

ICMR-CDSCO/IVD/GD/PROTOCOLS/01/2024

Indian Council of Medical Research and Central Drugs Standard Control Organization

Department of Health Research and Directorate General of Health Services

Ministry of Health and Family Welfare

Government of India

Document No.: ICMR-CDSCO/IVD/GD/PROTOCOLS/01/2024

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

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

1. Performance evaluation protocol for Chikungunya IgM ELISA
2. Performance evaluation protocol for Chikungunya IgM RDT
3. Performance evaluation protocol for Chikungunya real-time PCR
4. Performance evaluation protocol for Dengue NS1 RDT
5. Field evaluation protocol for Dengue NS1 RDT
6. Performance evaluation protocol for Dengue NS1 ELISA
7. Field evaluation protocol for Dengue NS1 ELISA
8. Performance evaluation protocol for Dengue IgM RDT
9. Performance evaluation protocol for Dengue IgM ELISA
10. Performance evaluation protocol for Dengue NS1/ IgM combo RDT
11. Field evaluation protocol for Dengue NS1/ IgM combo RDT
12. Performance evaluation protocol for Dengue real-time PCR
13. Field evaluation protocol for Dengue real-time PCR
14. Performance evaluation protocol for Zika virus real-time PCR

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

Dated: 31st December 2024

Place: New Delhi

STANDARD IVD PERFORMANCE EVALUATION PROTOCOL
STAKEHOLDER FEEDBACK FORM

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

Name: _____

Designation and Affiliation: _____



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STANDARD PERFORMANCE EVALUATION PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

ARBOVIRUS IN-VITRO DIAGNOSTICS

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

-Dengue virus, Chikungunya virus, Zika virus



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DECEMBER, 2024
New Delhi, India

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Arbovirus IVD Performance Evaluation Protocols

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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.

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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% [#]	15	20	Strong positive: 4 Moderate positive: 8 Weak positive: 8
95%	73	80	Strong positive: 18 Moderate positive: 31 Weak positive: 31
90%	138	140	Strong positive: 30 Moderate positive: 55 Weak positive: 55
85%	196	200	Strong positive: 42 Moderate positive: 79 Weak positive: 79
80%	246	250	Strong positive: 54 Moderate positive: 98 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% [#]	15	20	Rubella IgM positive: 1 Dengue IgM positive: 3 ^a Acute febrile illness cases: 8 ^b Healthy subjects from endemic regions: 8
95%	73	80	Rubella IgM positive: 5 Dengue IgM positive: 15 ^a Acute febrile illness cases: 30

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			^b Healthy subjects from endemic regions: 30
90%	138	140	Rubella IgM positive: 8 Dengue IgM positive: 26 ^a Acute febrile illness cases: 53 ^b Healthy subjects from endemic regions: 53
85%	196	200	Rubella IgM positive: 12 Dengue IgM positive: 38 ^a Acute febrile illness cases: 75 ^b Healthy subjects from endemic regions: 75
80%	246	250	Rubella IgM positive: 15 Dengue IgM positive: 47 ^a Acute febrile illness cases: 94 ^b Healthy subjects from endemic regions: 94
^a Acute febrile illness cases negative for above pathogens AND Chikungunya IgM & PCR			
^b 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>			

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97 **6. Test reproducibility**

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

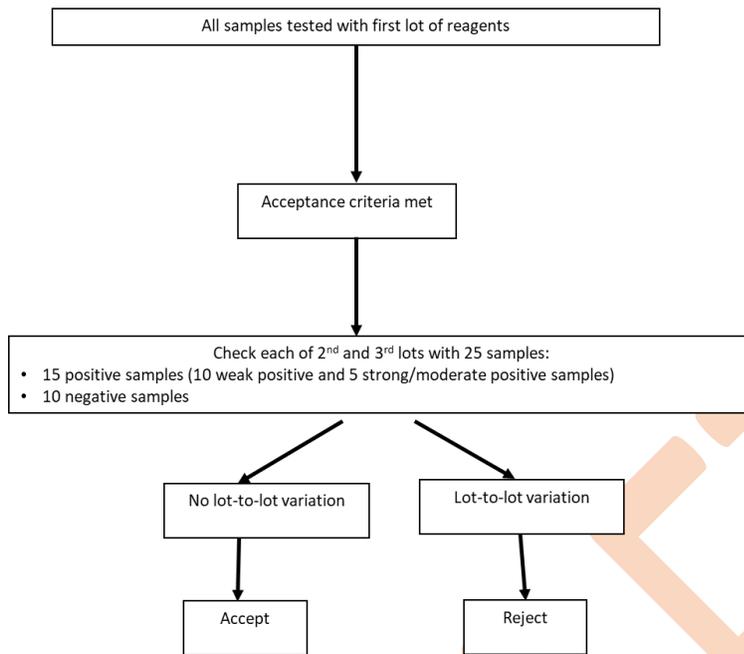
99 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
100 as follows:

- 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).

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109 Refer the flowchart below (Fig.1):

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



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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).

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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.**

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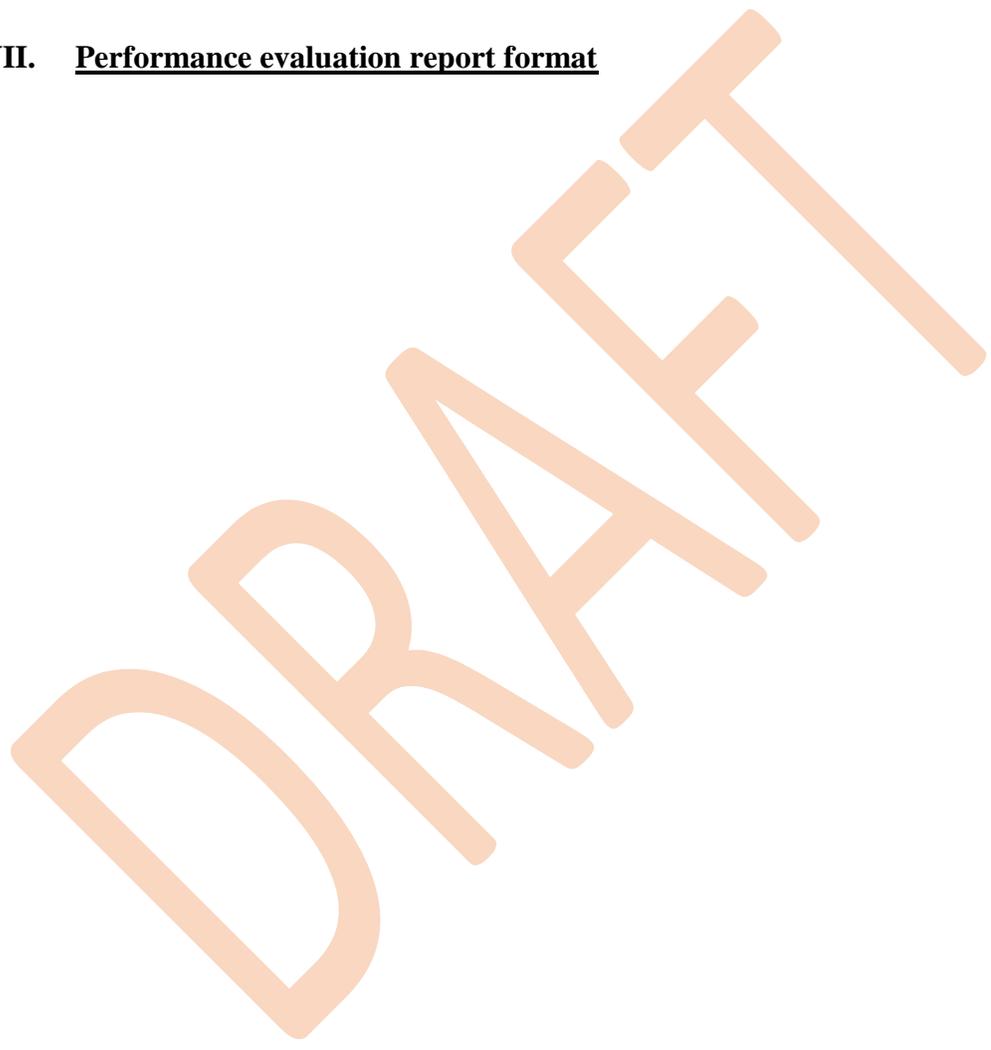
124 **VI. References:**

- 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.

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2. Johnson BW, Goodman CH, Holloway K, de Salazar PM, Valadere AM, Drebot MA. Evaluation of Commercially Available Chikungunya Virus Immunoglobulin M Detection Assays. *Am J Trop Med Hyg.* 2016 Jul 6;95(1):182-192. doi: 10.4269/ajtmh.16-0013. Epub 2016 Mar 14.
 3. 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>

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137 **VII. Performance evaluation report format**

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

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

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162 **Results:**

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

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	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.)*

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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

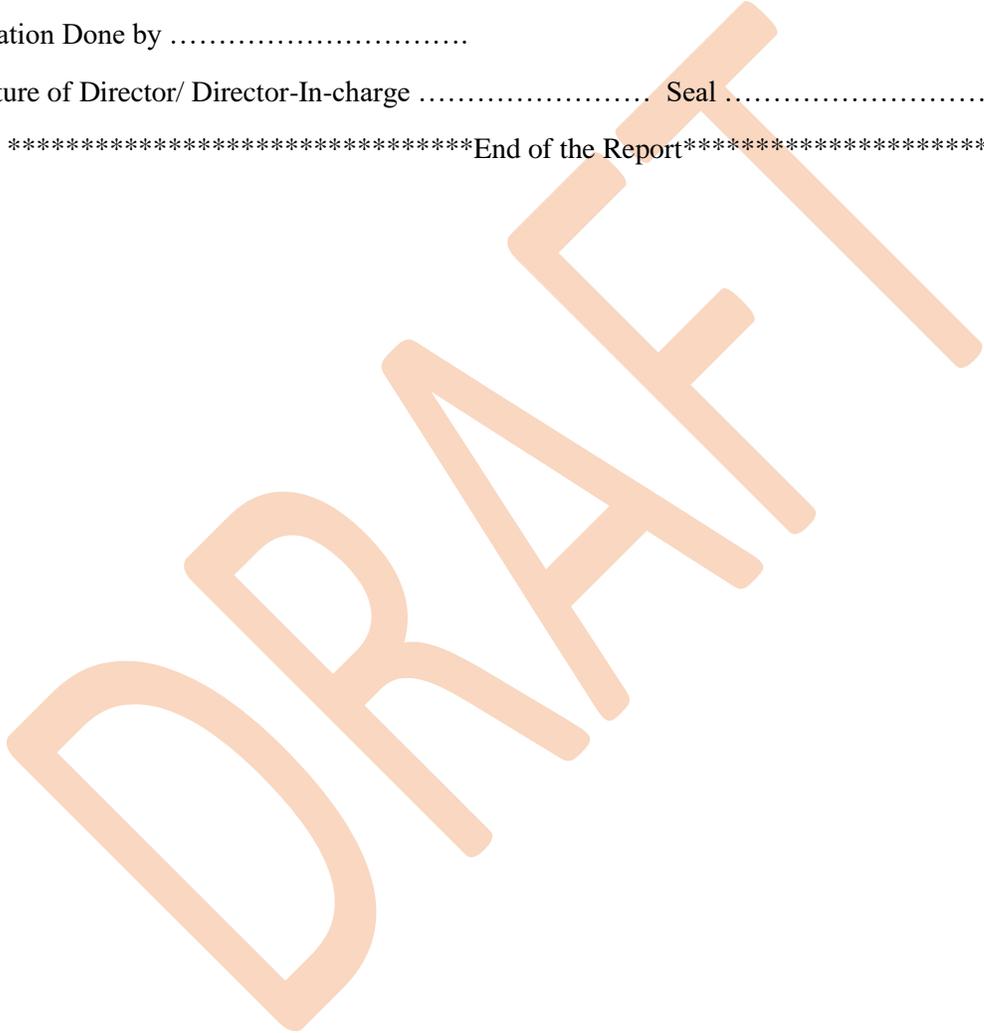
Note: This report is exclusively forKit (Lot No.....) manufactured by
(Supplied by)

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% [#]	16	20	Strong Positive: 6 Moderate Positive: 8 Weak Positive: 6
95%	77	80	Strong Positive: 23 Moderate Positive: 34 Weak Positive: 23
90%	145	150	Strong Positive: 43 Moderate Positive: 64 Weak Positive: 43
85%	206	210	Strong Positive: 61 Moderate Positive: 88 Weak Positive: 61
80%	258	260	Strong Positive: 75 Moderate Positive: 110 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% [#]	16	20	Rubella IgM positive: 1 Dengue IgM positive: 3 ^a Acute febrile illness cases: 12 ^b 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 ^a Acute febrile illness cases: 48 ^b Healthy subjects from endemic regions: 16
90%	145	150	Rubella IgM positive: 5 Dengue IgM positive: 25 ^a Acute febrile illness cases: 90 ^b Healthy subjects from endemic regions: 30
85%	206	210	Rubella IgM positive: 7 Dengue IgM positive: 35 ^a Acute febrile illness cases: 126 ^b Healthy subjects from endemic regions: 42
80%	258	260	Rubella IgM positive: 9 Dengue IgM positive: 43 ^a Acute febrile illness cases: 156 ^b Healthy subjects from endemic regions: 52
^a Acute febrile illness cases negative for above pathogens AND Chikungunya IgM & PCR ^b 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>			

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6. Test reproducibility

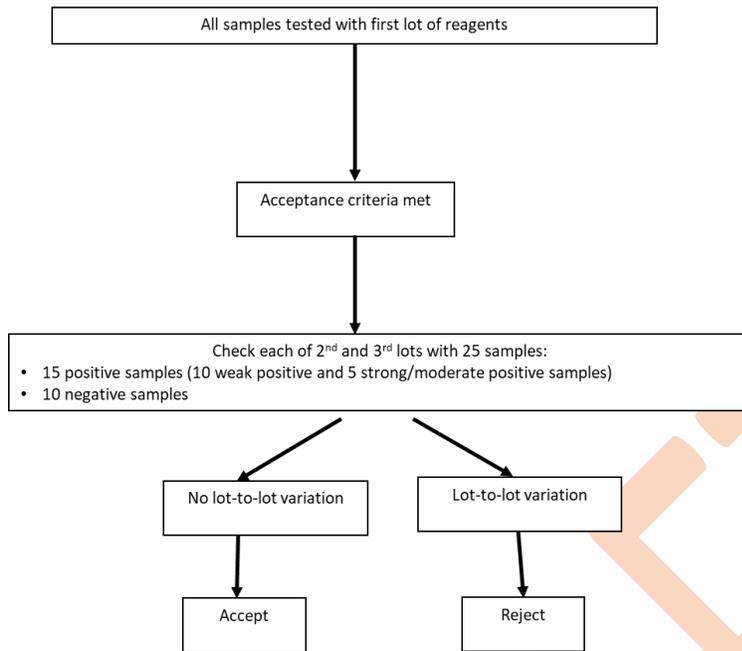
A. Sample size for lot-to-lot reproducibility

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



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B. Sample size for reader-to-reader reproducibility

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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).

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

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7. Acceptance criteria

300

Expected sensitivity: $\geq 80\%$

301

Expected specificity: $\geq 90\%$

302

Invalid test rate: $\leq 5\%$

303

8. Publication Rights:

304

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

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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)
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320 **VII. Performance evaluation report format**

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

341 **Results:**

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

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.)*

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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

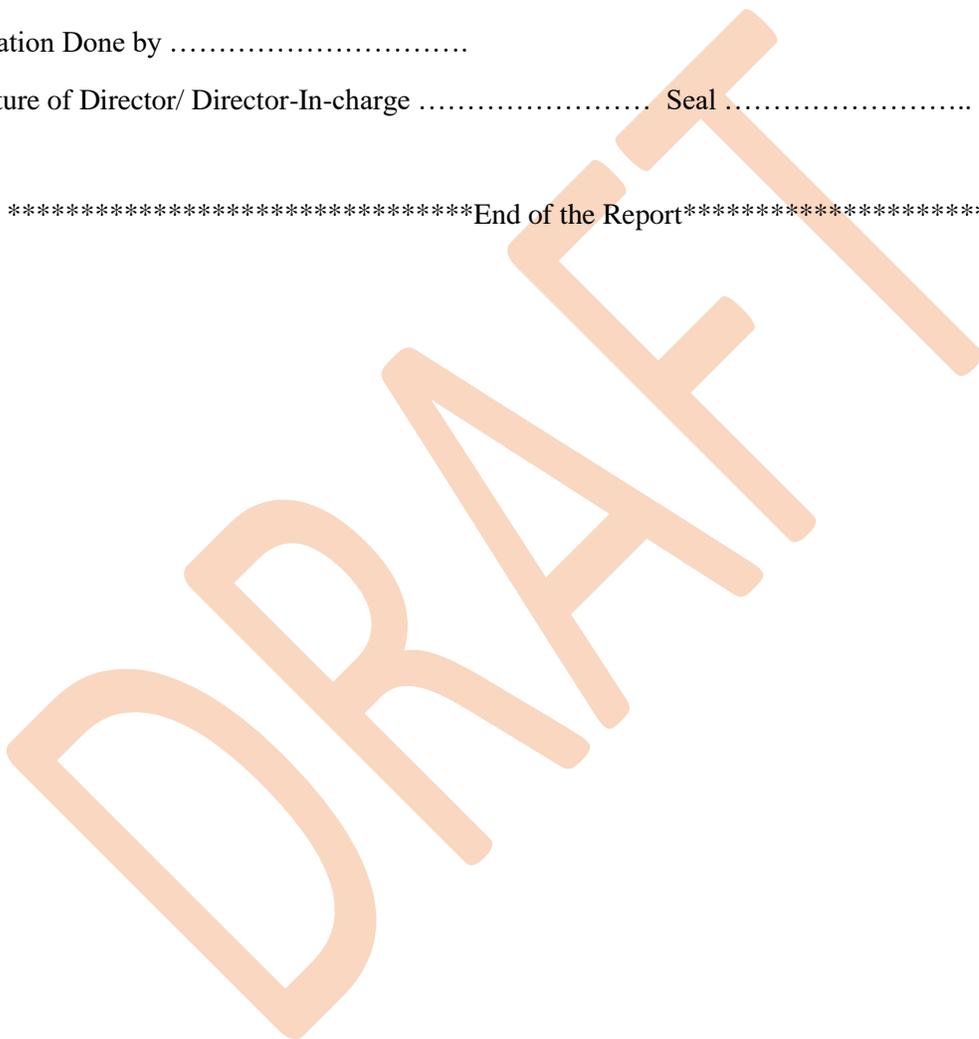
Note: This report is exclusively forKit (Lot No.....) manufactured by
(Supplied by)

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

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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% [#]	16	20	Strong Positive: 5 Moderate Positive: 10 Weak Positive: 5
95%	77	80	Strong Positive: 20 Moderate Positive: 40 Weak Positive: 20
90%	145	150	Strong Positive: 38 Moderate Positive: 74 Weak Positive: 38
85%	206	210	Strong Positive: 53 Moderate Positive: 104 Weak Positive: 53

453 [#]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% [#]	16	20	Rubella IgM positive: 1 Dengue IgM positive: 4 ^a Acute febrile illness cases: 10 ^b Healthy subjects from endemic regions: 5
95%	77	80	Rubella IgM positive: 5

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			Dengue IgM positive: 15 ^a Acute febrile illness cases: 40 ^b Healthy subjects from endemic regions: 20
90%	145	150	Rubella IgM positive: 9 Dengue IgM positive: 28 ^a Acute febrile illness cases: 75 ^b Healthy subjects from endemic regions: 38
85%	206	210	Rubella IgM positive: 13 Dengue IgM positive: 39 ^a Acute febrile illness cases: 105 ^b Healthy subjects from endemic regions: 53
^a Acute febrile illness cases negative for above pathogens AND Chikungunya IgM & PCR ^b 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**

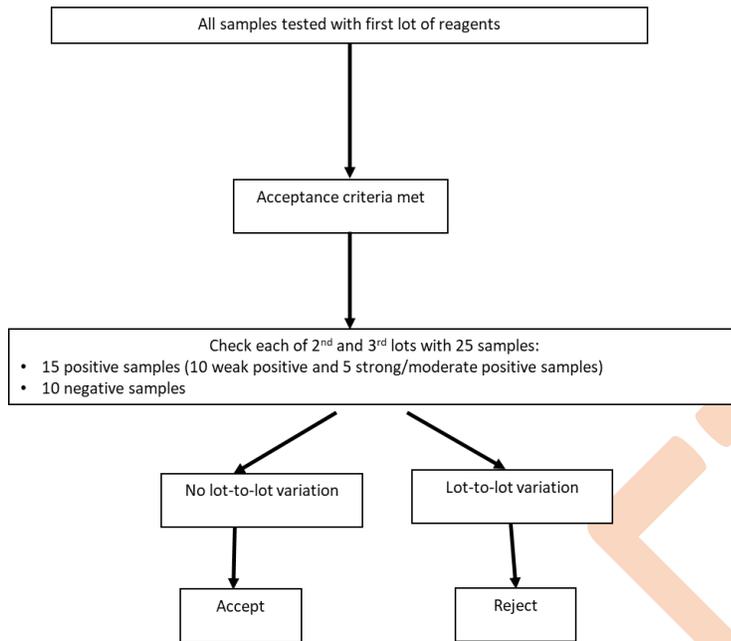
466 **A. Sample size for lot-to-lot reproducibility**

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).
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- 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)

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VII. Performance evaluation report format

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Arbovirus IVD Performance Evaluation Protocols
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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 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		
Regulatory Approval: Import license / Manufacturing license/ Test license		
License Number:Issue date:		
Valid Up to:		
Application No.		
Sample Panel	Positive samples (provide details: clinical/spiked, strong, moderate, weak)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel)	

528
529 **Results**

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

530

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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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*****

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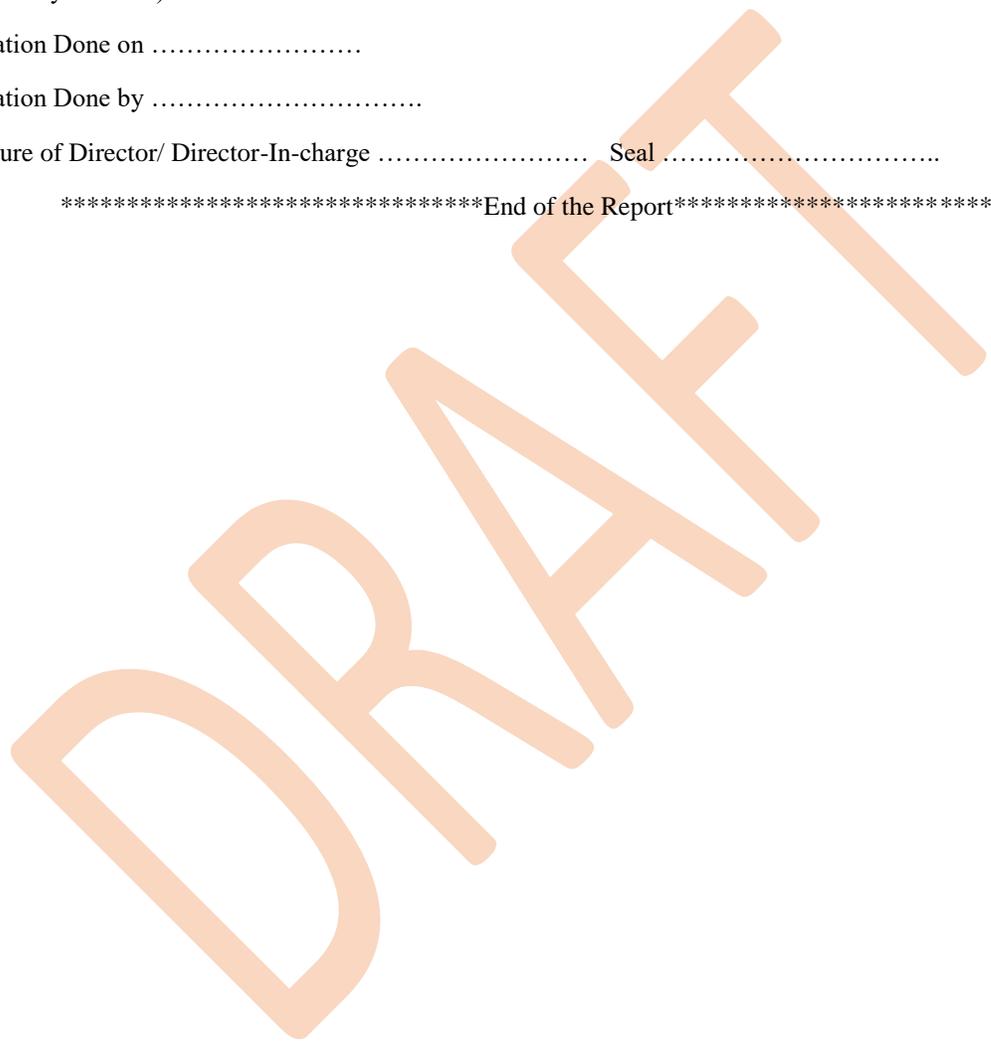
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566 **Performance evaluation protocol for Dengue NS1 RDT kits**

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>
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99% [#]	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% [#]	16	20	<p><u>-PCR/RT-PCR positive samples from other acute febrile illness cases</u> Chikungunya: 4 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 8</p> <p><u>-Samples from other flavivirus disease cases</u> *Japanese Encephalitis PCR/antigen positive: 1 *West Nile Virus PCR/antigen positive: 1 *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> Chikungunya: 15 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 30</p> <p><u>-Samples from other flavivirus disease cases</u> *Japanese Encephalitis PCR/antigen positive: 5 *West Nile Virus PCR/antigen positive: 5 *Zika Virus PCR/antigen positive: 5</p>

Arbovirus IVD Performance Evaluation Protocols
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			<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> Chikungunya: 28 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 57</p> <p><u>-Samples from other flavivirus disease cases</u> *Japanese Encephalitis PCR/antigen positive: 9 *West Nile Virus PCR/antigen positive: 9 *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> Chikungunya: 39 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 79</p> <p><u>-Samples from other flavivirus disease cases</u> *Japanese Encephalitis PCR/antigen positive: 13 *West Nile Virus PCR/antigen positive 13 *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> Chikungunya: 49 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 98</p> <p><u>-Samples from other flavivirus disease cases</u> *Japanese Encephalitis PCR/antigen positive: 16</p>

			<p>*West Nile Virus PCR/antigen positive: 16 *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). 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. 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>			

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6. Test reproducibility

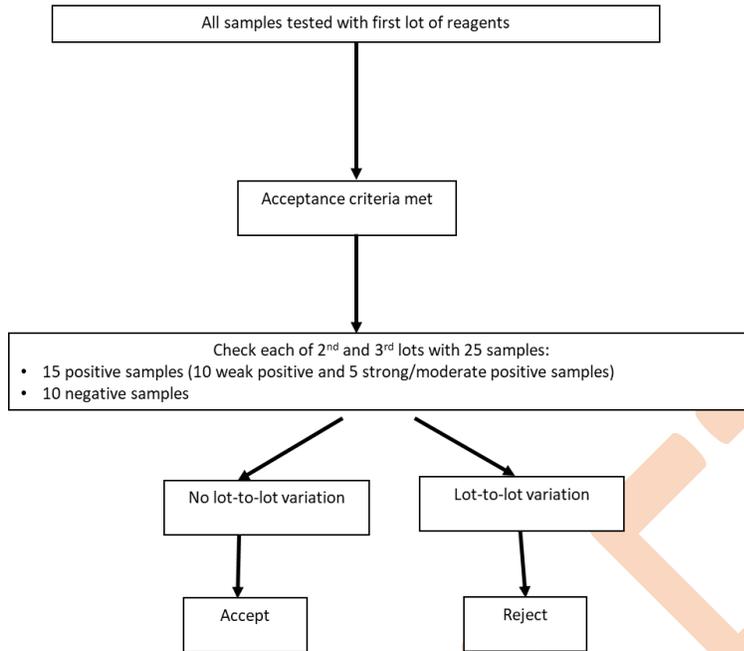
A. Sample size for lot-to-lot reproducibility

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



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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).

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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,
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678 sensitivity and specificity for the diagnosis of acute Dengue virus infection. *PLoS Negl*
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- 680 3. Yow KS, Aik J, Tan EY, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent
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684 Tests for Early Detection of Dengue in Clinical Samples. *J Trop Med*. 2017; 2017:
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688 Diagnostic Assessment TGS-3. 2017. Available at:
689 [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)
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695 Jun;58(2):159-164. doi: 10.4103/0972-9062.321747. PMID: 35074951.

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697 **VII. Performance evaluation report format**

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

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708

Results:

		Reference assay (name)		
		Positive	Negative	Total
Name of Dengue NS1 - based RDT kit	Positive			
	Negative			
	Total			

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	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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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*****

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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**

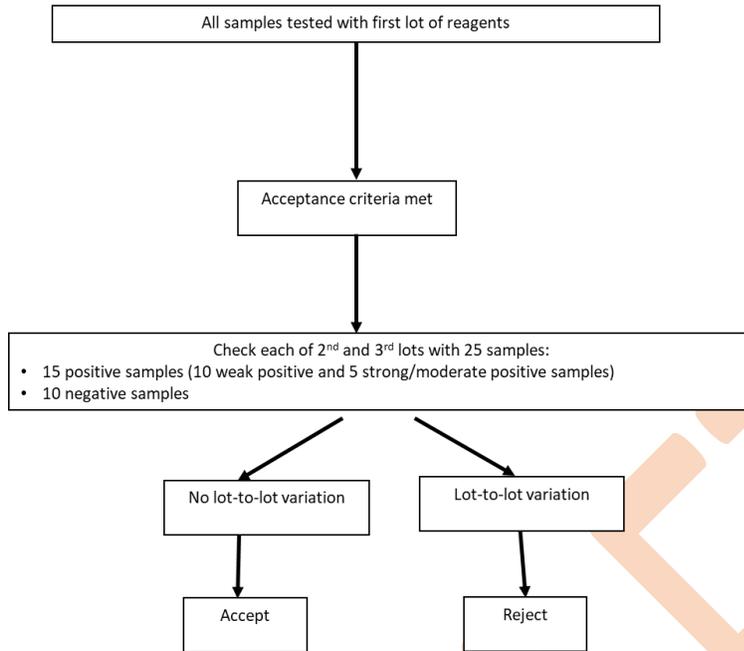
847 **A. Sample size for lot-to-lot reproducibility**

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.**

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878 **VI. References:**

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900 35074951.

901 **VII. Performance evaluation report format**

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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		
Regulatory Approval:		
Import license / Manufacturing license/ Test license		
License Number:Issue date:		
Valid Up to:		
Application No.		
Sample Panel	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:

		Reference assay (name)		
		Positive	Negative	Total
Name of Dengue NS1 - based RDT kit	Positive			
	Negative			
	Total			

918

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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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*****

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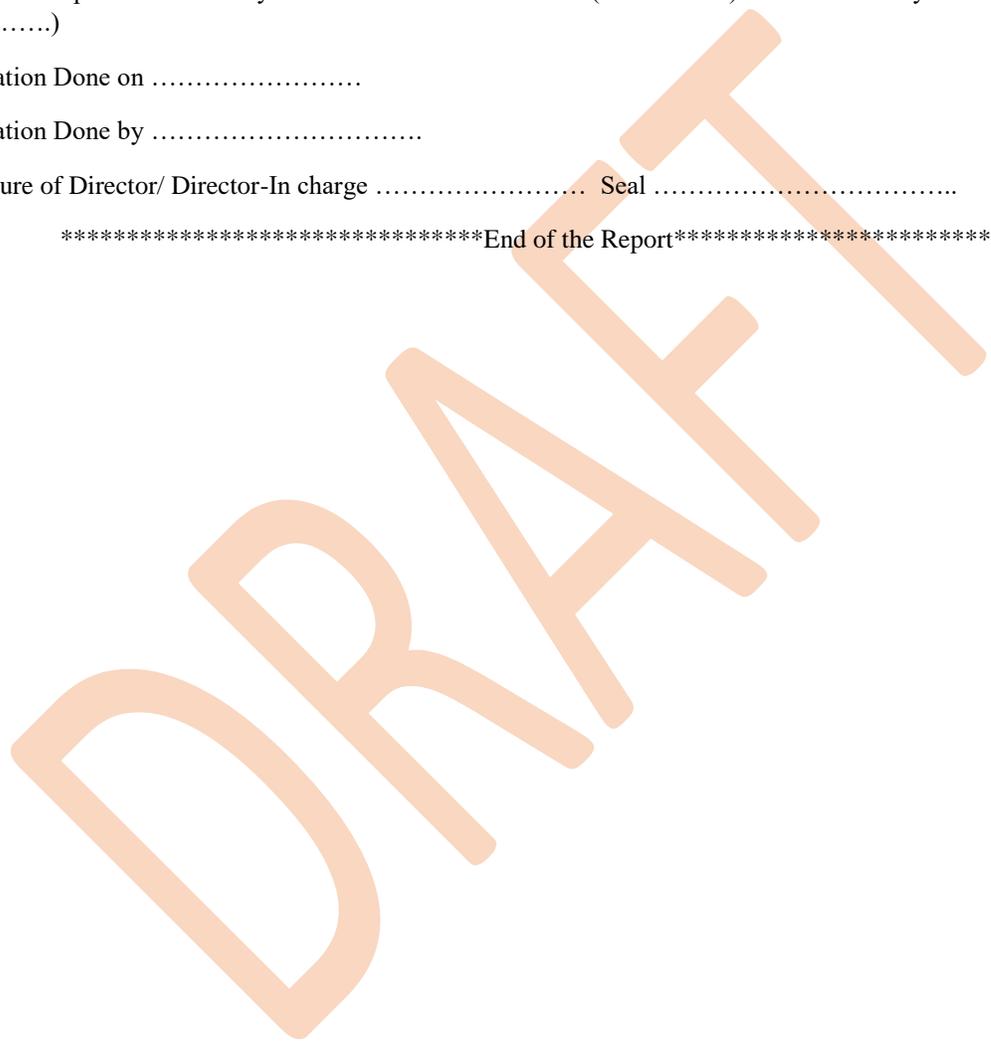
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956 **Performance evaluation protocol for Dengue NS1 ELISA kits**

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>
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		<i>[Sample size rounded off]</i>	
99% [#]	15	20	Strong Positive: 4 Moderate Positive: 8 Weak Positive: 8
95%	73	80	Strong Positive: 18 Moderate Positive: 31 Weak Positive: 31
90%	138	140	Strong Positive: 30 Moderate Positive: 55 Weak Positive: 55
85%	196	200	Strong Positive: 42 Moderate Positive: 79 Weak Positive: 79
80%	246	250	Strong Positive: 54 Moderate Positive: 98 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% [#]	15	20	Chikungunya positive: 4 ^a Acute febrile cases negative for Dengue: 8 *Japanese Encephalitis PCR/antigen positive: 1 *West Nile Virus PCR/antigen positive: 1 *Zika Virus PCR/antigen positive: 1 ^b Healthy subjects from endemic regions: 5
95%	73	80	Chikungunya positive: 15 ^a Acute febrile cases negative for Dengue: 30 *Japanese Encephalitis PCR/antigen positive: 5 *West Nile Virus PCR/antigen positive: 5 *Zika Virus PCR/antigen positive: 5 ^b Healthy subjects from endemic regions: 20
90%	138	140	Chikungunya positive: 26 ^a Acute febrile cases negative for Dengue: 52 *Japanese Encephalitis PCR/antigen positive: 9

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			*West Nile Virus PCR/antigen positive: 9 *Zika Virus PCR/antigen positive: 9 ^b Healthy subjects from endemic regions: 35
85%	196	200	Chikungunya positive: 37 ^a Acute febrile cases negative for Dengue: 74 *Japanese Encephalitis PCR/antigen positive: 13 *West Nile Virus PCR/antigen positive: 13 *Zika Virus PCR/antigen positive: 13 ^b Healthy subjects from endemic regions: 50
80%	246	250	Chikungunya positive: 46 ^a Acute febrile cases negative for Dengue: 94 *Japanese Encephalitis PCR/antigen positive: 16 *West Nile Virus PCR/antigen positive: 16 *Zika Virus PCR/antigen positive: 16 ^b Healthy subjects from endemic regions: 62
^a Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR) ^b Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid) <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**

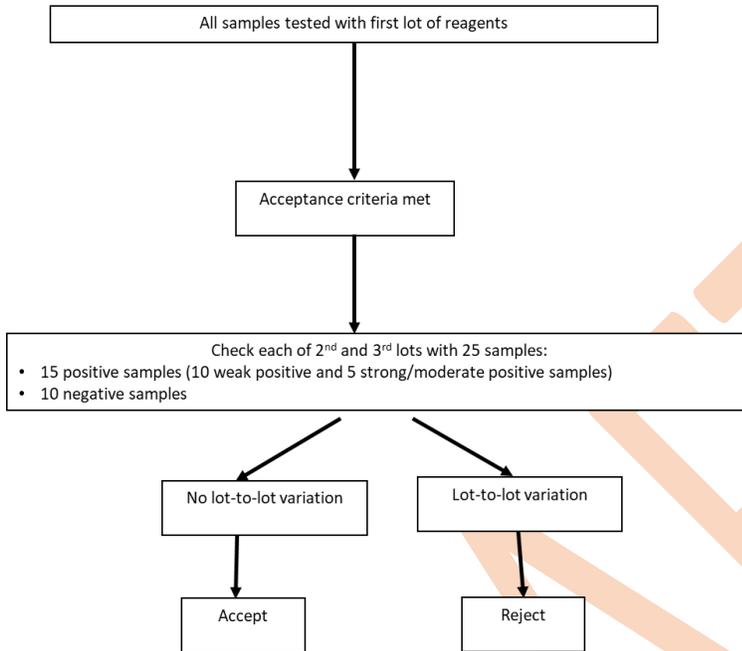
1037 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
1038 as follows:

- 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



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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.**

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

1102
1103 **Results**

		Reference assay (name)		
		Positive	Negative	Total
Name of Dengue NS1 - based ELISA kit	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**

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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*****

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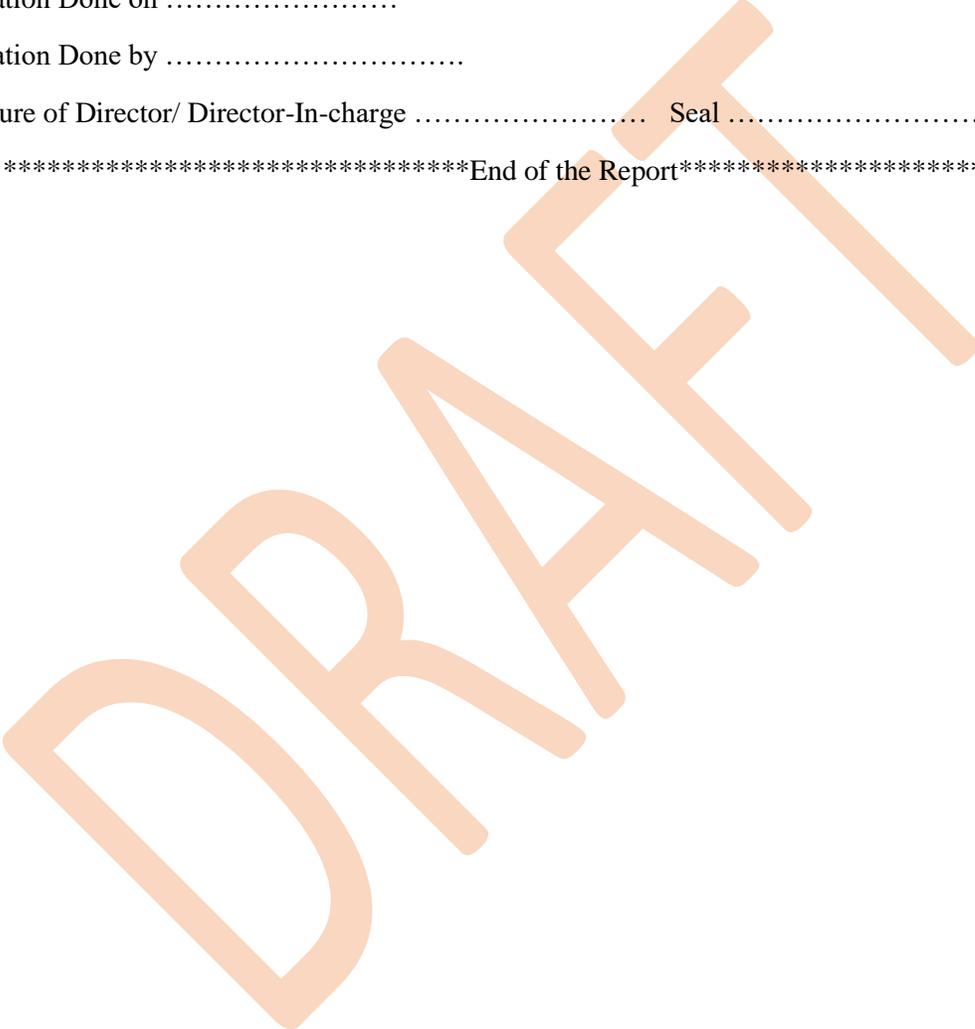
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1140 **Field evaluation protocol for Dengue NS1 ELISA kits**

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**

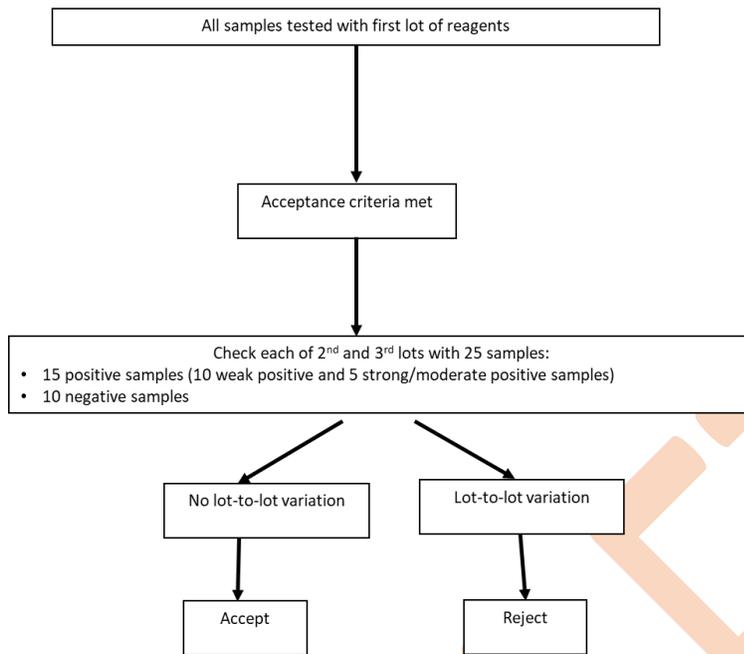
1244 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
1245 as follows:

- 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



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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, Olliaro 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,
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1288 10.1002/jmv.21814.
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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)
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1295 VII. Performance evaluation report format

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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		
Regulatory Approval: Import license / Manufacturing license/ Test license		
License Number:Issue date:		
Valid Up to:		
Application No.		
Sample Panel	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
Name of Dengue NS1 based ELISA kit	Positive			
	Negative			
	Total			

1313

	Estimate (%)	95% CI
Sensitivity		
Specificity		

1314

1315

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1318

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

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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*****

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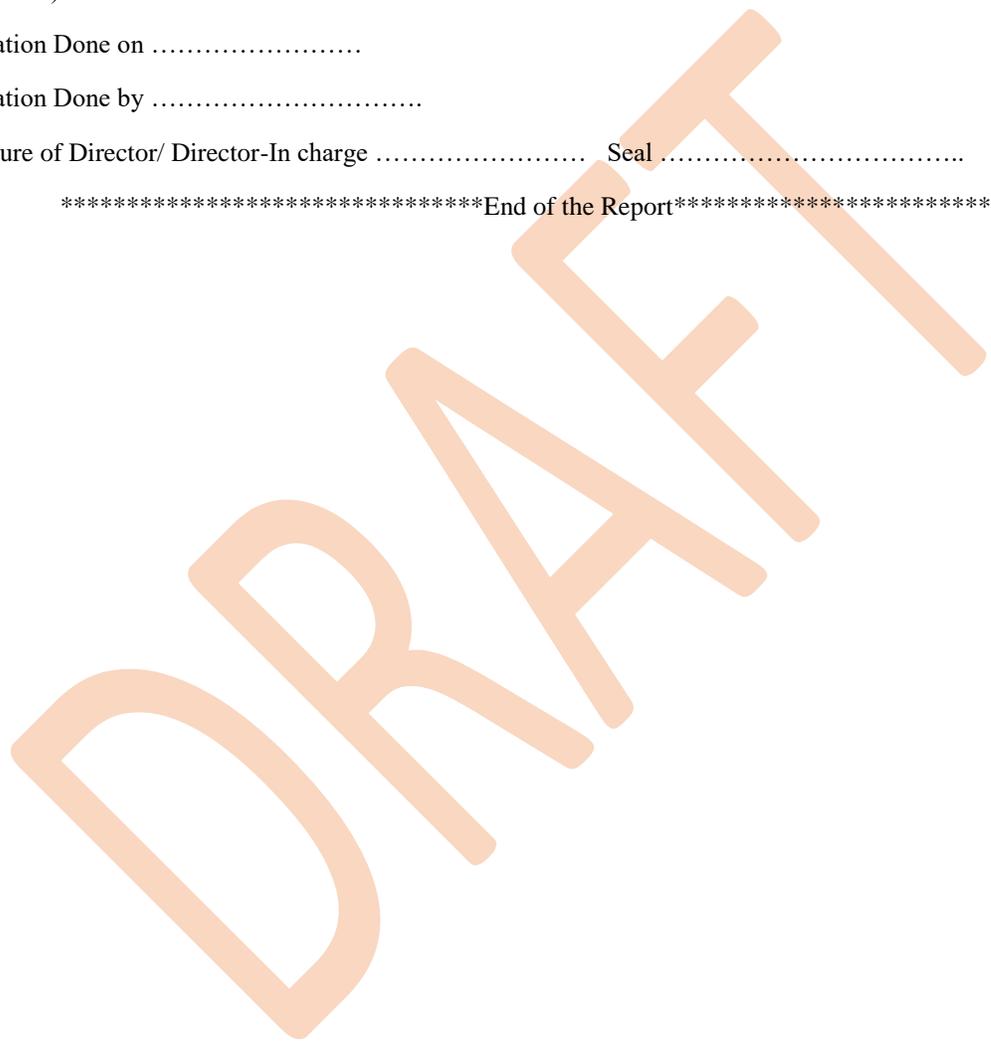
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1350 **Performance evaluation protocol for Dengue IgM RDT kits**

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.

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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% [#]	16	20	Strong Positive: 6 Moderate Positive: 8 Weak Positive: 6
95%	77	80	Strong Positive: 23 Moderate Positive: 34 Weak Positive: 23
90%	145	150	Strong Positive: 43 Moderate Positive: 64 Weak Positive: 43
85%	206	210	Strong Positive: 61 Moderate Positive: 88 Weak Positive: 61
80%	258	260	Strong Positive: 75 Moderate Positive: 110 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% [#]	16	20	Chikungunya positive: 4 ^a Acute febrile cases: 5 *Japanese Encephalitis IgM positive: 1 *West Nile Virus IgM positive: 1 *Zika Virus IgM positive: 1

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

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**

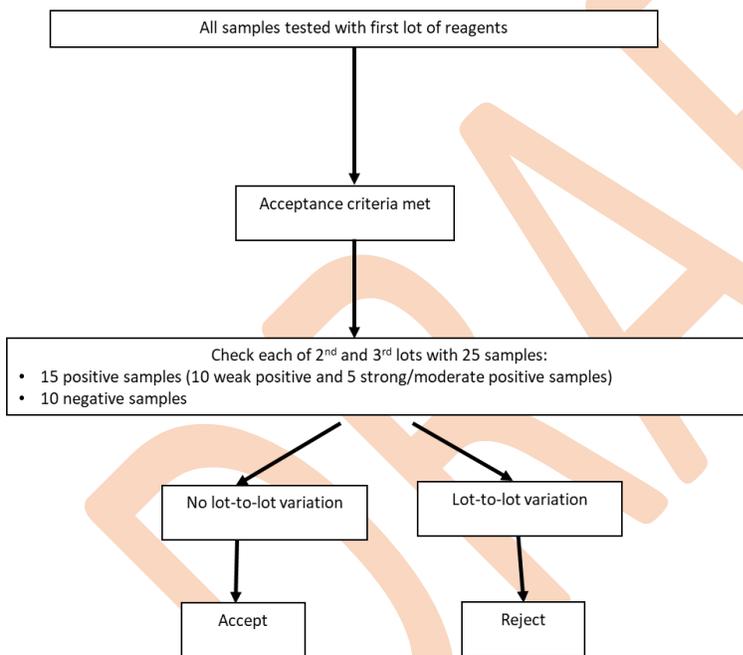
1437 **A. Sample size for lot-to-lot reproducibility**

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
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1483 [https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical
device/guidanceperformanceivd.pdf](https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical
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/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-
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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-
1492 emitting-products/Dengue-virus-serological-reagents-class-ii-special-controls-guideline-industry-
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-
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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**

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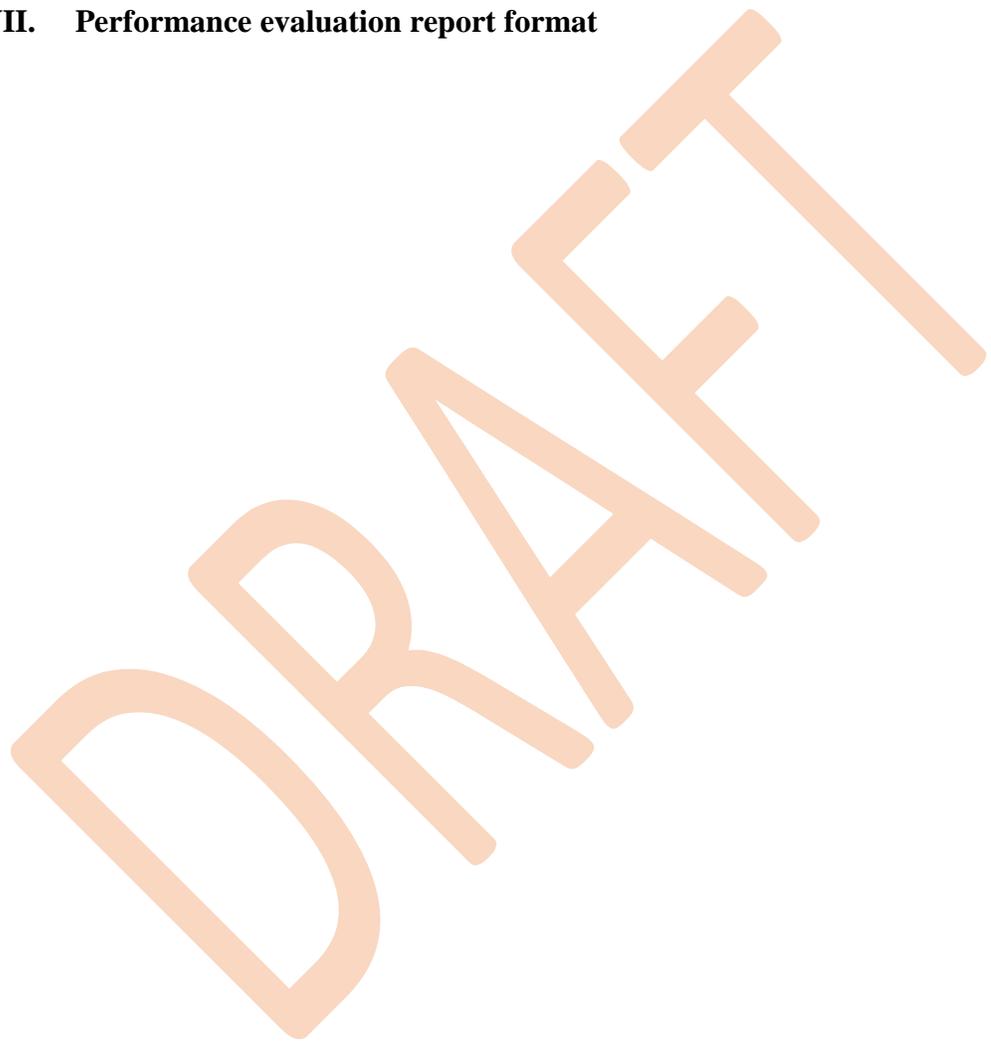
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PERFORMANCE EVALUATION REPORT FOR DENGUE IgM RDT KIT

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

1529

1530 **Results:**

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

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**

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- 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 forKit (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*****

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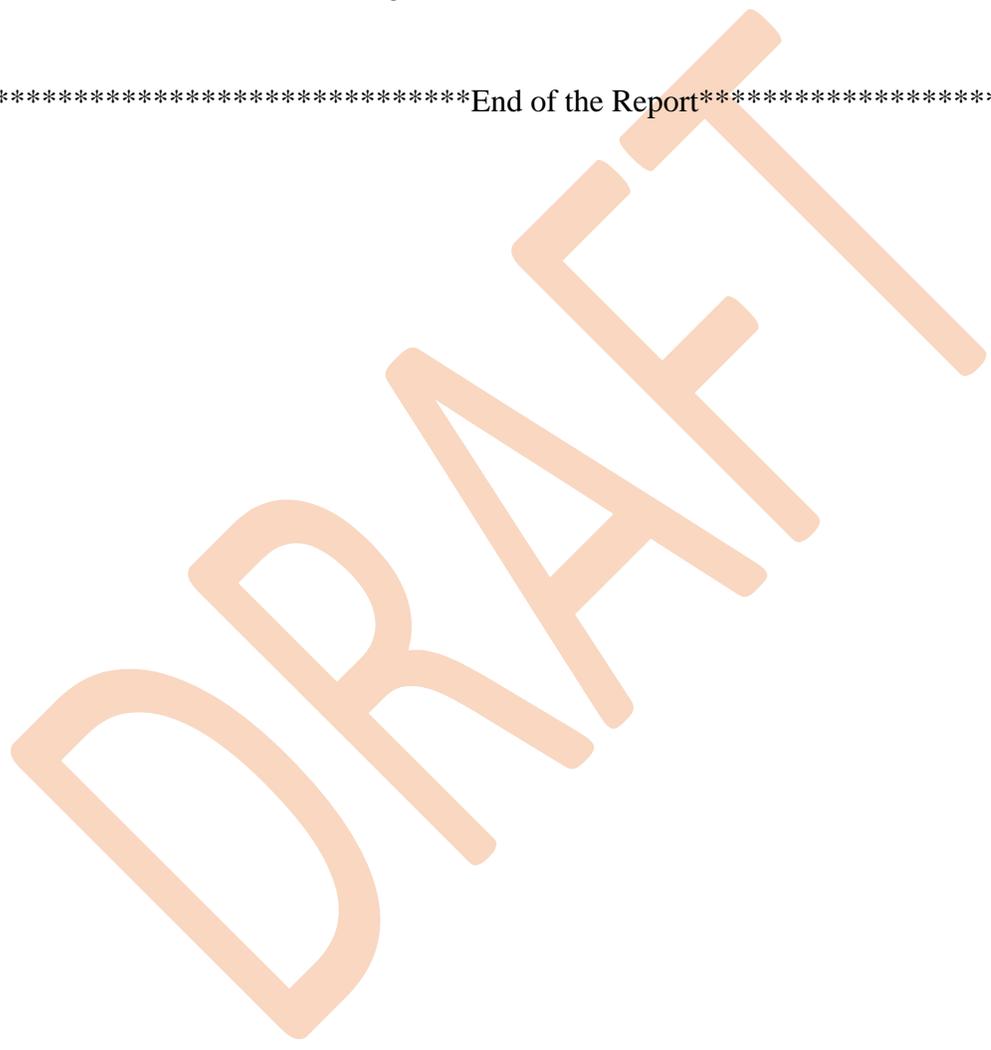
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1567 **Performance evaluation protocol for Dengue IgM ELISA kits**

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

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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% [#]	15	20	Strong Positive: 4 Moderate Positive: 8 Weak Positive: 8
95%	73	80	Strong Positive: 18 Moderate Positive: 31 Weak Positive: 31
90%	138	140	Strong Positive: 30 Moderate Positive: 55 Weak Positive: 55
85%	196	200	Strong Positive: 42 Moderate Positive: 79 Weak Positive: 79
80%	246	250	Strong Positive: 54 Moderate Positive: 98 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% [#]	15	20	Chikungunya positive: 3 ^a Acute febrile cases: 6 *Japanese Encephalitis IgM positive: 1 *West Nile Virus IgM positive: 1 *Zika Virus IgM positive: 1

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

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**

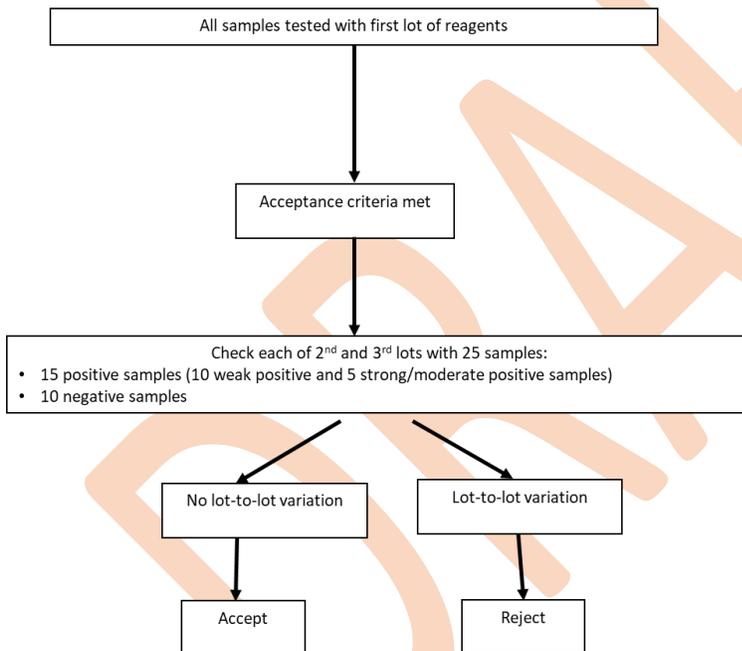
1652 **A. Sample size for lot-to-lot reproducibility**

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



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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:

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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.

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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. PLoSNegl Trop Dis. 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.

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***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

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

1714

1715 Results:

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

1716

	Estimate (%)	95% CI
Sensitivity		
Specificity		

1717 Conclusions:

1718 o Sensitivity, specificity

1719 o 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

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1725 Note: This report is exclusively forKit (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*****

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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.

1818

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

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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% [#]	16	28	*NS1 positive and IgM negative: 8 *NS1 and IgM positive: 12 *NS1 negative and IgM positive: 8
95%	77	84	*NS1 positive and IgM negative: 24 *NS1 and IgM positive: 36 *NS1 negative and IgM positive: 24
90%	145	160	*NS1 positive and IgM negative: 44 *NS1 and IgM positive: 72 *NS1 negative and IgM positive: 44
85%	206	220	*NS1 positive and IgM negative: 60 *NS1 and IgM positive: 100 *NS1 negative and IgM positive: 60
80%	258	260	*NS1 positive and IgM negative: 72 *NS1 and IgM positive: 116 *NS1 negative and IgM positive: 72
<p>* all 4 serotypes shall be represented</p> <p>Note: 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. 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.

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<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% [#]	16	28	Chikungunya positive: 1 ^a Acute febrile cases: 11 *Japanese Encephalitis IgM positive: 1 *West Nile Virus IgM positive: 1 *Zika Virus IgM positive: 1 **Japanese Encephalitis NS1 positive: 1 **West Nile Virus NS1 positive: 1 **Zika Virus NS1 positive: 1 Rheumatoid Arthritis/other autoimmune disease cases: 5 ^b Healthy subjects from endemic regions: 5
95%	77	84	Chikungunya positive: 3 ^a Acute febrile cases: 33 *Japanese Encephalitis IgM positive: 3 *West Nile Virus IgM positive: 3 *Zika Virus IgM positive: 3 **Japanese Encephalitis NS1 positive: 3 **West Nile Virus NS1 positive: 3 **Zika Virus NS1 positive: 3 Rheumatoid Arthritis/other autoimmune disease cases: 15 ^b Healthy subjects from endemic regions: 15
90%	145	160	Chikungunya positive: 5 ^a Acute febrile cases: 65 *Japanese Encephalitis IgM positive: 5 *West Nile Virus IgM positive: 5 *Zika Virus IgM positive: 5 **Japanese Encephalitis NS1 positive: 5 **West Nile Virus NS1 positive: 5 **Zika Virus NS1 positive: 5 Rheumatoid Arthritis/other autoimmune disease cases: 30 ^b Healthy subjects from endemic regions: 30
85%	206	220	Chikungunya positive: 7 ^a Acute febrile cases: 89 *Japanese Encephalitis IgM positive: 7 *West Nile Virus IgM positive: 7 *Zika Virus IgM positive: 7

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			**Japanese Encephalitis NS1 positive: 7 **West Nile Virus NS1 positive: 7 **Zika Virus NS1 positive: 7 Rheumatoid Arthritis/other autoimmune disease cases: 41 ^b Healthy subjects from endemic regions: 41
80%	258	260	Chikungunya positive: 8 ^a Acute febrile cases: 106 *Japanese Encephalitis IgM positive: 8 *West Nile Virus IgM positive: 8 *Zika Virus IgM positive: 8 **Japanese Encephalitis NS1 positive: 8 **West Nile Virus NS1 positive: 8 **Zika Virus NS1 positive: 8 Rheumatoid Arthritis/other autoimmune disease cases: 49 ^b Healthy subjects from endemic regions: 49
^a Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR) ^b Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, RNA) *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. **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. #Higher sample size should be used even for assays claiming 99% specificity.			

1827

1828 **5. Test reproducibility**

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

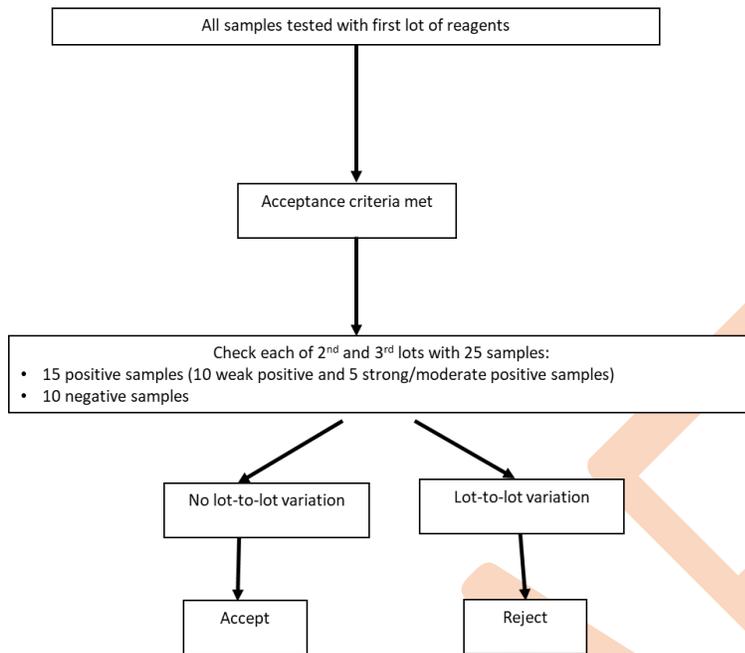
1830 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
 1831 as follows:

- 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

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+	-	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:**

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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**

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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		
Regulatory Approval: Import license / Manufacturing license/ Test license		
License Number:Issue date:		
Valid Up to:		
Application No.		
Sample Panel	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
Name of Dengue NS1 and IgM combo RDT kit	Positive			
	Negative			
	Total			

1921

	Estimate (%)	95% CI
Combined Sensitivity		
Combined Specificity		

1922

1923

- Details of cross reactivity with other flavivirus NS1 antigens:

1924

- **Conclusions:**

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- 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*****

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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

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 from 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
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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**

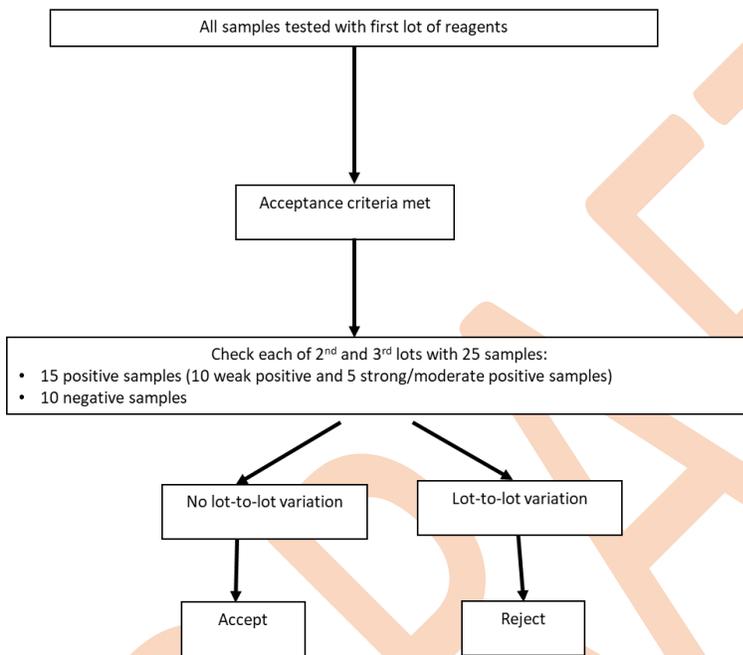
2076 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
2077 as follows:

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

- 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



2087

B. Sample size for reader-to-reader reproducibility

For reader-to-reader reproducibility, sample size should be 25 (15 positive samples comprising 10 low positive AND 5 moderate/high positive samples with adequate representation of NS1 and IgM, and 10 negative samples).

2092

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

C. Interpretation of results

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

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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 ≥90% each.

2104 Cross reactivity with other flavivirus antigens: Nil

2105 Invalid test rate: ≤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.
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2132 10.1371/journal.pntd.0006618.
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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.

2141 VII. Performance evaluation report format

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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	
Regulatory Approval: Import license / Manufacturing license/ Test license	
License Number:Issue date:	
Valid Up to:	
Application No.	
Sample Panel	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**

		Reference assay (name)		
		Positive	Negative	Total
Name of NS1 and IgM combo RDT kit	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

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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 forNS1 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*****

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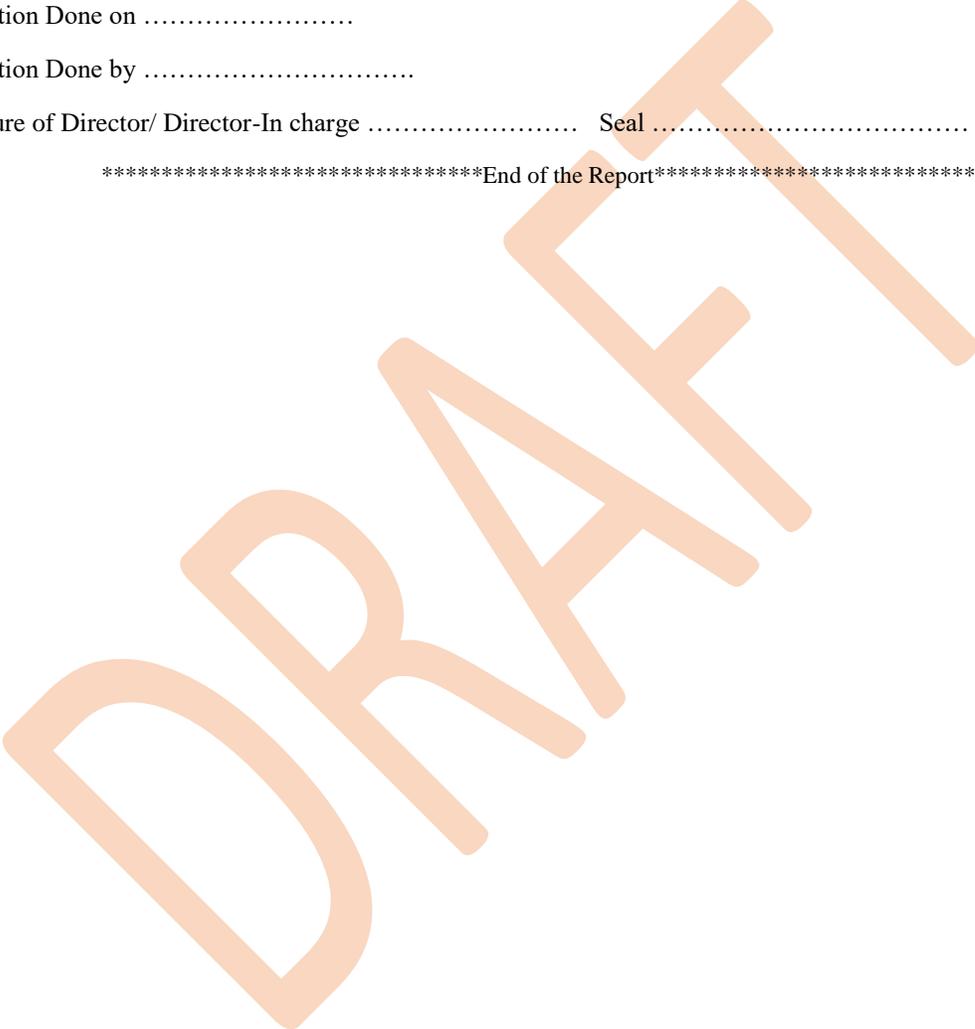
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2197 **Performance evaluation protocol for Dengue real-time PCR kit**

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% [#]	16	20	Strong positive (Ct value <25): 5 Moderate positive (Ct value between 25-30): 10 Weak positive (Ct value >30 to 34): 5
95%	77	80	Strong positive (Ct value <25): 20 Moderate positive (Ct value between 25-30): 40 Weak positive (Ct value >30 to 34): 20
90%	145	150	Strong positive (Ct value <25): 38 Moderate positive (Ct value between 25-30): 74 Weak positive (Ct value >30 to 34): 38
85%	206	210	Strong positive (Ct value <25): 53

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			Moderate positive (Ct value between 25-30): 104 Weak positive (Ct value >30 to 34): 53
80%	258	260	Strong positive (Ct value <25): 65 Moderate positive (Ct value between 25-30): 130 Weak positive (Ct value >30 to 34): 65
<p><u>Note:</u> If clinical samples positive for a particular serotype is not available, tissue culture fluid (5-10 different isolates with a plaque forming unit of 10⁵⁻⁶/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. 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% [#]	16	20	Chikungunya positive: 4 ^a Acute febrile cases: 8 [*] Japanese Encephalitis positive: 1 [*] West Nile Virus positive: 1 [*] Zika Virus positive: 1 ^b Healthy subjects from endemic regions: 5
95%	77	80	Chikungunya positive: 15 ^a Acute febrile cases: 30 [*] Japanese Encephalitis positive: 5 [*] West Nile Virus positive: 5 [*] Zika Virus positive: 5 ^b Healthy subjects from endemic regions: 20
90%	145	150	Chikungunya positive: 28 ^a Acute febrile cases: 57 [*] Japanese Encephalitis positive: 9 [*] West Nile Virus positive: 9 [*] Zika Virus positive: 9

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			^b Healthy subjects from endemic regions: 38
85%	206	210	Chikungunya positive: 39 ^a Acute febrile cases: 79 *Japanese Encephalitis positive: 13 *West Nile Virus positive: 13 *Zika Virus positive: 13 ^b Healthy subjects from endemic regions: 53
80%	258	260	Chikungunya positive: 49 ^a Acute febrile cases: 98 *Japanese Encephalitis positive: 16 *West Nile Virus positive: 16 *Zika Virus positive: 16 ^b Healthy subjects from endemic regions: 65
^a Acute febrile cases negative for all markers of Dengue (NS1 & IgM & IgG & RNA) ^b Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid) <u>* Note:</u> 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**

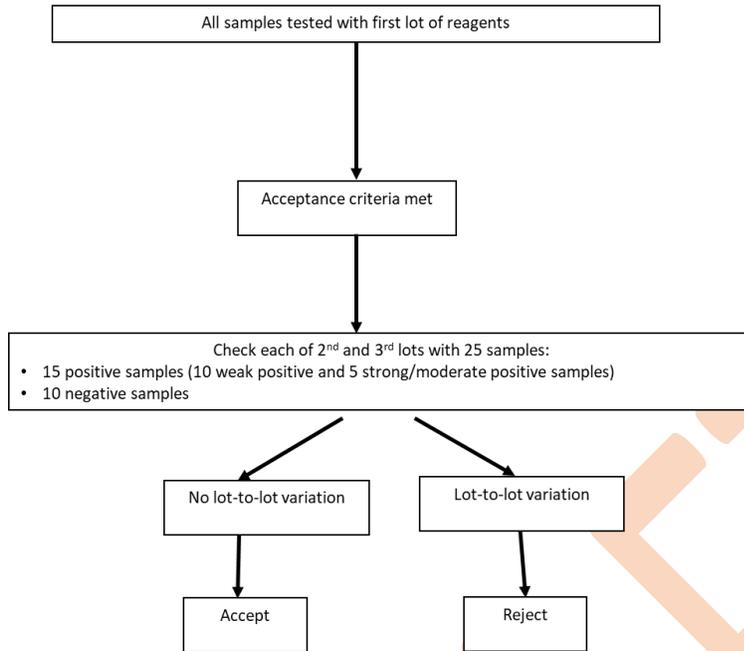
2292 **A. Sample size for lot-to-lot reproducibility**

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-
2328 eng.pdf;sequence=1)

2330 **VII. Performance evaluation report format**

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

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

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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*****

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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 μl isolate + 150 μl) 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**

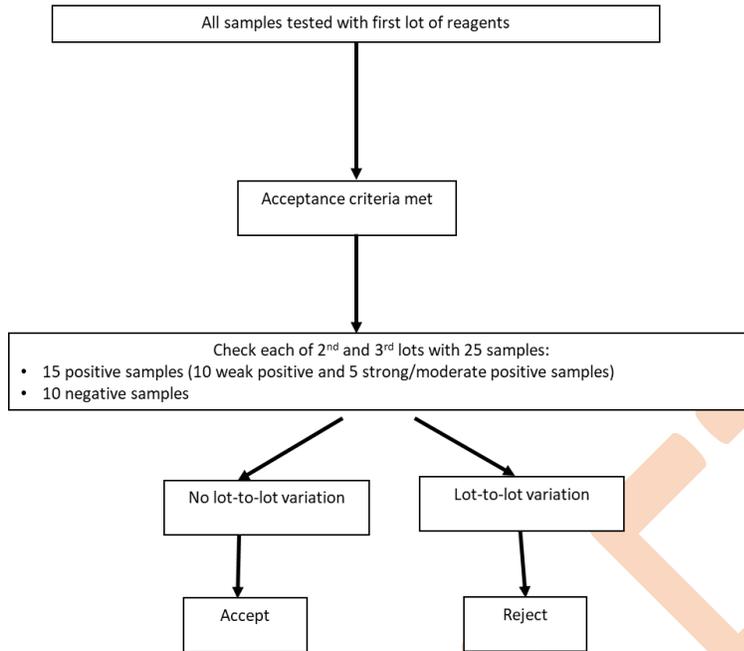
2500 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
2501 as follows:

- 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)

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2538 **VII. Performance evaluation report format**

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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		
Regulatory Approval: Import license / Manufacturing license/ Test license		
License Number:Issue date:		
Valid Up to:		
Application No.		
Sample Panel	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**

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

2565

	Estimate (%)	95% CI
Sensitivity		
Specificity		

2566

2567 ● Details of cross reactivity with other flaviviruses:

2568 ● **Conclusions:**

2569 ○ Sensitivity, specificity

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% [#]	16	20	Strong positive (Ct value <25): 5 Moderate positive (Ct value between 25-30): 10 Weak positive (Ct value >30 to 34): 5
95%	77	80	Strong positive (Ct value <25): 20 Moderate positive (Ct value between 25-30): 40

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			Weak positive (Ct value >30 to 34): 20
90%	145	150	Strong positive (Ct value <25): 38 Moderate positive (Ct value between 25-30): 74 Weak positive (Ct value >30 to 34): 38
85%	206	210	Strong positive (Ct value <25): 53 Moderate positive (Ct value between 25-30): 104 Weak positive (Ct value >30 to 34): 53
80%	258	260	Strong positive (Ct value <25): 65 Moderate positive (Ct value between 25-30): 130 Weak positive (Ct value >30 to 34): 65

Note 1: Representative positive samples from genotype (African, Asian/American) may be included, if feasible.

Note 2: Contrived positive samples – In absence of reference clinical samples, a contrived positive sample may be used.

Contrived positive samples should be prepared using spiking of diluted Zika virus culture isolate in unique negative samples, as follows:

Tissue culture fluid (3-5 different isolates with a plaque forming unit of 10^{5-6} /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.

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

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

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>
--------------------	-------------------------------	---	---------------------------------

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99% [#]	16	20	^a Acute febrile cases: 10 Dengue PCR positive: 4 Chikungunya PCR positive: 1 [*] Japanese Encephalitis positive: 1 [*] West Nile Virus positive: 1 Healthy subjects from endemic regions: 3
95%	77	80	^a Acute febrile cases: 40 Dengue PCR positive: 15 Chikungunya PCR positive: 5 [*] Japanese Encephalitis positive: 5 [*] West Nile Virus positive: 5 Healthy subjects from endemic regions: 10
90%	145	150	^a Acute febrile cases: 76 Dengue PCR positive: 28 Chikungunya PCR positive: 9 [*] Japanese Encephalitis positive: 9 [*] West Nile Virus positive: 9 Healthy subjects from endemic regions: 19
85%	206	210	^a Acute febrile cases: 105 Dengue PCR positive: 40 Chikungunya PCR positive: 13 [*] Japanese Encephalitis positive: 13 [*] West Nile Virus positive: 13 Healthy subjects from endemic regions: 26
80%	258	260	^a Acute febrile cases: 130 Dengue PCR positive: 49 Chikungunya PCR positive: 16 [*] Japanese Encephalitis positive: 16 [*] West Nile Virus positive: 16 Healthy subjects from endemic regions: 33
^a Acute febrile cases negative by Zika virus molecular reference assay [*] Positive samples / samples spiked with culture filtrate of Japanese Encephalitis and West Nile Virus <u>Note:</u> 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. <i>#Higher sample size should be used even for assays claiming 99% specificity.</i>			

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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**

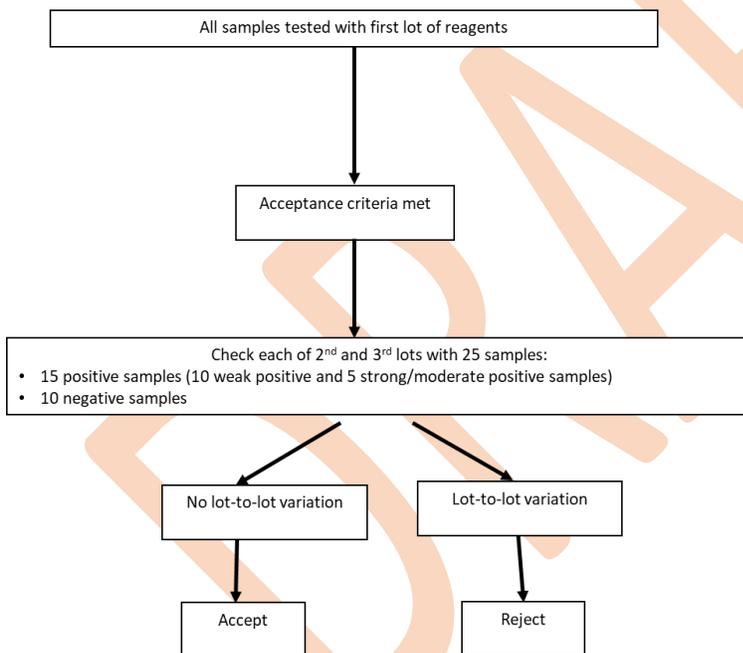
2697 Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be
2698 as follows:

- 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



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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).

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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.**

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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
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2737 Bowzard J, Villanueva JM, Muñoz-Jordan JL. Performance of the Triplex real-time RT-PCR assay
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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.

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2747 **VII. Performance evaluation report format**

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

2758 **Results**

		Reference assay (name)		
		Positive	Negative	Total
Name of Zika real-time PCR kit	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**

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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*****

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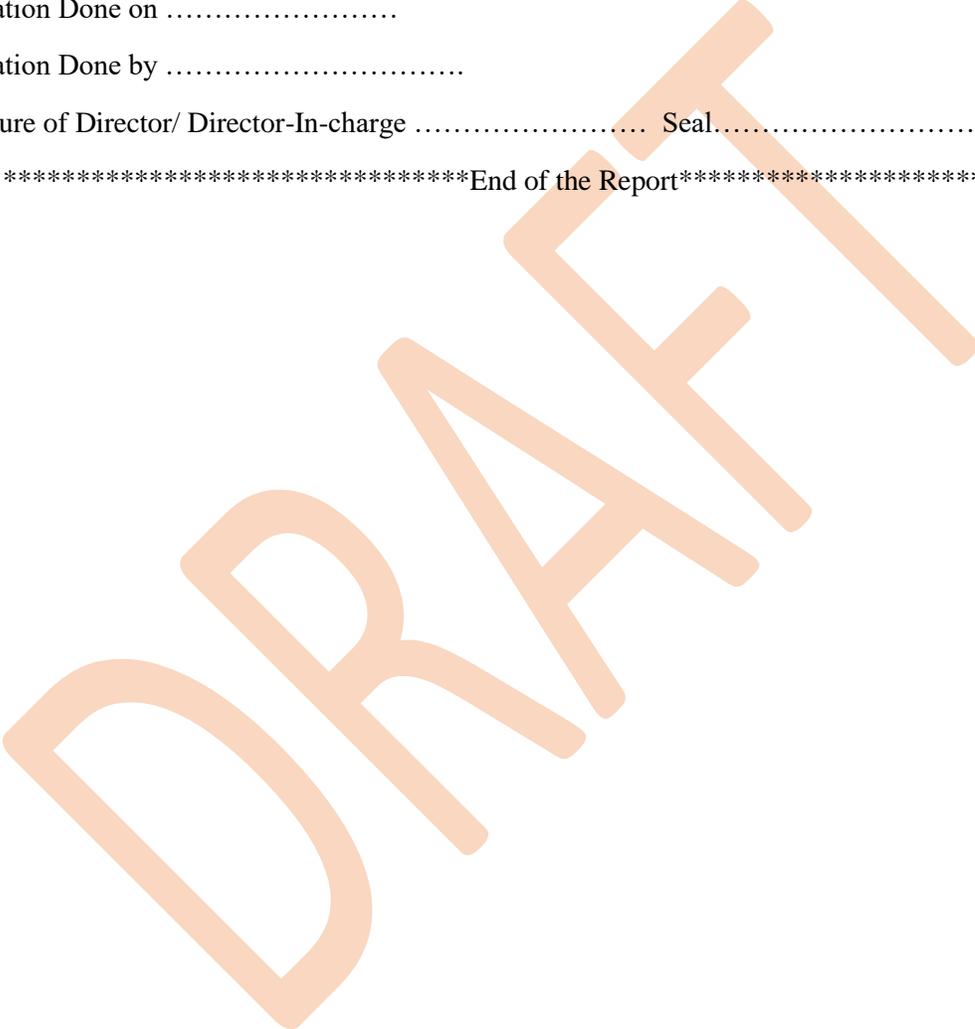
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2795 **Information on Operational and Test Performance Characteristics Required from Manufacturers for**
2796 **Dengue/Chikungunya/ Zika IVD**

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

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- 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