



1

---

# STANDARD PERFORMANCE EVALUATION PROTOCOLS

## DRAFT FOR STAKEHOLDER COMMENTS

---

### ARBOVIRUS IN-VITRO DIAGNOSTICS

2

ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

3

-Dengue virus, Chikungunya virus, Zika virus

4



5

6

DECEMBER, 2024  
New Delhi, India

7  
8

**Arbovirus IVD Performance Evaluation Protocols**

**Table of Contents**

| S.No. | Content   | Page Number |
|-------|---|-------------|
| 1.    | Chikungunya IgM ELISA – Performance evaluation protocol                                     | 2           |
| 2.    | Chikungunya IgM RDT – Performance evaluation protocol                                       | 10          |
| 3.    | Chikungunya real time PCR – Performance evaluation protocol                                 | 18          |
| 4.    | Dengue NS1 RDT – Performance evaluation protocol  | 26          |
| 5.    | Dengue NS1 RDT – Field evaluation protocol  | 35          |
| 6.    | Dengue NS1 ELISA – Performance evaluation protocol  | 43          |
| 7.    | Dengue NS1 ELISA – Field evaluation protocol  | 51          |
| 8.    | Dengue IgM RDT – Performance evaluation protocol  | 59          |
| 9.    | Dengue IgM ELISA – Performance evaluation protocol  | 68          |
| 10.   | Dengue NS1/IgM combo RDT – Performance evaluation protocol                                  | 76          |
| 11.   | Dengue NS1/IgM combo RDT – Field evaluation protocol  | 86          |
| 12.   | Dengue real time PCR – Performance evaluation protocol                                      | 95          |
| 13.   | Dengue real time PCR – Field evaluation protocol  | 104         |
| 14.   | Zika virus real time PCR – Performance evaluation protocol                                  | 112         |
| 15.   | Information on operational and test performance characteristics required from manufacturers | 121         |

9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19

20 **Performance evaluation protocol for Chikungunya IgM ELISA kits**

21 **I. Background:**

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

26 **II. Purpose:**

27 To evaluate the performance characteristics of Chikungunya IgM ELISA kits in the diagnosis of  
28 Chikungunya infection.

29 **III. Requirements:**

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

37 **IV. Ethical approvals:**

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

41 **V. Procedure:**

- 42 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 43 **2. Preparation of Evaluation sites/laboratories:**
  - 44 **Identified IVD kit evaluation laboratories should establish their proficiency through**
  - 45 A. Accreditation from NABL for at least one of the Quality management system (NABL  
46 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
47 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
  - 48 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
49 and competency testing on following
    - 50 ➤ Preparation & characterization of kit evaluation panel
    - 51 ➤ Handling of Chikungunya IgM ELISA kits received for performance evaluation  
52 (Verification/Storage/Unpacking etc).

- 53      ➤ Testing, interpreting, recording of results & reporting
- 54      ➤ Data handling, data safety & confidentiality

### 55      **3. Preparation of Chikungunya IgM ELISA IVD kit evaluation panel**

56      Well characterised Chikungunya IVD kit evaluation panel is a critical requirement for performance  
57      evaluation of IVD kits. Hence statistically significant number of sera samples should be available  
58      from Chikungunya confirmed cases. Further characterised for Chikungunya IgM positivity by  
59      using approved reference kits having high sensitivity and specificity.

60      Chikungunya IgM performance evaluation panel need to be tested again by the reference assays at  
61      the time of evaluating a particular index test to confirm the positive and negative status of the  
62      samples.

### 63      **4. Reference assay:**

64      All the samples will be tested by CDC/NIV real-time (RT-PCR) assay. *Samples which are positive*  
65      *by RT-PCR assay will be further tested by any two of the following IgM ELISA kits:*

- 66      i.      *ICMR-NIV MAC ELISA kit*
- 67      ii.     *Inbios CHIKjj Detect™ IgM ELISA*
- 68      iii.    *Anti-Chikungunya virus ELISA (IgM) Test (Euroimmun, Luebeck, Germany)*

69      Samples positive by at least two kits will be considered. If sufficient RT-PCR positive samples  
70      are not available, samples positive by at least 2 ELISA kits (of the kits mentioned above) can  
71      be considered as true positive samples.

72      *Samples which are negative by RT-PCR and at least two IgM ELISA kits mentioned above will be*  
73      *considered as Chikungunya negative samples.*

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

85      Positive samples: Positive samples should be positive by RT-PCR at least two ELISA kits from  
86      the three mentioned above. If sufficient RT-PCR positive samples are not available, samples  
87      positive by at least 2 ELISA kits (of the kits mentioned above) can be considered as true positive  
88      samples.

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

89 Negative samples: Samples which are negative by RT-PCR and at least two IgM ELISA kits  
90 mentioned above will be considered as Chikungunya negative samples.

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

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>                                   |
|--|-------------------------------|---|---|
| 99% <sup>#</sup>   | 15                            | 20  | Strong positive: 4<br>Moderate positive: 8<br>Weak positive: 8    |
| 95%  | 73                            | 80  | Strong positive: 18<br>Moderate positive: 31<br>Weak positive: 31 |
| 90%  | 138                           | 140   | Strong positive: 30<br>Moderate positive: 55<br>Weak positive: 55 |
| 85%  | 196                           | 200   | Strong positive: 42<br>Moderate positive: 79<br>Weak positive: 79 |
| 80%  | 246                           | 250   | Strong positive: 54<br>Moderate positive: 98<br>Weak positive: 98 |
| <i>The samples need to be classified as strong, moderate and weak positives based on ELISA units of the reference assay.</i> |                               |   |   |
| <i>#Higher sample size should be used even for assays claiming 99% sensitivity.</i>  |                               |   |   |

93  
94 Table 2. Sample sizes and panel composition of negative chikungunya samples for different values  
95 of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 15                            | 20  | Rubella IgM positive: 1<br>Dengue IgM positive: 3<br><sup>a</sup> Acute febrile illness cases: 8<br><sup>b</sup> Healthy subjects from endemic regions: 8 |
| 95%                | 73                            | 80  | Rubella IgM positive: 5<br>Dengue IgM positive: 15<br><sup>a</sup> Acute febrile illness cases: 30  |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|   |     |     |   |
|---|-----|-----|---|
|   |     |     | <sup>b</sup> Healthy subjects from endemic regions: 30  |
| 90%   | 138 | 140 | Rubella IgM positive: 8<br>Dengue IgM positive: 26<br><sup>a</sup> Acute febrile illness cases: 53<br><sup>b</sup> Healthy subjects from endemic regions: 53  |
| 85%   | 196 | 200 | Rubella IgM positive: 12<br>Dengue IgM positive: 38<br><sup>a</sup> Acute febrile illness cases: 75<br><sup>b</sup> Healthy subjects from endemic regions: 75 |
| 80%   | 246 | 250 | Rubella IgM positive: 15<br>Dengue IgM positive: 47<br><sup>a</sup> Acute febrile illness cases: 94<br><sup>b</sup> Healthy subjects from endemic regions: 94 |
| <sup>a</sup> Acute febrile illness cases negative for above pathogens AND Chikungunya IgM & PCR<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Chikungunya markers (IgM, RNA)<br><i>#Higher sample size should be used even for assays claiming 99% specificity.</i> |     |     |   |

96

97 **6. Test reproducibility**

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

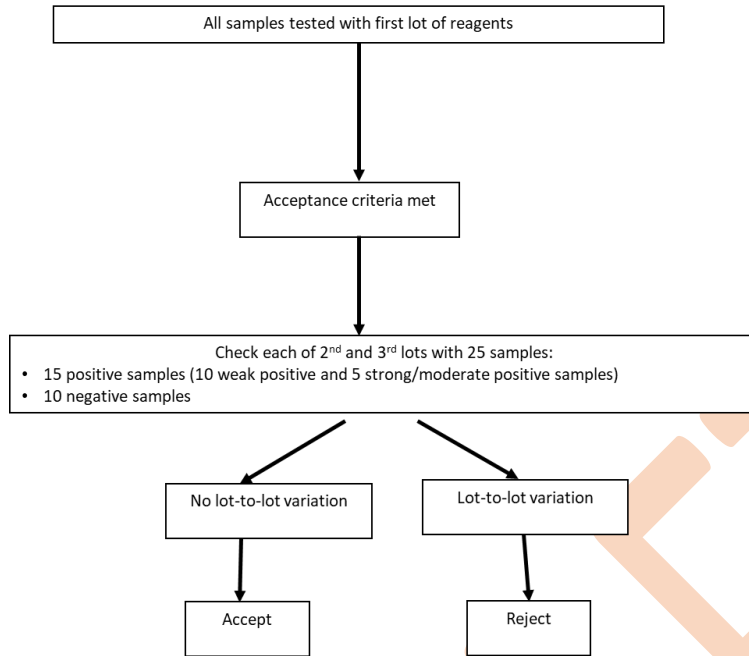
99 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
100 as follows:

- 101 • First lot of the assay: should be tested on statistically significant number of positive  
102 and negative samples as calculated in the protocol.
- 103 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
104 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
105 samples).
- 106 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
107 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

108

109 Refer the flowchart below (Fig.1):

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



110

111

112

### 113 **7. Acceptance Criteria**

114 Expected sensitivity:  $\geq 90\%$

115 Expected specificity:  $\geq 95\%$

### 116 **8. Publication Rights:**

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

118

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

123

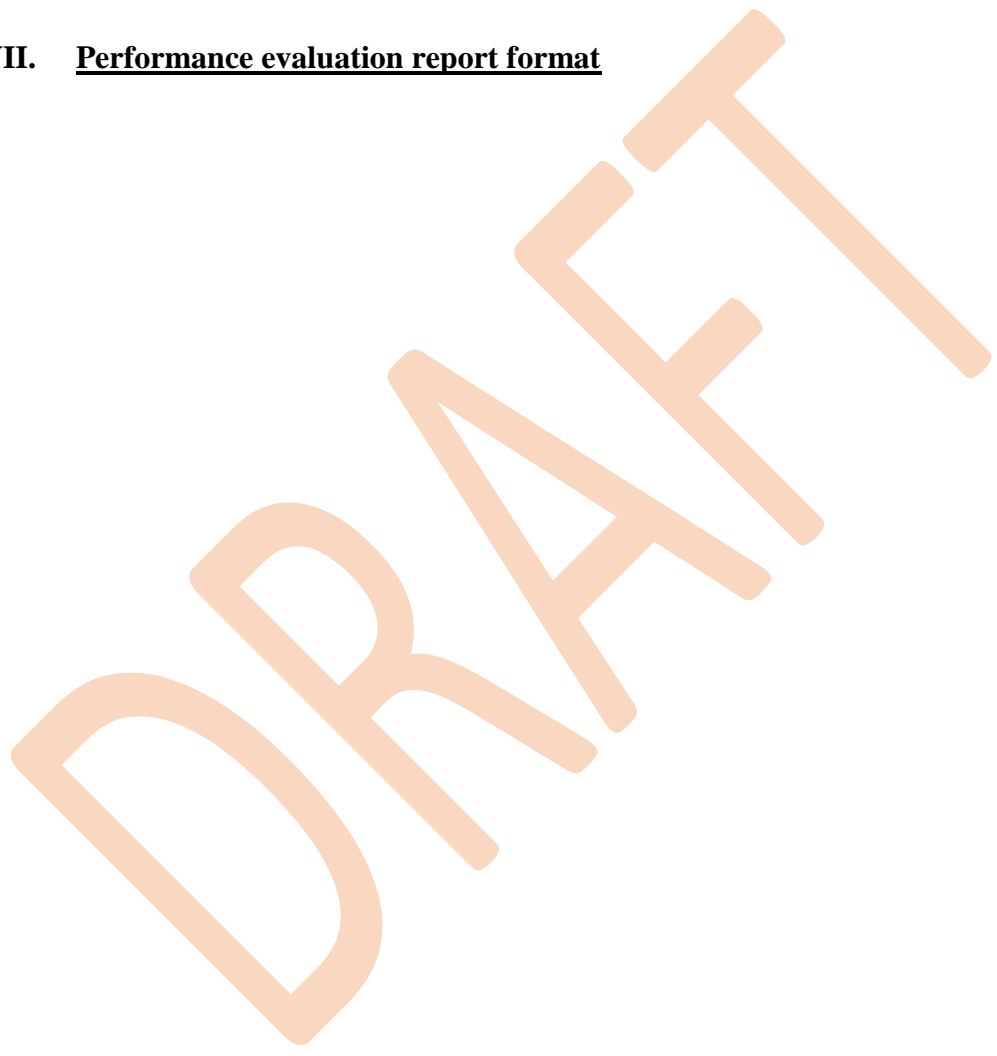
### 124 **VI. References:**

- 125 1. Kikuti M, Tauro LB, Moreira PSS, et al. Evaluation of two commercially available Chikungunya  
126 virus IgM enzyme-linked immunoassays (ELISA) in a setting of concomitant transmission of  
127 Chikungunya, Dengue and Zika viruses. Int J Infect Dis. 2020 Feb;91:38-43.

- 128 2. Johnson BW, Goodman CH, Holloway K, de Salazar PM, Valadere AM, Drebot MA. Evaluation of  
129 Commercially Available Chikungunya Virus Immunoglobulin M Detection Assays. Am J Trop  
130 Med Hyg. 2016 Jul 6;95(1):182-192. doi: 10.4269/ajtmh.16-0013. Epub 2016 Mar 14.  
131 3. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
132 Diagnostic Assessment TGS-3. 2017. Available at:  
133 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-  
135 eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-<br/>134 eng.pdf;sequence=1)

136  
137 **VII. Performance evaluation report format**

138  
139  
140  
141  
142  
143  
144  
145  
146  
147  
148  
149  
150  
151  
152  
153  
154  
155  
156  
157  
158





159 **PERFORMANCE EVALUATION REPORT FOR CHIKUNGUNYA IgM ELISA KIT**

160

|  |  |  |
|--|--|--|
| 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   |  |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license    |  |  |
| License Number:Issue date:   |  |  |
| Valid Up to:   |  |  |
| Application No.  |  |  |
| <b>Sample Panel</b>  | Positive samples (provide details: strong, moderate, weak)                           |  |
|  | Negative samples (provide detail: clinical/spiked, including cross reactivity panel) |  |

161

162 **Results:**

|  |              | <b>Reference assay ..... (name)</b> |          |       |
|--|--------------|-------------------------------------|----------|-------|
|  |              | Positive                            | Negative | Total |
| <b>Name of Chikungunya antibody -based ELISA kit</b> | Positive     |                                     |          |       |
|  | Negative     |                                     |          |       |
|  | <b>Total</b> |                                     |          |       |

163

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

164 **Conclusions:**

165 ○ Sensitivity, specificity

166 ○ Performance: **Satisfactory / Not satisfactory**

167 *(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.)*

168

169  
170  
171  
172  
173  
174  
175  
176  
177  
178  
179  
180  
181  
182  
183  
184  
185  
186  
187  
188  
189  
190  
191  
192  
193  
194  
195  
196  
197

**Disclaimers**

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

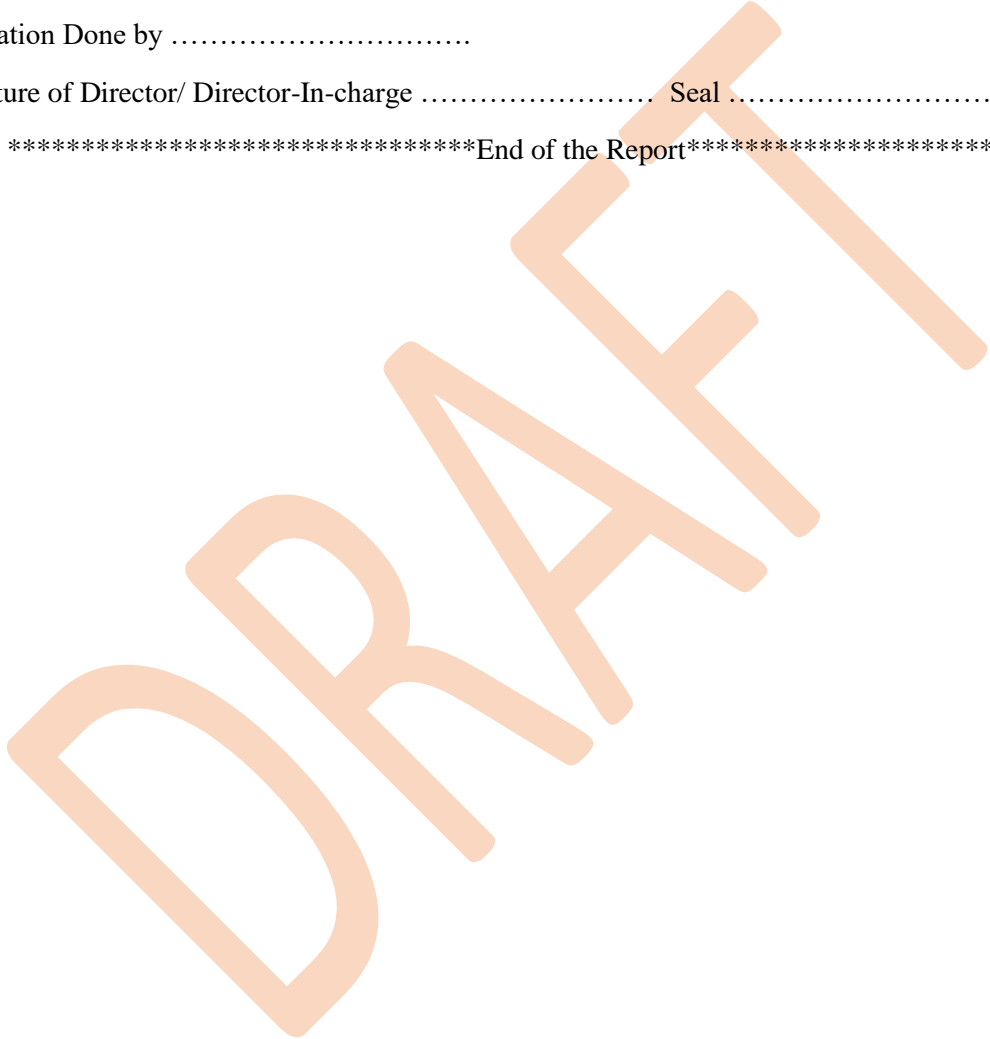
Note: This report is exclusively for .....Kit (Lot No.....) manufactured by .....  
(Supplied by .....)

Evaluation Done on .....

Evaluation Done by .....

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

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



198 **Performance evaluation protocol for Chikungunya IgM RDT kits**

199 **I. Background:**

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

204 **II. Purpose:**

205 To evaluate the performance characteristics of Chikungunya IgM RDT kits in the diagnosis of  
206 Chikungunya infection.

207 **III. Requirements:**

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

215 **IV. Ethical approvals:**

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

219 **V. Procedure:**

- 220 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 221 **2. Preparation of Evaluation sites/laboratories:**
  - 222 **Identified IVD kit evaluation laboratories should establish their proficiency through**
    - 223 A.Accreditation form NABL for at least one of the Quality management system (NABL  
224 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
225 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
    - 226 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
227 and competency testing on following
      - 228 ➤ Preparation & characterization of kit evaluation panel
      - 229 ➤ Handling of Chikungunya IgM RDT kits received for performance evaluation  
230 (Verification/Storage/Unpacking etc).

- 231 ➤ Testing, interpreting, recording of results & reporting
- 232 ➤ Data handling, data safety & confidentiality

### 233 3. Preparation of Chikungunya IgM Rapid IVD kit evaluation panel

234 Well characterised Chikungunya IVD kit evaluation panel is a critical requirement for performance  
235 evaluation of IVD kits. Hence statistically significant number of sera samples should be available  
236 from Chikungunya confirmed cases. Further characterised for Chikungunya IgM positivity by  
237 using approved reference kits having high sensitivity and specificity.

238 Chikungunya IgM performance evaluation panel need to be tested again by the reference assays at  
239 the time of evaluating a particular index test to confirm the positive and negative status of the  
240 samples.

#### 241 4. Reference assay:

242 All the samples will be tested by CDC/NIV real-time PCR assay. *Samples which are positive by*  
243 *RT-PCR assay will be further tested by any two of the following IgM ELISA kits:*

- 244 i. ICMR-NIV MAC ELISA kit
- 245 ii. Inbios CHIKjj Detect™ IgM ELISA
- 246 iii. Anti-Chikungunya virus ELISA (IgM) Test (Euroimmun, Luebeck, Germany)

247 Samples positive by at least two kits will be considered. If sufficient RT-PCR positive samples  
248 are not available, samples positive by at least 2 ELISA kits (of the kits mentioned above) can  
249 be considered as true positive samples.

250 *Samples which are negative by RT-PCR and at least two IgM ELISA kits mentioned above will be*  
251 *considered as Chikungunya negative samples.*

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

263 Positive samples: Positive samples should be positive by RT-PCR at least two ELISA kits from  
264 the three mentioned above. If sufficient RT-PCR positive samples are not available, samples  
265 positive by at least 2 ELISA kits (of the kits mentioned above) can be considered as true positive  
266 samples.

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

267 Negative samples: Samples which are negative by RT-PCR and at least two IgM ELISA kits  
268 mentioned above will be considered as Chikungunya negative samples.

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

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>                                    |
|--|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Strong Positive: 6<br>Moderate Positive: 8<br>Weak Positive: 6     |
| 95%  | 77                            | 80  | Strong Positive: 23<br>Moderate Positive: 34<br>Weak Positive: 23  |
| 90%  | 145                           | 150   | Strong Positive: 43<br>Moderate Positive: 64<br>Weak Positive: 43  |
| 85%  | 206                           | 210   | Strong Positive: 61<br>Moderate Positive: 88<br>Weak Positive: 61  |
| 80%  | 258                           | 260   | Strong Positive: 75<br>Moderate Positive: 110<br>Weak Positive: 75 |
| <i>The samples need to be classified as strong, moderate and weak positives based on ELISA units of the reference assay.</i> |                               |   |  |
| <i>#Higher sample size should be used even for assays claiming 99% sensitivity.</i>  |                               |   |  |

271

272

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

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>  |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Rubella IgM positive: 1<br>Dengue IgM positive: 3<br><sup>a</sup> Acute febrile illness cases: 12<br><sup>b</sup> Healthy subjects from endemic regions: 4 |
| 95%                | 77                            | 80  | Rubella IgM positive: 3  |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |   |
|--|-----|-----|---|
|  |     |     | Dengue IgM positive: 13<br><sup>a</sup> Acute febrile illness cases: 48<br><sup>b</sup> Healthy subjects from endemic regions: 16                             |
| 90%  | 145 | 150 | Rubella IgM positive: 5<br>Dengue IgM positive: 25<br><sup>a</sup> Acute febrile illness cases: 90<br><sup>b</sup> Healthy subjects from endemic regions: 30  |
| 85%  | 206 | 210 | Rubella IgM positive: 7<br>Dengue IgM positive: 35<br><sup>a</sup> Acute febrile illness cases: 126<br><sup>b</sup> Healthy subjects from endemic regions: 42 |
| 80%  | 258 | 260 | Rubella IgM positive: 9<br>Dengue IgM positive: 43<br><sup>a</sup> Acute febrile illness cases: 156<br><sup>b</sup> Healthy subjects from endemic regions: 52 |
| <sup>a</sup> Acute febrile illness cases negative for above pathogens AND Chikungunya IgM & PCR<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Chikungunya markers (IgM, RNA) |     |     |   |
| <i>#Higher sample size should be used even for assays claiming 99% specificity.</i>  |     |     |   |

275  
276  
277  
278  
279  
280  
281  
282  
283  
284  
285  
286  
287

**6. Test reproducibility**

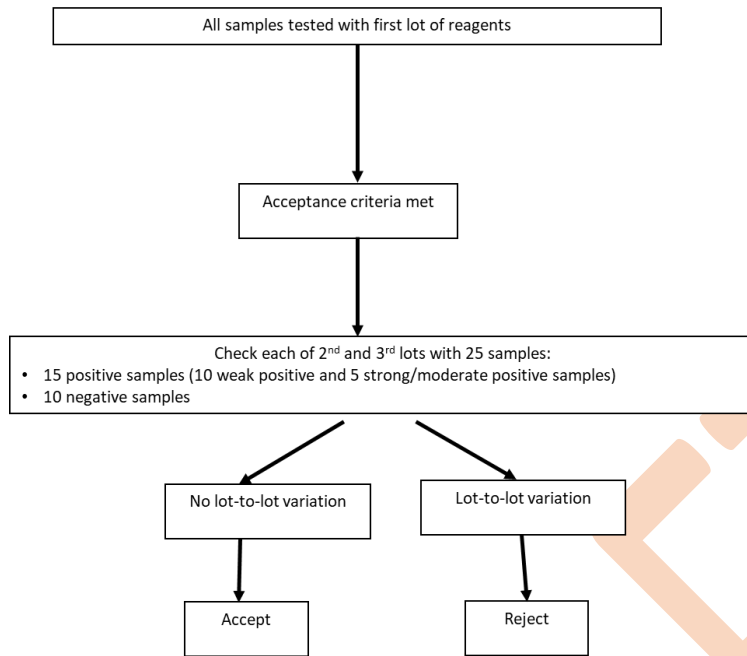
**A. Sample size for lot-to-lot reproducibility**

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

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

288 Refer the flowchart below (Fig. 1):

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



289

290

291

292

### B. Sample size for reader-to-reader reproducibility

293

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).

295

296

297

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

298

### 7. Acceptance criteria

299

Expected sensitivity:  $\geq 80\%$

300

Expected specificity:  $\geq 90\%$

301

Invalid test rate:  $\leq 5\%$

302

### 8. Publication Rights:

303

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

304

305

306

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

307

308 acceptable. Any request of re-validation from the same manufacturer for the same test type  
309 will only be entertained if valid proof of change in the kit composition is submitted.

310 **VI. References:**

- 311 1. Kikuti M, Tauro LB, Moreira PSS, et al. Evaluation of two commercially available  
312 Chikungunya virus IgM enzyme-linked immunoassays (ELISA) in a setting of  
313 concomitant transmission of Chikungunya, Dengue and Zika viruses. Int J Infect Dis.  
314 2020 Feb;91:38-43.
- 315 2. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
316 Diagnostic Assessment TGS-3. 2017. Available at:  
317 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
318 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
319

320 **VII. Performance evaluation report format**

321  
322  
323  
324  
325  
326  
327  
328  
329  
330  
331  
332  
333  
334  
335  
336  
337  
338  
339



340 **PERFORMANCE EVALUATION REPORT FOR CHIKUNGUNYA IgM 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 CDSO/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  |   |  |
| <b><u>Regulatory Approval:</u></b>  |   |  |
| Import license / Manufacturing license/ Test license                                  |   |  |
| License Number:Issue date:  |   |  |
| Valid Up to:  |   |  |
| Application No.   |   |  |
| <b>Sample Panel</b>   | Positive samples (provide details: strong, moderate, weak)                            |  |
|   | Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

341 **Results:**

|   |              | <b>Reference assay ..... (name)</b> |          |       |
|---|--------------|-------------------------------------|----------|-------|
|   |              | Positive                            | Negative | Total |
| <b>Name of Chikungunya antibody - based RDT kit</b> | Positive     |                                     |          |       |
|   | Negative     |                                     |          |       |
|   | <b>Total</b> |                                     |          |       |

342

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

343 **Conclusions:**

344     ○ Sensitivity, specificity

345     ○ Performance: **Satisfactory / Not satisfactory**

346 *(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.)*

348

349  
350  
351  
352  
353  
354  
355  
356  
357  
358  
359  
360  
361  
362  
363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378

**Disclaimers**

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

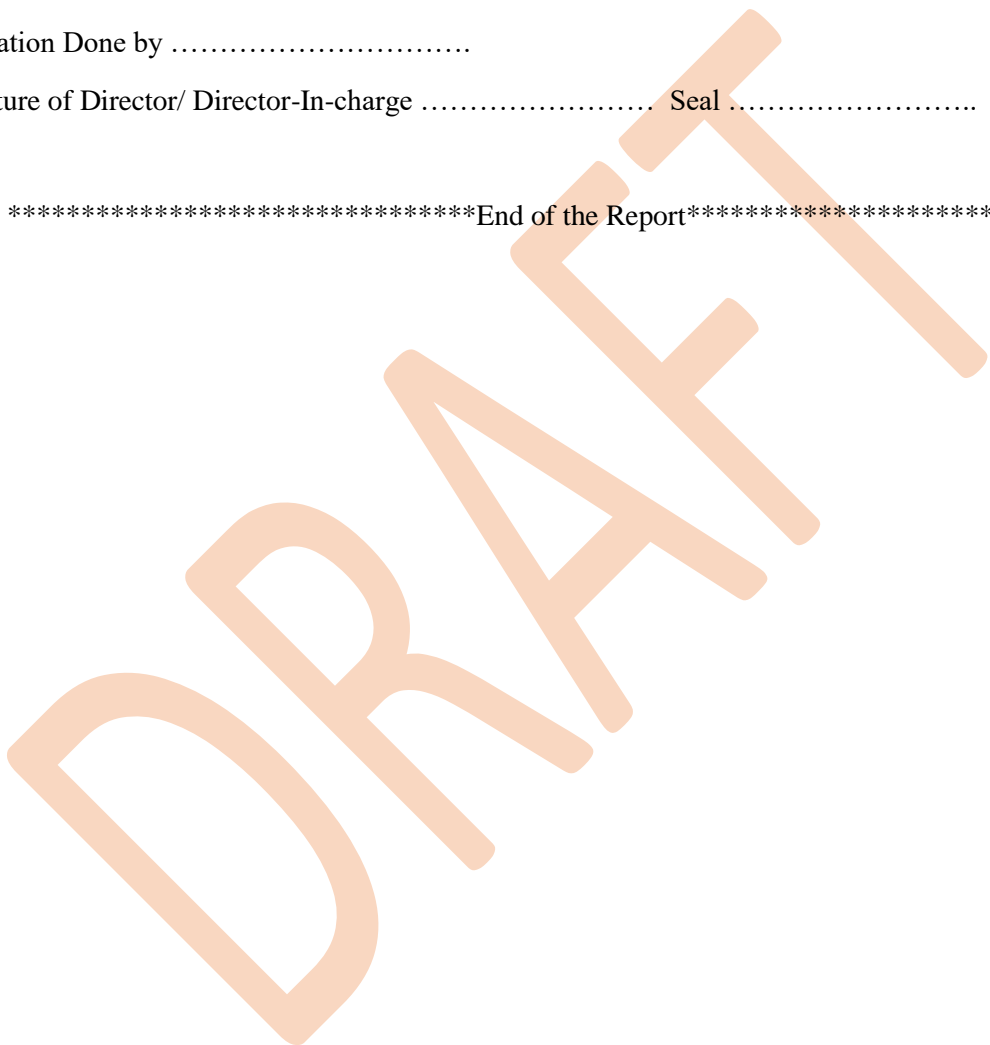
Note: This report is exclusively for .....Kit (Lot No.....) manufactured by .....  
(Supplied by .....)

Evaluation Done on .....

Evaluation Done by .....

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

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



379 **Performance evaluation protocol for Chikungunya real-time PCR kits**

380 **I. Background:**

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

385 **II. Purpose:**

386 To evaluate the performance characteristics of Chikungunya PCR kits in the diagnosis of  
387 Chikungunya infection.

388 **III. Requirements:**

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

396 **IV. Ethical approvals:**

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

400 **V. Procedure:**

401 **1. Study design/type:** Diagnostic accuracy study using archived/ leftover/spiked clinical  
402 samples.

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

404 **Identified IVD kit evaluation laboratories should establish their proficiency through**  
405 A.Accreditation form NABL for at least one of the Quality management system (NABL  
406 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
407 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

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

- 410 ➤ Preparation & characterization of kit evaluation panel
- 411 ➤ Handling of Chikungunya PCR kits received for performance evaluation  
412 (Verification/Storage/Unpacking etc).

- 413 ➤ Testing, interpreting, recording of results & reporting
- 414 ➤ Data handling, data safety & confidentiality

### 415 **3. Preparation of Chikungunya RNA evaluation panel**

416 Well characterised Chikungunya sample panel positive for RNA is a critical requirement for  
417 performance evaluation of IVD kits utilizing genome detection. Hence statistically significant  
418 number of sera/whole blood samples should be available from Chikungunya PCR confirmed cases.

### 419 **4. RNA extraction**

420 *RNA extraction should be performed using a standard RNA extraction kit using spin columns such*  
421 *as QIAamp Viral RNA Mini kitor MDI Viral Mini RNA Extraction Mini Prep Kit or magnetic*  
422 *bead-based extraction methods such as MagMax viral RNA isolation kit.*

423 If the manufacturer of the index test recommends a specific RNA extraction kit, the same needs to  
424 be provided by the manufacturer.

### 425 **5. Real-Time PCR System**

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

### 429 **6. Internal control/Extraction control**

430 The test under evaluation should have an internal control or extraction control (RNA added before  
431 extraction to a sample).

### 432 **7. Reference assay:**

433 Any FDA approved Chikungunya PCR assay or CDC/NIV protocol for detection of Chikungunya  
434 RNA should be used as the reference assay.

435 All positive samples should be confirmed positive for Chikungunya by reference assay.

436 All negative samples should be negative for all markers of Chikungunya infection (RNA using  
437 reference assay AND IgM using any two of the following kits - ICMR-NIV MAC ELISA  
438 kit/Inbios CHIKjj Detect™ IgM ELISA/Anti-Chikungunya virus ELISA (IgM) Test (Euroimmun,  
439 Luebeck, Germany).

440 **8. Sample size and sample panel composition:** Sample sizes of positive and negative  
441 samples and sample panel composition against different values of sensitivity and specificity are  
442 provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance,  
443 an absolute precision of 5%, and invalid test rate  $\leq 5\%$ . Appropriate sample size has to be chosen  
444 from the tables according to the values of sensitivity and specificity being claimed by the  
445 manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs  
446 to consider the sample size associated with the largest sensitivity/specificity provided in the table

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

447 that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity  
448 available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required  
449 to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would  
450 require usage of the sample size outlined for 85% specificity.

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

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>                                    |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Strong Positive: 5<br>Moderate Positive: 10<br>Weak Positive: 5    |
| 95%                | 77                            | 80  | Strong Positive: 20<br>Moderate Positive: 40<br>Weak Positive: 20  |
| 90%                | 145                           | 150   | Strong Positive: 38<br>Moderate Positive: 74<br>Weak Positive: 38  |
| 85%                | 206                           | 210   | Strong Positive: 53<br>Moderate Positive: 104<br>Weak Positive: 53 |

453 <sup>#</sup>Higher sample size should be used even for assays claiming 99% sensitivity.

454 Strong positive (Ct value between <25)

455 Moderate positive (Ct value between 25-30)

456 Weak positive (Ct value between >30 to 34)

457

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

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>  |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Rubella IgM positive: 1<br>Dengue IgM positive: 4<br><sup>a</sup> Acute febrile illness cases: 10<br><sup>b</sup> Healthy subjects from endemic regions: 5 |
| 95%                | 77                            | 80  | Rubella IgM positive: 5  |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|   |     |     |  |
|---|-----|-----|--|
|   |     |     | Dengue IgM positive: 15<br><sup>a</sup> Acute febrile illness cases: 40<br><sup>b</sup> Healthy subjects from endemic regions: 20                              |
| 90%   | 145 | 150 | Rubella IgM positive: 9<br>Dengue IgM positive: 28<br><sup>a</sup> Acute febrile illness cases: 75<br><sup>b</sup> Healthy subjects from endemic regions: 38   |
| 85%   | 206 | 210 | Rubella IgM positive: 13<br>Dengue IgM positive: 39<br><sup>a</sup> Acute febrile illness cases: 105<br><sup>b</sup> Healthy subjects from endemic regions: 53 |
| <sup>a</sup> Acute febrile illness cases negative for above pathogens <b>AND</b> Chikungunya IgM & PCR<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Chikungunya markers (IgM, RNA) |     |     |  |

460 #Higher sample size should be used even for assays claiming 99% specificity.

461 **9. Evaluation method:**

462 The index test and the reference tests should be run simultaneously on the sample panel to avoid  
 463 false negative results by index test due to free thawing of samples or deterioration of sample quality  
 464 on long term storage.

465 **10. Test reproducibility**

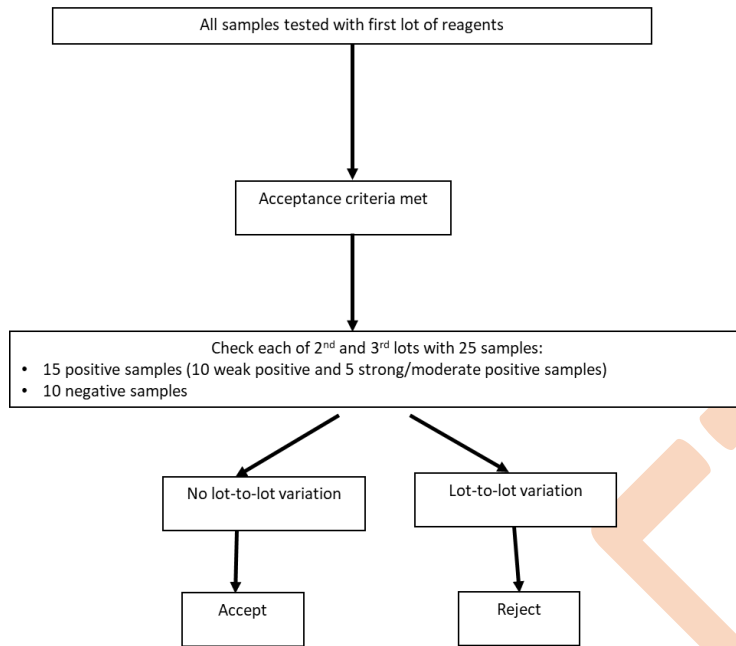
466 **A. Sample size for lot-to-lot reproducibility**

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

- 469 • First lot of the assay: should be tested on statistically significant number of positive  
 470 and negative samples as calculated in the protocol.
- 471 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
 472 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
 473 samples).
- 474 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
 475 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

476 Refer the flowchart below (Fig. 1):

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



477

478

## 479 **11. Acceptance criteria**

480 Expected sensitivity:  $\geq 95\%$

481 Expected specificity:  $\geq 98\%$

482 Cross reactivity with related viruses: NIL

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

## 484 **11. Publication Rights:**

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

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

490

## 491 **VI. References:**

- 492 1. Santiago, G.A., Vázquez, J., Courtney, S. et al. Performance of the Triplex real-time RT-PCR assay  
493 for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun 9, 1391 (2018).  
494 <https://doi.org/10.1038/s41467-018-03772-1>
- 495 2. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
496 Diagnostic Assessment TGS-3. 2017. Available at:

497 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
498 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)

499  
500  
501

**VII. Performance evaluation report format**

502  
503  
504  
505  
506  
507  
508  
509  
510  
511  
512  
513  
514  
515  
516  
517  
518  
519  
520  
521  
522  
523  
524  
525

DRAFT



**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

526 **PERFORMANCE EVALUATION REPORT FOR CHIKUNGUNYA REAL-TIME PCR**  
527 **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<br>CDSO/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   |   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license      |   |  |
| License Number:Issue date:   |   |  |
| Valid Up to:   |   |  |
| Application No.  |   |  |
| <b>Sample Panel</b>  | Positive samples (provide details: clinical/spiked, strong, moderate, weak)           |  |
|  | Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

528  
529 **Results**

|   |          | Reference assay ..... (name) |          |       |
|---|----------|------------------------------|----------|-------|
|   |          | Positive                     | Negative | Total |
| <b>Name of Chikungunya real-time PCR kits</b> | Positive |                              |          |       |
|   | Negative |                              |          |       |
|   | Total    |                              |          |       |

530

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

- 531  
532 ● **Conclusions:**  
533 ○ Cross reactivity with related viruses:  
534 ○ **Performance: Satisfactory / Not satisfactory**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

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

537

538 **Disclaimers**

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

541 Note: This report is exclusively for Chikungunya..... Kit (Lot No.....) manufactured by .....  
542 (supplied by .....)

543 Evaluation Done on .....

544 Evaluation Done by .....

545 Signature of Director/ Director-In-charge ..... Seal .....

546 \*\*\*\*\*End of the Report\*\*\*\*\*

547

548

549

550

551

552

553

554

555

556

557

558

559

560

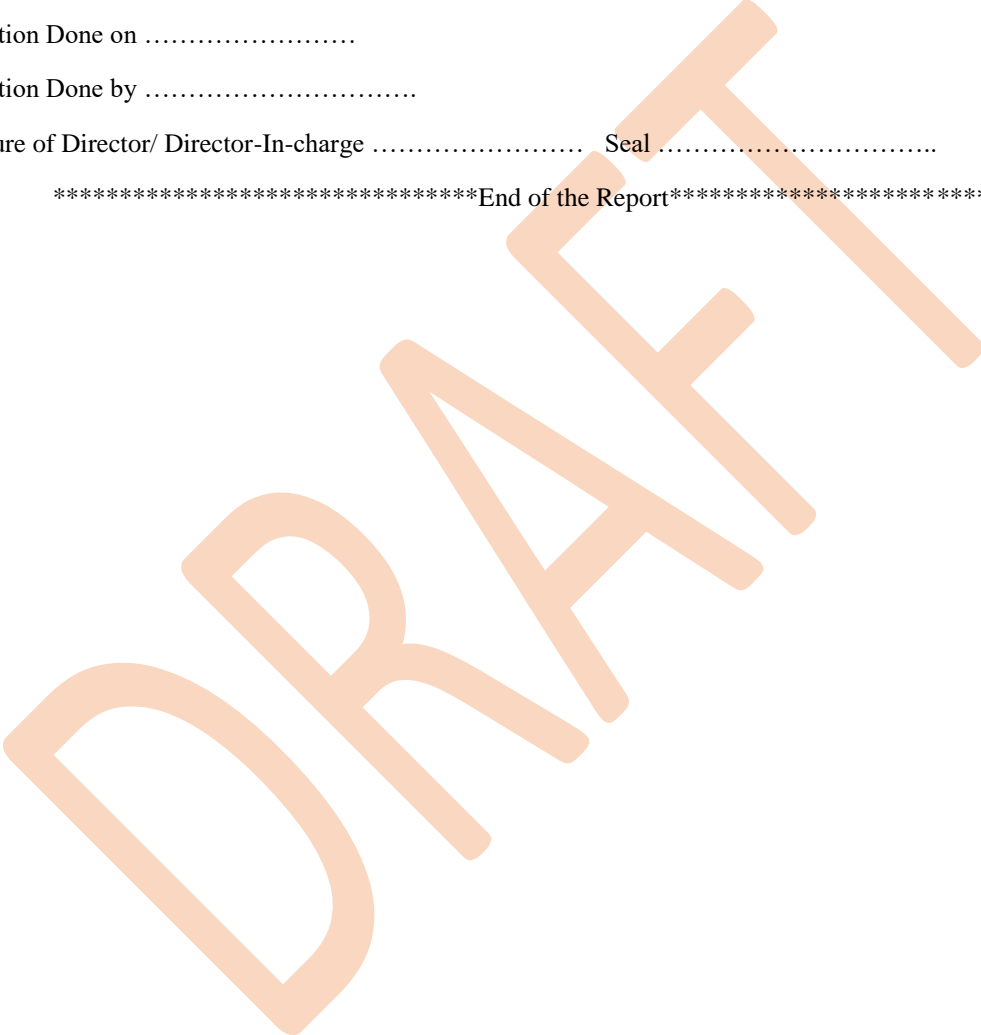
561

562

563

564

565



566 **Performance evaluation protocol for Dengue NS1 RDT kits**

567 **I. Background:**

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

572 **II. Purpose:**

573 To evaluate the performance characteristics of Dengue NS1 RDT kits in the diagnosis of Dengue  
574 infection.

575 **III. Requirements:**

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

583 **IV. Ethical approvals:**

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

587 **V. Procedure:**

- 588 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 589 **2. Preparation of Evaluation sites/laboratories:**
  - 590 **Identified IVD kit evaluation laboratories should establish their proficiency through**
    - 591 A. Accreditation form NABL for at least one of the Quality management system (NABL  
592 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
593 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
    - 594 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
595 and competency testing on following
      - 596 ➤ Preparation & characterization of kit evaluation panel
      - 597 ➤ Handling of Dengue NS1 Rapid IVD kits received for performance evaluation  
598 (Verification/Storage/Unpacking etc).

- 599 ➤ Testing, interpreting, recording of results & reporting
- 600 ➤ Data handling, data safety & confidentiality

601 **3. Preparation of Dengue RDT IVD kit evaluation panel**

602 Well characterised Dengue NS1 RDT IVD kit evaluation panel is a critical requirement for  
603 performance evaluation of IVD kits. Hence statistically significant number of sera samples should  
604 be available from Dengue confirmed cases. Further characterised for Dengue NS1 positivity by  
605 using approved reference kits having high sensitivity and specificity.

606 Dengue NS1 performance evaluation panel need to be tested again by the reference assays at the  
607 time of evaluating a particular index test to confirm the positive and negative status of the samples.

608 **4. Reference assay:**

609 US-FDA approved Dengue NS1 ELISA kit should be used as reference assay.

610 Serotype status to be assessed using CDC/NIV real-time PCR serotyping protocols.

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

622 Positive samples: The panel of positive samples should include samples positive by the reference  
623 assay and real-time PCR assay (True positives). Samples should be representative of all 4 serotypes  
624 and varying degrees of positivity. The samples should be classified as strong, moderate and weak  
625 positives based on ELISA units of the reference assay.

626

627 Negative samples: These should include samples negative by the reference NS1 ELISA assay and  
628 real-time PCR using CDC/NIV serotyping protocol (True negatives).

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

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i> |
|--------------------|-------------------------------|---|---------------------------------|
|--------------------|-------------------------------|---|---------------------------------|

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|                  |     |     |   |
|------------------|-----|-----|---|
| 99% <sup>#</sup> | 16  | 20  | Samples should be representative of all 4 serotypes and varying degrees of positivity, with at least 25% weak positive samples. |
| 95%              | 77  | 80  |   |
| 90%              | 145 | 150 |   |
| 85%              | 206 | 210 |   |
| 80%              | 258 | 260 |   |

631

632 #Higher sample size should be used even for assays claiming 99% sensitivity.

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

| Specificity      | Calculated sample size | No. of Negative Samples required [Sample size rounded off] | Sample Panel Composition   |
|------------------|------------------------|--|--|
| 99% <sup>#</sup> | 16                     | 20   | <p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u><br/>Chikungunya: 4<br/>Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR): 8</p> <p><u>-Samples from other flavivirus disease cases</u><br/>*Japanese Encephalitis PCR/antigen positive: 1<br/>*West Nile Virus PCR/antigen positive: 1<br/>*Zika Virus PCR/antigen positive: 1</p> <p>-Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 5</p> |
| 95%              | 77                     | 80   | <p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u><br/>Chikungunya: 15<br/>Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR): 30</p> <p><u>-Samples from other flavivirus disease cases</u><br/>*Japanese Encephalitis PCR/antigen positive: 5<br/>*West Nile Virus PCR/antigen positive: 5<br/>*Zika Virus PCR/antigen positive: 5</p>   |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|     |     |     |   |
|-----|-----|-----|---|
|     |     |     | <p>-Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 20</p>  |
| 90% | 145 | 150 | <p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u><br/>Chikungunya: 28<br/>Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR): 57</p> <p><u>-Samples from other flavivirus disease cases</u><br/>*Japanese Encephalitis PCR/antigen positive: 9<br/>*West Nile Virus PCR/antigen positive: 9<br/>*Zika Virus PCR/antigen positive: 9</p> <p>-Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 38</p>   |
| 85% | 206 | 210 | <p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u><br/>Chikungunya: 39<br/>Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR): 79</p> <p><u>-Samples from other flavivirus disease cases</u><br/>*Japanese Encephalitis PCR/antigen positive: 13<br/>*West Nile Virus PCR/antigen positive 13<br/>*Zika Virus PCR/antigen positive: 13</p> <p>-Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 53</p> |
| 80% | 258 | 260 | <p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u><br/>Chikungunya: 49<br/>Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR): 98</p> <p><u>-Samples from other flavivirus disease cases</u><br/>*Japanese Encephalitis PCR/antigen positive: 16</p>  |

|  |  |  |  |
|--|--|--|--|
|  |  |  | <p>*West Nile Virus PCR/antigen positive: 16<br/>*Zika Virus PCR/antigen positive: 16</p> <p>-Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 65</p> |
| <p>* In the absence of natural samples, spiked samples may be used, as per details provided in the note below.</p> <p>Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng -1 µg/ml) and diluted in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).<br/>Before used for evaluation, flavivirus NS1 reconstituted in serum samples needs to be tested by the dengue NS1 reference assay, and dilutions which are negative for dengue should be used for evaluation.<br/>The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.</p> <p><i>#Higher sample size should be used even for assays claiming 99% specificity.</i></p> |  |  |  |

635  
636  
637  
638  
639  
640  
641  
642  
643  
644  
645  
646  
647  
648

**6. Test reproducibility**

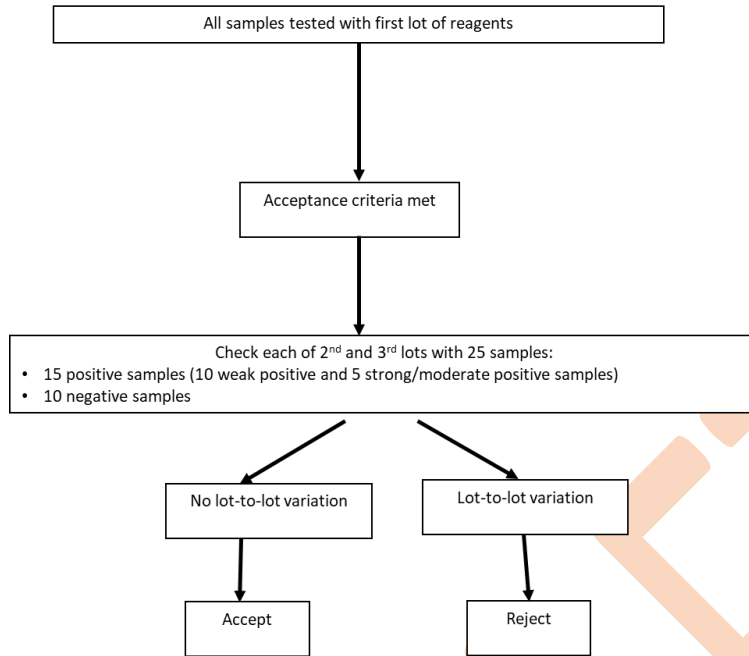
**A. Sample size for lot-to-lot reproducibility**

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

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

Refer the flowchart below (Fig. 1):

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



649

650

### 651 B. Sample size for reader-to-reader reproducibility

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

654

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

### 657 7. Criteria for approval of the Dengue NS1 RDT kits

658 Expected sensitivity:  $\geq 80\%$

659 Expected specificity:  $\geq 95\%$

660 Cross reactivity with other flavivirus antigens: Nil

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

### 662 9. Publication Rights:

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

664

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



667 acceptable. Any request of re-validation from the same manufacturer for the same test type  
668 will only be entertained if valid proof of change in the kit composition is submitted.

669

670 **VI. References:**

- 671 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian  
672 E, Pelegrino JL, Artsob H, Guzman MG, Oliario P, Zwang J, Guillerm M, Kliks S, Halstead S,  
673 Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection  
674 of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct  
675 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
- 676 2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanaroj S,  
677 Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay  
678 sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl  
679 Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
- 680 3. Yow KS, Aik J, Tan EY, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent  
681 Dengue infections: An evaluation of six kits on clinical specimens. PLoS One. 2021 Apr  
682 1;16(4): e0249602. doi: 10.1371/journal.pone.0249602.
- 683 4. Mat Jusoh TNA, Shueb RH. Performance Evaluation of Commercial Dengue Diagnostic  
684 Tests for Early Detection of Dengue in Clinical Samples. J Trop Med. 2017; 2017:  
685 4687182. doi: 10.1155/2017/4687182. Epub 2017 Dec 12. PMID: 29379526; PMCID:  
686 PMC5742879.
- 687 5. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
688 Diagnostic Assessment TGS-3. 2017. Available at:  
689 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-  
690 eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)
- 691 6. Mahajan R, Nair M, Saldanha AM, Harshana A, Pereira AL, Basu N, Goswami RP,  
692 Bhattacharya N, Bandyopadhyay B, SenGupta M, Day M, Flevaud L, Boelaert M,  
693 Burza S. Diagnostic accuracy of commercially available immunochromatographic  
694 rapid tests for diagnosis of dengue in India. J Vector Borne Dis. 2021 Apr-  
695 Jun;58(2):159-164. doi: 10.4103/0972-9062.321747. PMID: 35074951.

696

697 **VII. Performance evaluation report format**

698

699

700

701

702

703

704

705

706

**PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 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 CDSO/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  |   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |   |  |
| License Number:Issue date:  |   |  |
| Valid Up to:  |   |  |
| Application No.   |   |  |
| <b>Sample Panel</b>   | Positive samples (provide details: clinical/spiked, strong, moderate, weak)           |  |
|   | Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

707

708

**Results:**

|   |          | <b>Reference assay ..... (name)</b> |          |       |
|---|----------|-------------------------------------|----------|-------|
|   |          | Positive                            | Negative | Total |
| <b>Name of Dengue NS1 - based RDT kit</b> | Positive |                                     |          |       |
|   | Negative |                                     |          |       |
|   | Total    |                                     |          |       |

709

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

710

711

- Details of cross reactivity with other flavivirus NS1 antigens:

712

- **Conclusions:**

713

- Sensitivity, specificity

714

- Performance: **Satisfactory / Not satisfactory**

715

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

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

718

719 **Disclaimers**

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

722

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

724 Evaluation Done on .....

725 Evaluation Done by .....

726 Signature of Director/ Director-In-charge ..... Seal .....

727 \*\*\*\*\*End of the Report\*\*\*\*\*

728

729

730

731

732

733

734

735

736

737

738

739

740

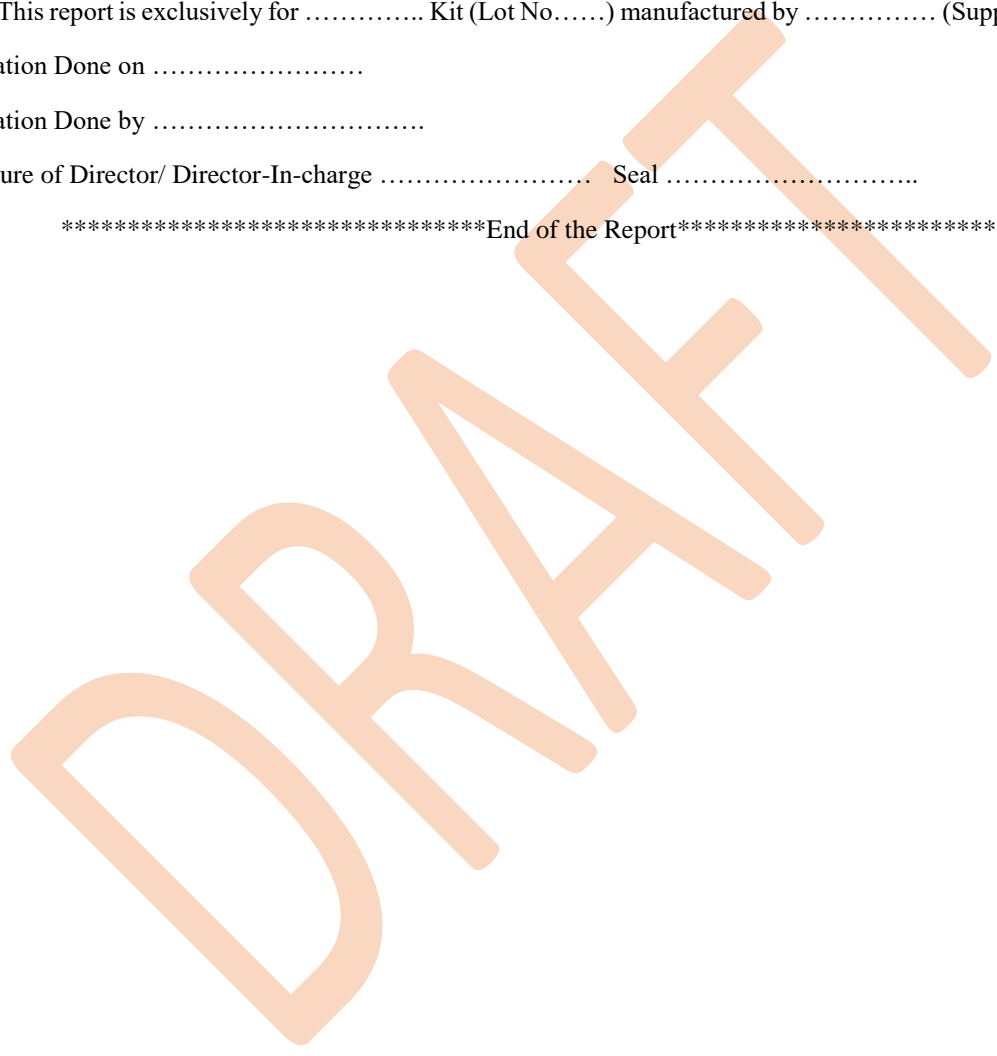
741

742

743

744

745



746 **Field evaluation protocol for Dengue NS1 RDT kits**

747 **I. Background:**

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

752 **II. Purpose:**

753 To evaluate the performance characteristics of Dengue NS1 RDT kits in the diagnosis of Dengue  
754 infection in individuals with unknown disease status.

755 **III. Requirements:**

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

762  
763 **IV. Ethical approval:**

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

765 **V. Procedure:**

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

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

768 **Identified IVD kit evaluation laboratories should establish their proficiency through**

769 A.Accreditation form NABL for at least one of the Quality management system (NABL  
770 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
771 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

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

- 774 ➤ Preparation & characterization of kit evaluation panel
- 775 ➤ Handling of Dengue NS1 RDT IVD kits received for performance evaluation  
776 (Verification/Storage/Unpacking etc).
- 777 ➤ Testing, interpreting, recording of results & reporting
- 778 ➤ Data handling, data safety & confidentiality

779 **3. Sample size for performance evaluation:**

780 Sample sizes of positive and negative samples of Dengue against different values of  
781 sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated  
782 assuming 95% level of significance, an absolute precision of 5%, and invalid test rate  $\leq 5\%$ .  
783 It is further assumed that 30% of the individuals attending the health care facilities for acute  
784 febrile illness and suspected for Dengue will be positive for Dengue. Appropriate sample  
785 size has to be chosen from the tables according to the values of sensitivity and specificity  
786 being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the  
787 table, the manufacturer needs to consider the sample size associated with the largest  
788 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as  
789 per the next smaller value of the sensitivity/ specificity available in the table). For example,  
790 if a manufacturer claims a sensitivity of 93%, they are required to use a sample size  
791 mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require  
792 usage of the sample size outlined for 85% specificity.

793 Sample size has to be calculated based on both the sensitivity and the specificity. The  
794 final sample size will be the maximum of the two. For example, at 95% sensitivity and  
795 95% specificity, the sample size required will be 260 (maximum of 260 and 110).  
796

797 Table 1. Sample sizes for different values of sensitivity claimed by the manufacturer.

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--|-------------------------------|--|
| 99%#   | 53                            | 60   |
| 95%  | 255                           | 260  |
| 90%  | 484                           | 490  |
| 85%  | 686                           | 690  |
| 80%  | 861                           | 870  |
| * Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria |                               |  |

798  
799 #Higher sample size should be used even for assays claiming 99% sensitivity.

800  
801 Table 2. Sample sizes for different values of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--------------------|-------------------------------|--|
| 99%#               | 23                            | 30   |
| 95%                | 109                           | 110  |
| 90%                | 207                           | 210  |
| 85%                | 294                           | 300  |
| 80%                | 369                           | 370  |

\* Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria

802

803 *#Higher sample size should be used even for assays claiming 99% specificity.*

804 Recruitment of cases shall be halted once desired number of positive and negative samples are  
805 reached.

806 **4. Inclusion criteria:**

807 Individuals with Dengue like illness (An individual with acute febrile illness of 2-7 days with two  
808 or more manifestations: Head ache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic  
809 manifestations)

810 **5. Exclusion criteria:**

811 Individuals with already known positive history for other pathogens

812 **6. Reference assay:**

813 *US-FDA approved Dengue NS1 ELISA kit should be used as reference assay.*

814 *Serotype status to be assessed using CDC/NIV real-time PCR serotyping protocols.*

815 **7. Study implementation:**

816 The individuals with Dengue like illness will be recruited into the study and five ml of whole blood  
817 will be collected in vacutainer tubes and the serum will be separated by centrifugation and used  
818 for the study. The serum sample will be subjected to the following reference tests and the index  
819 test.

820 It needs to be ensured that the samples are tested by reference tests and index test simultaneously.

821 **8. Positive samples:**

822 Samples positive by the reference NS1 ELISA assay and real-time PCR assay will be considered  
823 as true positive sample.

824 **9. Negative samples:**

825 Samples negative by the reference NS1 ELISA assay and real-time PCR using CDC/NIV  
826 *serotyping protocol* will be considered as true negative.

827 **A. Cross reactivity:**

828 Clinical samples or commercially available NS1 antigens from other flaviviruses will be used to  
829 test cross reactivity of the index test.

830 i. Japanese Encephalitis PCR/antigen positive: 5 samples\*

831 ii. West Nile Virus PCR/antigen: 5 samples\*

832           iii.    Zika Virus PCR/antigen: 5 samples\*

833           \*In the absence of natural samples, spiked samples may be used, as per details provided in the note below.

834           **Note:**

835           Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed  
836           in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng -1 µg/ml) and diluted  
837           in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).

838           Before used for evaluation, flavivirus NS1 reconstituted in serum samples needs to be tested by the dengue NS1  
839           reference assay, and dilutions which are negative for dengue should be used for evaluation.

840           The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.

841           **10. Statistical analysis:**

842           Sensitivity and specificity will be calculated.

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

846           **11. Test reproducibility**

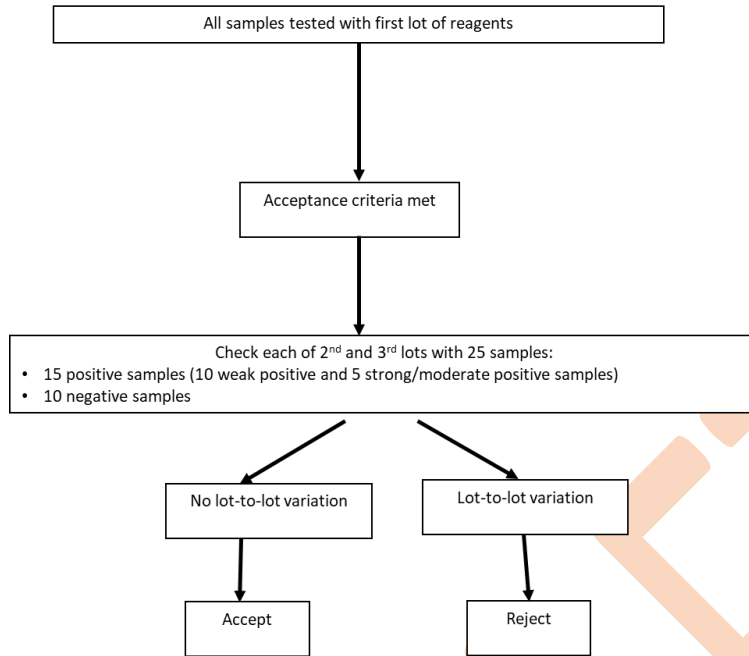
847           **A. Sample size for lot-to-lot reproducibility**

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

- 850           • First lot of the assay: should be tested on statistically significant number of positive  
851           and negative samples as calculated in the protocol.
- 852           • Second lot of the assay: should be tested on 25 samples (15 positive samples  
853           comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
854           samples).
- 855           • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
856           10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

857           Refer the flowchart below (Fig. 1):

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



858

### 859 B. Sample size for reader-to-reader reproducibility

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

862

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

### 865 12. Acceptance Criteria

866 Expected sensitivity:  $\geq 80\%$

867 Expected specificity:  $\geq 95\%$

868 Cross-reactivity with other flavivirus antigens: Nil

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

### 870 13. Publication Rights:

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

872

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



877

878 **VI. References:**

- 879 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E,  
880 Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling  
881 RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of  
882 Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct  
883 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
- 884 2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon  
885 IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for  
886 the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi:  
887 10.1371/journal.pntd.0003193.
- 888 3. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar  
889 A, John D, Mehendale SM. Dengue infection in India: A systematic review and meta-analysis.  
890 PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi: 10.1371/journal.pntd.0006618.
- 891
- 892 4. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
893 Diagnostic Assessment TGS-3. 2017. Available at:  
894 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
895 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)
- 896 5. Mahajan R, Nair M, Saldanha AM, Harshana A, Pereira AL, Basu N, Goswami RP, Bhattacharya  
897 N, Bandyopadhyay B, SenGupta M, Day M, Flevaud L, Boelaert M, Burza S. Diagnostic accuracy  
898 of commercially available immunochromatographic rapid tests for diagnosis of dengue in India.  
899 J Vector Borne Dis. 2021 Apr-Jun;58(2):159-164. doi: 10.4103/0972-9062.321747. PMID:  
900 35074951.

901 **VII. Performance evaluation report format**

902

903

904

905

906

907

908

909

910

911

912

913

914

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSCO/IVD/GD/PROTOCOLS/02/2024

915

**PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 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   |   |  |
| <b>Regulatory Approval:</b>  |   |  |
| Import license / Manufacturing license/ Test license                                   |   |  |
| License Number:Issue date:   |   |  |
| Valid Up to:   |   |  |
| Application No.  |   |  |
| <b>Sample Panel</b>  | Positive samples: Not applicable, may categorize cases as per duration of illness                 |  |
|  | Negative samples (may categorize as per duration of illness, must include cross reactivity panel) |  |

916

917

**Results:**

|   |          | <b>Reference assay ..... (name)</b> |          |       |
|---|----------|-------------------------------------|----------|-------|
|   |          | Positive                            | Negative | Total |
| <b>Name of Dengue NS1 - based RDT kit</b> | Positive |                                     |          |       |
|   | Negative |                                     |          |       |
|   | Total    |                                     |          |       |

918

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

919

920

921

922

923

924

- Details of cross reactivity with other flavivirus NS1 antigens:
- Conclusions:
  - Sensitivity, specificity
  - Performance: **Satisfactory / Not satisfactory**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

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

927

928 **Disclaimers**

- 929 1. This validation process does not approve / disapprove the kit design  
930 2. This validation process does not certify user friendliness of the kit / assay  
931

932 Note: This report is exclusively for NS1.....Kit (Lot No.....) manufactured by ..... (supplied  
933 by .....

934 Evaluation Done on .....

935 Evaluation Done by .....

936 Signature of Director/ Director-In charge ..... Seal .....

937 \*\*\*\*\*End of the Report\*\*\*\*\*

938

939

940

941

942

943

944

945

946

947

948

949

950

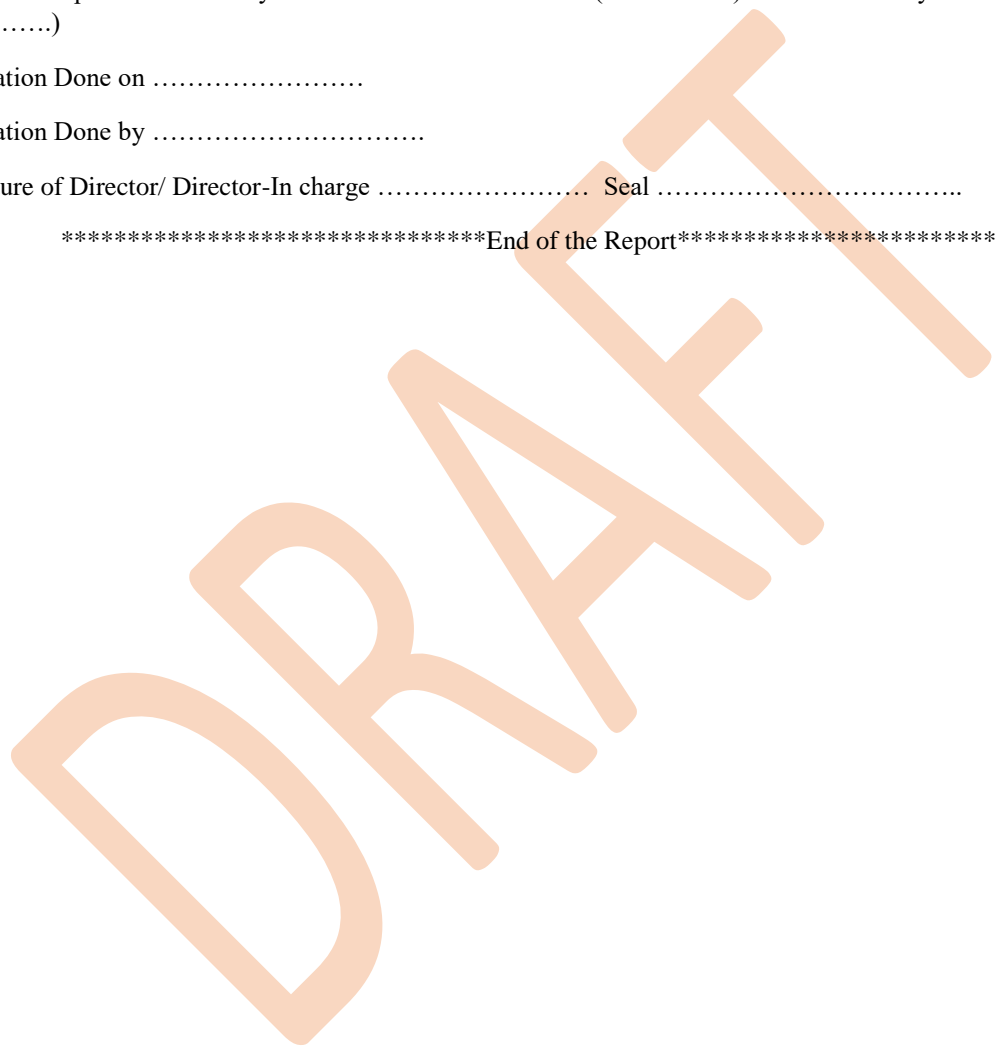
951

952

953

954

955



956 **Performance evaluation protocol for Dengue NS1 ELISA kits**

957 **I. Background:**

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

962 **II. Purpose:**

963 To evaluate the performance characteristics of Dengue NS1 ELISA kits in the diagnosis of Dengue  
964 infection.

965 **III. Requirements:**

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

973 **IV. Ethical approvals:**

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

977 **V. Procedure:**

- 978 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 979 **2. Preparation of Evaluation sites/laboratories:**
  - 980 **Identified IVD kit evaluation laboratories should establish their proficiency through**
    - 981 A.Accreditation form NABL for at least one of the Quality management system (NABL  
982 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
983 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
    - 984 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
985 and competency testing on following
      - 986 ➤ Preparation & characterization of kit evaluation panel
      - 987 ➤ Handling of Dengue NS1 ELISA kits received for performance evaluation  
988 (Verification/Storage/Unpacking etc).

- 989 ➤ Testing, interpreting, recording of results & reporting
- 990 ➤ Data handling, data safety & confidentiality

991 **3. Preparation of Dengue NS1 ELISA IVD kit evaluation panel**

992 Well characterised Dengue NS1 ELISA IVD kit evaluation panel is a critical requirement for  
993 performance evaluation of IVD kits. Hence statistically significant number of sera samples should  
994 be available from Dengue confirmed cases. Further characterised for Dengue NS1 positivity by  
995 using approved reference kits having high sensitivity and specificity.

996 Dengue NS1 performance evaluation panel need to be tested again by the reference assays at the  
997 time of evaluating a particular index test to confirm the positive and negative status of the samples.

998 **4. Reference assay:**

999 US-FDA approved Dengue NS1 ELISA kit should be used as reference assay.

1000 Serotype status to be assessed using CDC/NIV real-time PCR serotyping protocols.

1001 **5. Sample size and sample panel composition:** Sample sizes of positive and negative  
1002 samples and sample panel composition against different values of sensitivity and specificity are  
1003 provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance,  
1004 and an absolute precision of 5%. Appropriate sample size has to be chosen from the tables according  
1005 to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed  
1006 sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample size  
1007 associated with the largest sensitivity/specificity provided in the table that is smaller to the claimed  
1008 value (that is, as per the next smaller value of the sensitivity/ specificity available in the table). For  
1009 example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample size  
1010 mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage of the  
1011 sample size outlined for 85% specificity.

1012 Positive samples: The panel of positive samples should include samples positive by the reference  
1013 assay and real-time PCR assay (True positives). Samples should be representative of all 4 serotypes  
1014 and varying degrees of positivity. The samples should be classified as strong, moderate and weak  
1015 positives based on ELISA units of the reference assay.

1016

1017 Negative samples: These should include samples negative by the reference NS1 ELISA assay and  
1018 real-time PCR using CDC/NIV serotyping protocol (True negatives).

1019

1020 Table 1. Sample sizes and panel composition of positive Dengue samples for different values of  
1021 sensitivity claimed by the manufacturer.

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required</i> | <i>Sample Panel Composition</i> |
|--------------------|-------------------------------|---|---------------------------------|
|--------------------|-------------------------------|---|---------------------------------|

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|                  |     |                                  |   |
|------------------|-----|----------------------------------|---|
|                  |     | <i>[Sample size rounded off]</i> |   |
| 99% <sup>#</sup> | 15  | 20                               | Strong Positive: 4<br>Moderate Positive: 8<br>Weak Positive: 8    |
| 95%              | 73  | 80                               | Strong Positive: 18<br>Moderate Positive: 31<br>Weak Positive: 31 |
| 90%              | 138 | 140                              | Strong Positive: 30<br>Moderate Positive: 55<br>Weak Positive: 55 |
| 85%              | 196 | 200                              | Strong Positive: 42<br>Moderate Positive: 79<br>Weak Positive: 79 |
| 80%              | 246 | 250                              | Strong Positive: 54<br>Moderate Positive: 98<br>Weak Positive: 98 |

1022

1023 *#Higher sample size should be used even for assays claiming 99% sensitivity.*

1024 Table 2. Sample sizes and panel composition of negative Dengue samples for different values of  
1025 specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 15                            | 20  | Chikungunya positive: 4<br><sup>a</sup> Acute febrile cases negative for Dengue: 8<br>*Japanese Encephalitis PCR/antigen positive: 1<br>*West Nile Virus PCR/antigen positive: 1<br>*Zika Virus PCR/antigen positive: 1<br><sup>b</sup> Healthy subjects from endemic regions: 5    |
| 95%                | 73                            | 80  | Chikungunya positive: 15<br><sup>a</sup> Acute febrile cases negative for Dengue: 30<br>*Japanese Encephalitis PCR/antigen positive: 5<br>*West Nile Virus PCR/antigen positive: 5<br>*Zika Virus PCR/antigen positive: 5<br><sup>b</sup> Healthy subjects from endemic regions: 20 |
| 90%                | 138                           | 140   | Chikungunya positive: 26<br><sup>a</sup> Acute febrile cases negative for Dengue: 52<br>*Japanese Encephalitis PCR/antigen positive: 9  |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |  |
|--|-----|-----|--|
|  |     |     | *West Nile Virus PCR/antigen positive: 9<br>*Zika Virus PCR/antigen positive: 9<br><sup>b</sup> Healthy subjects from endemic regions: 35  |
| 85%  | 196 | 200 | Chikungunya positive: 37<br><sup>a</sup> Acute febrile cases negative for Dengue: 74<br>*Japanese Encephalitis PCR/antigen positive: 13<br>*West Nile Virus PCR/antigen positive: 13<br>*Zika Virus PCR/antigen positive: 13<br><sup>b</sup> Healthy subjects from endemic regions: 50 |
| 80%  | 246 | 250 | Chikungunya positive: 46<br><sup>a</sup> Acute febrile cases negative for Dengue: 94<br>*Japanese Encephalitis PCR/antigen positive: 16<br>*West Nile Virus PCR/antigen positive: 16<br>*Zika Virus PCR/antigen positive: 16<br><sup>b</sup> Healthy subjects from endemic regions: 62 |
| <sup>a</sup> Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR)<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid)<br><br><i>#Higher sample size should be used even for assays claiming 99% specificity.</i> |     |     |  |

1026

1027 \*In the absence of natural samples, spiked samples may be used, as per details provided in the note below.

1028 **Note:**

1029 Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed  
1030 in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng -1 µg/ml) and diluted  
1031 in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).

1032 Before used for evaluation, flavivirus NS1 reconstituted in serum samples needs to be tested by the dengue NS1  
1033 reference assay, and dilutions which are negative for dengue should be used for evaluation.

1034 The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.

1035 **6. Test reproducibility**

1036 **A. Sample size for lot-to-lot reproducibility**

1037 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
1038 as follows:

1039 • First lot of the assay: should be tested on statistically significant number of positive  
1040 and negative samples as calculated in the protocol.

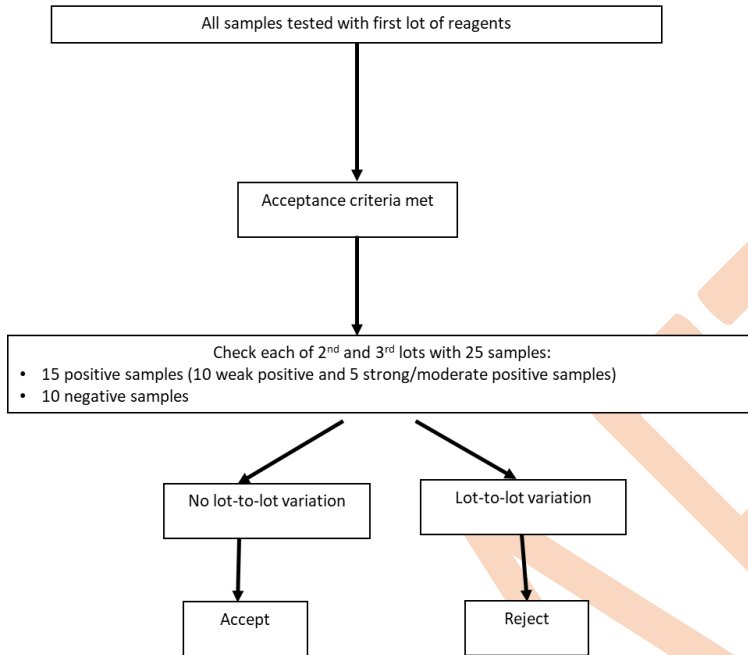
1041 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
1042 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
1043 samples).

1044 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
1045 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

1046

1047 Refer the flowchart below (Fig. 1):

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



1048

1049

1050

## 1051 **7. Acceptance Criteria**

1052 Expected sensitivity:  $\geq 90\%$

1053 Expected specificity:  $\geq 95\%$

1054 Cross reactivity with other flavivirus antigens: Nil

## 1055 **9. Publication Rights:**

1056 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

1057

1058

1059 **After following due procedure as defined in this document, once any kit is found to be Not**  
1060 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
1061 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
1062 **will only be entertained if valid proof of change in the kit composition is submitted.**

1063



1064  
1065  
1066  
1067  
1068  
1069  
1070  
1071  
1072  
1073  
1074  
1075  
1076  
1077  
1078  
1079  
1080  
1081  
1082  
1083  
1084  
1085  
1086  
1087  
1088  
1089  
1090  
1091  
1092  
1093  
1094  
1095  
1096  
1097  
1098  
1099  
1100

**VI. References:**

1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino JL, Artsob H, Guzman MG, Oliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoS Negl Trop Dis. 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanaroj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoS Negl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
3. 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](https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf)
4. 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>
5. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment TGS-3. 2017. Available at: <https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1>

**VII. Performance evaluation report format**

1101 **PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 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 CDSO/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   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license                |  |
| License Number:Issue date:   |  |
| Valid Up to:   |  |
| Application No.  |  |
| <b>Sample</b> Positive samples (provide details: strong, moderate, weak)                           |  |
| <b>Panel</b> Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

1102  
1103 **Results**

|   |          | Reference assay ..... (name) |          |       |
|---|----------|------------------------------|----------|-------|
|   |          | Positive                     | Negative | Total |
| <b>Name of Dengue NS1 - based ELISA kit</b> | Positive |                              |          |       |
|   | Negative |                              |          |       |
|   | Total    |                              |          |       |

1104

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

- 1105  
1106 • Details of cross reactivity with other flavivirus NS1 antigens:  
1107 • **Conclusions:**  
1108 ○ Sensitivity, specificity  
1109 ○ Performance: **Satisfactory / Not satisfactory**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

1110 *(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from the batch*  
1111 *mentioned above using ..... sample. Results should not be extrapolated to other sample types.)*

1112 **Disclaimers**

- 1113 1. This validation process does not approve / disapprove the kit design
- 1114 2. This validation process does not certify user friendliness of the kit / assay

1115 Note: This report is exclusively for ..... Kit (Lot No.....) manufactured by ..... (Supplied  
1116 by .....)

1117 Evaluation Done on .....

1118 Evaluation Done by .....

1119 Signature of Director/ Director-In-charge ..... Seal .....

1120 \*\*\*\*\*End of the Report\*\*\*\*\*

1121

1122

1123

1124

1125

1126

1127

1128

1129

1130

1131

1132

1133

1134

1135

1136

1137

1138

1139

1140 **Field evaluation protocol for Dengue NS1 ELISA kits**

1141 **I. Background:**

1142 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
1143 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
1144 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
1145 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

1146 **II. Purpose:**

1147 To evaluate the performance characteristics of Dengue NS1 ELISA kits in the diagnosis of Dengue  
1148 infection in individuals with unknown disease status.

1149 **III. Requirements:**

- 1150 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
1151 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
1152 the required equipment.
- 1153 2. Evaluation sites/laboratories (With required equipment)
- 1154 3. Reference test kits
- 1155 4. Laboratory supplies

1156  
1157 **IV. Ethical approval:**

1158 *The study will be initiated after approval from the institutional human ethics committee.*

1159 **V. Procedure:**

1160 **1. Study design/type:** Cross-sectional study

1161 **2. Preparation of Evaluation sites/laboratories:**

1162 **Identified IVD kit evaluation laboratories should establish their proficiency through**

1163 A.Accreditation form NABL for at least one of the Quality management system (NABL  
1164 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
1165 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

1166 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
1167 and competency testing on following

- 1168 ➤ Preparation & characterization of kit evaluation panel
- 1169 ➤ Handling of Dengue NS1 ELISA kits received for performance evaluation  
1170 (Verification/Storage/Unpacking etc).
- 1171 ➤ Testing, interpreting, recording of results & reporting
- 1172 ➤ Data handling, data safety & confidentiality

1173 **3. Sample size for performance evaluation:**

1174 Sample sizes of positive and negative samples of Dengue against different values of  
1175 sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated  
1176 assuming 95% level of significance, and an absolute precision of 5%. It is further assumed  
1177 that 30% of the individuals attending the health care facilities for acute febrile illness and  
1178 suspected for Dengue will be positive for Dengue. Appropriate sample size has to be chosen  
1179 from the tables according to the values of sensitivity and specificity being claimed by the  
1180 manufacturer. If a claimed sensitivity/specificity is not present in the table, the  
1181 manufacturer needs to consider the sample size associated with the largest  
1182 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as  
1183 per the next smaller value of the sensitivity/ specificity available in the table). For example,  
1184 if a manufacturer claims a sensitivity of 93%, they are required to use a sample size  
1185 mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require  
1186 usage of the sample size outlined for 85% specificity.

1187 Sample size has to be calculated based on both the sensitivity and the specificity. The  
1188 final sample size will be the maximum of the two. For example, at 95% sensitivity and  
1189 95% specificity, the sample size required will be 245 (maximum of 245 and 105).

1190  
1191 Table 1. Sample sizes for different values of sensitivity claimed by the manufacturer.

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--|-------------------------------|--|
| 99%#   | 51                            | 55   |
| 95%  | 243                           | 245  |
| 90%  | 461                           | 465  |
| 85%  | 653                           | 655  |
| 80%  | 820                           | 820  |
| * Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria |                               |  |

1192  
1193 #Higher sample size should be used even for assays claiming 99% sensitivity.

1194  
1195 Table 2. Sample sizes for different values of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--------------------|-------------------------------|--|
| 99%#               | 22                            | 25   |
| 95%                | 104                           | 105  |
| 90%                | 198                           | 200  |
| 85%                | 280                           | 280  |
| 80%                | 351                           | 355  |

\* Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria

1196

1197 *#Higher sample size should be used even for assays claiming 99% specificity.*

1198 Recruitment of cases shall be halted once desired number of positive and negative samples are  
1199 reached.

1200

1201 **4. Inclusion criteria:**

1202 Individuals with Dengue like illness (A patient with acute febrile illness of 2-7 days with two or  
1203 more manifestations: Head ache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic  
1204 manifestations)

1205 **5. Exclusion criteria**

1206 Individuals with already known positive history for other pathogens

1207 **6. Reference assay:**

1208 *US-FDA approved Dengue NS1 ELISA kit should be used as reference assay.*

1209 *Serotype status to be assessed using CDC / NIV real-time PCR serotyping protocols.*

1210 **7. Study implementation:**

1211 *The individuals with Dengue like illness will be recruited into the study and five ml of whole blood*  
1212 *will be collected in vacutainer tubes and the serum will be separated by centrifugation and used*  
1213 *for the study. The serum sample will be subjected to the following reference tests and the index*  
1214 *test.*

1215 *It needs to be ensured that the samples are tested by reference tests and index test simultaneously.*

1216 **8. Positive samples:**

1217 Samples positive by the reference NS1 ELISA assay and real-time PCR assay (True positives).  
1218 will be considered as true positive sample.

1219 **9. Negative samples:**

1220 Samples negative by the reference NS1 ELISA assay and real-time PCR using CDC/NIV  
1221 *serotyping protocol* will be considered as true negative.

1222 **A. Cross reactivity:**

1223 Clinical samples or commercially available NS1 antigens from other flaviviruses will be used to  
1224 test cross reactivity of the index test.

- 1225 1. Japanese Encephalitis PCR/antigen positive: 5 samples  
1226 2. West Nile Virus PCR/antigen: 5 samples  
1227 3. Zika Virus PCR/antigen: 5 samples

1228 \*In the absence of natural samples, spiked samples may be used, as per details provided in the note below.

1229 **Note:**

1230 Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed  
1231 in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng -1 µg/ml) and diluted  
1232 in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).

1233 Before used for evaluation, NS1 reconstituted in serum samples needs to be tested by the reference assay and dilution  
1234 which are positive only should be used for evaluation.

1235 The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.

1236 **10. Statistical analysis:**

1237 Sensitivity and specificity will be calculated.

1238 Interim analysis of data shall be conducted on completing evaluation of 25%, 50% and 75% of  
1239 samples. If, at any point, the performance of the assay is found to be not satisfactory, the assay  
1240 shall not be evaluated further. Evaluation fee shall be charged accordingly.

1241

1242 **11. Test reproducibility**

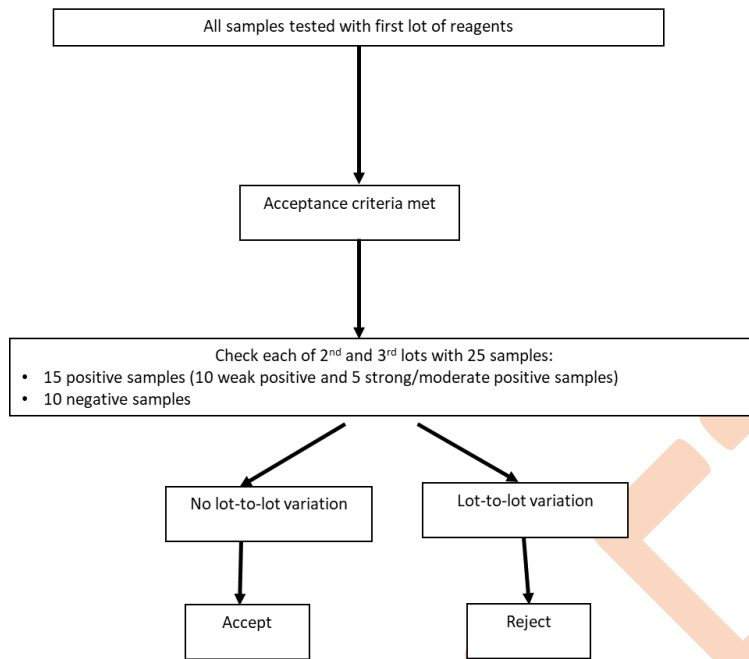
1243 **a. Sample size for lot-to-lot reproducibility**

1244 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
1245 as follows:

- 1246 • First lot of the assay: should be tested on statistically significant number of positive  
1247 and negative samples as calculated in the protocol.  
1248 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
1249 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
1250 samples).  
1251 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
1252 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).  
1253

1254 Refer the flowchart below (Fig. 1):

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



1255

1256

1257

## 1258 **12. Acceptance Criteria**

1259 Expected sensitivity:  $\geq 90\%$

1260 Expected specificity:  $\geq 95\%$

1261 Cross-reactivity with other flavivirus antigens: Nil

## 1262 **13. Publication Rights:**

1263 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

1264

1265 **After following due procedure as defined in this document, once any kit is found to be Not**  
1266 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
1267 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
1268 **will only be entertained if valid proof of change in the kit composition is submitted.**

1269

## 1270 **VI. References:**

- 1271 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian  
1272 E, Pelegrino JL, Artsob H, Guzman MG, Oliario P, Zwang J, Guillerm M, Kliks S, Halstead S,  
1273 Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection



- 1274 of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct  
1275 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
- 1276 2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S,  
1277 Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay  
1278 sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl  
1279 Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
- 1280 3. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K,  
1281 Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic  
1282 review and meta-analysis. PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi:  
1283 10.1371/journal.pntd.0006618.  
1284
- 1285 4. Castro-Jorge LA, Machado PR, Fávero CA, Borges MC, Passos LM, de Oliveira RM,  
1286 Fonseca BA. Clinical evaluation of the NS1 antigen-capture ELISA for early diagnosis of  
1287 Dengue virus infection in Brazil. J Med Virol. 2010 Aug;82(8):1400-5. doi:  
1288 10.1002/jmv.21814.  
1289
- 1290 5. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
1291 Diagnostic Assessment TGS-3. 2017. Available at:  
1292 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
1293 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
1294

## 1295 VII. Performance evaluation report format

1296  
1297  
1298  
1299  
1300  
1301  
1302  
1303  
1304  
1305  
1306  
1307  
1308  
1309

1310

**PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 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 CDSO/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  |   |  |
| <b>Regulatory Approval:</b>   |   |  |
| Import license / Manufacturing license/ Test license                                  |   |  |
| License Number:Issue date:  |   |  |
| Valid Up to:  |   |  |
| Application No.   |   |  |
| <b>Sample Panel</b>   | Positive samples: Not applicable, may categorize cases as per duration of illness                 |  |
|   | Negative samples (may categorize as per duration of illness, must include cross reactivity panel) |  |

1311

**1312 Results**

|   |          | Reference assay ..... (name) |          |       |
|---|----------|------------------------------|----------|-------|
|   |          | Positive                     | Negative | Total |
| <b>Name of Dengue NS1 based ELISA kit</b> | Positive |                              |          |       |
|   | Negative |                              |          |       |
|   | Total    |                              |          |       |

1313

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

1314

1315

1316

1317

1318

- Details of cross reactivity with other flavivirus NS1 antigens:
- Conclusions:
  - Sensitivity, specificity
  - Performance: **Satisfactory / Not satisfactory**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

1319 *(Sensitivity and specificity have been assessed in using kits provided by the manufacturer from the batch mentioned above using*  
1320 *..... sample in controlled lab setting. Results should not be extrapolated to other sample types.)*

1321

1322 **Disclaimers**

- 1323 1. This validation process does not approve / disapprove the kit design
- 1324 2. This validation process does not certify user friendliness of the kit / assay

1325 Note: This report is exclusively for NS1.....Kit (Lot No.....) manufactured by ..... (supplied  
1326 by .....)

1327 Evaluation Done on .....

1328 Evaluation Done by .....

1329 Signature of Director/ Director-In charge ..... Seal .....

1330 \*\*\*\*\*End of the Report\*\*\*\*\*

1331

1332

1333

1334

1335

1336

1337

1338

1339

1340

1341

1342

1343

1344

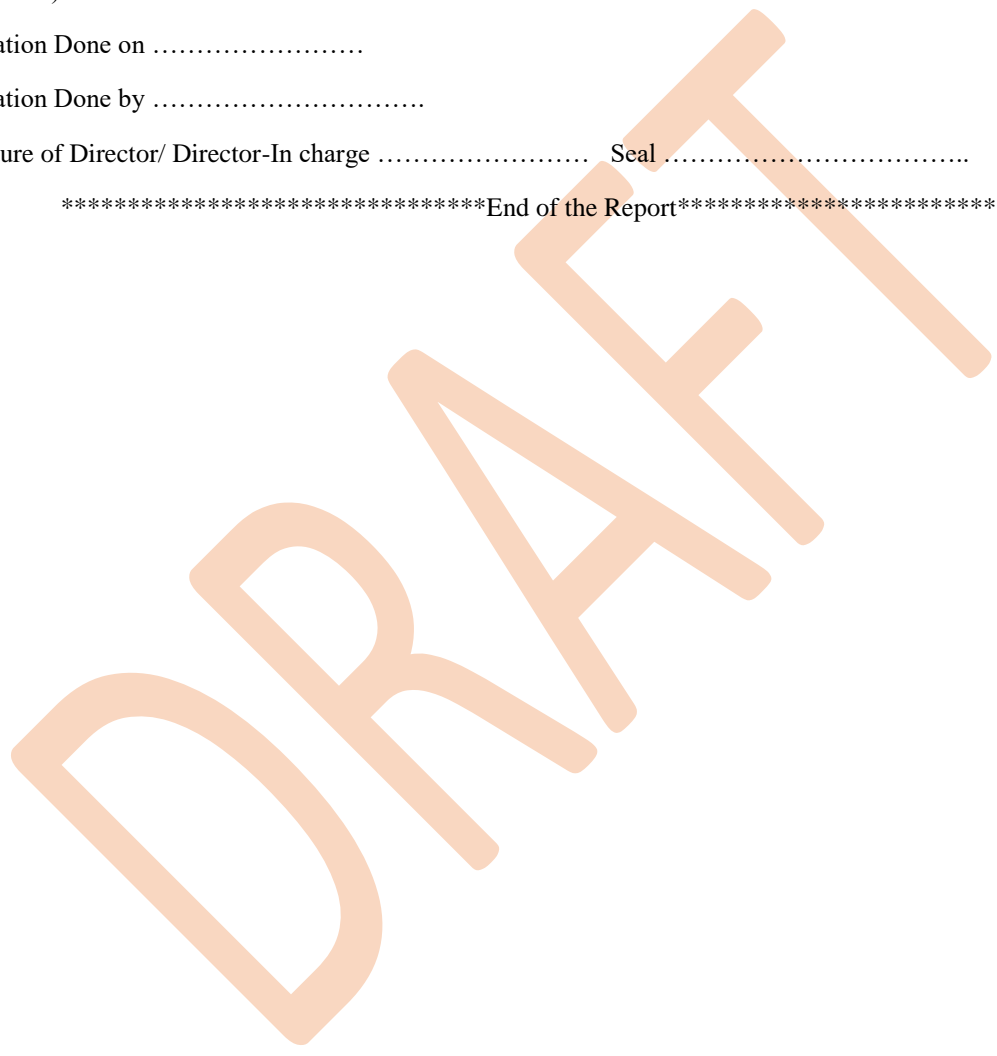
1345

1346

1347

1348

1349



1350 **Performance evaluation protocol for Dengue IgM RDT kits**

1351 **I. Background:**

1352 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
1353 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
1354 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
1355 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

1356 **II. Purpose:**

1357 To evaluate the performance characteristics of Dengue IgM RDT kits in the diagnosis of Dengue  
1358 infection.

1359 **III. Requirements:**

1360 a) Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
1361 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
1362 the required equipment.

1363 b) Evaluation sites/laboratories (With required equipment)

1364 c) Reference test kits

1365 d) Characterised Evaluation panel

1366 e) Laboratory supplies

1367 **IV. Ethical approvals:**

1368 Exempted from Ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory  
1369 Validation Testing, 2024. A self-declaration form as provided in ICMR guidelines to be submitted  
1370 by the investigators to the institutional authorities and ethics committee for information.

1371 **V. Procedure:**

1372 **1. Study design/type:** Diagnostic accuracy study using archived/ leftover clinical samples

1373 **2. Preparation of Evaluation sites/laboratories:**

1374 **Identified IVD kit evaluation laboratories should establish their proficiency through**

1375 A.Accreditation form NABL for at least one of the Quality management system (NABL  
1376 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
1377 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

1378 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
1379 and competency testing on following

1380 ➤ Preparation & characterization of kit evaluation panel

1381 ➤ Handling of Dengue IgM Rapid IVD kits received for performance evaluation  
1382 (Verification/Storage/Unpacking etc).

1383 ➤ Testing, interpreting, recording of results & reporting

1384 ➤ Data handling, data safety & confidentiality

1385

### 1386 **3. Preparation of Dengue IgM Rapid IVD kit evaluation panel**

1387 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance  
1388 evaluation of IVD kits. Hence statistically significant number of sera samples should be  
1389 available from Dengue confirmed cases. Further characterised for Dengue IgM positivity by  
1390 using approved reference kits having high sensitivity and specificity.

1391 Dengue IgM performance evaluation panel need to be tested again by the reference assays at  
1392 the time of evaluating a particular index test to confirm the positive and negative status of the  
1393 samples.

### 1394 **4. Reference assay:**

1395 US-FDA approved Dengue IgM ELISA kit should be used as reference assay.

1396 NS1 antigen status to be assessed using US FDA approved NS1 ELISA kit.

1397 Serotype status to be assessed using a combination of CDC/NIV real-time PCR serotyping  
1398 protocols.

1399 At least 50% of the samples should be positive by real-time PCR or NS1 antigen and IgM  
1400 ELISA.

1401 Primary and Secondary status to be assessed by Panbio Dengue IgG capture ELISA kit.

1402

1403 **5. Sample size and sample panel composition:** Sample sizes of positive and negative  
1404 samples of Dengue against different values of sensitivity and specificity are provided in Tables  
1405 1 and 2. Sample sizes have been calculated assuming 95% level of significance, an absolute  
1406 precision of 5%, and invalid test rate  $\leq 5\%$ . Appropriate sample size has to be chosen from the  
1407 tables according to the values of sensitivity and specificity being claimed by the manufacturer.  
1408 If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider  
1409 the sample size associated with the largest sensitivity/specificity provided in the table that is  
1410 smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity  
1411 available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are  
1412 required to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87%  
1413 specificity would require usage of the sample size outlined for 85% specificity.

1414

1415 Positive samples: The panel of positive samples should include samples positive by the reference  
1416 assay, with 50% samples positive for Dengue NS1/RT-PCR assay (True positives). Samples should  
1417 be representative of all 4 serotypes and varying degrees of positivity. The samples should be  
1418 classified as strong, moderate and weak positives based on ELISA units of the reference assay.

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

1419

1420 Negative samples: These should include samples negative by the reference assay, NS1 ELISA  
1421 assay and/or real-time PCR using CDC/NIV serotyping protocol (True negatives).

1422

1423 Table 1. Sample sizes and panel composition of positive Dengue samples for different values of  
1424 sensitivity claimed by the manufacturer.

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>                                    |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Strong Positive: 6<br>Moderate Positive: 8<br>Weak Positive: 6     |
| 95%                | 77                            | 80  | Strong Positive: 23<br>Moderate Positive: 34<br>Weak Positive: 23  |
| 90%                | 145                           | 150   | Strong Positive: 43<br>Moderate Positive: 64<br>Weak Positive: 43  |
| 85%                | 206                           | 210   | Strong Positive: 61<br>Moderate Positive: 88<br>Weak Positive: 61  |
| 80%                | 258                           | 260   | Strong Positive: 75<br>Moderate Positive: 110<br>Weak Positive: 75 |

1425

1426 *#Higher sample size should be used even for assays claiming 99% sensitivity.*

1427

1428 Table 2. Sample sizes and panel composition of negative Dengue samples for different values of  
1429 specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 16                            | 20  | Chikungunya positive: 4<br><sup>a</sup> Acute febrile cases: 5<br>*Japanese Encephalitis IgM positive: 1<br>*West Nile Virus IgM positive: 1<br>*Zika Virus IgM positive: 1 |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |  |
|--|-----|-----|--|
|  |     |     | Rheumatoid Arthritis/other autoimmune disease cases: 4<br><sup>b</sup> Healthy subjects from endemic regions: 4  |
| 95%  | 77  | 80  | Chikungunya positive: 16<br><sup>a</sup> Acute febrile cases: 23<br>*Japanese Encephalitis IgM positive: 3<br>*West Nile Virus IgM positive: 3<br>*Zika Virus IgM positive: 3<br>Rheumatoid Arthritis/other autoimmune disease cases: 16<br><sup>b</sup> Healthy subjects from endemic regions: 16 |
| 90%  | 145 | 150 | Chikungunya positive: 30<br><sup>a</sup> Acute febrile cases: 45<br>*Japanese Encephalitis IgM positive: 5<br>*West Nile Virus IgM positive: 5<br>*Zika Virus IgM positive: 5<br>Rheumatoid Arthritis/other autoimmune disease cases: 30<br><sup>b</sup> Healthy subjects from endemic regions: 30 |
| 85%  | 206 | 210 | Chikungunya positive: 42<br><sup>a</sup> Acute febrile cases: 63<br>*Japanese Encephalitis IgM positive: 7<br>*West Nile Virus IgM positive: 7<br>*Zika Virus IgM positive: 7<br>Rheumatoid Arthritis/other autoimmune disease cases: 42<br><sup>b</sup> Healthy subjects from endemic regions: 42 |
| 80%  | 258 | 260 | Chikungunya positive: 52<br><sup>a</sup> Acute febrile cases: 77<br>*Japanese Encephalitis IgM positive: 9<br>*West Nile Virus IgM positive: 9<br>*Zika Virus IgM positive: 9<br>Rheumatoid Arthritis/other autoimmune disease cases: 52<br><sup>b</sup> Healthy subjects from endemic regions: 52 |
| <sup>a</sup> Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR)<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA) |     |     |  |

1430

1431 *#Higher sample size should be used even for assays claiming 99% specificity.*

1432 \*Note: Depending on the availability of IgM positive samples for cross reactive flaviviruses, the requirement of  
 1433 samples for each virus may be increased or decreased accordingly to reach the total number of samples. If IgM positive  
 1434 samples for cross reactive flaviviruses are not available, commercially available IgM sera panel for different viruses  
 1435 can be procured and used to test cross reactivity.

1436 **6. Test reproducibility**

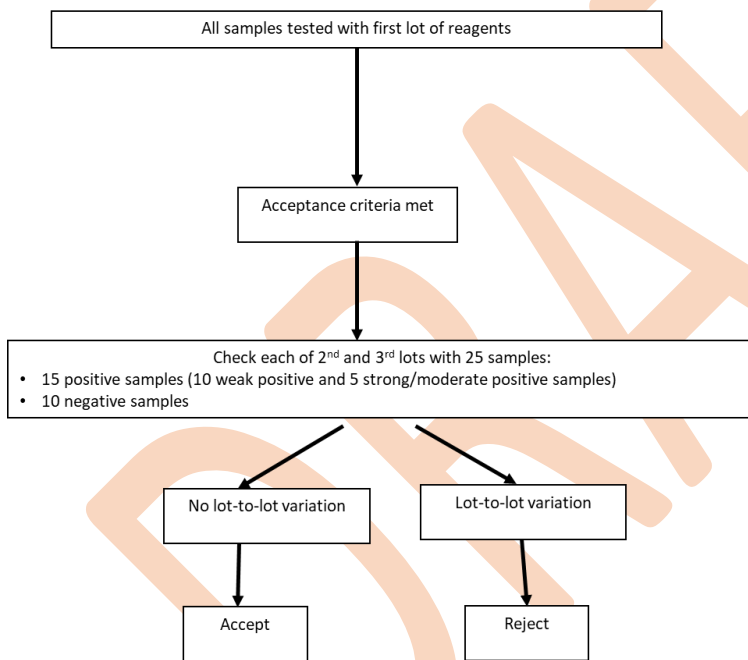
1437 **A. Sample size for lot-to-lot reproducibility**

1438 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
1439 as follows:

- 1440 • First lot of the assay: should be tested on statistically significant number of positive  
1441 and negative samples as calculated in the protocol.
- 1442 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
1443 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
1444 samples).
- 1445 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
1446 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- 1447

1448 Refer the flowchart below (Fig. 1):

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



1449

1450

1451

1452 **B. Sample size for reader-to-reader reproducibility**

1453 For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10  
1454 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

1455

1456 Two operators will be reading the test results independently as per manufacturer’s instruction.

1457 Agreement should be 100% between the operators.

1458 **7. Acceptance Criteria**



1459 Expected sensitivity:  $\geq 80\%$

1460 Expected specificity:  $\geq 90\%$

1461 Invalid test rate:  $\leq 5\%$

1462 **8. Publication Rights:**

1463 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

1464

1465 **After following due procedure as defined in this document, once any kit is found to be Not**  
1466 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
1467 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
1468 **will only be entertained if valid proof of change in the kit composition is submitted.**

1469 **VI. References:**

- 1470 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vázquez S,  
1471 Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo Lic  
1472 NR, Bessoff KE, Kliks S, Peeling RW. Evaluation of commercially available anti-Dengue virus  
1473 immunoglobulin M tests. *Emerg Infect Dis*. 2009 Mar;15(3):436-40. doi: 10.3201/eid1503.080923.
- 1474 2. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E,  
1475 Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling  
1476 RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of  
1477 Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. *PLoS Negl Trop Dis*. 2014 Oct  
1478 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
- 1479 3. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests.**  
1480 **(Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.**
- 1481 4. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro  
1482 Diagnostic Medical Devices. 2018. Available at:  
1483 [https://cdsco.gov.in/opencms/export/sites/CDSO\\_WEB/Pdf-documents/medical  
device/guidanceperformanceivd.pdf](https://cdsco.gov.in/opencms/export/sites/CDSO_WEB/Pdf-documents/medical<br/>1484 device/guidanceperformanceivd.pdf)
- 1485 5. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently  
1486 Asked Questions. 2022. Available at:  
1487 [https://cdsco.gov.in/opencms/export/sites/CDSO\\_WEB/Pdf-documents/IVD/FAQs/CDSO-IVD-  
FAQ-03-2022-.pdf](https://cdsco.gov.in/opencms/export/sites/CDSO_WEB/Pdf-documents/IVD/FAQs/CDSO-IVD-<br/>1488 FAQ-03-2022-.pdf)
- 1489 6. U.S. Food and Drug Administration. Dengue Virus Serological Reagents - Class II Special Controls  
1490 Guideline for Industry and Food and Drug Administration Staff. 2014. Available at:  
1491 [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](https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-<br/>1492 emitting-products/Dengue-virus-serological-reagents-class-ii-special-controls-guideline-industry-<br/>1493 and-food-and-drug)
- 1494 7. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
1495 Diagnostic Assessment TGS-3. 2017. Available at:  
1496 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-  
eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-<br/>1497 eng.pdf;sequence=1)

1498 8. Yow KS, Aik J, Tan EY, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent Dengue  
1499 infections: An evaluation of six kits on clinical specimens. PLoS One. 2021 Apr 1;16(4):e0249602.  
1500 doi: 10.1371/journal.pone.0249602.

1501

1502 **\*The validation protocols need to be revisited after introduction of Dengue vaccines and the**  
1503 **acceptance criteria needs revisiting every year so as to enable the availability of best**  
1504 **diagnostic kits.**

1505 **VII. Performance evaluation report format**

1506

1507

1508

1509

1510

1511

1512

1513

1514

1515

1516

1517

1518

1519

1520

1521

1522

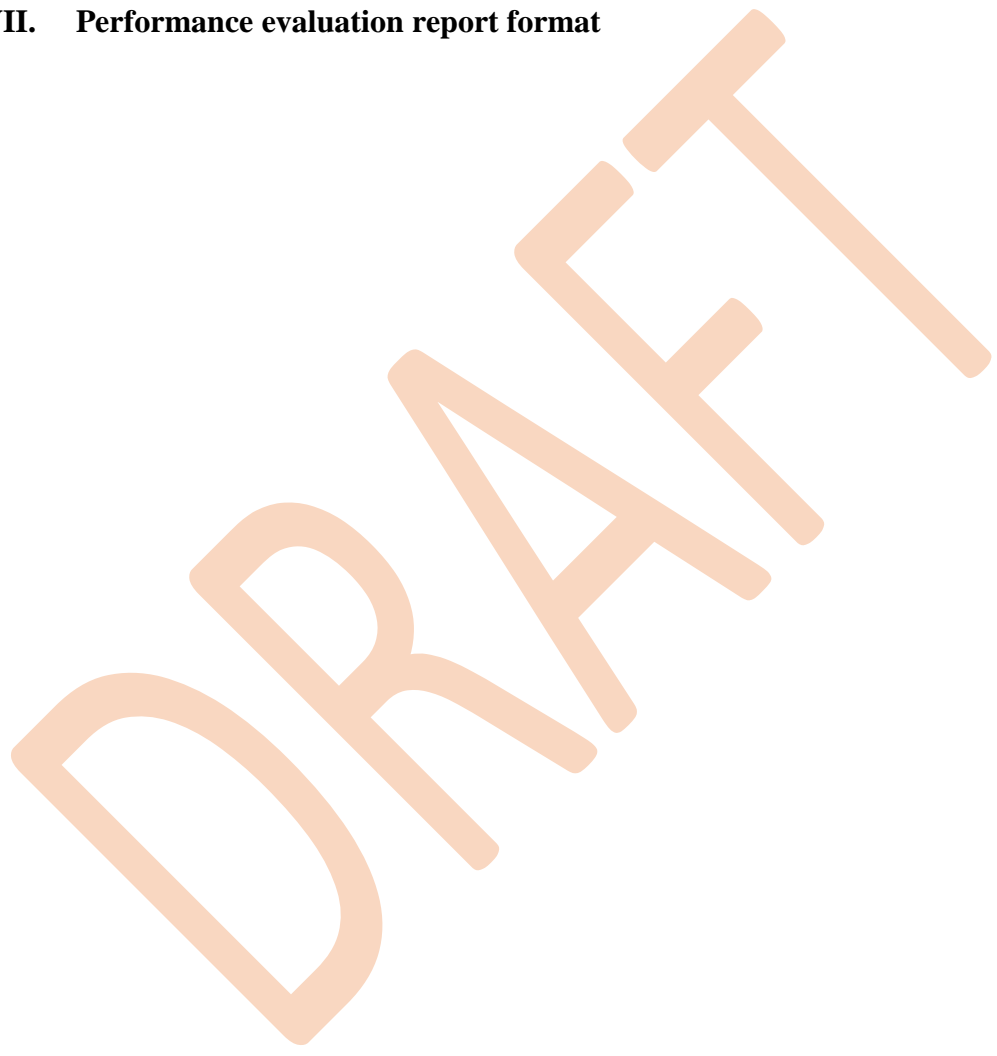
1523

1524

1525

1526

1527



1528

**PERFORMANCE EVALUATION REPORT FOR DENGUE IgM 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 CDSO/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   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license                |  |
| License Number:Issue date:   |  |
| Valid Up to:   |  |
| Application No.  |  |
| <b>Sample</b> Positive samples (provide details: strong, moderate, weak)                           |  |
| <b>Panel</b> Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

1529

1530 **Results:**

|  |              | <b>Reference assay ..... (name)</b> |          |       |
|--|--------------|-------------------------------------|----------|-------|
|  |              | Positive                            | Negative | Total |
| <b>Name of Dengue antibody - based RDT kit</b> | Positive     |                                     |          |       |
|  | Negative     |                                     |          |       |
|  | <b>Total</b> |                                     |          |       |

1531

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

1532 **Conclusions:**

1533 ○ Sensitivity, specificity

1534 ○ Performance: **Satisfactory / Not satisfactory**

1535 *(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.)*

1537 **Disclaimers**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

- 1538 1. This validation process does not approve / disapprove the kit design
- 1539 2. This validation process does not certify user friendliness of the kit / assay

1540 Note: This report is exclusively for .....Kit (Lot No.....) manufactured by .....  
1541 (Supplied by .....)

1542 Evaluation Done on .....

1543 Evaluation Done by .....

1544 Signature of Director/ Director-In-charge ..... Seal .....

1545

1546 \*\*\*\*\*End of the Report\*\*\*\*\*

1547

1548

1549

1550

1551

1552

1553

1554

1555

1556

1557

1558

1559

1560

1561

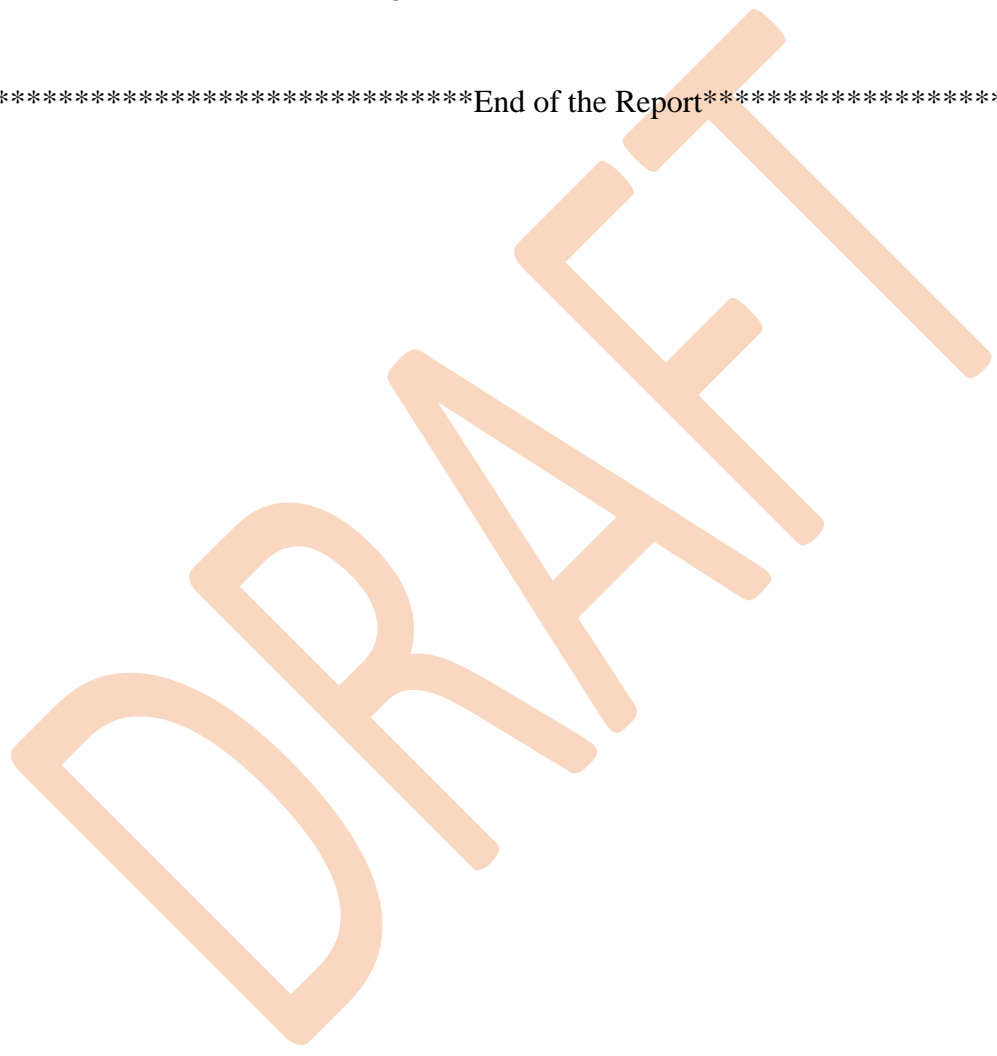
1562

1563

1564

1565

1566



1567 **Performance evaluation protocol for Dengue IgM ELISA kits**

1568 **I. Background:**

1569 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
1570 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
1571 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
1572 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

1573 **II. Purpose:**

1574 To evaluate the performance characteristics of Dengue IgM ELISA kits in the diagnosis of Dengue  
1575 infection.

1576 **III. Requirements:**

- 1577 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
1578 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
1579 the required equipment.
- 1580 2. Evaluation sites/laboratories (With required equipment)
- 1581 3. Reference test kits
- 1582 4. Characterised Evaluation panel
- 1583 5. Laboratory supplies

1584 **IV. Ethical approval:**

1585 Exempted from Ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory  
1586 Validation Testing, 2024. A self-declaration form as provided in ICMR guidelines to be submitted  
1587 by the investigators to the institutional authorities and ethics committee for information.

1588 **V. Procedure:**

- 1589 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 1590 **2. Preparation of Evaluation sites/laboratories:**
  - 1591 **Identified IVD kit evaluation laboratories should establish their proficiency through**
    - 1592 A.Accreditation form NABL for at least one of the Quality management system (NABL  
1593 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
1594 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
    - 1595 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
1596 and competency testing on following
      - 1597 ➤ Preparation & characterization of kit evaluation panel
      - 1598 ➤ Handling of Dengue IgM ELISA IVD kits received for performance evaluation  
1599 (Verification/Storage/Unpacking etc).

1600 ➤ Testing, interpreting, recording of results & reporting

1601 ➤ Data handling, data safety & confidentiality

### 1602 **3. Preparation of Dengue IgM ELISA IVD kit evaluation panel**

1603 Well characterised Dengue IVD kit evaluation panel is a critical requirement for performance  
1604 evaluation of IVD kits. Hence statistically significant number of sera samples should be  
1605 available from Dengue confirmed cases. Further characterised for Dengue IgM positivity by  
1606 using approved reference kits having high sensitivity and specificity.

1607 Dengue IgM performance evaluation panel need to be tested again by the reference assays at  
1608 the time of evaluating a particular index test to confirm the positive and negative status of the  
1609 samples.

### 1610 **4. Reference assay:**

1611 US-FDA approved Dengue IgM ELISA kit should be used as reference assay.

1612 NS1 antigen status to be assessed using US FDA approved NS1 ELISA kit.

1613 Serotype status to be assessed using a combination of CDC/NIV real-time PCR serotyping  
1614 protocols.

1615 At least 50% of the samples should be positive by real-time PCR or NS1 antigen and IgM  
1616 ELISA.

1617 Primary and Secondary status to be assessed by Panbio Dengue IgG capture ELISA kit.

1618 **5. Sample size and sample panel composition:** Sample sizes of positive and negative  
1619 samples and sample panel composition against different values of sensitivity and specificity are  
1620 provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of  
1621 significance, and an absolute precision of 5%. Appropriate sample size has to be chosen from  
1622 the tables according to the values of sensitivity and specificity being claimed by the  
1623 manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer  
1624 needs to consider the sample size associated with the largest sensitivity/specificity provided in  
1625 the table that is smaller to the claimed value (that is, as per the next smaller value of the  
1626 sensitivity/ specificity available in the table). For example, if a manufacturer claims a sensitivity  
1627 of 93%, they are required to use a sample size mentioned against 90% sensitivity. Similarly, a  
1628 claim of 87% specificity would require usage of the sample size outlined for 85% specificity.

1629  
1630 Positive samples: The panel of positive samples should include samples positive by the reference  
1631 assay, with 50% samples positive for Dengue NS1/ RT-PCR assay (True positives). Samples  
1632 should be representative of primary/secondary Dengue and all 4 Dengue virus serotypes, with  
1633 varying degrees of positivity. The samples should be classified as strong, moderate and weak  
1634 positives based on ELISA units of the reference assay.

1635

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

1636 Negative samples: These should include samples negative by the reference assay, NS1 ELISA  
1637 and/or real-time PCR using CDC and/or NIV serotyping protocols. (True negatives).

1638

1639 Table 1. Sample sizes and panel composition of positive Dengue samples for different values of  
1640 sensitivity claimed by the manufacturer.

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>                                   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 15                            | 20  | Strong Positive: 4<br>Moderate Positive: 8<br>Weak Positive: 8    |
| 95%                | 73                            | 80  | Strong Positive: 18<br>Moderate Positive: 31<br>Weak Positive: 31 |
| 90%                | 138                           | 140   | Strong Positive: 30<br>Moderate Positive: 55<br>Weak Positive: 55 |
| 85%                | 196                           | 200   | Strong Positive: 42<br>Moderate Positive: 79<br>Weak Positive: 79 |
| 80%                | 246                           | 250   | Strong Positive: 54<br>Moderate Positive: 98<br>Weak Positive: 98 |

1641

1642 *#Higher sample size should be used even for assays claiming 99% sensitivity.*

1643 Table 2. Sample sizes and panel composition of negative Dengue samples for different values of  
1644 specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 15                            | 20  | Chikungunya positive: 3<br><sup>a</sup> Acute febrile cases: 6<br>*Japanese Encephalitis IgM positive: 1<br>*West Nile Virus IgM positive: 1<br>*Zika Virus IgM positive: 1 |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |   |
|--|-----|-----|---|
|  |     |     | Rheumatoid Arthritis/other autoimmune disease cases: 4<br><sup>b</sup> Healthy subjects from endemic regions: 4   |
| 95%  | 73  | 80  | Chikungunya positive: 10<br><sup>a</sup> Acute febrile cases: 25<br>*Japanese Encephalitis IgM positive: 5<br>*West Nile Virus IgM positive: 5<br>*Zika Virus IgM positive: 5<br>Rheumatoid Arthritis/other autoimmune disease cases: 15<br><sup>b</sup> Healthy subjects from endemic regions: 15    |
| 90%  | 138 | 140 | Chikungunya positive: 18<br><sup>a</sup> Acute febrile cases: 43<br>*Japanese Encephalitis IgM positive: 9<br>*West Nile Virus IgM positive: 9<br>*Zika Virus IgM positive: 9<br>Rheumatoid Arthritis/other autoimmune disease cases: 26<br><sup>b</sup> Healthy subjects from endemic regions: 26    |
| 85%  | 196 | 200 | Chikungunya positive: 25<br><sup>a</sup> Acute febrile cases: 63<br>*Japanese Encephalitis IgM positive: 12<br>*West Nile Virus IgM positive: 12<br>*Zika Virus IgM positive: 12<br>Rheumatoid Arthritis/other autoimmune disease cases: 38<br><sup>b</sup> Healthy subjects from endemic regions: 38 |
| 80%  | 246 | 250 | Chikungunya positive: 31<br><sup>a</sup> Acute febrile cases: 77<br>*Japanese Encephalitis IgM positive: 16<br>*West Nile Virus IgM positive: 16<br>*Zika Virus IgM positive: 16<br>Rheumatoid Arthritis/other autoimmune disease cases: 47<br><sup>b</sup> Healthy subjects from endemic regions: 47 |
| <sup>a</sup> Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR)<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA) |     |     |   |

1645

1646 *#Higher sample size should be used even for assays claiming 99% specificity.*

1647 \*Note: Depending on the availability of IgM positive samples for cross reactive flaviviruses, the requirement of  
 1648 samples for each virus may be increased or decreased accordingly to reach the total number of samples. If IgM positive  
 1649 samples for cross reactive flaviviruses are not available, commercially available IgM sera panel for different viruses  
 1650 can be procured and used to test cross reactivity.

1651 **6. Test reproducibility**



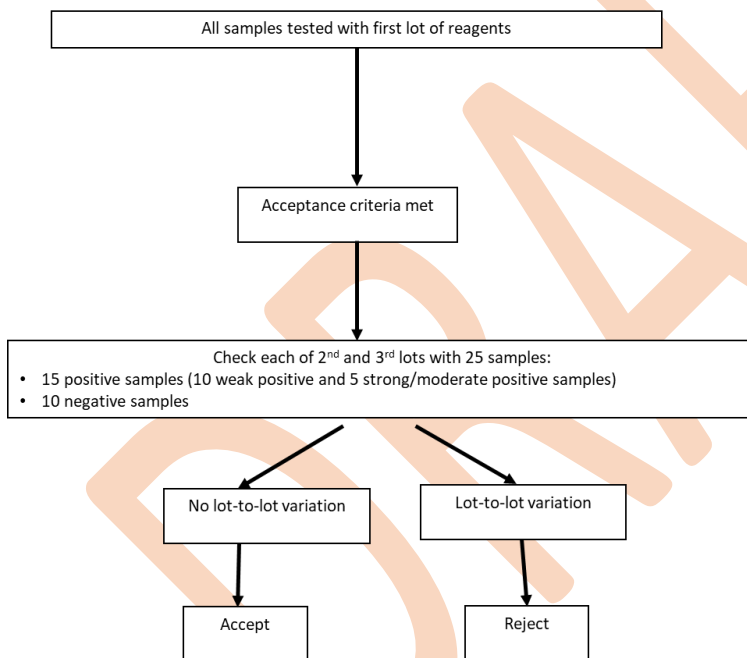
1652 **A. Sample size for lot-to-lot reproducibility**

1653 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
1654 as follows:

- 1655 • First lot of the assay: should be tested on statistically significant number of positive  
1656 and negative samples as calculated in the protocol.
- 1657 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
1658 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
1659 samples).
- 1660 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
1661 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- 1662

1663 Refer the flowchart below (Fig. 1):

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



1664

1665

1666

1667 **7. Acceptance criteria**

1668 Expected sensitivity:  $\geq 90\%$

1669 Expected specificity:  $\geq 95\%$

1670 **8. Publication Rights:**

1671 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

1672

1673

1674 **After following due procedure as defined in this document, once any kit is found to be Not**  
1675 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
1676 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
1677 **will only be entertained if valid proof of change in the kit composition is submitted.**

1678

## VI. References:

1679

1680

1681

1682

1683

1684

1685

1686

1687

1688

1689

1690

1691

1692

1693

1694

1695

1696

1697

1698

1699

1700

1701

1702

1703

1704

1705

1706

1707

1708

1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vázquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo Lic NR, Bessoff KE, Kliks S, Peeling RW. Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. *Emerg Infect Dis.* 2009 Mar;15(3):436-40. doi: 10.3201/eid1503.080923.
2. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. *PLoS Negl Trop Dis.* 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
3. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.
4. 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/CDSKO\\_WEB/Pdf-documents/medical\\_device/guidanceperformanceivd.pdf](https://cdsco.gov.in/opencms/export/sites/CDSKO_WEB/Pdf-documents/medical_device/guidanceperformanceivd.pdf)
5. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently Asked Questions. 2022. Available at: [https://cdsco.gov.in/opencms/export/sites/CDSKO\\_WEB/Pdf-documents/IVD/FAQs/CDSKO-IVD-FAQ-03-2022-.pdf](https://cdsco.gov.in/opencms/export/sites/CDSKO_WEB/Pdf-documents/IVD/FAQs/CDSKO-IVD-FAQ-03-2022-.pdf)
6. 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>
7. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment TGS-3. 2017. Available at: <https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1>

1709

1710

1711

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

1712

## VII. Performance evaluation report format

1713

**PERFORMANCE EVALUATION REPORT FOR DENGUE IgM 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 CDSO/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  |   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |   |  |
| License Number:Issue date:  |   |  |
| Valid Up to:  |   |  |
| Application No.   |   |  |
| <b>Sample</b>   | Positive samples (provide details: strong, moderate, weak)                            |  |
| <b>Panel</b>  | Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

1714

**1715 Results:**

|   |              | Reference assay ..... (name) |          |       |
|---|--------------|------------------------------|----------|-------|
|   |              | Positive                     | Negative | Total |
| <b>Name of Dengue antibody -based ELISA kit</b> | Positive     |                              |          |       |
|   | Negative     |                              |          |       |
|   | <b>Total</b> |                              |          |       |

1716

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

**1717 Conclusions:**

1718 ○ Sensitivity, specificity

1719 ○ Performance: **Satisfactory / Not satisfactory**

1720 *(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.)*

**1722 Disclaimers**

1723 1. This validation process does not approve / disapprove the kit design

1724 2. This validation process does not certify user friendliness of the kit / assay

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSCO/IVD/GD/PROTOCOLS/02/2024**

1725 Note: This report is exclusively for .....Kit (Lot No.....) manufactured by ..... (Supplied  
1726 by .....)

1727 Evaluation Done on .....

1728 Evaluation Done by .....

1729 Signature of Director/ Director-In-charge ..... Seal.....

1730

1731 \*\*\*\*\*End of the Report\*\*\*\*\*

1732

1733

1734

1735

1736

1737

1738

1739

1740

1741

1742

1743

1744

1745

1746

1747

1748

1749

1750

1751

1752

1753

DRAFT

1754 **Performance evaluation protocol for Dengue NS1/IgM combo RDT kits**

1755 **I. Background:**

1756 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
1757 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
1758 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
1759 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

1760 **II. Purpose:**

1761 To evaluate the performance characteristics of Dengue NS1/IgM combo RDT kits in the diagnosis  
1762 of Dengue infection.

1763 **III. Requirements:**

- 1764 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
1765 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
1766 the required equipment.
- 1767 2. Evaluation sites/laboratories (With required equipment)
- 1768 3. Reference test kits
- 1769 4. Characterised Evaluation panel
- 1770 5. Laboratory supplies

1771 **IV. Ethical approvals:**

1772 Exempted from Ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory  
1773 Validation Testing, 2024. A self-declaration form as provided in ICMR guidelines to be submitted  
1774 by the investigators to the institutional authorities and ethics committee for information.

1775 **V. Procedure:**

- 1776 **1. Study design/type:** Diagnostic accuracy study using archived/leftover clinical samples.
- 1777 **2. Preparation of Evaluation sites/laboratories:**
  - 1778 **Identified IVD kit evaluation laboratories should establish their proficiency through**
    - 1779 A. Accreditation form NABL for at least one of the Quality management system (NABL  
1780 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
1781 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.
    - 1782 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
1783 and competency testing on following
      - 1784 ➤ Preparation & characterization of kit evaluation panel
      - 1785 ➤ Handling of Dengue NS1/IgM combo IVD kits received for performance evaluation  
1786 (Verification/Storage/Unpacking etc).

- 1787 ➤ Testing, interpreting, recording of results & reporting
- 1788 ➤ Data handling, data safety & confidentiality

1789 **3. Preparation of Dengue RDT IVD kit evaluation panel**

1790 Well characterised Dengue RDT IVD kit evaluation panel is a critical requirement for performance  
1791 evaluation of IVD kits. Hence statistically significant number of sera samples should be available  
1792 from Dengue confirmed cases. Further characterised for Dengue NS1 and IgM positivity by using  
1793 approved reference kits having high sensitivity and specificity.

1794 Dengue NS1/IgM performance evaluation panel need to be tested again by the reference assays at  
1795 the time of evaluating a particular index test to confirm the positive and negative status of the  
1796 samples.

1797 **4. Reference assay:**

1798 Anti-DENV IgM detection ELISA US-FDA approved kit

1799 **AND/OR**

1800 DENV NS1 ELISA US-FDA approved kit

1801 Serotype status to be assessed using a combination of CDC and/or NIV real-time PCR serotyping  
1802 protocols.

1803 All positive samples need confirmation reference NS1/IgM ELISA assay and real-time PCR assay.

1804 **Sample size and sample panel composition:** Sample sizes of positive and negative samples of  
1805 Dengue against different values of sensitivity and specificity are provided in Tables 1 and 2.  
1806 Sample sizes have been calculated assuming 95% level of significance, an absolute precision of  
1807 5%, and invalid test rate  $\leq 5\%$ . Appropriate sample size has to be chosen from the tables according  
1808 to the values of sensitivity and specificity being claimed by the manufacturer. If a claimed  
1809 sensitivity/specificity is not present in the table, the manufacturer needs to consider the sample  
1810 size associated with the largest sensitivity/specificity provided in the table that is smaller to the  
1811 claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in the  
1812 table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a sample  
1813 size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require usage  
1814 of the sample size outlined for 85% specificity.

1815 Positive samples: Samples which are positive for IgM or NS1 or both by the reference assays will  
1816 be considered as true positive samples. There should be representation of samples positive for all  
1817 four serotypes.

1819 Negative samples: These should include samples negative by all the reference assays and real-time  
1820 PCR using CDC and/or NIV serotyping protocol (True negatives).

1821

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

1822 Table 1. Sample sizes and panel composition of positive Dengue samples for different values of  
1823 sensitivity claimed by the manufacturer.

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off for balanced allocation]</i> | <i>Sample Panel Composition</i>  |
|--|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 28  | *NS1 positive and IgM negative: 8<br>*NS1 and IgM positive: 12<br>*NS1 negative and IgM positive: 8    |
| 95%  | 77                            | 84  | *NS1 positive and IgM negative: 24<br>*NS1 and IgM positive: 36<br>*NS1 negative and IgM positive: 24  |
| 90%  | 145                           | 160   | *NS1 positive and IgM negative: 44<br>*NS1 and IgM positive: 72<br>*NS1 negative and IgM positive: 44  |
| 85%  | 206                           | 220   | *NS1 positive and IgM negative: 60<br>*NS1 and IgM positive: 100<br>*NS1 negative and IgM positive: 60 |
| 80%  | 258                           | 260   | *NS1 positive and IgM negative: 72<br>*NS1 and IgM positive: 116<br>*NS1 negative and IgM positive: 72 |
| <p><b>* all 4 serotypes shall be represented</b></p> <p><b>Note:</b><br/>In the absence of natural samples, spiked samples may be used as per details provided below:</p> <p>Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng - 1 µg/ml) and diluted in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).</p> <p>Before used for evaluation, flavivirus NS1 reconstituted in serum samples needs to be tested by the dengue NS1 reference assay, and dilutions which are negative for dengue should be used for evaluation.</p> <p>The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.</p> <p><i>#Higher sample size should be used even for assays claiming 99% sensitivity.</i></p> |                               |   |  |

1824  
1825 Table 2. Sample sizes and panel composition of negative Dengue samples for different values of  
1826 specificity claimed by the manufacturer.

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off for balanced allocation]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 16                            | 28  | Chikungunya positive: 1<br><sup>a</sup> Acute febrile cases: 11<br>*Japanese Encephalitis IgM positive: 1<br>*West Nile Virus IgM positive: 1<br>*Zika Virus IgM positive: 1<br>**Japanese Encephalitis NS1 positive: 1<br>**West Nile Virus NS1 positive: 1<br>**Zika Virus NS1 positive: 1<br>Rheumatoid Arthritis/other autoimmune disease cases: 5<br><sup>b</sup> Healthy subjects from endemic regions: 5   |
| 95%                | 77                            | 84  | Chikungunya positive: 3<br><sup>a</sup> Acute febrile cases: 33<br>*Japanese Encephalitis IgM positive: 3<br>*West Nile Virus IgM positive: 3<br>*Zika Virus IgM positive: 3<br>**Japanese Encephalitis NS1 positive: 3<br>**West Nile Virus NS1 positive: 3<br>**Zika Virus NS1 positive: 3<br>Rheumatoid Arthritis/other autoimmune disease cases: 15<br><sup>b</sup> Healthy subjects from endemic regions: 15 |
| 90%                | 145                           | 160   | Chikungunya positive: 5<br><sup>a</sup> Acute febrile cases: 65<br>*Japanese Encephalitis IgM positive: 5<br>*West Nile Virus IgM positive: 5<br>*Zika Virus IgM positive: 5<br>**Japanese Encephalitis NS1 positive: 5<br>**West Nile Virus NS1 positive: 5<br>**Zika Virus NS1 positive: 5<br>Rheumatoid Arthritis/other autoimmune disease cases: 30<br><sup>b</sup> Healthy subjects from endemic regions: 30 |
| 85%                | 206                           | 220   | Chikungunya positive: 7<br><sup>a</sup> Acute febrile cases: 89<br>*Japanese Encephalitis IgM positive: 7<br>*West Nile Virus IgM positive: 7<br>*Zika Virus IgM positive: 7  |



**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |   |
|--|-----|-----|---|
|  |     |     | <p>**Japanese Encephalitis NS1 positive: 7</p> <p>**West Nile Virus NS1 positive: 7</p> <p>**Zika Virus NS1 positive: 7</p> <p>Rheumatoid Arthritis/other autoimmune disease cases: 41</p> <p><sup>b</sup>Healthy subjects from endemic regions: 41</p>   |
| 80%  | 258 | 260 | <p>Chikungunya positive: 8</p> <p><sup>a</sup>Acute febrile cases: 106</p> <p>*Japanese Encephalitis IgM positive: 8</p> <p>*West Nile Virus IgM positive: 8</p> <p>*Zika Virus IgM positive: 8</p> <p>**Japanese Encephalitis NS1 positive: 8</p> <p>**West Nile Virus NS1 positive: 8</p> <p>**Zika Virus NS1 positive: 8</p> <p>Rheumatoid Arthritis/other autoimmune disease cases: 49</p> <p><sup>b</sup>Healthy subjects from endemic regions: 49</p> |
| <p><sup>a</sup> Acute febrile cases negative for Dengue (NS1 &amp; IgM &amp; IgG &amp; PCR)</p> <p><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA)</p> <p>*Depending on the availability of IgM positive samples for cross reactive flaviviruses, the requirement of samples for each virus may be increased or decreased accordingly to reach the total number of samples. If IgM positive samples for cross reactive flaviviruses are not available, commercially available IgM sera panel for different viruses can be procured and used to test cross reactivity.</p> <p>**Before used for evaluation, the NS1 reconstituted in serum samples needs to be tested by the reference assay and dilution which are positive only should be used for evaluation. The serum sample used for spiking or reconstitution should be negative for Dengue NS1, RNA and IgM antibody.</p> <p><i>#Higher sample size should be used even for assays claiming 99% specificity.</i></p> |     |     |   |

1827

1828 **5. Test reproducibility**

1829 **A. Sample size for lot-to-lot reproducibility**

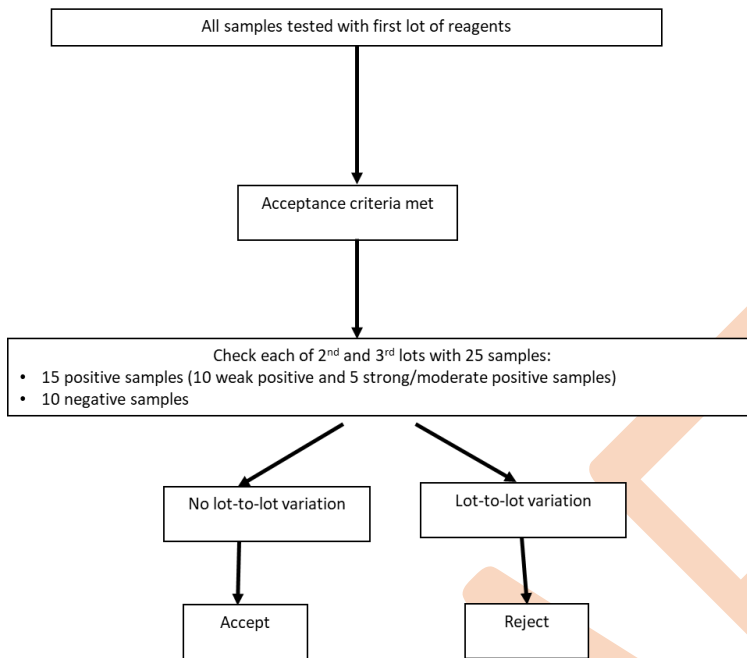
1830 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
1831 as follows:

- 1832 • First lot of the assay: should be tested on statistically significant number of positive  
1833 and negative samples as calculated in the protocol.
- 1834 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
1835 comprising 10 low positive **AND** 5 moderate/high positive samples with adequate  
1836 representation of NS1 and IgM, and 10 negative samples).
- 1837 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
1838 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

1839

1840 Refer the flowchart below (Fig. 1):

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



1841

1842

1843

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

1845 For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10  
1846 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

1847

1848 Two operators will be reading the test results independently as per manufacturer’s instruction.  
1849 Agreement should be 100% between the operators.

**C. Interpretation of results**

1851 Since the kits have been provided in combo format, concordance has to be calculated separately  
1852 for NS1 and IgM, and the overall sensitivity and specificity have to be calculated based on the  
1853 combined results of NS1 and IgM. If the sample is positive for any one or both analytes (NS1 or  
1854 IgM or both), then the sample is considered positive. Refer the table below for interpretation:

| NS1 Reference test result | IgM reference test result | Final Reference test result | NS1 Index test result | IgM Index test result | Final index test result | Interpretation |
|---------------------------|---------------------------|-----------------------------|-----------------------|-----------------------|-------------------------|----------------|
| +                         | +                         | Positive                    | +                     | -                     | Positive                | True Positive  |
| +                         | +                         | Positive                    | -                     | +                     | Positive                | True Positive  |
| +                         | +                         | Positive                    | -                     | -                     | Negative                | False Negative |
| +                         | +                         | Positive                    | +                     | +                     | Positive                | True Positive  |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|   |   |          |   |   |          |                |
|---|---|----------|---|---|----------|----------------|
| + | - | Positive | + | - | Positive | True Positive  |
| + | - | Positive | - | + | Positive | True Positive  |
| + | - | Positive | - | - | Negative | False Negative |
| - | + | Positive | + | - | Positive | True Positive  |
| - | + | Positive | - | + | Positive | True Positive  |
| - | + | Positive | - | - | Negative | False Negative |
| - | - | Negative | - | + | Positive | False Positive |
| - | - | Negative | + | - | Positive | False Positive |

1855

1856 **6. Acceptance criteria:**

1857 A minimum concordance of 80% for NS1 and 80% for IgM should be achieved with the reference  
1858 assay, and an overall combined sensitivity\* and specificity\$ of  $\geq 90\%$  each.

1859 Cross reactivity with other flavivirus antigens: Nil

1860 Invalid test rate:  $\leq 5\%$

1861 \* Samples which are positive for NS1 or IgM or both by the kit under evaluation (irrespective of the  
1862 reference assay results) will be considered as positive and used for sensitivity calculation

1863 \$ Sample which are negative for both NS1 and IgM by kit under evaluation (irrespective of the reference  
1864 assay results) will be considered as negative and used for specificity calculation

1865 **9. Publication Rights:**

1866 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

1867

1868 **After following due procedure as defined in this document, once any kit is found to be Not**  
1869 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
1870 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
1871 **will only be entertained if valid proof of change in the kit composition is submitted.**

1872

1873 **VI. References:**

- 1874 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino  
1875 JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS.  
1876 Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-  
1877 Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct 16;8(10):e3171. doi:  
1878 10.1371/journal.pntd.0003171.
- 1879 2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK,  
1880 Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis  
1881 of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi:  
1882 10.1371/journal.pntd.0003193.
- 1883 3. Yow KS, Aik J, Tan EY, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent Dengue infections:  
1884 An evaluation of six kits on clinical specimens. PLoS One. 2021 Apr 1;16(4):e0249602. doi:  
1885 10.1371/journal.pone.0249602.

- 1886 4. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic  
1887 Assessment TGS-3. 2017. Available at: [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-  
1889 RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-<br/>1888 RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
1890 5. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics  
1891 evaluation series, 3). ISBN 978 92 4 159775 3.

1892 **VII. Performance evaluation report format**

1893  
1894  
1895  
1896  
1897  
1898  
1899  
1900  
1901  
1902  
1903  
1904  
1905  
1906  
1907  
1908  
1909  
1910  
1911  
1912  
1913  
1914  
1915



**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSCO/IVD/GD/PROTOCOLS/02/2024

1916 **PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 and IgM COMBO RDT**  
1917 **KIT**

1918

|  |   |  |
|--|---|--|
| 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   |   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license    |   |  |
| License Number:Issue date:   |   |  |
| Valid Up to:   |   |  |
| Application No.  |   |  |
| <b>Sample Panel</b>  | Positive samples (provide details: strong, moderate, weak)                            |  |
|  | Negative samples (provide details: clinical/spiked, including cross reactivity panel) |  |

1919

1920

**Results:**

|   |          | Reference assay ..... (name) |          |       |
|---|----------|------------------------------|----------|-------|
|   |          | Positive                     | Negative | Total |
| <b>Name of Dengue NS1 and IgM combo RDT kit</b> | Positive |                              |          |       |
|   | Negative |                              |          |       |
|   | Total    |                              |          |       |

1921

|                      | Estimate (%) | 95% CI |
|----------------------|--------------|--------|
| Combined Sensitivity |              |        |
| Combined Specificity |              |        |

1922

1923

- Details of cross reactivity with other flavivirus NS1 antigens:

1924

- **Conclusions:**

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

- 1925 ○ Concordance for NS1, Concordance for IgM
- 1926 ○ Sensitivity, specificity
- 1927 ○ Performance: **Satisfactory / Not satisfactory**

1928 *(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from*  
1929 *the batch mentioned above using ..... sample. Results should not be extrapolated to other sample types.)*

1930 **Disclaimers**

- 1931 1. This validation process does not approve / disapprove the kit design
- 1932 2. This validation process does not certify user friendliness of the kit / assay
- 1933

1934 Note: This report is exclusively for ..... Kit (Lot No.....) manufactured by ..... (Supplied by .....)

1935 Evaluation Done on .....

1936 Evaluation Done by .....

1937 Signature of Director/ Director-In-charge ..... Seal .....

1938 \*\*\*\*\*End of the Report\*\*\*\*\*

1939

1940

1941

1942

1943

1944

1945

1946

1947

1948

1949

1950

1951

1952

1953

1954

1955

1956

1957 **Field evaluation protocol for Dengue NS1 and IgM combo RDT kits**

1958 **I. Background:**

1959 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
1960 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
1961 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
1962 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

1963 **II. Purpose:**

1964 To evaluate the performance characteristics of Dengue NS1/IgM RDT combo kits in the diagnosis  
1965 of Dengue infection in individuals with unknown disease status.

1966 **III. Requirements:**

- 1967 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
1968 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
1969 the required equipment.
- 1970 2. Evaluation sites/laboratories (With required equipment)
- 1971 3. Reference test kits
- 1972 4. Laboratory supplies

1973  
1974 **IV. Ethical approval:**

1975 *The study will be initiated after approval from the institutional human ethics committee.*

1976 **V. Procedure:**

1977 **1. Study design/type:** Cross-sectional study

1978 **2. Preparation of Evaluation sites/laboratories:**

1979 **Identified IVD kit evaluation laboratories should establish their proficiency through**

1980 A.Accreditation form NABL for at least one of the Quality management system (NABL  
1981 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
1982 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

1983 B.Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
1984 and competency testing on following

- 1985 ➤ Preparation & characterization of kit evaluation panel
- 1986 ➤ Handling of Dengue NS1 RDT/IgM RDT IVD kits received for performance evaluation  
1987 (Verification/Storage/Unpacking etc).
- 1988 ➤ Testing, interpreting, recording of results & reporting
- 1989 ➤ Data handling, data safety & confidentiality

1990 **3. Sample size for performance evaluation:**

1991 Sample sizes of positive and negative samples of Dengue against different values of  
1992 sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated  
1993 assuming 95% level of significance, an absolute precision of 5%, and invalid test rate  $\leq 5\%$ .  
1994 It is further assumed that 30% of the individuals attending the health care facilities for acute  
1995 febrile illness and suspected for Dengue will be positive for Dengue. Appropriate sample  
1996 size has to be chosen from the tables according to the values of sensitivity and specificity  
1997 being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the  
1998 table, the manufacturer needs to consider the sample size associated with the largest  
1999 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as  
2000 per the next smaller value of the sensitivity/ specificity available in the table). For example,  
2001 if a manufacturer claims a sensitivity of 93%, they are required to use a sample size  
2002 mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require  
2003 usage of the sample size outlined for 85% specificity.  
2004 Sample size has to be calculated based on both the sensitivity and the specificity. The final  
2005 sample size will be the maximum of the two. For example, at 95% sensitivity and 95%  
2006 specificity, the sample size required will be 260 (maximum of 260 and 110). It is desirable  
2007 to cover at least one Dengue season so that adequate samples are available for evaluation.  
2008  
2009

Table 1. Sample sizes for different values of sensitivity claimed by the manufacturer.

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--|-------------------------------|--|
| 99%#   | 53                            | 60   |
| 95%  | 255                           | 260  |
| 90%  | 484                           | 490  |
| 85%  | 686                           | 690  |
| 80%  | 861                           | 870  |
| * Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria |                               |  |

2010  
2011 *#Higher sample size should be used even for assays claiming 99% sensitivity.*  
2012

2013 Table 2. Sample sizes for different values of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--------------------|-------------------------------|--|
| 99%#               | 23                            | 30   |
| 95%                | 109                           | 110  |
| 90%                | 207                           | 210  |
| 85%                | 294                           | 300  |



**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

|  |     |     |
|--|-----|-----|
| 80%  | 369 | 370 |
| * Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria |     |     |

2014  
2015 *#Higher sample size should be used even for assays claiming 99% specificity.*  
2016 Recruitment of cases shall be halted once desired number of positive and negative samples are  
2017 reached.

2018  
2019 **4. Inclusion criteria:**  
2020 Patient with Dengue like illness (A patient with acute febrile illness of 1-14 days with two or more  
2021 manifestations: Head ache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic  
2022 manifestations etc. The 1-14 days disease duration shall cover viraemic as well as convalescent  
2023 phase of Dengue infection, so that both Dengue NS1 and IgM positive cases are enrolled.)

2024 **5. Exclusion criteria:**  
2025 Individuals with already known positive history for other pathogens

2026 **6. Reference assay:**  
2027 Anti-DENV IgM detection ELISA US-FDA approved kit

2028 **AND/OR**  
2029 DENV NS1 ELISA US-FDA approved kit  
2030 Serotype status to be assessed using a combination of CDC and/or NIV real-time PCR serotyping  
2031 protocols.

2032 **7. Study implementation:**  
2033 The individuals with Dengue like illness will be recruited into the study and five ml of whole blood  
2034 will be collected in vacutainer tubes and the serum will be separated by centrifugation and used  
2035 for the study.

2036 It needs to be ensured that the samples are tested by reference tests and index test simultaneously.

2037 **8. Positive samples:**  
2038 Samples which are positive for IgM or NS1 or both by the reference assays will be considered as  
2039 true positive samples.

2040 **9. Negative samples:**  
2041 Samples which are negative by the reference assay will be considered as negative.

2042 **A. Cross reactivity (other flavivirus infections):**

2043 A.1 NS1:

2044 Clinical samples or commercially available NS1 antigens from other flaviviruses will be used  
2045 to test cross reactivity of the NS1 component of index test.

- 2046 i. Japanese Encephalitis PCR/antigen positive: 5 samples\*
- 2047 ii. West Nile Virus PCR/antigen: 5 samples\*
- 2048 iii. Zika Virus PCR/antigen: 5 samples\*

2049 \*In the absence of natural samples, spiked samples may be used, as per details provided in the note below.

2050 **Note:**

2051 Recombinant NS1 antigen of cross reactive flaviviruses (Zika, West Nile and Japanese Encephalitis viruses) expressed  
2052 in mammalian cells can be obtained commercially and reconstituted in serum samples (100 ng -1 µg/ml) and diluted  
2053 in the ratio of 1:2 and used accordingly (at least five dilutions for each virus specific NS1).

2054 Before used for evaluation, NS1 reconstituted in serum samples needs to be tested by the reference assay and dilution  
2055 which are positive only should be used for evaluation.

2056 The serum samples used for reconstitution should be negative for Dengue NS1, RNA and IgM antibody.

2057 A.2 IgM:

2058 Clinical samples positive for IgM for other flaviviruses will be used to test cross reactivity of  
2059 the IgM component of index test.

- 2060 i. Japanese Encephalitis IgM positive: 5 samples
- 2061 ii. West Nile Virus IgM positive: 5 samples
- 2062 iii. Zika Virus IgM positive: 5 samples

2063 **Note:** Depending on the availability of IgM positive samples for cross reactive flaviviruses, the requirement of samples  
2064 for each virus may be increased or decreased accordingly to reach the total number of samples. If IgM positive samples  
2065 for cross reactive flaviviruses are not available, commercially available IgM sera panel for different viruses can be  
2066 procured and used to test cross reactivity.

2067 **10. Statistical analysis:**

2068 Concordance will be calculated separately for Dengue NS1 and IgM. Combined sensitivity and  
2069 specificity will also be calculated.

2070 Interim analysis of data shall be conducted on completing evaluation of 25%, 50% and 75% of  
2071 samples. If, at any point, the performance of the assay is found to be not satisfactory, the assay  
2072 shall not be evaluated further. Evaluation fee shall be charged accordingly.

2073

2074 **11. Test reproducibility**

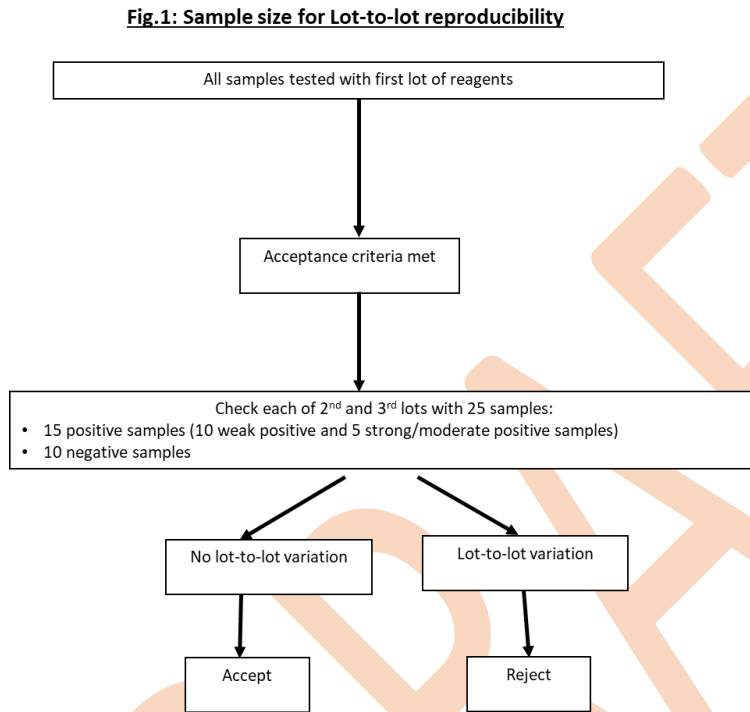
2075 **A. Sample size for lot-to-lot reproducibility**

2076 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
2077 as follows:

- 2078 • First lot of the assay: should be tested on statistically significant number of positive  
2079 and negative samples as calculated in the protocol.

- 2080 • Second lot of the assay: should be tested on 25 samples (15 positive samples
- 2081 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative
- 2082 samples).
- 2083 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising
- 2084 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- 2085

2086 Refer the flowchart below (Fig. 1):



2087

### 2088 **B. Sample size for reader-to-reader reproducibility**

2089 For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10

2090 low positive **AND** 5 moderate/high positive samples with adequate representation of NS1 and

2091 IgM, and 10 negative samples).

2092

2093 Two operators will be reading the test results independently as per manufacturer’s instruction.

2094 Agreement should be 100% between the operators.

### 2095 **C. Interpretation of results**

2096 Since the kits have been provided in a combo format, the sensitivity and specificity has to be

2097 calculated based on the combined results of the NS1 and IgM. If the sample is positive for any one

2098 or both analytes (NS1 or IgM or both), then the sample is considered positive. Refer the table

2099 below:

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

| NS1 Reference test result | IgM reference test result | Final Reference test result | NS1 Index test result | IgM Index test result | Final index test result | Interpretation |
|---------------------------|---------------------------|-----------------------------|-----------------------|-----------------------|-------------------------|----------------|
| +                         | +                         | Positive                    | +                     | -                     | Positive                | True Positive  |
| +                         | +                         | Positive                    | -                     | +                     | Positive                | True Positive  |
| +                         | +                         | Positive                    | -                     | -                     | Negative                | False Negative |
| +                         | +                         | Positive                    | +                     | +                     | Positive                | True Positive  |
| +                         | -                         | Positive                    | +                     | -                     | Positive                | True Positive  |
| +                         | -                         | Positive                    | -                     | +                     | Positive                | True Positive  |
| +                         | -                         | Positive                    | -                     | -                     | Negative                | False Negative |
| -                         | +                         | Positive                    | +                     | -                     | Positive                | True Positive  |
| -                         | +                         | Positive                    | -                     | +                     | Positive                | True Positive  |
| -                         | +                         | Positive                    | -                     | -                     | Negative                | False Negative |
| -                         | -                         | Negative                    | -                     | +                     | Positive                | False Positive |
| -                         | -                         | Negative                    | +                     | -                     | Positive                | False Positive |

2100

2101 **12. Acceptance criteria:**

2102 A minimum concordance of 80% for NS1 and 80% for IgM should be achieved with the reference  
2103 assay, and an overall combined sensitivity\* and specificity\$ of  $\geq 90\%$  each.

2104 Cross reactivity with other flavivirus antigens: Nil

2105 Invalid test rate:  $\leq 5\%$

2106 \* Samples which are positive for NS1 or IgM or both by the kit under evaluation (index test) irrespective  
2107 of the reference assay results will be considered as positive and used for sensitivity calculation

2108 \$ Samples which are negative for both NS1 and IgM by kit under evaluation only will be considered as  
2109 negative and used for specificity calculation

2110 **13. Publication Rights:**

2111 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

2112

2113 **After following due procedure as defined in this document, once any kit is found to be Not**  
2114 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
2115 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
2116 **will only be entertained if valid proof of change in the kit composition is submitted.**

2117

2118

2119 **VI. References:**

2120 1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian  
2121 E, Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S,

- 2122 Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection  
2123 of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct  
2124 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
- 2125 2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S,  
2126 Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay  
2127 sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl  
2128 Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
- 2129 3. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K,  
2130 Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic  
2131 review and meta-analysis. PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi:  
2132 10.1371/journal.pntd.0006618.
- 2133 4. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
2134 Diagnostic Assessment TGS-3. 2017. Available at:  
2135 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
2136 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)
- 2137 5. WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests.  
2138 (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.

2139  
2140  
2141 **VII. Performance evaluation report format**  
2142

2143  
2144  
2145  
2146  
2147  
2148  
2149  
2150  
2151  
2152  
2153  
2154  
2155  
2156  
2157

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

2158 **PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 and IgM COMBO RDT**  
2159 **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 CDSO/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  |   |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |   |
| License Number:Issue date:  |   |
| Valid Up to:  |   |
| Application No.   |   |
| <b>Sample Panel</b>   | Positive samples: Not applicable, may categorize cases as per duration of illness                 |
|   | Negative samples (may categorize as per duration of illness, must include cross reactivity panel) |

2160 **Results**

|  |          | <b>Reference assay ..... (name)</b> |          |       |
|--|----------|-------------------------------------|----------|-------|
|  |          | Positive                            | Negative | Total |
| <b>Name of NS1 and IgM combo RDT kit</b> | Positive |                                     |          |       |
|  | Negative |                                     |          |       |
|  | Total    |                                     |          |       |

2161

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

2162

2163 ● Details of cross reactivity with other flavivirus NS1 antigens:

2164 ● Conclusions:

2165 ○ Sensitivity, specificity

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

2166                   ○ Performance: **Satisfactory / Not satisfactory**  
2167                   (*Sensitivity and specificity have been assessed in using kits provided by the manufacturer from the batch mentioned above using*  
2168                   ..... sample in ..... (field/controlled lab). Results should not be extrapolated to other sample types.)

2169                   **Disclaimers**

- 2170                   1. This validation process does not approve / disapprove the kit design
- 2171                   2. This validation process does not certify user friendliness of the kit / assay

2172                   Note: This report is exclusively for .....NS1 and IgM combo Kit (Lot No.....) manufactured by  
2173                   ..... (supplied by .....)

2174                   Evaluation Done on .....

2175                   Evaluation Done by .....

2176                   Signature of Director/ Director-In charge ..... Seal .....

2177                   \*\*\*\*\*End of the Report\*\*\*\*\*

2178

2179

2180

2181

2182

2183

2184

2185

2186

2187

2188

2189

2190

2191

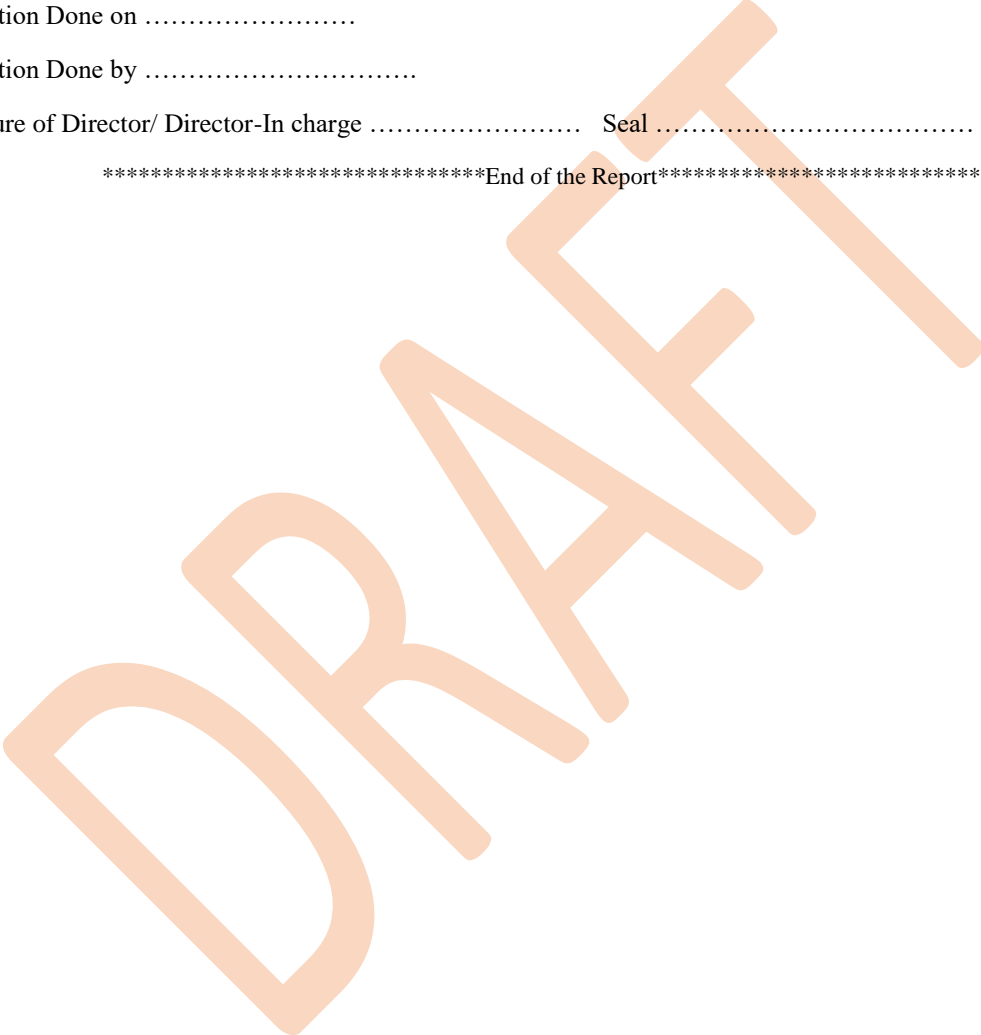
2192

2193

2194

2195

2196



2197 **Performance evaluation protocol for Dengue real-time PCR kit**

2198 **I. Background:**

2199 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
2200 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
2201 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
2202 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

2203 This recommendation focuses on the laboratory performance evaluation of Dengue virus  
2204 molecular diagnostic test. All clinical samples tested in the study should be evaluated in  
2205 accordance with the candidate test's instructions for use.

2206

2207 **II. Purpose:**

2208 To evaluate the performance characteristics of Dengue real-time PCR kits in the diagnosis of  
2209 Dengue infection.

2210 **III. Requirements:**

2211 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
2212 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
2213 the required equipment.

2214 2. Evaluation sites/laboratories (With required equipment)

2215 3. Reference test kits

2216 4. Characterised Evaluation panel

2217 5. Laboratory supplies

2218 **IV. Ethical approvals:**

2219 Exempted from Ethics approval as per ICMR's Guidance on Ethical Requirements for Laboratory  
2220 Validation Testing, 2024. A self-declaration form as provided in ICMR guidelines to be submitted  
2221 by the investigators to the institutional authorities and ethics committee for information.

2222 **V. Procedure:**

2223 **1. Study design/type:** Diagnostic accuracy study using archived/ leftover/ spiked clinical  
2224 samples.

2225 **2. Preparation of Evaluation sites/laboratories:**

2226 **Identified IVD kit evaluation laboratories should establish their proficiency through**

2227 A. Accreditation form NABL for at least one of the Quality management system (NABL  
2228 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
2229 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.



2230 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
2231 and competency testing on following

2232 ➤ Preparation & characterization of kit evaluation panel

2233 ➤ Handling of Dengue RT-PCR kits received for performance evaluation  
2234 (Verification/Storage/Unpacking etc).

2235 ➤ Testing, interpreting, recording of results & reporting

2236 ➤ Data handling, data safety & confidentiality

### 2237 **3. Preparation of Dengue RNA evaluation panel**

2238 Well characterised Dengue serum/plasma panel positive for RNA by RT-PCR is a critical  
2239 requirement for performance evaluation of IVD kits utilizing genome detection. Hence statistically  
2240 significant number of sera/plasma samples should be available from Dengue PCR confirmed cases.

### 2241 **4. RNA extraction**

2242 RNA extraction shall be performed using standard techniques. If the manufacturer of the index test  
2243 recommends a specific RNA extraction kit, the same needs to be provided by the manufacturer.

### 2244 **5. Real-Time PCR System**

2245 PCR shall be performed using IVD-approved machines. If any equipment(s) is specified in the  
2246 IFU of the index test, it shall be used for the evaluation, and it shall be provided by the  
2247 manufacturer if not available within the lab's IVD evaluation scope.

### 2248 **6. Internal control/Extraction control**

2249 The test under evaluation should have an internal control or extraction control (RNA added before  
2250 extraction to a sample).

### 2251 **7. Reference assay:**

2252 Any FDA approved Dengue PCR assay or CDC/NIV protocol for detection of Dengue virus RNA  
2253 should be used as the reference assay.

2254 All positive samples should be confirmed positive for at least one serotype by real-time PCR assay  
2255 using CDC/NIV protocol.

2256 All negative samples should be negative for all the markers of Dengue infection (NS1, IgM, and  
2257 RNA).

2258

2259 **8. Sample size and sample panel composition:** Sample sizes of positive and negative  
2260 samples and sample panel composition against different values of sensitivity and specificity are  
2261 provided in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance,

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

2262 an absolute precision of 5%, and invalid test rate  $\leq 5\%$ . Appropriate sample size has to be chosen  
 2263 from the tables according to the values of sensitivity and specificity being claimed by the  
 2264 manufacturer. If a claimed sensitivity/specificity is not present in the table, the manufacturer needs  
 2265 to consider the sample size associated with the largest sensitivity/specificity provided in the table  
 2266 that is smaller to the claimed value (that is, as per the next smaller value of the sensitivity/ specificity  
 2267 available in the table). For example, if a manufacturer claims a sensitivity of 93%, they are required  
 2268 to use a sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would  
 2269 require usage of the sample size outlined for 85% specificity.

2270 Positive samples: These include samples positive by the reference real-time PCR assay (True  
 2271 positives) and representative of all four serotypes.

2273 Negative samples: All negative samples should be negative by reference real-time PCR assay, US-  
 2274 FDA approved NS1 antigen ELISA kit-and US FDA approved IgM Capture ELISA.

2275

2276

2277 Table 1. Sample sizes and panel composition of positive Dengue samples for different values of  
 2278 sensitivity claimed by the manufacturer.

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>  |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Strong positive (Ct value <25): 5<br>Moderate positive (Ct value between 25-30): 10<br>Weak positive (Ct value >30 to 34): 5   |
| 95%                | 77                            | 80  | Strong positive (Ct value <25): 20<br>Moderate positive (Ct value between 25-30): 40<br>Weak positive (Ct value >30 to 34): 20 |
| 90%                | 145                           | 150   | Strong positive (Ct value <25): 38<br>Moderate positive (Ct value between 25-30): 74<br>Weak positive (Ct value >30 to 34): 38 |
| 85%                | 206                           | 210   | Strong positive (Ct value <25): 53   |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |   |
|--|-----|-----|---|
|  |     |     | Moderate positive (Ct value between 25-30): 104<br>Weak positive (Ct value >30 to 34): 53                                       |
| 80%  | 258 | 260 | Strong positive (Ct value <25): 65<br>Moderate positive (Ct value between 25-30): 130<br>Weak positive (Ct value >30 to 34): 65 |
| <p><u>Note:</u><br/>If clinical samples positive for a particular serotype is not available, tissue culture fluid (5-10 different isolates with a plaque forming unit of 10<sup>5-6</sup>/ml) (Heat-inactivated) from reference laboratories can be obtained, spiked in serum samples (15 µl isolate + 150 µl) and can be further diluted in the ratio of 1:10, frozen at -80°C, and tested by the reference assay when needed and the positive samples can be used for evaluation.<br/>The serum used for spiking isolate should be negative for Dengue virus RNA, and NS1.</p> |     |     |   |

2279

2280 *#Higher sample size should be used even for assays claiming 99% sensitivity.*

2281

2282 Table 2. Sample sizes and panel composition of negative Dengue samples for different values of  
2283 specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>   |
|--------------------|-------------------------------|---|---|
| 99% <sup>#</sup>   | 16                            | 20  | Chikungunya positive: 4<br><sup>a</sup> Acute febrile cases: 8<br><sup>*</sup> Japanese Encephalitis positive: 1<br><sup>*</sup> West Nile Virus positive: 1<br><sup>*</sup> Zika Virus positive: 1<br><sup>b</sup> Healthy subjects from endemic regions: 5    |
| 95%                | 77                            | 80  | Chikungunya positive: 15<br><sup>a</sup> Acute febrile cases: 30<br><sup>*</sup> Japanese Encephalitis positive: 5<br><sup>*</sup> West Nile Virus positive: 5<br><sup>*</sup> Zika Virus positive: 5<br><sup>b</sup> Healthy subjects from endemic regions: 20 |
| 90%                | 145                           | 150   | Chikungunya positive: 28<br><sup>a</sup> Acute febrile cases: 57<br><sup>*</sup> Japanese Encephalitis positive: 9<br><sup>*</sup> West Nile Virus positive: 9<br><sup>*</sup> Zika Virus positive: 9   |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|   |     |     |  |
|---|-----|-----|--|
|   |     |     | <sup>b</sup> Healthy subjects from endemic regions: 38   |
| 85%   | 206 | 210 | Chikungunya positive: 39<br><sup>a</sup> Acute febrile cases: 79<br>*Japanese Encephalitis positive: 13<br>*West Nile Virus positive: 13<br>*Zika Virus positive: 13<br><sup>b</sup> Healthy subjects from endemic regions: 53 |
| 80%   | 258 | 260 | Chikungunya positive: 49<br><sup>a</sup> Acute febrile cases: 98<br>*Japanese Encephalitis positive: 16<br>*West Nile Virus positive: 16<br>*Zika Virus positive: 16<br><sup>b</sup> Healthy subjects from endemic regions: 65 |
| <sup>a</sup> Acute febrile cases negative for all markers of Dengue (NS1 & IgM & IgG & RNA)<br><sup>b</sup> Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid)<br><br><u>* Note:</u><br>If PCR positive samples for cross reactive flaviviruses not available, commercially available RNA panels should be used to test cross reactivity. |     |     |  |

2284

2285 *#Higher sample size should be used even for assays claiming 99% specificity.*

2286 **9. Evaluation method:**

2287 The index test and the reference tests should be run simultaneously on the sample panel to  
 2288 avoid false negative results by index test due to free thawing of samples or deterioration of  
 2289 sample quality on long term storage. Both the index and reference tests should be run on  
 2290 the sample plate for each of the panel samples.

2291 **10. Test reproducibility**

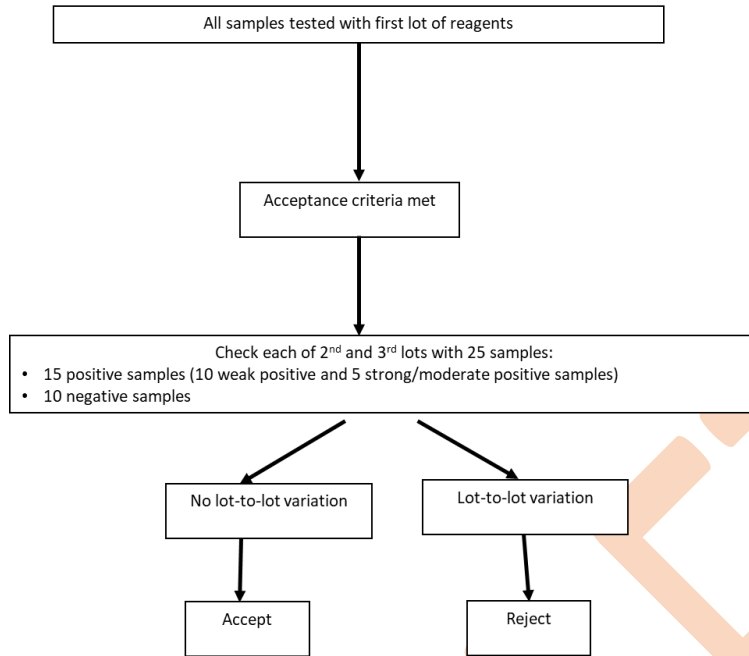
2292 **A. Sample size for lot-to-lot reproducibility**

2293 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
 2294 as follows:

- 2295 • First lot of the assay: should be tested on statistically significant number of positive  
 2296 and negative samples as calculated in the protocol.
- 2297 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
 2298 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
 2299 samples).
- 2300 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
 2301 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).  
 2302

2303 Refer the flowchart below (Fig. 1):

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



2304

2305

2306

### 2307 **11. Acceptance Criteria**

2308 Expected sensitivity:  $\geq 95\%$

2309 Expected specificity:  $\geq 98\%$

2310 Cross reactivity with other flavivirus: Nil

2311 Invalid test rate:  $\leq 5\%$

2312

### 2313 **13. Publication Rights:**

2314 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

2315

2316 **After following due procedure as defined in this document, once any kit is found to be Not**  
2317 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
2318 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
2319 **will only be entertained if valid proof of change in the kit composition is submitted.**

2320

### 2321 **VI. References:**

2322 1. Santiago, G.A., Vázquez, J., Courtney, S. et al. Performance of the Triplex real-time RT-PCR assay  
2323 for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun 9, 1391 (2018).  
2324 <https://doi.org/10.1038/s41467-018-03772-1>  
2325 2. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
2326 Diagnostic Assessment TGS-3. 2017. Available at:  
2327 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-  
2329 eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-<br/>2328 eng.pdf;sequence=1)

2330 **VII. Performance evaluation report format**

2331  
2332  
2333  
2334  
2335  
2336  
2337  
2338  
2339  
2340  
2341  
2342  
2343  
2344  
2345  
2346  
2347  
2348  
2349  
2350  
2351  
2352



2353 **PERFORMANCE EVALUATION REPORT FOR DENGUE 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 CDSO/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  |   |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |   |
| License Number:Issue date:  |   |
| Valid Up to:  |   |
| Application No.   |   |
| <b>Sample Panel</b>   | Positive samples (provide details: clinical/ spiked, strong, moderate, weak)          |
|   | Negative samples (provide details clinical/ spiked, including cross reactivity panel) |

2354

2355 **Results**

|                              |          | Reference assay ..... (name) |          |       |
|------------------------------|----------|------------------------------|----------|-------|
|                              |          | Positive                     | Negative | Total |
| Name of Dengue real-time PCR | Positive |                              |          |       |
|                              | Negative |                              |          |       |
|                              | Total    |                              |          |       |

2356

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

2357

2358

- Details of cross reactivity with other flaviviruses:

2359

- **Conclusions:**

2360

- Sensitivity, specificity

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

2361           ○ Performance: **Satisfactory / Not satisfactory**  
2362   *(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from*  
2363   *the batch mentioned above using ..... sample. Results should not be extrapolated to other sample types.)*

2364    **Disclaimers**

- 2365           1. This validation process does not approve / disapprove the kit design  
2366           2. This validation process does not certify user friendliness of the kit / assay

2367    Note: This report is exclusively for Dengue..... Kit (Lot No.....) manufactured by .....  
2368    (supplied by .....)

2369    Evaluation Done on .....

2370    Evaluation Done by .....

2371    Signature of Director/ Director-In-charge ..... Seal .....

2372    \*\*\*\*\*End of the Report\*\*\*\*\*

2373

2374

2375

2376

2377

2378

2379

2380

2381

2382

2383

2384

2385

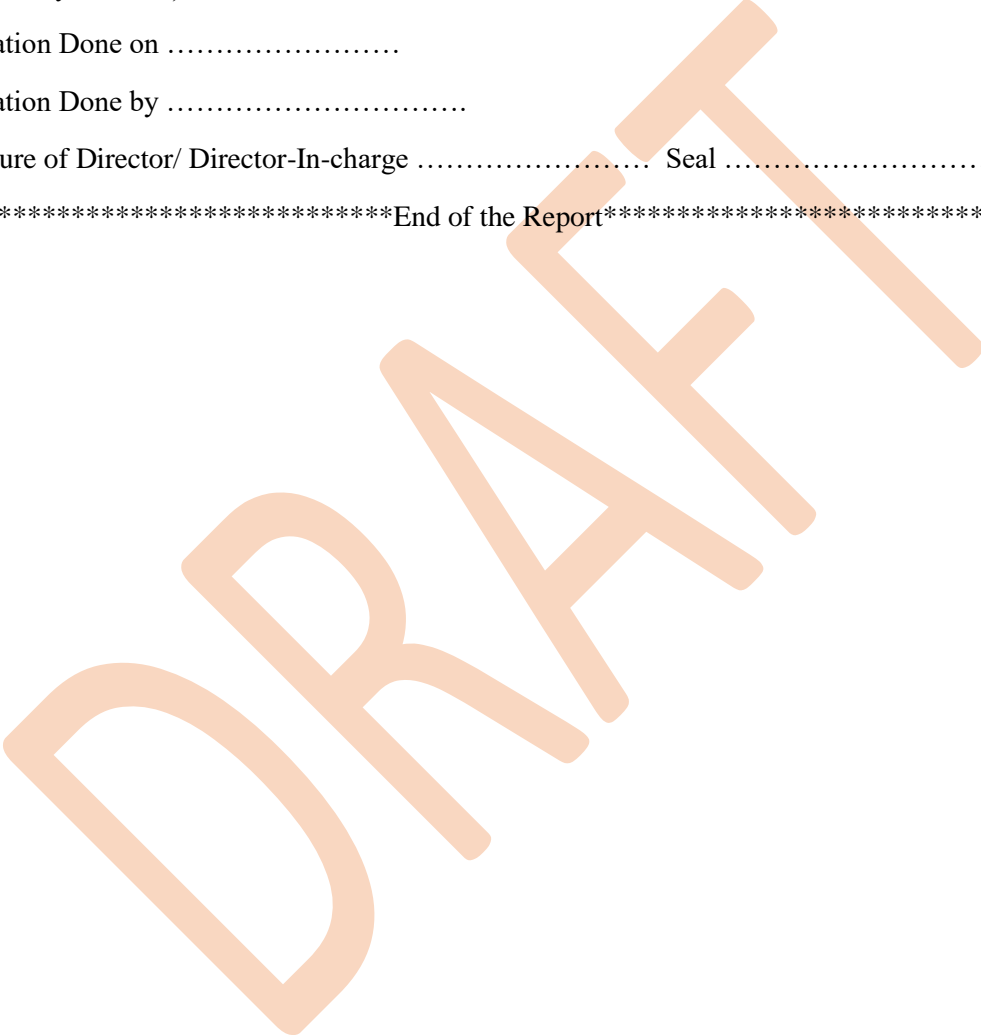
2386

2387

2388

2389

2390





2391 **Field evaluation protocol for Dengue real-time PCR kits**

2392 **I. Background:**

2393 CDSCO and ICMR, New Delhi, have aimed at facilitating the availability of Quality-Assured  
2394 Diagnostics kits appropriate for use in India. Hence the following guidelines shall establish the  
2395 uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The performance  
2396 evaluation is to independently verify the manufacturer's claim regarding IVD performance.

2397 **II. Purpose:**

2398 To evaluate the performance characteristics of Dengue real-time PCR kits in the diagnosis of  
2399 Dengue infection in individuals with unknown disease status.

2400 **III. Requirements:**

- 2401 1. Supply of kits under evaluation (Along with batch/lot No. Expiry & required details). If  
2402 the kit to be evaluated works in a closed system format, the manufacturer needs to supply  
2403 the required equipment.
- 2404 2. Evaluation sites/laboratories (With required equipment)
- 2405 3. Reference test kits
- 2406 4. Laboratory supplies

2407  
2408 **IV. Ethical approvals:**

2409 The study will be initiated after approval from the institutional human ethics committee.

2410 **V. Procedure:**

2411 **1. Study design/type:** Cross-sectional study

2412 **2. Preparation of Evaluation sites/laboratories:**

2413 **Identified IVD kit evaluation laboratories should establish their proficiency through**

2414 A. Accreditation from NABL for at least one of the Quality management system (NABL  
2415 accreditation for testing Lab / calibration lab (ISO/IES 17025), Medical Lab (ISO 15189), PT  
2416 provider ISO/IEC 17043 or CDSCO approved Reference laboratory.

2417 B. Staff training: All the staff involved in IVD kit evaluation should undergo hands on training  
2418 and competency testing on following

- 2419 ➤ Preparation & characterization of kit evaluation panel
- 2420 ➤ Handling of Dengue RT-PCR kits received for performance evaluation  
2421 (Verification/Storage/Unpacking etc).
- 2422 ➤ Testing, interpreting, recording of results & reporting
- 2423 ➤ Data handling, data safety & confidentiality

2424 **3. Sample size for performance evaluation:**

2425 Sample sizes of positive and negative samples of Dengue against different values of  
 2426 sensitivity and specificity are provided in Tables 1 and 2. Sample sizes have been calculated  
 2427 assuming 95% level of significance, an absolute precision of 5%, and invalid test rate  $\leq 5\%$ .  
 2428 It is further assumed that 30% of the individuals attending the health care facilities for acute  
 2429 febrile illness and suspected for Dengue will be positive for Dengue. Appropriate sample  
 2430 size has to be chosen from the tables according to the values of sensitivity and specificity  
 2431 being claimed by the manufacturer. If a claimed sensitivity/specificity is not present in the  
 2432 table, the manufacturer needs to consider the sample size associated with the largest  
 2433 sensitivity/specificity provided in the table that is smaller to the claimed value (that is, as  
 2434 per the next smaller value of the sensitivity/ specificity available in the table). For example,  
 2435 if a manufacturer claims a sensitivity of 93%, they are required to use a sample size  
 2436 mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require  
 2437 usage of the sample size outlined for 85% specificity.  
 2438 Sample size has to be determined based on both the sensitivity and the specificity. The  
 2439 required sample size will be the maximum of the two. For example, at 95% sensitivity  
 2440 and 95% specificity, the sample size required will be 260 (maximum of 260 and 110).

2441  
2442 Table 1. Sample sizes for different values of sensitivity claimed by the manufacturer.

| <i>Sensitivity</i>   | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--|-------------------------------|--|
| 99%#   | 53                            | 60   |
| 95%  | 255                           | 260  |
| 90%  | 484                           | 490  |
| 85%  | 686                           | 690  |
| 80%  | 861                           | 870  |
| * Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria |                               |  |

2443  
2444 #Higher sample size should be used even for assays claiming 99% sensitivity.

2445  
2446 Table 2. Sample sizes for different values of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of individuals* [Sample size rounded off]</i> |
|--------------------|-------------------------------|--|
| 99%#               | 23                            | 30   |
| 95%                | 109                           | 110  |
| 90%                | 207                           | 210  |
| 85%                | 294                           | 300  |
| 80%                | 369                           | 370  |

\* Individuals attending the health care facilities for acute febrile illness and suspected for Dengue meeting the inclusion criteria

2447

2448 *#Higher sample size should be used even for assays claiming 99% specificity.*

2449 Recruitment of cases shall be halted once desired number of positive and negative samples are  
2450 reached.

2451 **4. Inclusion criteria:**

2452 Individuals with Dengue like illness (A patient with acute febrile illness of 2-7 days with two or  
2453 more manifestations: Head ache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic  
2454 manifestations)

2455 **5. Exclusion criteria:**

2456 Individuals with already known positive history for other pathogens

2457 **6. RNA extraction**

2458 RNA extraction shall be performed using standard techniques. If any extraction system is specified  
2459 in the IFU, that shall be used for the test and shall be provided by the manufacturer.

2460 **7. Real-Time PCR System**

2461 PCR shall be performed using IVD-approved machines. If any equipment(s) is specified in the  
2462 IFU, that shall be used for the test and shall be provided by the manufacturer.

2463 **8. Internal control/Extraction control**

2464 The test under evaluation should have an internal control or extraction control (RNA added before  
2465 extraction to a sample).

2466 **9. Reference assay:**

2467 Any FDA approved Dengue PCR assay or CDC/NIV protocol for detection of Dengue RNA  
2468 should be used as the reference assay.

2469 All positive samples should be confirmed positive for at least one serotype by real-time PCR assay  
2470 using CDC/NIV protocol.

2471 All negative samples should be negative for all the markers of Dengue infection (NS1 & IgM &  
2472 IgG and RNA).

2473 **10. Study implementation:**

2474 The individuals with Dengue like illness will be recruited into the study and five ml of whole blood  
2475 will be collected in vacutainer tubes and the serum will be separated by centrifugation and used  
2476 for the study.

2477 It needs to be ensured that the samples are tested by reference tests and index test simultaneously.

2478 **11. Positive samples:**

2479 Samples which are positive by reference real-time PCR assay will be considered as true positive  
2480 sample.

2481 **12. Negative samples:**

2482 Samples which are negative by the reference assay will be considered as negative.

2483 **A. Cross reactivity:**

2484 Clinical samples or commercially available Viral RNA genome of other flaviviruses/RNA from  
2485 sequence confirmed virus isolates will be used to test cross reactivity of the index test.

- 2486 a. Japanese Encephalitis PCR positive: 5 samples
- 2487 b. West Nile Virus PCR positive: 5 samples
- 2488 c. Zika Virus PCR positive: 5 samples

2489 Alternatively, tissue culture fluid of cross reactive flaviviruses (with a plaque forming unit of  $10^{5-6}/\text{ml}$ )(Heat  
2490 inactivated) from reference laboratories can be obtained, spiked in serum samples (15  $\mu\text{l}$  isolate + 150  $\mu\text{l}$ ) and can be  
2491 further diluted in the ratio of 1:10, tested by the reference assay and the negative samples can be used for evaluation.

2492 The serum used for spiking isolate should be negative for Dengue virus RNA, and NS1.

2493 **13. Statistical analysis:**

2494 Sensitivity and specificity will be calculated.

2495 Interim analysis of data shall be conducted on completing evaluation of 25%, 50% and 75% of  
2496 samples. If, at any point, the performance of the assay is found to be not satisfactory, the assay  
2497 shall not be evaluated further. Evaluation fee shall be charged accordingly.

2498 **14. Test reproducibility**

2499 **A. Sample size for lot-to-lot reproducibility**

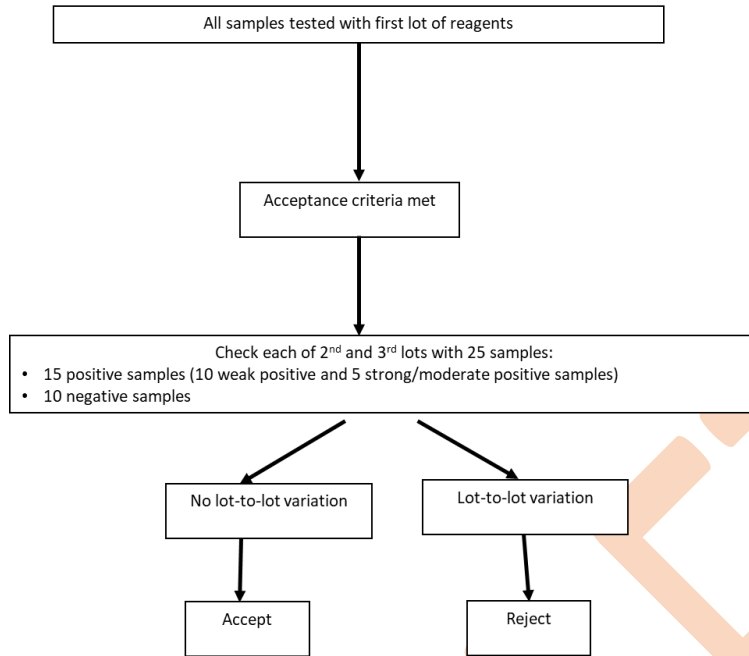
2500 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
2501 as follows:

- 2502 • First lot of the assay: should be tested on statistically significant number of positive  
2503 and negative samples as calculated in the protocol.
- 2504 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
2505 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
2506 samples).
- 2507 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
2508 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

2509

2510 Refer the flowchart below (Fig. 1):

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



2511

## 2512 **15. Acceptance Criteria**

2513 Sensitivity:  $\geq 95\%$

2514 Specificity:  $\geq 98\%$

2515 Cross reactivity with other flavivirus: Nil

2516 Invalid test rate:  $\leq 5\%$

## 2517 **16. Publication Rights:**

2518 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

2519

2520 **After following due procedure as defined in this document, once any kit is found to be Not**  
2521 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
2522 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
2523 **will only be entertained if valid proof of change in the kit composition is submitted.**

2524

## 2525 **VI. References:**

2526 1. Santiago, G.A., Vázquez, J., Courtney, S. et al. Performance of the Trioplex real-time RT-PCR  
2527 assay for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun 9, 1391(2018).  
2528 <https://doi.org/10.1038/s41467-018-03772-1>

2529 2. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K,  
2530 Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic

2531 review and meta-analysis. PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi:  
2532 10.1371/journal.pntd.0006618.

2533 3. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
2534 Diagnostic Assessment TGS-3. 2017. Available at:  
2535 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
2536 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)

2537

2538 **VII. Performance evaluation report format**

2539

2540

2541

2542

2543

2544

2545

2546

2547

2548

2549

2550

2551

2552

2553

2554

2555

2556

2557

2558

2559

2560

2561



2562 **PERFORMANCE EVALUATION REPORT FOR DENGUE 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 CDSO/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  |   |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |   |  |
| License Number:Issue date:  |   |  |
| Valid Up to:  |   |  |
| Application No.   |   |  |
| <b>Sample Panel</b>   | Positive samples: Not applicable, may categorize cases as per duration of illness                 |  |
|   | Negative samples (may categorize as per duration of illness, must include cross reactivity panel) |  |

2563

2564 **Results**

|   |          | <b>Reference assay ..... (name)</b> |          |       |
|---|----------|-------------------------------------|----------|-------|
|   |          | Positive                            | Negative | Total |
| <b>Name of Dengue real-time PCR kit</b> | Positive |                                     |          |       |
|   | Negative |                                     |          |       |
|   | Total    |                                     |          |       |

2565

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

2566

2567

- Details of cross reactivity with other flaviviruses:

2568

- **Conclusions:**

2569

- Sensitivity, specificity

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

2570           ○ Performance: **Satisfactory / Not satisfactory**  
2571           *(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from*  
2572           *the batch mentioned above using ..... sample. Results should not be extrapolated to other sample types.)*

2573           **Disclaimers**

- 2574           1. This validation process does not approve / disapprove the kit design  
2575           2. This validation process does not certify user friendliness of the kit / assay  
2576

2577           Note: This report is exclusively for Dengue..... Kit (Lot No.....) manufactured by ..... (supplied by  
2578           .....)

2579           Evaluation Done on .....

2580           Evaluation Done by .....

2581           Signature of Director/ Director-In-charge ..... Seal .....

2582           \*\*\*\*\*End of the Report\*\*\*\*\*

2583

2584

2585

2586

2587

2588

2589

2590

2591

2592

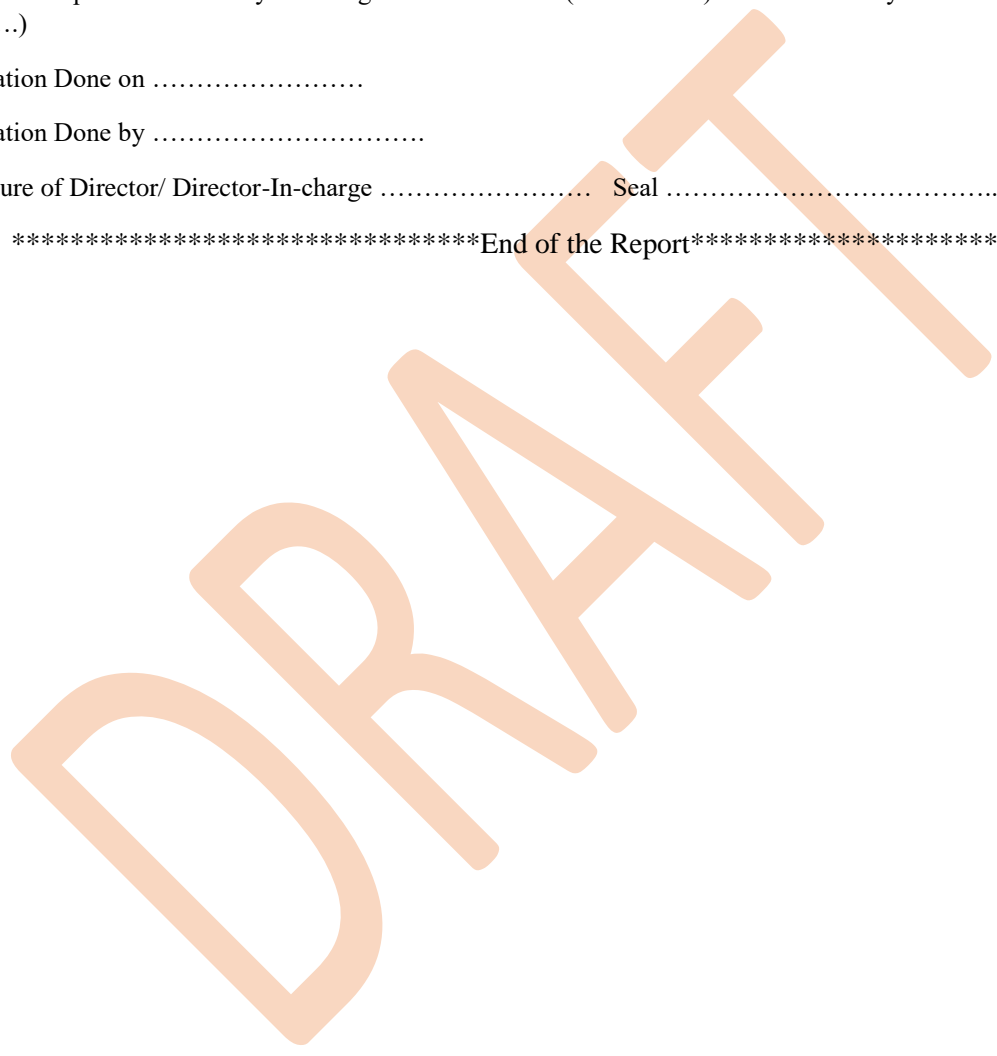
2593

2594

2595

2596

2597





2598 **Performance evaluation protocol for Real-time PCR tests for Zika virus**

2599 **I. Background:**

2600 CDSCO and ICMR, New Delhi, aimed at facilitating the evaluation and deployment of Quality-  
2601 Assured Diagnostics kits appropriate for use in India. Hence the following guidelines shall  
2602 establish the uniformity in performance evaluation of in-vitro diagnostic kits (IVD). The  
2603 performance evaluation is to independently verify the manufacturer's claim regarding in-vitro  
2604 diagnostic kit (IVD) performance.

2605 This recommendation focuses on the laboratory performance evaluation of Zika virus molecular  
2606 diagnostic test. All clinical samples tested in the study should be evaluated in accordance with the  
2607 candidate test's proposed diagnostic algorithm (i.e., tested using the procedure in the instructions  
2608 for use), including retesting when appropriate.

2609 **II. Purpose:** To evaluate the performance characteristics of Zika virus RT-PCR test for diagnosis  
2610 of Zika infection.

2611 **III. Requirements:**

- 2612 1. Supply of kits under evaluation (along with batch/lot No. Expiry & required details)
- 2613 2. Evaluation site/laboratory should be equipped with necessary equipment and supplies for  
2614 molecular testing. Any essential equipment and consumables for closed system to be  
2615 supplied and maintained from the manufacturer, during the period of evaluation.
- 2616 3. Reference test kits
- 2617 4. Characterized evaluation panel
- 2618 5. Laboratory supplies

2619 **IV. Ethics approval:** Exempted from Ethics approval as per ICMR's Guidance on Ethical  
2620 Requirements for Laboratory Validation Testing, 2024. A self-declaration form as provided in  
2621 ICMR guidelines to be submitted by the investigators to the institutional authorities and ethics  
2622 committee for information.

2623 **V. Procedure:**

2624 **1. Study design:** Diagnostic accuracy study using archived/leftover/spiked clinical samples.

2625 **2. Preparation of Evaluation site/laboratory:** Performance evaluation performance and report  
2626 to be issued only from designated reference testing laboratory/ NABL accredited laboratory, as  
2627 specified by state or central licensing authority.

2628 **3. Identified IVD kit evaluation laboratories should establish their proficiency through**

2629 A.NABL accreditation for at least one of the Quality management system (NABL accreditation  
2630 for testing laboratory/ calibration laboratory (ISO/IES 17025), Medical Laboratory (ISO  
2631 15189), PT provider ISO/IEC 17043 or CDSO approved Reference laboratory.

2632 B.Staff training: All the staff involved in the IVD kit evaluation should undergo hands on  
2633 training and competency testing on following

- 2634 ➤ Preparation & characterization of evaluation panel
- 2635 ➤ Handling of Zika molecular diagnostic kits received for performance evaluation  
2636 (Verification/Storage/Unpacking etc.)
- 2637 ➤ Testing, interpretation, recording of results & reporting
- 2638 ➤ Data handling, data safety & confidentiality

2639

#### 2640 **4. Preparation of Zika reference evaluation panel**

2641 Well characterized Zika molecular evaluation panel is a critical requirement for performance  
2642 evaluation of IVD kits. Hence, statistically significant number of clinical samples should be used  
2643 for evaluation.

- 2644 ● Frozen samples ( $\leq -70^{\circ}\text{C}$ ) may be used, if stored appropriately and analytical data  
2645 demonstrate that accuracy of test results is not affected.
- 2646 ● Samples that previously tested positive by FDA approved PCR and/or CDC/NIV  
2647 approved protocols may be used.
- 2648 ● In the absence of natural samples, spiked clinical samples may be used.

#### 2649 **5. RNA extraction**

2650 RNA extraction shall be performed using standard techniques. If the manufacturer of the index test  
2651 recommends a specific RNA extraction kit, the same needs to be provided by the manufacturer.

#### 2652 **6. Real-Time PCR System**

2653 PCR shall be performed using IVD-approved machines. If any equipment(s) is specified in the  
2654 IFU of the index test, it shall be used for the evaluation, and it shall be provided by the  
2655 manufacturer if not available within the lab's IVD evaluation scope.

#### 2656 **7. Internal control/Extraction control**

2657 The test under evaluation should have an internal control or extraction control (RNA added before  
2658 extraction to a sample).

#### 2659 **8. Reference assay:**

2660 Any FDA approved Zika PCR assay or CDC/NIV protocol for detection of Zika RNA should be  
2661 used as the reference assay.

2662 Evaluations with the reference test should be conducted as per the manufacturer’s instructions  
2663 for use.

2664  
2665 Positive and negative samples should be subjected to both the reference test and test under  
2666 evaluation.

2667 **9. Sample size and sample panel composition:** Sample sizes of positive and negative  
2668 samples and panel composition against different values of sensitivity and specificity are provided  
2669 in Tables 1 and 2. Sample sizes have been calculated assuming 95% level of significance, an  
2670 absolute precision of 5%, and invalid test rate  $\leq 5\%$ . Appropriate sample size has to be chosen from  
2671 the tables according to the values of sensitivity and specificity being claimed by the manufacturer.  
2672 If a claimed sensitivity/specificity is not present in the table, the manufacturer needs to consider the  
2673 sample size associated with the largest sensitivity/specificity provided in the table that is smaller to  
2674 the claimed value (that is, as per the next smaller value of the sensitivity/ specificity available in  
2675 the table). For example, if a manufacturer claims a sensitivity of 93%, they are required to use a  
2676 sample size mentioned against 90% sensitivity. Similarly, a claim of 87% specificity would require  
2677 usage of the sample size outlined for 85% specificity.

2678 **Positive Samples:**

- 2679 • Clinical positive samples: Sample tested positive by Zika virus molecular reference assay
- 2680 from clinically suspect cases.
- 2681 • Contrived positive samples: In absence of reference clinical samples, a contrived positive
- 2682 sample may be used.

2683 Contrived positive samples should be prepared using spiking of diluted Zika virus culture isolate  
2684 in unique negative samples, as per the note below:

2685 Table 1. Sample sizes and panel composition of positive Zika virus samples for different values  
2686 of sensitivity claimed by the manufacturer.

| <i>Sensitivity</i> | <i>Calculated sample size</i> | <i>No. of Positive Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i>  |
|--------------------|-------------------------------|---|--|
| 99% <sup>#</sup>   | 16                            | 20  | Strong positive (Ct value <25): 5<br>Moderate positive (Ct value between 25-30): 10<br>Weak positive (Ct value >30 to 34): 5 |
| 95%                | 77                            | 80  | Strong positive (Ct value <25): 20<br>Moderate positive (Ct value between 25-30): 40   |

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|   |     |     |   |
|---|-----|-----|---|
|   |     |     | Weak positive (Ct value >30 to 34): 20  |
| 90%   | 145 | 150 | Strong positive (Ct value <25): 38<br>Moderate positive (Ct value between 25-30): 74<br>Weak positive (Ct value >30 to 34): 38  |
| 85%   | 206 | 210 | Strong positive (Ct value <25): 53<br>Moderate positive (Ct value between 25-30): 104<br>Weak positive (Ct value >30 to 34): 53 |
| 80%   | 258 | 260 | Strong positive (Ct value <25): 65<br>Moderate positive (Ct value between 25-30): 130<br>Weak positive (Ct value >30 to 34): 65 |
| <p>Note 1: Representative positive samples from genotype (African, Asian/American) may be included, if feasible.</p> <p>Note 2: <u>Contrived positive samples</u> – In absence of reference clinical samples, a contrived positive sample may be used.</p> <p>Contrived positive samples should be prepared using spiking of diluted Zika virus culture isolate in unique negative samples, as follows:</p> <p>Tissue culture fluid (3-5 different isolates with a plaque forming unit of <math>10^{5-6}</math>/ml) (Heat inactivated) from reference laboratories can be obtained, spiked in serum samples (15 µl isolate + 150 µl) and can be further diluted in the ratio of 1:10, tested by the reference assay and the positive samples can be used for evaluation.</p> <p>The serum used for spiking isolate should be negative for Dengue virus RNA, and NS1.</p> <p><i>#Higher sample size should be used even for assays claiming 99% sensitivity.</i></p> |     |     |   |

2687

2688 Table 2. Sample sizes and panel composition of negative Zika virus samples for different values  
2689 of specificity claimed by the manufacturer.

| <i>Specificity</i> | <i>Calculated sample size</i> | <i>No. of Negative Samples required [Sample size rounded off]</i> | <i>Sample Panel Composition</i> |
|--------------------|-------------------------------|---|---------------------------------|
|--------------------|-------------------------------|---|---------------------------------|

**Arbovirus IVD Performance Evaluation Protocols**  
ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024

|  |     |     |   |
|--|-----|-----|---|
| 99% <sup>#</sup>   | 16  | 20  | <sup>a</sup> Acute febrile cases: 10<br>Dengue PCR positive: 4<br>Chikungunya PCR positive: 1<br><sup>*</sup> Japanese Encephalitis positive: 1<br><sup>*</sup> West Nile Virus positive: 1<br>Healthy subjects from endemic regions: 3       |
| 95%  | 77  | 80  | <sup>a</sup> Acute febrile cases: 40<br>Dengue PCR positive: 15<br>Chikungunya PCR positive: 5<br><sup>*</sup> Japanese Encephalitis positive: 5<br><sup>*</sup> West Nile Virus positive: 5<br>Healthy subjects from endemic regions: 10     |
| 90%  | 145 | 150 | <sup>a</sup> Acute febrile cases: 76<br>Dengue PCR positive: 28<br>Chikungunya PCR positive: 9<br><sup>*</sup> Japanese Encephalitis positive: 9<br><sup>*</sup> West Nile Virus positive: 9<br>Healthy subjects from endemic regions: 19     |
| 85%  | 206 | 210 | <sup>a</sup> Acute febrile cases: 105<br>Dengue PCR positive: 40<br>Chikungunya PCR positive: 13<br><sup>*</sup> Japanese Encephalitis positive: 13<br><sup>*</sup> West Nile Virus positive: 13<br>Healthy subjects from endemic regions: 26 |
| 80%  | 258 | 260 | <sup>a</sup> Acute febrile cases: 130<br>Dengue PCR positive: 49<br>Chikungunya PCR positive: 16<br><sup>*</sup> Japanese Encephalitis positive: 16<br><sup>*</sup> West Nile Virus positive: 16<br>Healthy subjects from endemic regions: 33 |
| <sup>a</sup> Acute febrile cases negative by Zika virus molecular reference assay<br><sup>*</sup> Positive samples / samples spiked with culture filtrate of Japanese Encephalitis and West Nile Virus<br><br><u>Note:</u><br>If PCR positive samples for cross reactive flaviviruses are not available, commercially available RNA panels/RNA from virus isolates should be used to test cross reactivity.<br><br><i>#Higher sample size should be used even for assays claiming 99% specificity.</i> |     |     |   |

2690

2691 **10. Evaluation method:**

2692 The index test and the reference tests should be run simultaneously on the sample panel to avoid  
 2693 false negative results by index test due to free thawing of samples or deterioration of sample quality  
 2694 on long term storage.

2695 **11. Test reproducibility**

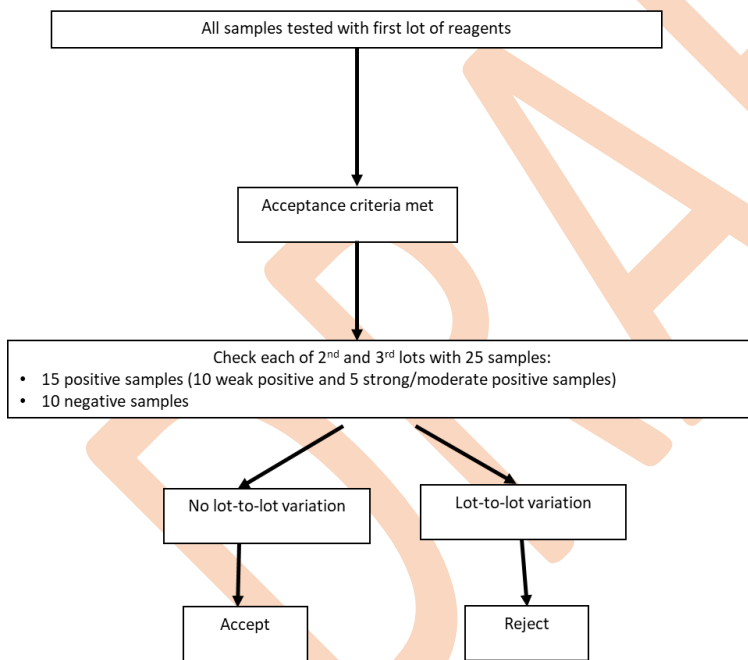
2696 **A. Sample size for lot-to-lot reproducibility**

2697 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be  
2698 as follows:

- 2699 • First lot of the assay: should be tested on statistically significant number of positive  
2700 and negative samples as calculated in the protocol.
- 2701 • Second lot of the assay: should be tested on 25 samples (15 positive samples  
2702 comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative  
2703 samples).
- 2704 • Third lot of the assay: should be tested on 25 samples (15 positive samples comprising  
2705 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).
- 2706

2707 Refer the flowchart below (Fig. 1):

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



2708

2709

2710

2711 **12. Acceptance criteria**

2712 Sensitivity:  $\geq 95\%$

2713 Specificity:  $\geq 98\%$

2714 Cross reactivity with other pathogens: Nil

2715 Invalid test rate:  $\leq 5\%$

2716  
2717 Agreement between sample types– Candidate tests meant for testing multiple sample matrices  
2718 should demonstrate a minimum of 95% positive percent agreement (PPA) and negative percent  
2719 agreement (NPA) for all specimen types.

2720

2721 **14. Publication Rights:**

2722 The PI(s) of the evaluating labs shall retain publication rights of the evaluation as lead author(s).

2723

2724 **After following due procedure as defined in this document, once any kit is found to be Not**  
2725 **of Standard Quality, thereafter, no request for repeat testing of the same kit will be**  
2726 **acceptable. Any request of re-validation from the same manufacturer for the same test type**  
2727 **will only be entertained if valid proof of change in the kit composition is submitted.**

2728

2729

2730 **VI. References:**

- 2731 1. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification –  
2732 Diagnostic Assessment TGS-3. 2017. Available at:  
2733 [https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
2734 [eng.pdf;sequence=1](https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1)  
2735
- 2736 2. Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, Butler AE, Roulo R,  
2737 Bowzard J, Villanueva JM, Muñoz-Jordan JL. Performance of the Trioplex real-time RT-PCR assay  
2738 for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun. 2018 Apr 11;9(1):1391.  
2739 doi: 10.1038/s41467-018-03772-1.
- 2740
- 2741 3. Stone M, Bakkour S, Grebe E, Emperador DM, Escadafal C, Deng X, Dave H, Kelly-Cirino C,  
2742 Lackritz E, Rojas DP, Simmons G, Rabe IB, Busch MP. Standardized evaluation of Zika nucleic acid  
2743 tests used in clinical settings and blood screening. PLoS Negl Trop Dis. 2023 Mar  
2744 17;17(3):e0011157.

2745

2746

2747 **VII. Performance evaluation report format**

2748

2749

2750

2751

2752

2753

2754

2755

2756

2757

**PERFORMANCE EVALUATION REPORT FOR ZIKA REAL-TIME PCR 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 CDSO/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  |  |
| <b>Regulatory Approval:</b><br>Import license / Manufacturing license/ Test license   |  |
| License Number:Issue date:  |  |
| Valid Up to:  |  |
| Application No.   |  |
| <b>Sample Panel</b>   | Positive samples (provide details: clinical/spiked, strong, moderate, weak)          |
|   | Negative samples (provide details clinical/spiked, including cross reactivity panel) |

2758 **Results**

|                                       |          | Reference assay ..... (name) |          |       |
|---------------------------------------|----------|------------------------------|----------|-------|
|                                       |          | Positive                     | Negative | Total |
| <b>Name of Zika real-time PCR kit</b> | Positive |                              |          |       |
|                                       | Negative |                              |          |       |
|                                       | Total    |                              |          |       |

2759

|             | Estimate (%) | 95% CI |
|-------------|--------------|--------|
| Sensitivity |              |        |
| Specificity |              |        |

2760

2761 ● Details of cross reactivity with other flaviviruses:

2762

2763 **FINAL CONCLUSION**

2764 **Performance: Satisfactory / Not satisfactory**



**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

2765 *(Sensitivity and specificity have been assessed in controlled lab setting using kits provided by the manufacturer from*  
2766 *the batch mentioned above using ..... sample. Results should not be extrapolated to other sample types.)*

2767 **Disclaimers**

- 2768 1. This validation process does not approve / disapprove the kit design
- 2769 2. This validation process does not certify user friendliness of the kit / assay

2770 Note: This report is exclusively for ..... Kit (Lot No.....) manufactured by ..... (supplied  
2771 by .....)

2772 Evaluation Done on .....

2773 Evaluation Done by .....

2774 Signature of Director/ Director-In-charge ..... Seal.....

2775 \*\*\*\*\*End of the Report\*\*\*\*\*

2776

2777

2778

2779

2780

2781

2782

2783

2784

2785

2786

2787

2788

2789

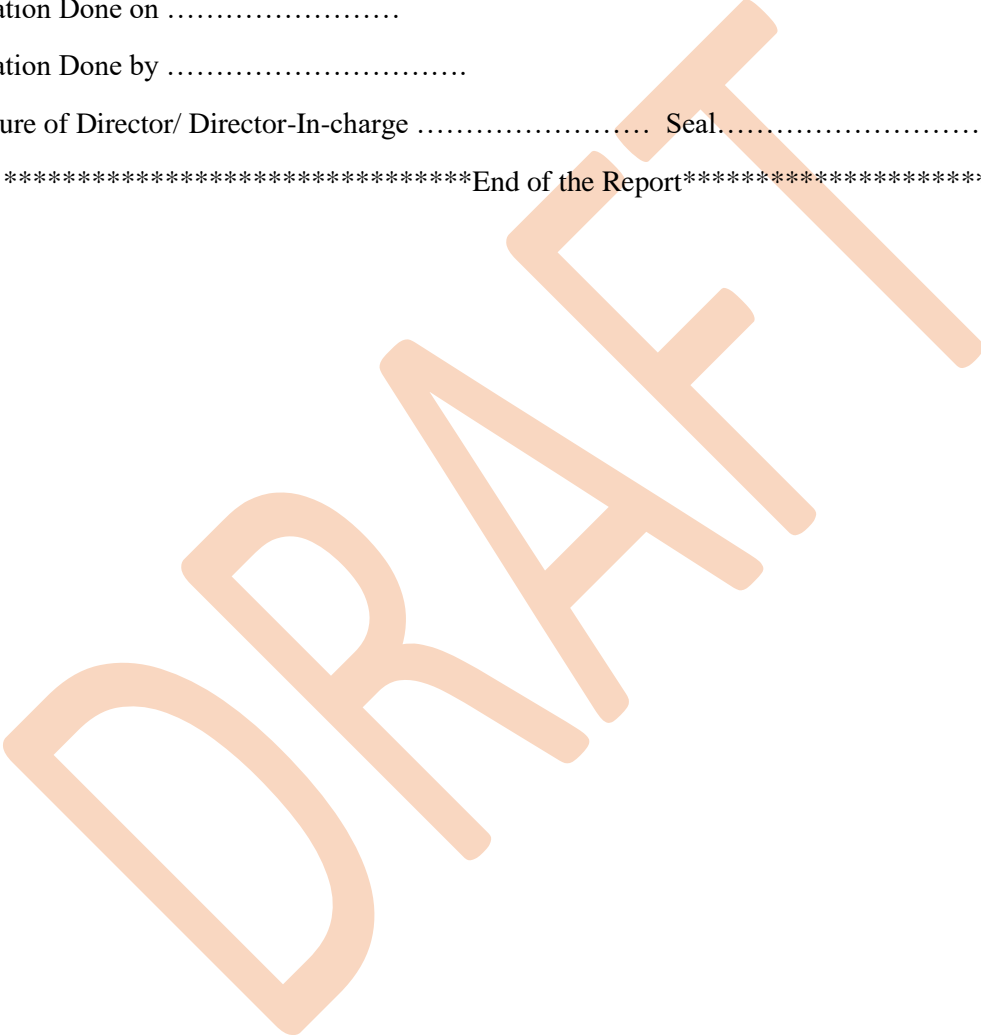
2790

2791

2792

2793

2794



2795 **Information on Operational and Test Performance Characteristics Required from Manufacturers for**  
2796 **Dengue/Chikungunya/ Zika IVD**

2797 The manufacturer should provide the following details about the IVD:

- 2798 1. Instructions for Use
- 2799 2. Scope of the IVD: to diagnose Dengue and/or/Chikungunya and/or Zika virus
- 2800 3. Intended Use Statement
- 2801 4. Principle of the assay
- 2802 5. Intended testing population(cases of acute febrile illness/suspected cases of Dengue and/or
- 2803 Chikungunya and/or Zika virus infection)
- 2804 6. Intended user(laboratory professional and/or health care worker at point-of-care)
- 2805 7. Detailed test protocol
- 2806 8. Lot/batch No.
- 2807 9. Date of manufacture
- 2808 10. Date of Expiry
- 2809 11. Information on operational Characteristics
  - 2810 i. Configuration of the kit/device
  - 2811 ii. Requirement of any additional equipment, device
  - 2812 iii. Requirement of any additional reagents
  - 2813 iv. Operation conditions
  - 2814 v. Storage and stability before and after opening
  - 2815 vi. Internal control provided or not
  - 2816 vii. Quality control and batch testing data
  - 2817 viii. Biosafety aspects- waste disposal requirements
- 2818 10. Information on Test Performance Characteristics
  - 2819 i. Type of sample-serum/plasma/whole blood/other specimen (specify)
  - 2820 ii. Volume of sample
  - 2821 iii. Sample rejection criteria (if any)
  - 2822 iv. Any additional sample processing required
  - 2823 v. Any additional device/consumable like sample transfer device, pipette, tube, etc required

**Arbovirus IVD Performance Evaluation Protocols**  
**ICMR-CDSO/IVD/GD/PROTOCOLS/02/2024**

- 2824 vi. Name of analyte to be detected
- 2825 vii. Pathogens targeted by the kit
- 2826 viii. Time taken for testing
- 2827 ix. Time for result reading and interpretation
- 2828 x. Manual or automated(equipment)reading
- 2829 xi. Limit of detection
- 2830 xii. Diagnostic sensitivity
- 2831 xiii. Diagnostic specificity
- 2832 xiv. Stability and reproducibility (including data)
- 2833 xv. Training required for testing (if any)
- 2834 xvi. If yes, duration
- 2835 xvii. Details of Cut-off and /or Equivocal Zone for interpretation of test
- 2836 xviii. Details of cross reactivity, if any
- 2837 xix. Interpretation of invalid and indeterminate results to be provided
- 2838 xx. It is recommended to provide data demonstrating the precision
- 2839
- 2840 \*Please mention “Not applicable” against sections not pertaining to the kit.
- 2841
- 2842
- 2843 \*\*\*\*\*End of the Document\*\*\*\*\*
- 2844