**

37 **I. Background:**

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
41 evaluation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit
42 (IVD) performance.

43 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:**

47 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:**

- 51 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If
52 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
- 57 5. Laboratory supplies

58 **IV. Ethical approvals:**

59 Performance evaluation activities using irreversibly de-identified leftover clinical samples are
60 exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory
61 Validation Testing, 2024.

62 Investigators are required to submit a self-declaration form, as outlined in the ICMR
63 guidelines, to the institutional authorities and ethics committee for information.

64 **V. Procedure:**

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

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

70

71 A. Availability of BSL-4 facility for handling of Nipah virus positive specimens

72 B. Accreditation for at least one Quality management system for at least one respiratory viral

73 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

75 approved Reference laboratory.

76 C. Staff training: All the staff involved in Nipah virus IVD evaluation should undergo hands on

77 training and competency testing on following

78 ➤ BSL-4 practices

79 ➤ Nipah virus culture and handling

80 ➤ Preparation & characterization of reference sample panel

81 ➤ Handling of Nipah virus RT-PCR kits received for performance evaluation

82 (Verification/Storage/Unpacking etc).

83 ➤ Testing, interpreting, recording of results & reporting

84 ➤ Data handling, data safety & confidentiality

3. Preparation of Nipah virus RNA evaluation panel

86 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

88 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

91 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.

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).

101 **7. Reference assay:**

102 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.

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

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

107

108 **8. Sample size for performance evaluation:** Sample size is calculated assuming 95%
109 sensitivity and specificity of the index test, 95% confidence level, absolute precision of 5% and
110 $\leq 5\%$ invalid test rate. A minimum of 77 (rounded to 80) positive clinical samples and a minimum
111 of 77 (rounded to 80) negative clinical samples are required. Sample sizes are calculated using the
112 formulae:

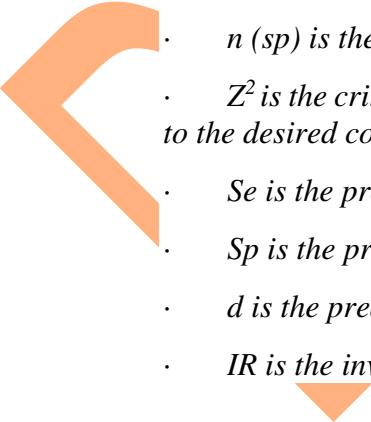
113

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

115

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

116

- 117  · *n (se) is the minimum number of positive samples.*
- 118 · *n (sp) is the minimum number of negative samples.*
- 119 · *Z² is the critical value from the standard normal distribution corresponding*
120 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*
- 121 · *S_e is the predetermined sensitivity.*
- 122 · *S_p is the predetermined specificity.*
- 123 · *d is the predetermined marginal error (5%)*
- 124 · *IR is the invalid test rate*

126

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

133

134

135

136 **9. Sample panel composition:**

137

A. Human samples

138

A.1 Positive samples (Minimum n=80 for each sample type): Clinical/ Spiked samples positive by the reference real-time PCR assay

140

A.1.1 Strong positive (Ct value <25) = 24 samples

141

A.1.2. Moderate positive (Ct value between 25-30) = 28 samples

142

A.1.3 Weak positive (Ct value >30 to 34) = 28 samples

143

The sample type should be as per the index test IFU. If an assay claims to detect Nipah virus RNA in several sample types, attempt should be made to use 80 positive samples across each sample type, or at least the sample types available with the evaluating lab. This relaxation is provided since clinical samples are scarce and obtained only during outbreaks occurring every few years in India, which necessitates using spiked clinical samples. The latter is difficult since Nipah virus is a BSL-4 level pathogen and its handling requires sophisticated laboratory setup and trained manpower.

150

In case the requisite number of specimens for a particular sample type are not available and a smaller number of samples are used for performance evaluation (i.e., sample size calculated assuming higher performance characteristics), it is necessary to ensure that the study has adequate power for acceptance of the evaluation results in case the assay falls short of the assumed performance characteristics.

155

Note:

156

If clinical samples positive for Nipah virus are not available, tissue culture fluid (Heat-inactivated) from reference laboratories can be used, spiked in serum/urine/Throat swab samples to obtain the panel with Ct value <25, 25-30 and >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 samples should be as follows

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

<p>A.2.1 Samples from cases having similar illness/ spiked samples which are RT-PCR positive for common pathogens but negative for Nipah virus</p>	<p>Samples from individuals presenting with ARI/ILI/SARI (n=45):</p> <p>5 positive clinical/ spiked samples from each of the following diseases:</p> <ol style="list-style-type: none"> 1. Influenza A virus @ 2. Influenza B virus @ 3. SARS-CoV-2 @ 4. RSV A/B @ 5. HPIV @ 6. HMPV @ 7. Adenovirus @ 8. Seasonal Coronaviruses * 9. Rhinovirus/Enterovirus* <p><i>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.</i></p>	<p>Samples from cases of AES (n=35):</p> <p>5 positive clinical/ spiked samples from each of the following diseases:</p> <ol style="list-style-type: none"> 1. Japanese Encephalitis @ 2. Dengue @ 3. HSV @ 4. VZV @ 5. West Nile Virus * 6. Chandipura virus * 7. Rabies virus * <p><i>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.</i></p>	<p>5 positive clinical/ spiked samples from each of the following diseases, presenting with respiratory and/or encephalitis symptoms (n=20):</p> <ol style="list-style-type: none"> 1. Measles 2. Rubella 3. Mumps 4. SARS-CoV-2
<p>A.2.2 Samples from cases with acute respiratory disease/ acute encephalitis/ acute febrile illness and RT-PCR negative for the above-mentioned pathogens and Nipah virus</p>	<p>25</p>	<p>35</p>	<p>40</p>

A.2.3 Healthy/ asymptomatic cases from endemic regions negative for Nipah virus	10	10	20
Serum/ throat swab/ urine samples collected from the same case may be used for evaluation.			

164 **10. Evaluation method:**

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

167

168 **11. Interpretation of results:**

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

170

171

172 **12. Resolution of discrepant results:**

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

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

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

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

178

180 **13. Test reproducibility**

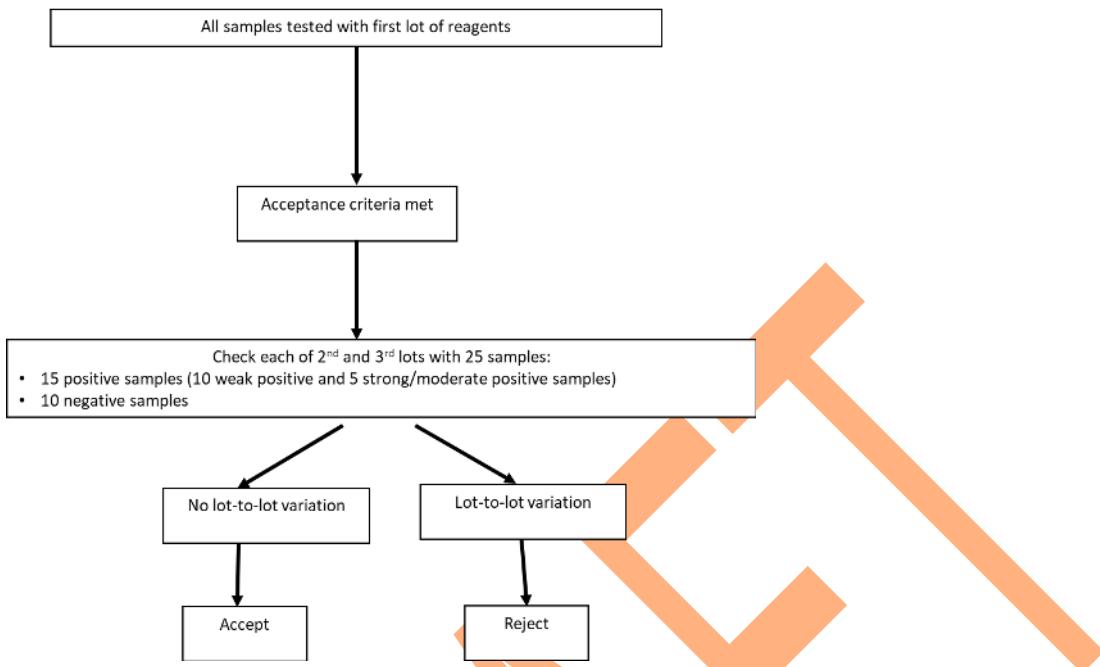
181 **A. Sample size for lot-to-lot reproducibility**

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

- 184 • First lot of the assay: should be tested on statistically significant number of positive
185 and negative samples as calculated in the protocol.
- 186 • Second lot of the assay: should be tested on 25 samples (15 positive samples
187 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative
188 samples).
- 189 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising
190 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

192 Refer the flowchart below (Fig. 1):

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



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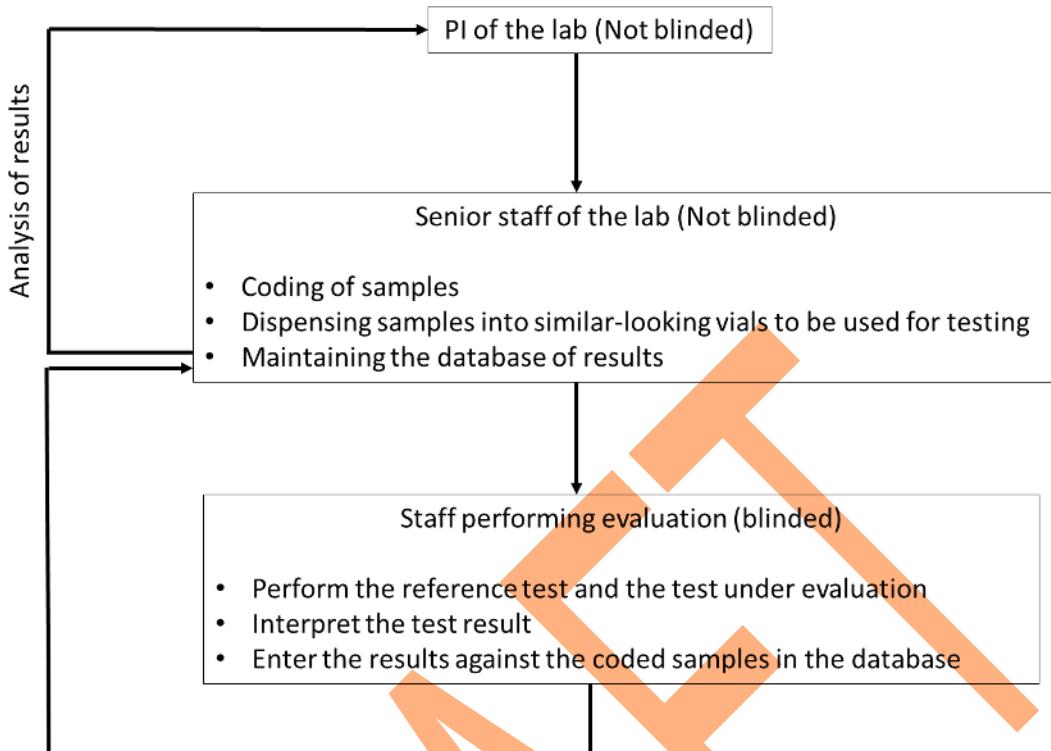
14. Blinding of laboratory staff

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

205

206

Fig.2: Blinding in evaluation exercise



207

208

209 15. Acceptance Criteria

210 Expected sensitivity: $\geq 95\%$

211 Expected specificity: $\geq 98\%$

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

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

214

215 16. Publication Rights:

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

217

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 acceptable.**

221 **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.**

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

229

230 **VI. References:**

- 231 1. Yadav PD, Majumdar T, Gupta N, Kumar MA, Shete A, Pardeshi P, Sultana S, Sahay RR, Manoj
232 MN, Patil S, Floura S, Gangakhedkar R, Mourya DT. Standardization & validation of Truenat™
233 point-of-care test for rapid diagnosis of Nipah. Indian J Med Res. 2021 Apr;154(4):645-649. doi:
234 10.4103/ijmr.IJMR_4717_20. PMID: 34854433; PMCID: PMC9205002.
- 235 2. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –
236 Diagnostic Assessment TGS-3. 2017. Available at:
237 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-
238 eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)

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240 **VII. Performance evaluation report format**

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257 **PERFORMANCE EVALUATION REPORT FOR NIPAH VIRUS REAL-TIME PCR**
258 **KITS**

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

259

260 **Results**

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

261

	Estimate (%)	95% CI
Sensitivity		
Specificity		

262

263

- Details of cross reactivity with other Paramyxoviruses:

264 **Conclusions:**

- 265 ○ Sensitivity, specificity
- 266 ○ Cross reactivity
- 267 ○ Invalid test rate
- 268 ○ Performance: **Satisfactory / Not satisfactory**

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

271 **Disclaimers**

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

274 Note: This report is exclusively for Nipah virus..... Kit (Lot No.....) manufactured by

275 (supplied by)

276 Evaluation Done on

277 Evaluation Done by

278 Signature of Director/ Director-In-charge Seal

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

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

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 *****End of the Document*****



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2 STANDARD PERFORMANCE 3 EVALUATION PROTOCOL 4 DRAFT FOR STAKEHOLDER COMMENTS

5
6 **CHANDIPURA VIRUS REAL TIME PCR
7 KIT**

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



9
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AUGUST, 2025
New Delhi, India

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DRAFT

37 **Performance evaluation protocol for Chandipura virus real-time PCR kits**

38 **I. Background**

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
42 evaluation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit
43 (IVD) performance.

44 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
46 accordance with the candidate test's instructions for use.

47 **II. Purpose:**

48 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.

50 **III. Requirements:**

51 1. **Kits Under Evaluation:** Include detailed information such as batch number, lot number, expiry
52 date, and other relevant specifications. For kits designed to operate within a closed system,
53 manufacturers must provide the necessary equipment and consumables for testing.

54 2. **Evaluation Sites/Laboratories:** Identify laboratories equipped with the required instruments
55 and infrastructure to conduct the evaluation.

56 3. **Reference Test Kits:** Use reference kits or in-house kits developed by the reference laboratory,
57 which have been validated to demonstrate satisfactory performance.

58 4. **Evaluation Panel:** Prepare a panel of well-characterised clinical samples from confirmed cases
59 or spiked samples for a comprehensive evaluation.

60 5. **Laboratory Supplies:** Ensure all necessary laboratory materials and supplies are available for
61 the evaluation process.

62 **IV. Ethical Approvals:**

63 Performance evaluation activities using irreversibly de-identified clinical samples are exempt from
64 ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory Validation
65 Testing, 2024.

66 Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
67 to the institutional authorities and ethics committee for information.

70

71 **V. Procedure:**

72 **1. Study design/type:** Diagnostic accuracy study using irreversibly de-identified archived
73 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 B) Staff training: All staff involved in IVD kit evaluation process should undergo hands on training
83 and competency assessment in the following areas:

- 84 • Preparation and characterization of kit evaluation panel
- 85 • Handling of Chandipura real-time PCR kits received for performance evaluation
(verification/storage/unpacking etc.).
- 86 • Testing procedures, interpretation and recording of results, and reporting
- 87 • Data handling, data safety & confidentiality

89 **3. Preparation of Chandipura RNA evaluation panel:**

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

93 The sample type for CHPV detection is Cerebrospinal fluid (CSF) and serum. If a kit claims to
94 detect CHPV in both sample types, attempt should be made to evaluate the assay across both serum
95 and CSF using statistically significant sample size for each sample type. In case all the sample
96 types mentioned in the IFU are not available with the lab, the performance evaluation report should
97 clearly mention the sample type against which the kit is evaluated, ensuring statistical rigor. There
98 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
101 as the assay under evaluation. If the manufacturer of the index test recommends a specific RNA

102 extraction kit, it needs to be provided by the manufacturer if the evaluation lab is unable to procure
103 the same.

104 **5. Real-time PCR system:**

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

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

110 **6. Internal Control/Extraction Control:**

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

113 **7. Reference assay:**

114 A WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved real time CHPV PCR
115 assay/ ICMR-National Institute of Virology, Pune developed protocol for detection of Chandipura
116 virus RNA will serve as the reference assay.

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

118 All negative samples should be confirmed negative by the reference assay and CHPV IgM.

119 **8. Sample size for performance evaluation:**

120 1. Sample size is calculated assuming 95% sensitivity and specificity of the index test, 95%
121 confidence level, absolute precision of 5% and $\leq 5\%$ invalid test rate. A minimum of 77 (rounded
122 to 80) positive clinical samples and a minimum of 77 (rounded to 80) negative clinical samples for
123 each sample type are required for performance evaluation. Sample sizes are calculated using the
124 formulae:

$$125 \\ 126 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

$$127 n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

128

129

130

- *n (se) is the minimum number of positive samples.*
- *n (sp) is the minimum number of negative samples.*
- *Z² is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to Z² = 1.96).*

134 · *Se* is the predetermined sensitivity.
 135 · *Sp* is the predetermined specificity.
 136 · *d* is the predetermined marginal error (5%)
 137 · *IR* is the invalid test rate

138

139 **9. Sample panel composition:**

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

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

143

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

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

152

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

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

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)	30 5 positive clinical/ spiked samples from each of the following diseases (confirmed by PCR): 1. Dengue virus @ 2. Japanese Encephalitis @ 3. HSV 1/2 * 4. West Nile Virus* 5. VSV *	35 1. Seven (07) positive clinical/ spiked samples from each of the following diseases: a) Japanese Encephalitis @ 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.		
<p><i>Cross reactivity panel is arranged in descending order of priority.</i> <i>The pathogens marked @ are essentially to be tested.</i> <i>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.</i></p> <p><i>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.</i></p>		

156

157 10. Evaluation method:

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

161 11. Interpretation of results:

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

163 **12. Resolution of discrepant results:**

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

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

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

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

168

169 **13. Test reproducibility:**

170 A) Sample size for lot-to-lot reproducibility:

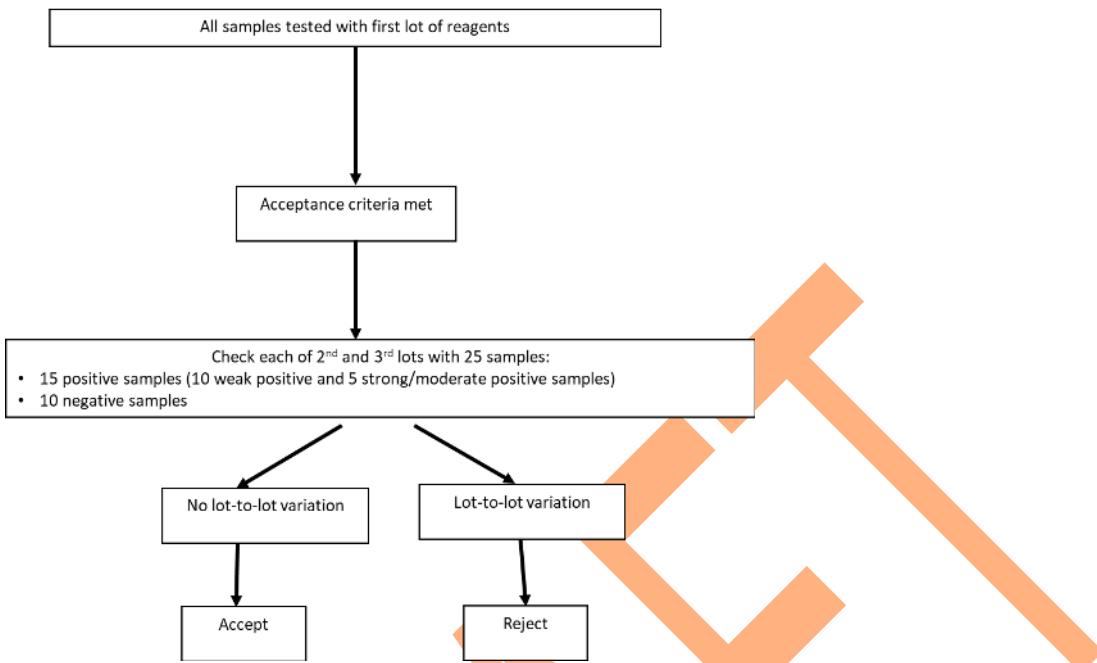
171 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be as
172 follows:

- 173 • First lot of the assay: should be tested on statistically significant number of positive and
174 negative samples as calculated in the protocol above
- 175 • Second lot of the assay: should be tested on 25 samples (15 positive samples comprising
176 10 low positives and 5 moderate/high positives, and 10 negative samples)
- 177 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising 10
178 low positives and 5 moderate/high positives, and 10 negative samples)

179 If there is no lot-to-lot variation, accept the assay.

180 Refer the flowchart below (Fig. 1):

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



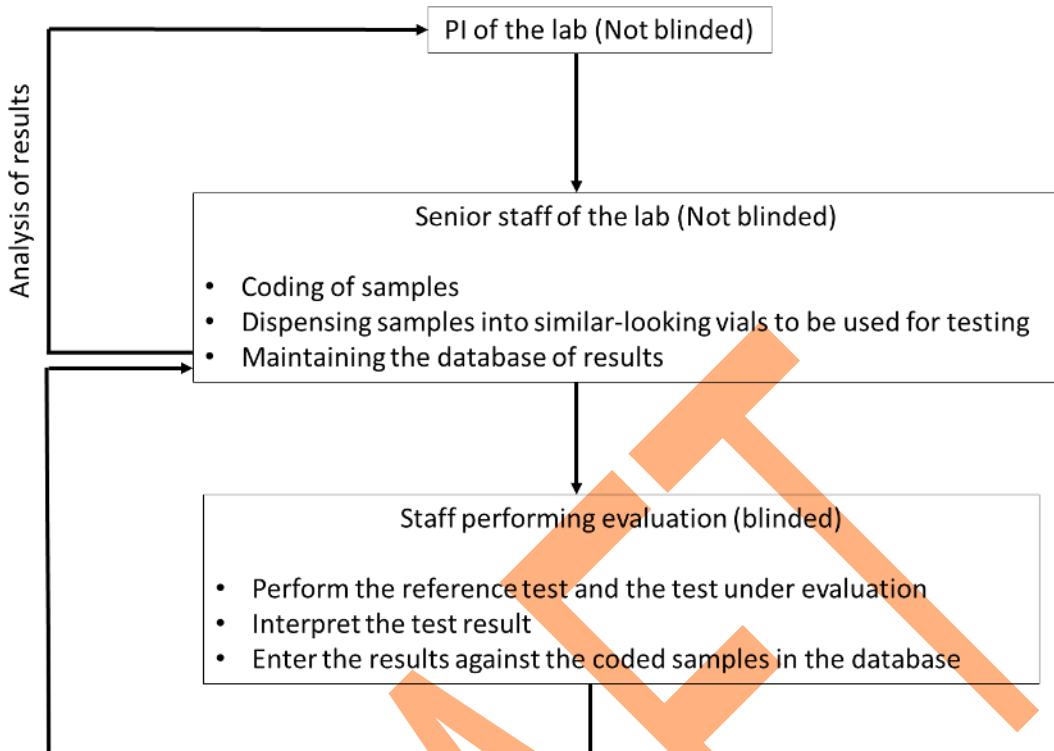
181

182 **14. Blinding of laboratory staff**

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

192

193 **Fig.2: Blinding in evaluation exercise**



194

195

15. Acceptance criteria:

197 Expected sensitivity: $\geq 95\%$

198 Expected specificity: $\geq 98\%$

199 Cross-reactivity with other rhabdoviruses: Nil

200 Invalid test rate $\leq 5\%$

201

16. Publication Rights:

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

205

206 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.

209 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.

213 Clinical samples are precious, therefore, repeat evaluation of a kit using the same/ different
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),
216 but which fail the performance evaluation by a margin of 5%.

217

218 **VI. References**

219 1. Sudeep AB, Gurav YK, Bondre VP. Changing clinical scenario in Chandipura virus
220 infection. *Indian J Med Res.* 2016;143(6):712-721. doi:10.4103/0971-5916.191929.

221 2. Sapkal GN, Sawant PM, Mourya DT. Chandipura Viral Encephalitis: A Brief Review. *Open*
222 *Virol J.* 2018 Aug 31;12:44-51. doi: 10.2174/1874357901812010044. PMID: 30288194; PMCID:
223 PMC6142667.

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

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229 **VII. Performance Evaluation Report Format**

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244 **PERFORMANCE EVALUATION REPORT FOR CHANDIPURA VIRUS REAL-TIME**
245 **PCR KITS**

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

246

247

Results

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

248

	Estimate (%)	95% CI
Sensitivity		
Specificity		

249

250

● **Conclusions:**

251

- Cross reactivity with related viruses:

252

- Invalid test rate:

253

- **Performance: Satisfactory / Not satisfactory**

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

257

258 **Disclaimers**

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

261 Note: This report is exclusively for Chandipura..... Kit (Lot No.....) manufactured by

262 (supplied by)

263 Evaluation Done on

264 Evaluation Done by

265 Signature of Director/ Director-In-charge Seal

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

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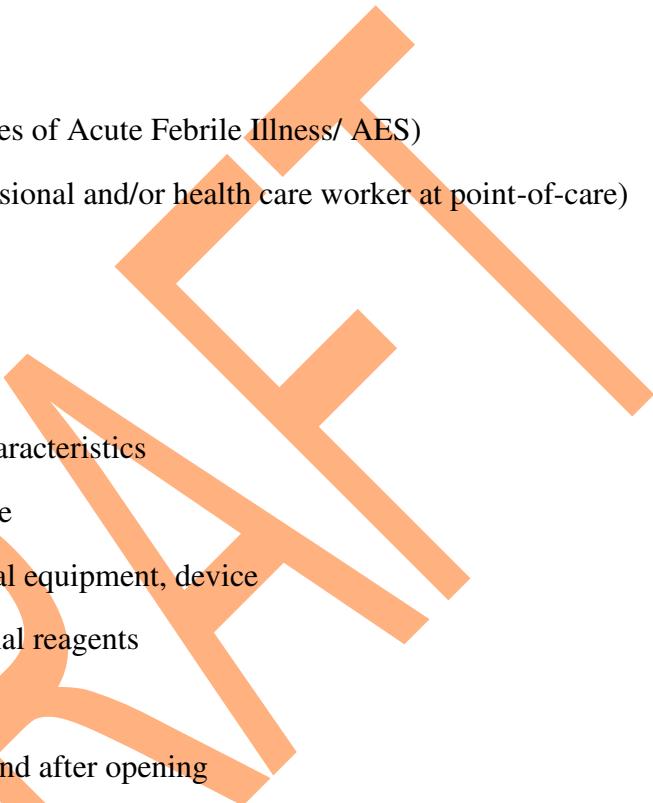
282

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

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 *****End of the Document*****

1

STANDARD PERFORMANCE EVALUATION PROTOCOL

2

DRAFT FOR STAKEHOLDER COMMENTS

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MULTIPLEX RESPIRATORY VIRUS REAL TIME PCR

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

AUGUST, 2025
New Delhi, India

12

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DRAFT

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

39 **I. Background:**

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
43 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

44 This recommendation focuses on the laboratory performance evaluation of multiplex respiratory
45 virus real time PCR kit. All clinical samples tested in the study should be evaluated in accordance
46 with the candidate test's instructions for use.

47 **II. Purpose:**

48 To evaluate the performance characteristics of multiplex respiratory virus real-time PCR kits using
49 irreversibly de-identified leftover archived clinical/spiked samples.

50 **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
55 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. Human metapneumovirus
8. Measles virus
9. Rhinovirus
10. Human Bocavirus
11. Enterovirus
12. Cytomegalovirus

57

58 **IV. Requirements:**

59 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If
60 the kit to be evaluated works in a closed system format, the manufacturer needs to supply
61 the required equipment.

62 2. Evaluation sites/laboratories (With required equipment)

63 3. Reference test kits

64 4. Characterised Evaluation panel

65 5. Laboratory supplies

66 **V. Ethical approvals:**

67 Performance evaluation activities using irreversibly de-identified leftover clinical samples are
68 exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for
69 Laboratory Validation Testing, 2024.

70 Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
71 to the institutional authorities and ethics committee for information.

72 **VI. Procedure:**

73 1. **Study design/type:** Diagnostic accuracy study using irreversibly de-identified archived/
74 spiked clinical samples

75 2. **Preparation of Evaluation sites/laboratories:**

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

78 A. Accreditation at least one of the Quality management systems for at least one respiratory viral
79 pathogen molecular testing (accreditation for Testing Lab / Calibration Lab as per ISO 17025,
80 Medical Lab as per ISO 15189, PT provider as per ISO/IEC 17043), or CDSCO approved
81 Reference laboratory.

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

84 ➤ Preparation & characterization of reference sample panel

85 ➤ Handling of multiplex respiratory virus RT-PCR kits received for performance evaluation
86 (Verification/Storage/Unpacking etc).

87 ➤ Testing

88 ➤ Data handling, data safety & confidentiality

89 3. **Preparation of multiplex respiratory virus evaluation panel**

90 A well characterised panel of positive and negative clinical samples is a critical requirement for
91 evaluation of these RT-PCR IVD kits. Also, a statistically significant number of clinical samples
92 should be used for the evaluation.

93 The sample type for respiratory virus detection is usually nasopharyngeal/oropharyngeal swab. If
94 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
96 available with the lab, the performance evaluation report should clearly mention the sample type
97 against which the kit is evaluated. There should be no ambiguity about the type of sample used for
98 evaluation.

99 **4. Nucleic acid extraction**

100 Nucleic acid extraction should be performed using standard techniques. If the manufacturer of the
101 index test recommends a specific nucleic acid extraction kit, it needs to be provided by the
102 manufacturer if the evaluation lab is unable to procure the same.

103 **Caution is advised in the selection of a nucleic acid extraction kit since the target pathogens comprise
104 both RNA and DNA viruses.*

105 **5. Real-Time PCR System**

106 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
108 manufacturer if not available within the lab's IVD evaluation scope.

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

111 **6. Internal control/Extraction control**

112 The test under evaluation (index test) must have an internal control (housekeeping gene), with or
113 without an extraction control (nucleic acid added before extraction to a sample).

114 **7. Reference assay:**

115 The following points are to be noted:

116 i. A WHO Pre-Qualified/ US FDA/ ATAGI Australia/ PMDA Japan approved single plex (for a
117 particular target pathogen) or multiplex real-time PCR assay/ ICMR-NIV Pune in-house single
118 plex (for a particular target pathogen) or multiplex Real Time PCR Assay should be used as the
119 reference assay.

120 ii. Since the list of target pathogens is extensive, a combination of single plex and/or multiplex
121 assays may be used as the reference assay(s), as long as these reference assays satisfy the criteria
122 outlined in point 7(i).

123 All samples positive for a particular pathogen should be confirmed positive by the reference assay.

124 All samples negative for a particular pathogen should be confirmed negative by the reference
125 assay.

126

127 **8. Sample size for performance evaluation:** The 2009 FDA guidance document
128 “*Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class II Special Controls*
129 *Guidance for Industry and FDA Staff*”, recommends including a sufficient number of
130 prospectively collected samples for each specimen type to generate a result with at least
131 90% sensitivity with a lower bound of the two-sided 95% confidence interval (CI) greater
132 than 80, and demonstrate specificity with a lower bound of the two-sided 95% CI greater
133 than 90%. In accordance with these guidelines and for feasibility of evaluation of these
134 extensive multiplex panels, sample size for each pathogen is calculated assuming $\geq 90\%$
135 sensitivity and specificity of the index test, 95% confidence level, absolute precision of
136 7.5%, and $\leq 5\%$ invalid test rate. A minimum of 65 positive clinical samples (rounded to
137 70) and a minimum of 65 negative clinical samples for each target pathogen are required
138 for performance evaluation of the assay. However, 120 negative samples are recommended
139 per pathogen to account for an extensive cross reactivity panel. Sample sizes are calculated
140 using the formulae:

$$142 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

$$143 n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

- 144
- 145
- 146 · *n (se) is the minimum number of positive samples.*
- 147 · *n (sp) is the minimum number of negative samples.*
- 148 · *Z² is the critical value from the standard normal distribution corresponding*
149 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*
- 150 · *S_e is the predetermined sensitivity.*
- 151 · *S_p is the predetermined specificity.*
- 152 · *d is the predetermined marginal error (5%)*
- 153 · *IR is the invalid test rate*

154 The details of sample requirement are outlined in Table 2.

155
156 *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.

157

158

Notes for Table 2:

159

160

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.

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9. Sample panel composition:

166

A. Human samples

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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 **A.2 Negative samples for each pathogen/ type or subtype of pathogen (Minimum**
174 **n=120):** All negative samples should be negative for the target pathogen/ its type or
175 subtype by the reference real-time PCR assay. Distribution of the negative samples should
176 be as follows:

177 A.2.1 NP/OP swab from individuals with respiratory infection that are negative for the
178 target pathogen/its type or subtype = 35 samples **

179 A.2.2 NP/OP swab from apparently healthy individuals with no respiratory symptoms =
180 23 samples **

181 A.2.3 Cross reactivity panel (Table 3): Samples negative for the target pathogen but
182 positive for other common respiratory viruses = 62 samples ***

183 Archived frozen sample aliquots if used for the evaluation, should not be thawed more than
184 once.

185 *** If samples are available with the evaluating lab that satisfy these criteria and are negative for*
186 *all the pathogens targeted by the kit, the same samples may be included in the negative sample*
187 *panel for all target pathogens to prevent wastage of resources.*

188 **** Same positive samples may be included in the cross-reactivity panel of several target*
189 *pathogens to prevent wastage of resources e.g.: the same Influenza A virus positive sample may*
190 *be included in the cross-reactivity panel for RSV, Human Metapneumovirus, SARS-CoV-2 etc.*

191
192 Table 3: Cross reactivity panel for performance evaluation of multiplex respiratory virus
193 real time PCR kit

Multiplex Respiratory Virus Real Time PCR Kit Performance Evaluation Protocol
ICMR-CDSCO/IVD/GD/PROTOCOLS/09/2025

Target Pathogen	Virus-wise no. of samples needed for cross reactivity analysis													Total no. of cross reactive samples per pathogen	
	Influenza virus A *	Influenza virus B *	SARS Coronaviru ^s -2 *	Respiratory syncytial virus *	Adenovirus @	Human Respiroviruses 1 and 3, Human Rubulaviruses 2 and 4 #	Human metapneumovirus @	Measles virus *	Rhinovirus @ \$	Human Bocavirus	Enterovirus \$	Cytomegalovirus	Seasonal coronaviruses*	Rubella	
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 Respiroviruses 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.

DRF

194 **B. Contrived samples:**
195 Contrived positive and negative samples may be used for evaluation in case of
196 paucity/unavailability of human clinical samples. Positive contrived samples should be
197 positive and negative contrived samples should be negative for the target
198 pathogen/type/subtype using the reference assay. The number and distribution of positive
199 and negative samples, including the cross reactivity panel, should remain the same.
200 Contrived positive samples (as part of positive sample panel/ cross-reactivity panel) should
201 be prepared by spiking a sample matrix negative for the pathogen with a pathogen-infected
202 cell line, genomic DNA plasmids or RNA transcripts.
203 It is recommended to demonstrate equivalence between contrived and clinical specimens.
204 Serial dilutions of clinical sample and serial dilutions of contrived sample with targeted
205 levels of analyte should be compared for demonstrating equivalence.

206 **10. Evaluation method:**

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

209 **11. Interpretation of results:**

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

212 **12. Resolution of discrepant results:**

213 True positive samples: These are samples positive by reference assay and index test.
214 True negative samples: These are samples negative by reference assay and index test.
215 False positive samples: These are samples negative by reference assay and positive by
216 index test.
217 False negative samples: These are samples positive by reference assay and negative by
218 index test.

219 **13. Test reproducibility**

220 **A. Sample size for lot-to-lot reproducibility**

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

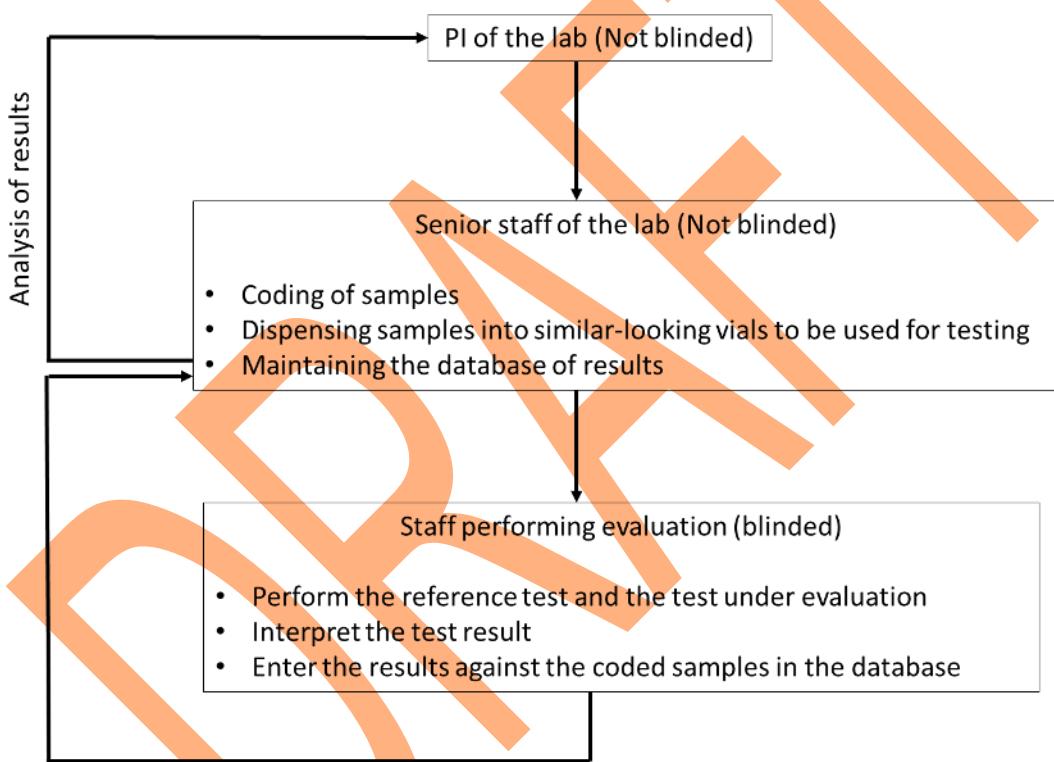
223

- 224 • First lot of the assay: should be tested on statistically significant number of positive
225 and negative samples for each pathogen/type of pathogen as calculated in the protocol.
- 226 • Second lot of the assay: should be tested on 25 samples for each pathogen/type of
227 pathogen (15 positive samples comprising 10 low positive **AND** 5 moderate/high
228 positive samples, and 10 negative samples).
- 229 • Third lot of the assay: should be tested on 25 samples for each pathogen/type of
230 pathogen (15 positive samples comprising 10 low positive **AND** 5 moderate/high
231 positive samples, and 10 negative samples).
- 232 • There should be no lot-to-lot variability.

233 **14. Blinding of laboratory staff**

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

243
244 Fig.2: Blinding in evaluation exercise



245
246
247

248 **15. Acceptance Criteria**

249 Expected sensitivity for each pathogen/type/subtype: $\geq 90\%$
250 Expected specificity for each pathogen/type/subtype: $\geq 95\%$
251 Cross reactivity with other viruses as outlined in the negative sample panel: Nil
252 Invalid test rate: $\leq 5\%$

253

254 **16. Publication Rights:**

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

256

257 **After following due procedure as defined in this document, once any kit is found to be Not**
258 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
259 **acceptable.**

260 **Any request of re-validation from the same manufacturer for the same test type will only be**
261 **entertained after a minimum of 3 months and only if a high-level technical summary of**
262 **modifications or functional improvements to the kit design is submitted, without explicit**
263 **disclosure of proprietary information.**

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

268

269 **VII. References:**

- 270 1. Food and Drug Administration. Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class
271 II Special Controls Guidance for Industry and FDA Staff. Available at:
272 <https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/respiratory-viral-panel-multiplex-nucleic-acid-assay-class-ii-special-controls-guidance-industry-and> [Accessed on 22nd January, 2025].
- 275 2. Food and Drug Administration. 510(k) Substantial Equivalence Determination Decision
276 Summary, Biofire Diagnostics LLC, FilmArray Pneumonia Panel. Available at:
277 https://www.accessdata.fda.gov/cdrh_docs/reviews/K180966.pdf [Accessed on 19th January
278 2025]
- 279 3. Food and Drug Administration: Testing for Human Metapneumovirus (hMPV) Using Nucleic
280 Acid Assays - Class II Special Controls Guidance for Industry and FDA Staff. 2009. Available
281 at: <https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/testing-human-metapneumovirus-hmpv-using-nucleic-acid-assays-class-ii-special-controls-guidance#3> [Accessed on January 11, 2025]

284

285 **VIII. Performance evaluation report format**

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290 **PERFORMANCE EVALUATION REPORT FOR MULTIPLEX RESPIRATORY VIRUS**
291 **REAL-TIME PCR KITS**

Name of the product (Brand /generic)	
Name and address of the legal manufacturer	
Name and address of the actual manufacturing site	
Name and address of the Importer	
Name of supplier: Manufacturer/Importer/Port office of CDSCO/State licensing Authority	
Lot No / Batch No.:	
Product Reference No/ Catalogue No	
Type of Assay	
Kit components	
Manufacturing Date	
Expiry Date	
Pack size (Number of tests per kit)	
Pathogens detected by the assay	
Intended Use	
Number of Tests Received	
Regulatory Approval:	
Import license / Manufacturing license/ Test license	
License Number:Issue date:	
Valid Up to:	
Application 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 **Results:** Tables 1 and 2 should be made for each pathogen/type of pathogen targeted by the kit
294 under evaluation

295

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

298

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

299

300 *Table 2: Sensitivity and specificity*
301

	Estimate (%)	95% CI
Sensitivity		
Specificity		

302 ● Details of cross reactivity with other viruses in the cross-reactivity panel:

303 ● **Conclusions:**

304 ○ Sensitivity, specificity
305 ○ Cross reactivity
306 ○ Invalid test rate
307 ○ Performance: **Satisfactory / Not satisfactory**

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

311 **Disclaimers**

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

314 **Note:**

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

317 Evaluation Done on

318 Evaluation Done by

319 Signature of Director/ Director-In-charge Seal

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

321

322

323

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331

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

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 *****End of the Document*****

1



STANDARD PERFORMANCE EVALUATION PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

2

DENGUE IgG BASED ASSAYS

3

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

4



5
6

AUGUST, 2025
New Delhi, India

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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

31 Performance evaluation protocol for Dengue IgG RDT kits

I. Background:

33 CDSO/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
36 evaluation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit
37 (IVD) performance.

II. Purpose:

39 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.

III. Requirements:

43 a) Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If
44 the kit to be evaluated works in a closed system format, the manufacturer needs to supply
45 the required equipment.

46 b) Evaluation sites/laboratories (With required equipment)

47 c) Reference test kits

48 d) Characterised Evaluation panel

49 e) Laboratory supplies

IV. Ethical approvals:

51 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
53 Validation Testing, 2024.

54 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.

V. Procedure:

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

65 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training
66 and competency testing on following

67 ➤ Preparation & characterization of kit evaluation panel
68 ➤ Handling of Dengue IgG Rapid IVD kits received for performance evaluation
69 (Verification/Storage/Unpacking etc).
70 ➤ Testing, interpreting, recording of results & reporting
71 ➤ Data handling, data safety & confidentiality

72

73 **3. Preparation of Dengue IgG Rapid IVD kit evaluation panel**

74 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance
75 evaluation of IVD kits. Hence statistically significant number of sera samples should be
76 collected from Dengue NS1/PCR/IgM confirmed cases. Further characterised for Dengue IgG
77 positivity by using approved reference kits having high sensitivity and specificity.

78 Dengue IgG performance evaluation panel need to be tested again by the reference assays at
79 the time of evaluating a particular index test to confirm the positive and negative status of the
80 samples.

81 **4. Reference assay:**

82 Positive and negative samples should be characterized using composite reference standard
83 of Dengue IgG AND one additional marker of Dengue (NS1 or IgM or PCR). The
84 following kits should be used for characterization of the sample panel:

- 85 • *Panbio Dengue IgG capture ELISA kit*
- 86 • *WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue IgM*
87 *ELISA kit*
- 88 • *NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI Australia/*
89 *PMDA Japan approved NS1 ELISA kit*
- 90 • *Serotype status to be assessed using a combination of CDC/NIV real-time PCR serotyping*
91 *protocols.*

92 **5. Sample size for performance evaluation:**

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

$$97 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

100
$$n_{sp} \geq \frac{Z^2 \times S_p (1 - S_p)}{d^2 \times (1 - IR)}$$

101

102

103

- *n (se) is the minimum number of positive samples.*
- *n (sp) is the minimum number of negative samples.*
- *Z² is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to Z² = 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*

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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.

121

122

123

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.

124

125

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

126

127

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

<i>Sensitivity</i>	<i>Calculated sample size</i>	<i>Minimum no. of Positive Samples required [Sample size rounded off] #</i>	<i>Sample Panel Composition</i>
99%	16	20	Strong Positive: 6 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 Table 2. Sample sizes and panel composition of negative dengue IgG samples for different values
131 of specificity claimed by the manufacturer.

Specificity	Calculated sample size	Minimum no. of Negative Samples required [Sample size rounded off] #	Sample Panel Composition
99%#	16	20	1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 7 2. Acute febrile illness cases: 8 <ul style="list-style-type: none"> • Chikungunya positive samples: 2 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 6 3. Samples from other flavivirus disease cases (cross-reactive panel): 3 <ul style="list-style-type: none"> • 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
95%	77	80	1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 27 2. Acute febrile illness cases: 32

			<ul style="list-style-type: none"> • Chikungunya positive samples:8 • Dengue (NS1 & IgM & IgG & PCR) negative samples:24 <p>3.Samples from other flavivirus disease cases(cross-reactive panel): 9</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 3 @ • West Nile Virus IgM/IgG positive: 3 * • Zika Virus IgM/IgG positive: 3 * <p>4. ^aHealthy subjects from endemic regions: 12</p>
90%	145	150	<p>1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 50</p> <p>2.Acute febrile illness cases: 60</p> <ul style="list-style-type: none"> • Chikungunya positive samples:15 • Dengue (NS1 & IgM & IgG & PCR) negative samples:45 <p>3.Samples from other flavivirus disease cases(cross-reactive panel): 15</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 5 @ • West Nile Virus IgM/IgG positive: 5 * • Zika Virus IgM/IgG positive: 5 * <p>4. ^aHealthy subjects from endemic regions: 25</p>
85%	206	210	<p>1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 70</p> <p>2.Acute febrile illness cases: 84</p> <ul style="list-style-type: none"> • Chikungunya positive samples:21 • Dengue (NS1 & IgM & IgG & PCR) negative samples:63 <p>3.Samples from other flavivirus disease cases(cross-reactive panel): 21</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 7 @ • West Nile Virus IgM/IgG positive: 7 * • Zika Virus IgM/IgG positive: 7 * <p>4. ^aHealthy subjects from endemic regions: 35</p>
80%	258	260	<p>1.Samples positive for dengue IgM/NS1/RNA but negative for IgG: 85</p> <p>2.Acute febrile illness cases: 104</p> <ul style="list-style-type: none"> • Chikungunya positive samples:26 • Dengue (NS1 & IgM & IgG & PCR) negative samples:78 <p>3.Samples from other flavivirus disease cases(cross-reactive panel): 27</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 9 @ • West Nile Virus IgM/IgG positive: 9 * • Zika Virus IgM/IgG positive: 9 * <p>4. ^aHealthy subjects from endemic regions: 44</p>

^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.

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6. Evaluation method:

135

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

136

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7. Interpretation of results:

140

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

141

8. Resolution of discrepant results:

142

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

143

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

144

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

145

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

146

147

148

9. Test reproducibility

149

A. Sample size for lot-to-lot reproducibility

150

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

151

152

- First lot of the assay: should be tested on statistically significant number of positive and negative samples as calculated in the protocol.

155 • 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).

156 • 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).

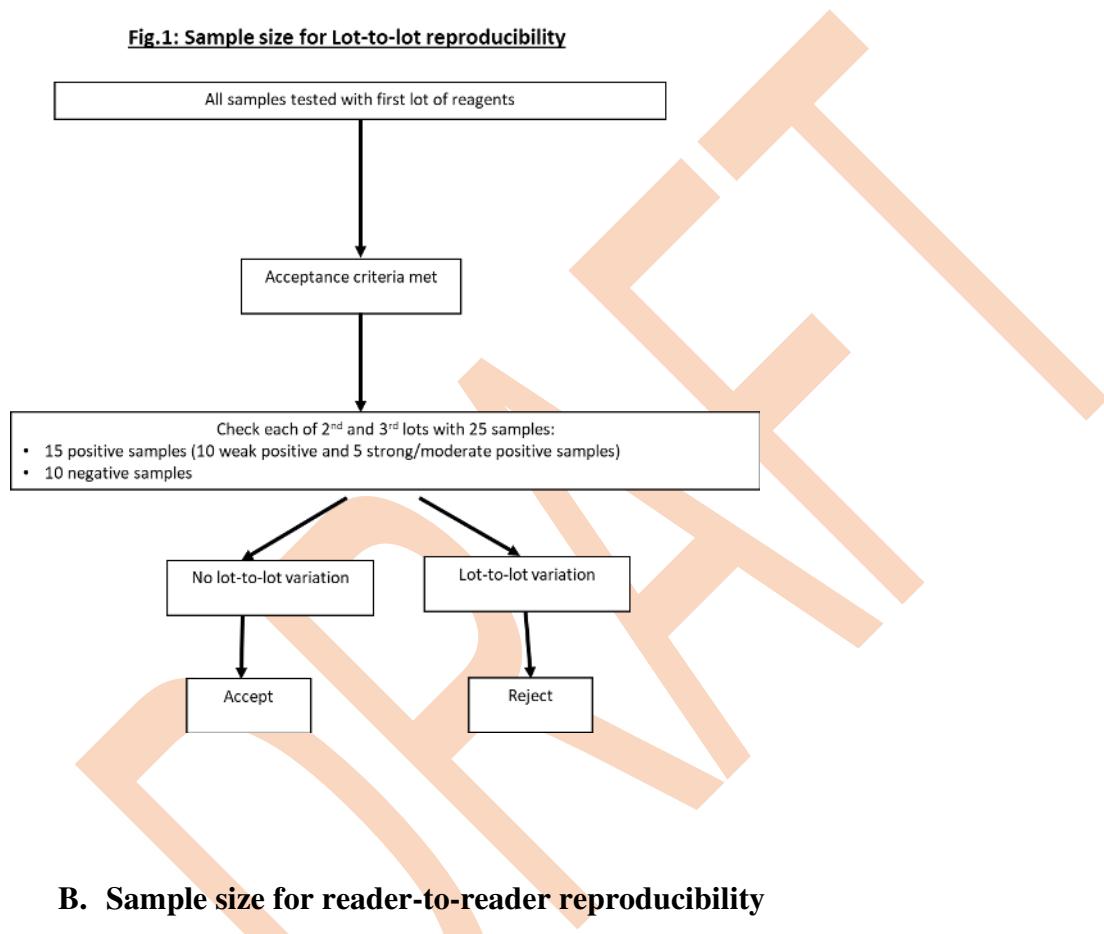
157

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159

160

161 Refer the flowchart below (Fig. 1):



162

163

164

165 **B. Sample size for reader-to-reader reproducibility**

166 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.

170 Agreement should be 100% between the operators.

171 **10. Blinding of laboratory staff**

172 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

173

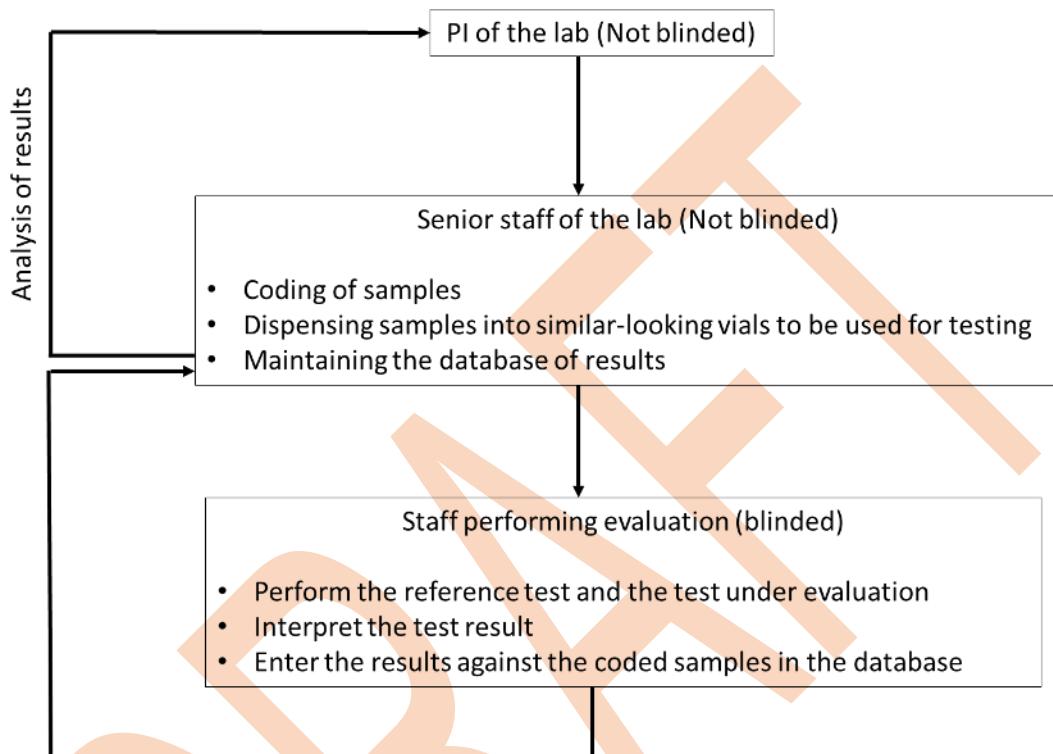
174

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176

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

181
182 Fig.2: Blinding in evaluation exercise



183
184
185

11. Acceptance Criteria

186 Expected sensitivity: $\geq 80\%$
187 Expected specificity: $\geq 90\%$
188 Cross reactivity: Nil
189 Invalid test rate: $\leq 5\%$
190 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 260 positive samples and ≥ 150 negative samples should be used for evaluation.

12. Publication Rights:

191 The PI (s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).
192
193

194
195

196 After following due procedure as defined in this document, once any kit is found to be Not
197 of Standard Quality, thereafter, no request for repeat testing of the same kit will be
198 acceptable.

199 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
204 well-characterized sample panel at a different laboratory may be considered only for kits
205 which claim high performance characteristics (sensitivity and specificity 95% and above),
206 but which fail the performance evaluation by a margin of 5%.

207

208 **VI. References:**

209 1. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G capture
210 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
213 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-documents/medical_device/guidanceperformanceivd.pdf

216 4. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently
217 Asked Questions. 2022. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf

218 5. U.S. Food and Drug Administration. Dengue Virus Serological Reagents - Class II Special Controls
219 Guideline for Industry and Food and Drug Administration Staff. 2014. Available at:
220 <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>

221 6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic
222 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>

223

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

227

228 **VII. Performance evaluation report format**

229

230

231

236

PERFORMANCE EVALUATION REPORT FOR DENGUE IgG RDT KIT

Name of the product (Brand /generic)	
Name and address of the legal manufacturer	
Name and address of the actual manufacturing site	
Name and address of the Importer	
Name of supplier: Manufacturer/Importer/Port office of CDSCO/State licensing Authority	
Lot No / Batch No.:	
Product Reference No/ Catalogue No	
Type of Assay	
Kit components	
Manufacturing Date	
Expiry Date	
Pack size (Number of tests per kit)	
Intended Use	
Number of Tests Received	
Regulatory Approval:	
Import license / Manufacturing license/ Test license	
License Number:Issue date:	
Valid Up to:	
Application No.	
Sample	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)		
		Positive	Negative	Total
Name of Dengue IgG antibody - based RDT kit	Positive			
	Negative			
	Total			

239

	Estimate (%)	95% CI
Sensitivity		
Specificity		

240 **Conclusions:**

- 241 ○ Sensitivity, specificity
- 242 ○ Cross-reactivity:
- 243 ○ Invalid test rate:
- 244
- 245 ○ Performance: **Satisfactory / Not satisfactory**

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

248 **Disclaimers**

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

251 Note: This report is exclusively forKit (Lot No.....) manufactured by
252 (Supplied by

253 Evaluation Done on

254 Evaluation Done by

255 Signature of Director/ Director-In-charge Seal

256

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

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276 **Performance evaluation protocol for Dengue IgM and IgG RDT combo kits**

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
281 evaluation is to independently verify the manufacturer's claim regarding in-vitro diagnostic kit
282 (IVD) performance.

283 **II. Purpose:**

284 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:**

- 288 f) Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If
289 the kit to be evaluated works in a closed system format, the manufacturer needs to supply
290 the required equipment.
- 291 g) Evaluation sites/laboratories (With required equipment)
- 292 h) Reference test kits
- 293 i) Characterised Evaluation panel
- 294 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.

299 Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
300 to the institutional authorities and ethics committee for information.

301 **V. Procedure:**

302 1. **Study design/type:** Diagnostic accuracy study using irreversibly de-identified archived/
303 spiked leftover clinical samples

304 2. **Preparation of Evaluation sites/laboratories:**

305 **Identified IVD kit evaluation laboratories should establish their proficiency through
306 ALL of the following:**

307 A. Accreditation for at least one of the Quality management systems (accreditation for Testing
308 Lab / Calibration Lab (ISO: 17025), Medical Lab (ISO: 15189), PT provider (ISO: 17043) or
309 CDSCO approved Reference laboratory.

310 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training
311 and competency testing on following

312 ➤ Preparation & characterization of kit evaluation panel
313 ➤ Handling of Dengue IgM and IgG Rapid IVD kits received for performance evaluation
314 (Verification/Storage/Unpacking etc).
315 ➤ Testing, interpreting, recording of results & reporting
316 ➤ Data handling, data safety & confidentiality

317

318 **3. Preparation of Dengue IgM and IgG Rapid IVD kit evaluation panel**

319 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance
320 evaluation of IVD kits. Hence statistically significant number of sera samples should be
321 collected from Dengue NS1/PCR/IgM confirmed cases. Further characterised for Dengue IgG
322 positivity by using approved reference kits having high sensitivity and specificity.

323 Dengue IgG performance evaluation panel need to be tested again by the reference assays at
324 the time of evaluating a particular index test to confirm the positive and negative status of the
325 samples.

326 **4. Reference assay:**

327 Positive and negative samples should be characterized using reference standard for Dengue
328 IgG (and one additional marker of Dengue - NS1 or IgM or PCR) AND IgM. The following
329 kits should be used for characterization of the sample panel:

- 330 • *Panbio Dengue IgG capture ELISA kit*
- 331 • *WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue*
332 *IgM ELISA kit*
- 333 • *NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI*
334 *Australia/ PMDA Japan approved NS1 ELISA kit*
- 335 • *Serotype status to be assessed using a combination of CDC/NIV real-time PCR*
336 *serotyping protocols.*

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

341

$$342 n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2 \times (1 - IR)}$$

343

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

344

345

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

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

348 · *Z² is the critical value from the standard normal distribution corresponding*
 349 *to the desired confidence level (95% CI corresponds to Z² = 1.96).*

350 · *Se is the predetermined sensitivity.*

351 · *Sp is the predetermined specificity.*

352 · *d is the predetermined marginal error (5%)*

353 · *IR is the invalid test rate*

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

362

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

366

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

369

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

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

Dengue IgG Based Assays Performance Evaluation Protocols

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

			<ul style="list-style-type: none"> • Weak positive: 4 <p>2. 10 samples positive for both Dengue IgM and IgG</p> <ul style="list-style-type: none"> • Strong positive IgG: 3 • Moderate positive IgG: 3 • Weak positive IgG: 4
95%	77	80	<p>40 samples positive for Dengue IgM</p> <ul style="list-style-type: none"> • Strong positive: 12 • Moderate positive: 14 • Weak positive: 14 <p>40 samples positive for both Dengue IgM and IgG</p> <ul style="list-style-type: none"> • Strong positive IgG: 12 • Moderate positive IgG: 14 • Weak positive IgG: 14
90%	145	150	<p>75 samples positive for Dengue IgM</p> <ul style="list-style-type: none"> • Strong positive: 23 • Moderate positive: 26 • Weak positive: 26 <p>75 samples positive for both Dengue IgM and IgG</p> <ul style="list-style-type: none"> • Strong positive IgG: 23 • Moderate positive IgG: 26 • Weak positive IgG: 26
85%	206	210	<p>105 samples positive for Dengue IgM</p> <ul style="list-style-type: none"> • Strong positive: 31 • Moderate positive: 37 • Weak positive: 37 <p>105 samples positive for both Dengue IgM and IgG</p> <ul style="list-style-type: none"> • Strong positive IgG: 31 • Moderate positive IgG: 37 • Weak positive IgG: 37
80%	258	260	<p>130 samples positive for Dengue IgM</p> <ul style="list-style-type: none"> • Strong positive: 38 • Moderate positive: 46 • Weak positive: 46

			130 samples positive for both Dengue IgM and IgG <ul style="list-style-type: none"> • Strong positive IgG: 38 • Moderate positive IgG: 46 • Weak positive IgG: 46
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372 #It is recommended to calculate the sample size as per manufacturer's claims of sensitivity
 373 and specificity; however, a higher sample size is suggested to ensure adequate power of
 374 the study in case the kit falls short of claimed performance characteristics.

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

Specificity	Calculated sample size	Minimum no. of Negative Samples required [Sample size rounded off for balanced allocation] #	Sample Panel Composition
99%	16	20	1. ^a Samples from acute febrile illness cases negative for dengue: 9 <ul style="list-style-type: none"> • Samples positive for chikungunya: 2 • Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):7 2. Samples from other flavivirus disease cases (cross-reactive panel): 3 <ul style="list-style-type: none"> • 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
95%	77	80	1. ^a Samples from acute febrile illness cases negative for dengue: 44 <ul style="list-style-type: none"> • Samples positive for chikungunya: 8 • Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):36 2. Samples from other flavivirus disease cases (cross-reactive panel): 6

			<ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 2@ • West Nile Virus IgM/IgG positive: 2* • Zika Virus IgM/IgG positive: 2 * <p>3. ^bHealthy subjects from endemic regions: 30</p>
90%	145	150	<p>1.^aSamples from acute febrile illness cases negative for dengue: 80</p> <ul style="list-style-type: none"> • Samples positive for chikungunya: 15 • Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):65 <p>2.Samples from other flavivirus disease cases (cross-reactive panel): 15</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 5 @ • West Nile Virus IgM/IgG positive:5* • Zika Virus IgM/IgG positive: 5* <p>3. ^bHealthy subjects from endemic regions: 55</p>
85%	206	210	<p>1.^aSamples from acute febrile illness cases negative for dengue: 110</p> <ul style="list-style-type: none"> • Samples positive for chikungunya: 21 • Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):89 <p>2.Samples from other flavivirus disease cases (cross-reactive panel): 24</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 8 @ • West Nile Virus IgM/IgG positive:8* • Zika Virus IgM/IgG positive: 8* <p>3. ^bHealthy subjects from endemic regions: 76</p>
80%	258	260	<p>1.^aSamples from acute febrile illness cases negative for dengue: 138</p>

			<ul style="list-style-type: none">• Samples positive for chikungunya: 26• Other Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR):112 <p>2. Samples from other flavivirus disease cases (cross-reactive panel): 27</p> <ul style="list-style-type: none">• Japanese Encephalitis IgM/IgG positive: 9 @• West Nile Virus IgM/IgG positive: 9*• Zika Virus IgM/IgG positive: 9* <p>3. ^bHealthy subjects from endemic regions: 95</p>
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^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)

#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.

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5. Evaluation method:

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

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6. Resolution of discrepant results:

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

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

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

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

390

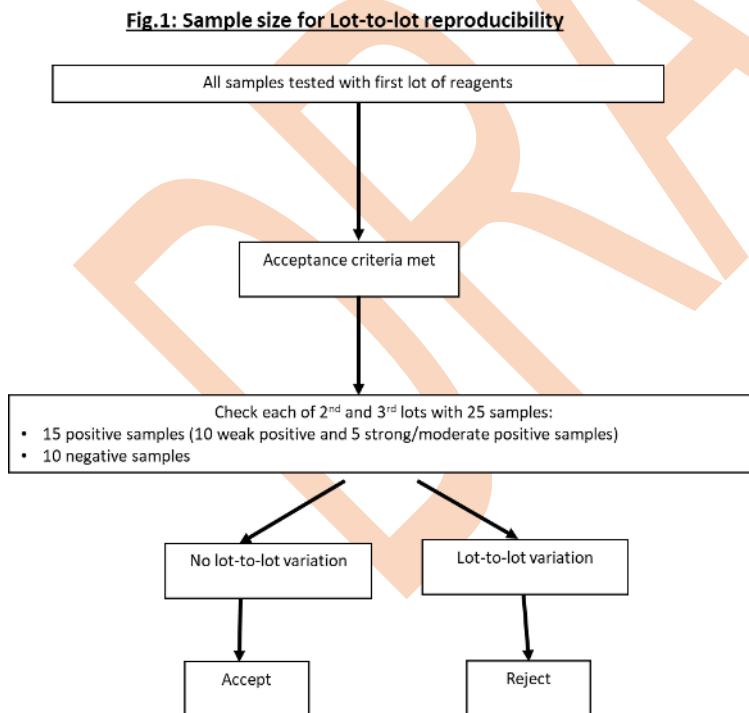
391 **7. Test reproducibility**

392 **C. Sample size for lot-to-lot reproducibility**

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

- 395 396 First lot of the assay: should be tested on statistically significant number of positive
397 and negative samples as calculated in the protocol.
- 398 399 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).
- 400 401 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).

402 Refer the flowchart below (Fig. 1):



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407 **D. Sample size for reader-to-reader reproducibility**

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

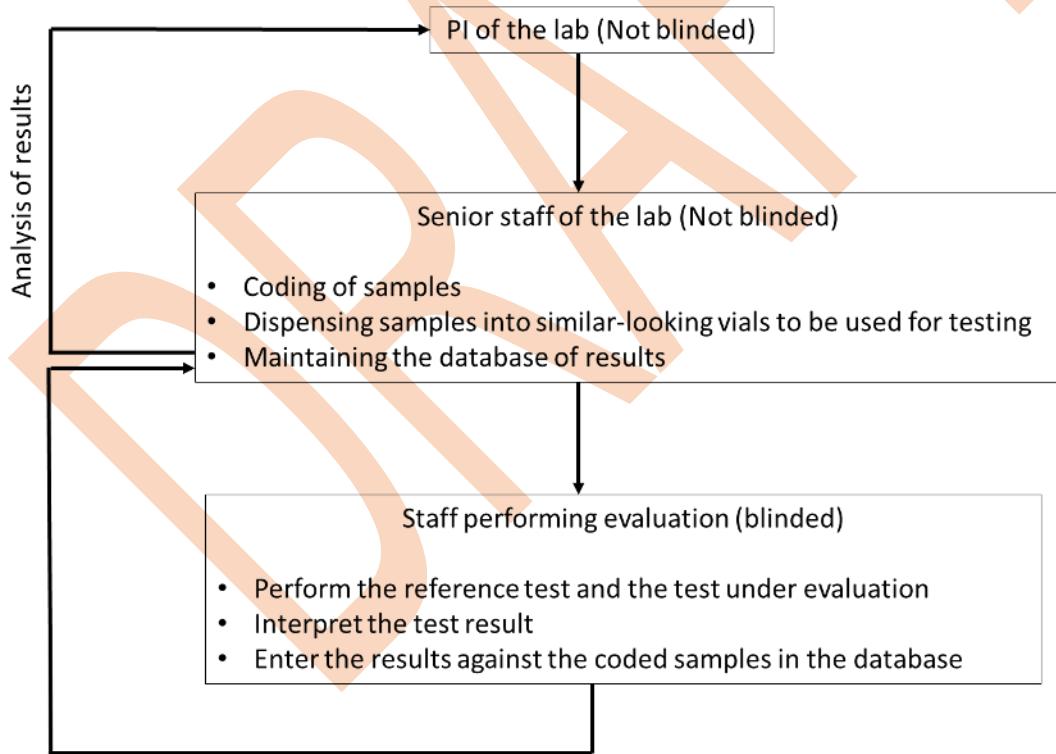
410
411 Two operators will be reading the test results independently as per manufacturer's instruction.
412 Agreement should be 100% between the operators.

413 **8. Blinding of laboratory staff**

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

423
424

Fig.2: Blinding in evaluation exercise



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9. Acceptance Criteria

428 Expected sensitivity for each analyte: $\geq 80\%$

429 Expected specificity for each analyte: $\geq 90\%$

430 Cross-reactivity: Nil
431 Invalid test rate: $\leq 5\%$
432 To achieve at least the performance characteristics outlined in the acceptance criteria, ≥ 260
433 positive samples and ≥ 150 negative samples should be used for evaluation.

434 **10. Publication Rights:**

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

436

437 **After following due procedure as defined in this document, once any kit is found to be Not**
438 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**
439 **acceptable.**

440 **Any request of re-validation from the same manufacturer for the same test type will only be**
441 **entertained after a minimum of 3 months and only if a high-level technical summary of**
442 **modifications or functional improvements to the kit design is submitted, without explicit**
443 **disclosure of proprietary information.**

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

448

449 **VI. References:**

- 450 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..
- 453 2. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.
- 455 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
- 459 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
- 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: <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>

467 6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic
468 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>

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

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475 **VII. Performance evaluation report format**

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

499

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

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501

Results for IgM:

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

502

503

Results for IgG:

		Reference assay (name)		
		Positive	Negative	Total

Name of Dengue antibody combo RDT kit	Positive			
	Negative			
	Total			

504

505

- Details of cross reactivity with other flavivirus antibodies:

507

- Invalid test rate:

508

- **Conclusions:**

510

- Sensitivity, specificity for dengue IgM:

511

- Sensitivity, specificity for dengue IgG:

512

- Performance:
 - **Satisfactory / Not satisfactory for Dengue IgM**
 - **Satisfactory / Not satisfactory for Dengue IgG**

513

514

515

(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.)

518

Disclaimers

519

1. This validation process does not approve / disapprove the kit design

520

2. This validation process does not certify user friendliness of the kit / assay

521

522

Note: This report is exclusively for Kit (Lot No.....) manufactured by (Supplied by

523

Evaluation Done on

524

Evaluation Done by

525

Signature of Director/ Director-In-charge Seal

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

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536 Performance evaluation protocol for Dengue IgG ELISA kits

I. Background:

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

II. Purpose:

544 To evaluate the performance characteristics of Dengue IgG ELISA kits in the diagnosis of Dengue
545 infection using irreversibly de-identified leftover archived clinical samples.

546 III. Requirements:

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

IV. Ethical approval:

555 Performance evaluation activities using irreversibly de-identified leftover clinical samples are
556 exempt from ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory
557 Validation Testing, 2024.

558 Investigators are required to submit a self-declaration form, as outlined in the ICMR guidelines,
559 to the institutional authorities and ethics committee for information.

560 V. Procedure:

- 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 CDSCO approved Reference laboratory.

569 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training
570 and competency testing on following

571 ➤ Preparation & characterization of kit evaluation panel
572 ➤ Handling of Dengue IgG ELISA IVD kits received for performance evaluation
573 (Verification/Storage/Unpacking etc).
574 ➤ Testing, interpreting, recording of results & reporting
575 ➤ Data handling, data safety & confidentiality

576 **3. Preparation of Dengue IgG ELISA IVD kit evaluation panel**

577 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance
578 evaluation of IVD kits. Hence statistically significant number of sera samples should be
579 collected from Dengue NS1/PCR/IgG confirmed cases. Further characterised for Dengue IgM
580 positivity by using approved reference kits having high sensitivity and specificity.

581 Dengue IgG performance evaluation panel need to be tested again by the reference assays at
582 the time of evaluating a particular index test to confirm the positive and negative status of the
583 samples.

584 **4. Reference assay:**

585 Positive and negative samples should be characterized using composite reference standard
586 of Dengue IgG AND one additional marker of Dengue (NS1 or IgM or PCR). The
587 following kits should be used for characterization of the sample panel:

- 588 • *Panbio Dengue IgG capture ELISA kit*
- 589 • *WHO Pre-Qualified/ US-FDA/ ATAGI Australia/ PMDA Japan approved Dengue*
590 *IgM ELISA kit*
- 591 • *NS1 antigen status to be assessed using WHO Pre-Qualified/ US-FDA/ ATAGI*
592 *Australia/ PMDA Japan approved NS1 ELISA kit*
- 593 • *Serotype status to be assessed using a combination of CDC/NIV real-time PCR*
594 *serotyping protocols.*

595

596 **5. Sample size for performance evaluation:**

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

601

602
$$n_{se} \geq \frac{Z^2 \times S_e (1 - S_e)}{d^2}$$

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

603

604

605

606

- *n (se) is the minimum number of positive samples.*
- *n (sp) is the minimum number of negative samples.*
- *Z² is the critical value from the standard normal distribution corresponding to the desired confidence level (95% CI corresponds to Z² = 1.96).*
- *Se is the predetermined sensitivity.*
- *Sp is the predetermined specificity.*
- *d is the predetermined marginal error (5%)*
-

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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.

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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. Samples should be representative of varying degrees of positivity:

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Negative samples: These should include samples negative by the reference assays for dengue IgG.

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

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

631

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

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

Specificity	Calculated sample size	Minimum no. of Negative Samples required [Sample size rounded off]#	Sample Panel Composition
99%	15	20	1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 7 2. Acute febrile illness cases: 8 <ul style="list-style-type: none"> • Chikungunya positive samples: 2 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 6 3. Samples from other flavivirus disease cases (cross-reactive panel): 3 <ul style="list-style-type: none"> • 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
95%	73	80	1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 27 2. Acute febrile illness cases: 32 <ul style="list-style-type: none"> • Chikungunya positive samples: 8 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 24

			<p>3. Samples from other flavivirus disease cases(cross-reactive panel): 9</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 3 @ • West Nile Virus IgM/IgG positive: 3 * • Zika Virus IgM/IgG positive: 3 * <p>4. ^aHealthy subjects from endemic regions: 12</p>
90%	138	140	<p>1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 45</p> <p>2. Acute febrile illness cases: 60</p> <ul style="list-style-type: none"> • Chikungunya positive samples: 15 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 45 <p>3. Samples from other flavivirus disease cases(cross-reactive panel): 15</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 5 @ • West Nile Virus IgM/IgG positive: 5 * • Zika Virus IgM/IgG positive: 5 * <p>4. ^aHealthy subjects from endemic regions: 20</p>
85%	196	200	<p>1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 65</p> <p>2. Acute febrile illness cases: 84</p> <ul style="list-style-type: none"> • Chikungunya positive samples: 21 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 63 <p>3. Samples from other flavivirus disease cases(cross-reactive panel): 21</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 7 @ • West Nile Virus IgM/IgG positive: 7 * • Zika Virus IgM/IgG positive: 7 * <p>4. ^aHealthy subjects from endemic regions: 30</p>
80%	246	250	<p>1. Samples positive for dengue IgM/NS1/RNA but negative for IgG: 80</p> <p>2. Acute febrile illness cases: 104</p> <ul style="list-style-type: none"> • Chikungunya positive samples: 26 • Dengue (NS1 & IgM & IgG & PCR) negative samples: 78 <p>3. Samples from other flavivirus disease cases(cross-reactive panel): 27</p> <ul style="list-style-type: none"> • Japanese Encephalitis IgM/IgG positive: 9 @ • West Nile Virus IgM/IgG positive: 9 * • Zika Virus IgM/IgG positive: 9 *

			4. ^a Healthy subjects from endemic regions: 39
^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)		
<p><i>#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.</i></p>			
<p><i>Cross reactivity panel is arranged in descending order of priority. The pathogens marked @ are essentially to be tested.</i></p>			
<p><i>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.</i></p>			
<p>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.</p>			

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640 6. Evaluation method:

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

643

644 7. Interpretation of results:

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

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647 8. Resolution of discrepant results:

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

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

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

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

653

654 9. Test reproducibility

A. Sample size for lot-to-lot reproducibility

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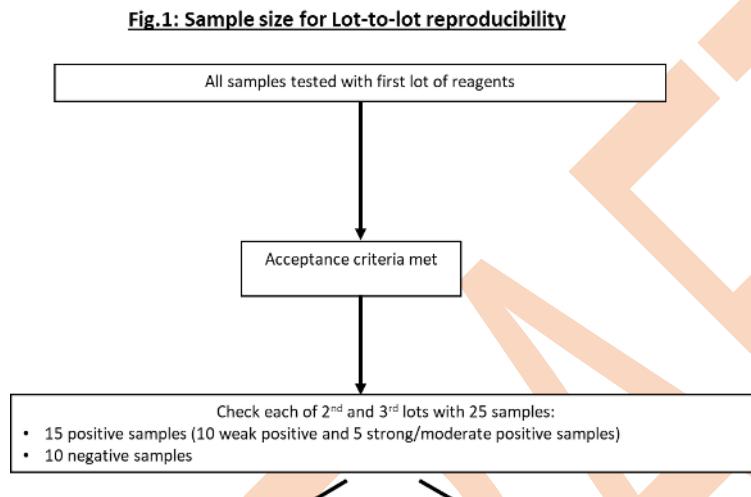
656 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
657 as follows:

658

- 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).

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666 Refer the flowchart below (Fig. 1):



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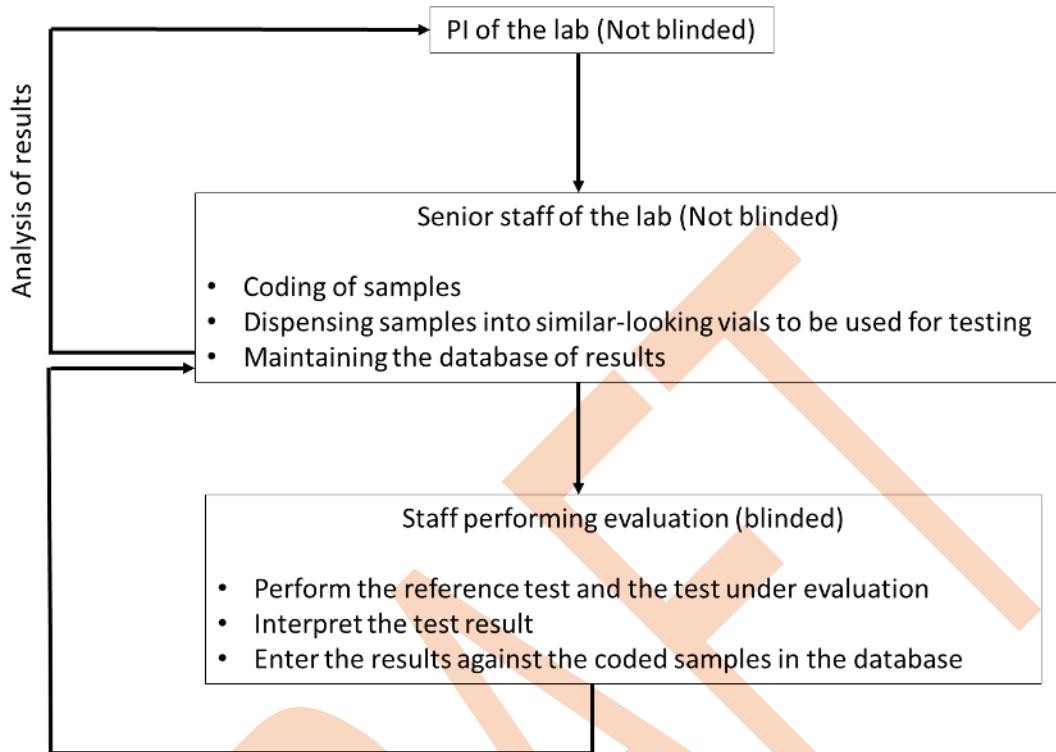
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671 **10. Blinding of laboratory staff**

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

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Fig.2: Blinding in evaluation exercise



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11. Acceptance criteria

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Expected sensitivity: $\geq 90\%$

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Expected specificity: $\geq 95\%$

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Cross-reactivity: Nil

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

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12. Publication Rights:

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

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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.

699 **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**
704 **well-characterized sample panel at a different laboratory may be considered only for kits**
705 **which claim high performance characteristics (sensitivity and specificity 95% and above),**
706 **but which fail the performance evaluation by a margin of 5%.**

707

708 **VI. References:**

- 709 1. Vazquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M
710 and G capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue
711 infections. *J Clin Virol.* 2007 Jul;39(3):194-8. doi: 10.1016/j.jcv.2007.04.003..
- 712 2. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests.**
(Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.
- 713 3. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro
714 Diagnostic Medical Devices. 2018. Available at:
https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical_device/guidanceperformanceivd.pdf
- 715 4. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices
716 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
- 717 5. U.S. Food and Drug Administration. Dengue Virus Serological Reagents - Class II Special Controls
718 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>
- 719 6. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –
720 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>

721
722 *The validation protocols need to be revisited after introduction of Dengue vaccines and the
723 acceptance criteria needs revisiting every year so as to enable the availability of best
724 diagnostic kits.

725

726 **VII. Performance evaluation report format**

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PERFORMANCE EVALUATION REPORT FOR DENGUE IgG ELISA KIT

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

740

741 **Results:**

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

742

	Estimate (%)	95% CI
Sensitivity		
Specificity		

743 **Conclusions:**

744 Sensitivity, specificity

745 Cross-reactivity:

746 Invalid test rate:

747 Performance: **Satisfactory / Not satisfactory**

748 (*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 **Disclaimers**

751 1. This validation process does not approve / disapprove the kit design

752 2. This validation process does not certify user friendliness of the kit / assay

753 Note: This report is exclusively forKit (Lot No.....) manufactured by (Supplied
754 by

755 Evaluation Done on

756 Evaluation Done by

757 Signature of Director/ Director-In-charge Seal.....

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

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781 **Information on Operational and Test Performance Characteristics Required from Manufacturers**
782 **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.

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827
828 *****End of the Document*****