STANDARD PERFORMANCE EVALUATION PROTOCOLS

DRAFT FOR STAKEHOLDER COMMENTS

ARBOVIRUS IN-VITRO DIAGNOSTICS

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

-Dengue virus, Chikungunya virus, Zika virus

DECEMBER, 2024

New Delhi, India

Arbovirus IVD Performance Evaluation Protocols

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Performance evaluation protocol for Chikungunya IgM ELISA kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Chikungunya IgM ELISA kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Chikungunya IgM ELISA IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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
The samples need to be classified as strong, moderate and weak positives based on ELISA units of the reference assay. *#Higher sample size should be used even for assays claiming 99% sensitivity.*			

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

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):

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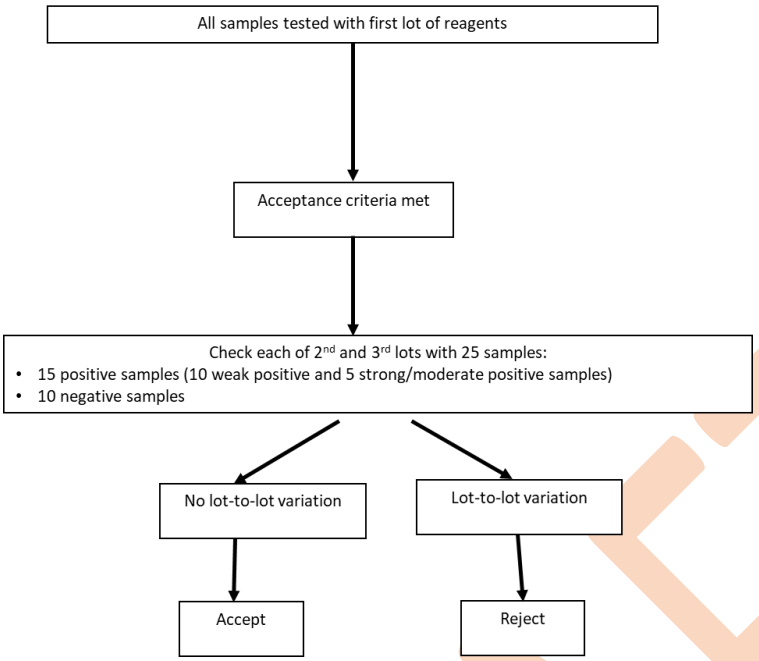
Fig.1: Sample size for Lot-to-lot reproducibility



7. Acceptance Criteria

Expected sensitivity: ≥90%

Expected specificity: ≥95%

8. Publication Rights:

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

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

VI. References:

1. **Kikuti M, Tauro LB, Moreira PSS, et al. Evaluation of two commercially available Chikungunya virus IgM enzyme-linked immunoassays (ELISA) in a setting of concomitant transmission of 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

VII. Performance evaluation report format

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PERFORMANCE EVALUATION REPORT FOR CHIKUNGUNYA 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 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)	

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

Conclusions:

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

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

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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 for ……Kit (Lot No……) manufactured by …… (Supplied by …….)

Evaluation Done on ……

Evaluation Done by …….

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

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

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Performance evaluation protocol for Chikungunya IgM RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Chikungunya IgM RDT kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Chikungunya IgM Rapid IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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
The samples need to be classified as strong, moderate and weak positives based on ELISA units of the reference assay. *#Higher sample size should be used even for assays claiming 99% sensitivity.*			

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

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):

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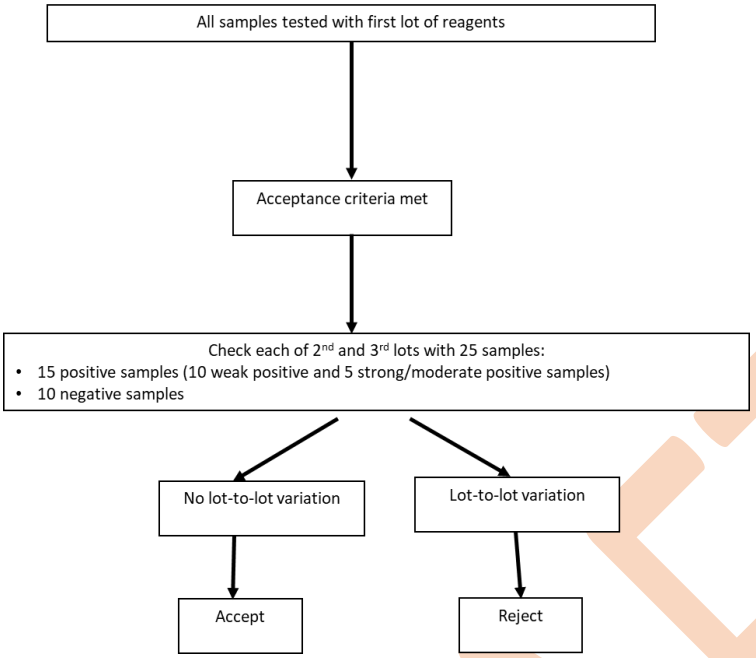
Fig.1: Sample size for Lot-to-lot reproducibility



B. Sample size for reader-to-reader reproducibility

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

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

7. Acceptance criteria

Expected sensitivity: ≥80%

Expected specificity: ≥90%

Invalid test rate: ≤5%

8. Publication Rights:

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

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

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

VI. References:

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

VII. Performance evaluation report format

DRAFT

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 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)	

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

Conclusions:

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

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

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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 for ……Kit (Lot No……) manufactured by …… (Supplied by …….)

Evaluation Done on ……

Evaluation Done by …….

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

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

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Performance evaluation protocol for Chikungunya real-time PCR kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/ leftover/spiked clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

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

3. Preparation of Chikungunya RNA evaluation panel

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

4. *RNA extraction*

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

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

5. Real-Time PCR System

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

6. Internal control/Extraction control

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

7. Reference assay:

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

Strong positive (Ct value between <25)

Moderate positive (Ct value between 25-30)

Weak positive (Ct value between >30 to 34)

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

			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)			

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

9. Evaluation method:

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

10. Test reproducibility

A. Sample size for lot-to-lot reproducibility

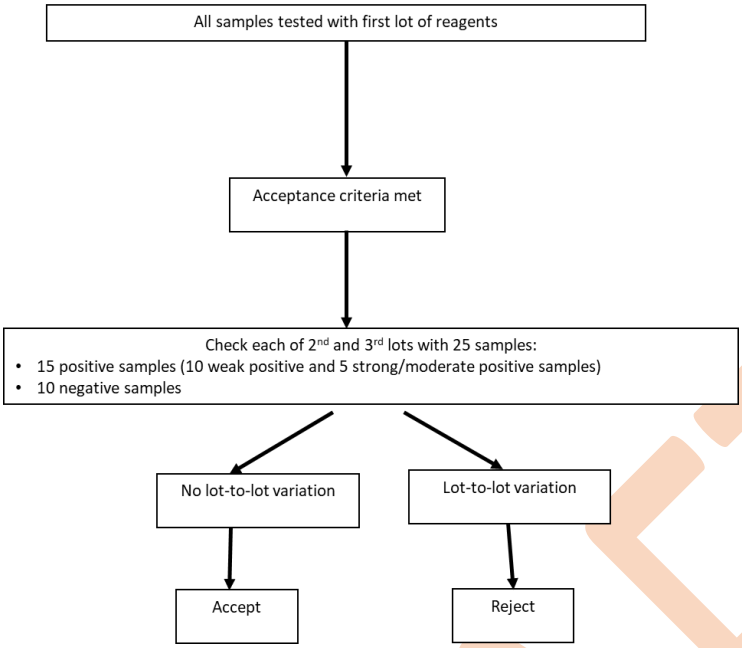
Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be as follows:

- 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):

DRAFT

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



11. Acceptance criteria

Expected sensitivity: ≥95%

Expected specificity: ≥98%

Cross reactivity with related viruses: NIL

Invalid test rate: ≤5%

11. Publication Rights:

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

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

VI. References:

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

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

VII. Performance evaluation report format

DRAFT

PERFORMANCE EVALUATION REPORT FOR CHIKUNGUNYA REAL-TIME PCR KITS

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

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- **Conclusions:**
  - Cross reactivity with related viruses:
  - **Performance: Satisfactory / Not satisfactory**

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

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Performance evaluation protocol for Dengue NS1 RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Dengue NS1 Rapid IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Dengue RDT IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*

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	-PCR/RT-PCR positive samples from other acute febrile illness cases Chikungunya: 4 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 8 -Samples from other flavivirus disease cases [*]Japanese Encephalitis PCR/antigen positive: 1 [*]West Nile Virus PCR/antigen positive: 1 [*]Zika Virus PCR/antigen positive: 1 -Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 5
95%	77	80	-PCR/RT-PCR positive samples from other acute febrile illness cases Chikungunya: 15 Acute febrile cases negative for Dengue (NS1 & IgM & IgG & PCR): 30 -Samples from other flavivirus disease cases [*]Japanese Encephalitis PCR/antigen positive: 5 [*]West Nile Virus PCR/antigen positive: 5 [*]Zika Virus PCR/antigen positive: 5

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

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			*West Nile Virus PCR/antigen positive: 16 *Zika Virus PCR/antigen positive: 16 -Samples from healthy subjects from endemic regions negative for all Dengue markers (NS1, IgM, IgG, nucleic acid): 65

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

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.

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

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):

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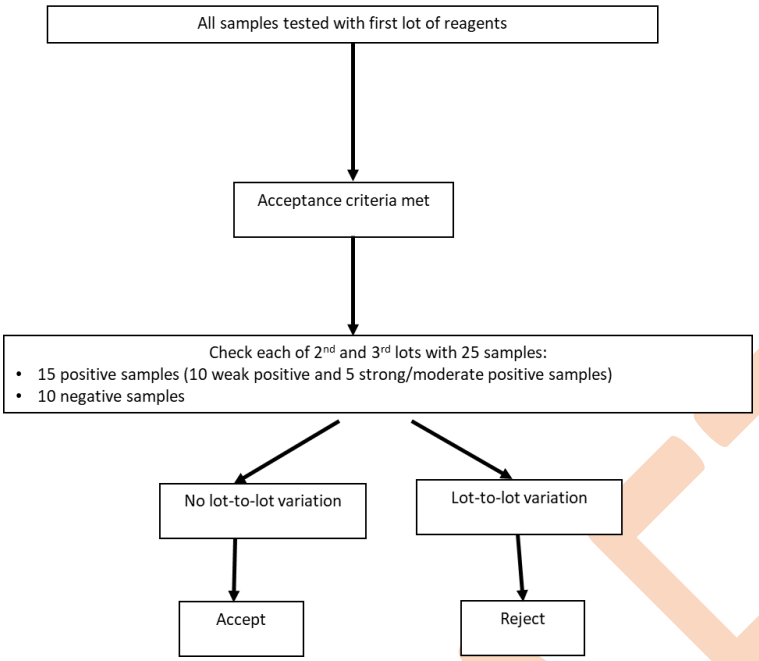
Fig.1: Sample size for Lot-to-lot reproducibility



B. Sample size for reader-to-reader reproducibility

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

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

7. Criteria for approval of the Dengue NS1 RDT kits

Expected sensitivity: ≥80%

Expected specificity: ≥95%

Cross reactivity with other flavivirus antigens: Nil

Invalid test rate: ≤5%

9. Publication Rights:

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

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

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

VI. References:

1. 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.
2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
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4. Mat Jusoh TNA, Shueb RH. Performance Evaluation of Commercial Dengue Diagnostic Tests for Early Detection of Dengue in Clinical Samples. J Trop Med. 2017; 2017: 4687182. doi: 10.1155/2017/4687182. Epub 2017 Dec 12. PMID: 29379526; PMCID: PMC5742879.
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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 (provide details: clinical/spiked, strong, moderate, weak)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel)	

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

DRAFT

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Field evaluation protocol for Dengue NS1 RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approval:

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

V. Procedure:

1. **Study design/type**: Cross-sectional study
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

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3. Sample size for performance evaluation:

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

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

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

Sensitivity	*Calculated sample size*	*No. of individuals* [Sample size rounded off]*
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		

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

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

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

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

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

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

4. Inclusion criteria:

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

5. Exclusion criteria:

Individuals with already known positive history for other pathogens

6. Reference assay:

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

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

7. Study implementation:

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

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

8. Positive samples:

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

9. Negative samples:

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

A. Cross reactivity:

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

i. Japanese Encephalitis PCR/antigen positive: 5 samples*
ii. West Nile Virus PCR/antigen: 5 samples*

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iii. Zika Virus PCR/antigen: 5 samples*

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

Note:

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.

10. Statistical analysis:

Sensitivity and specificity will be calculated.

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

11. 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):

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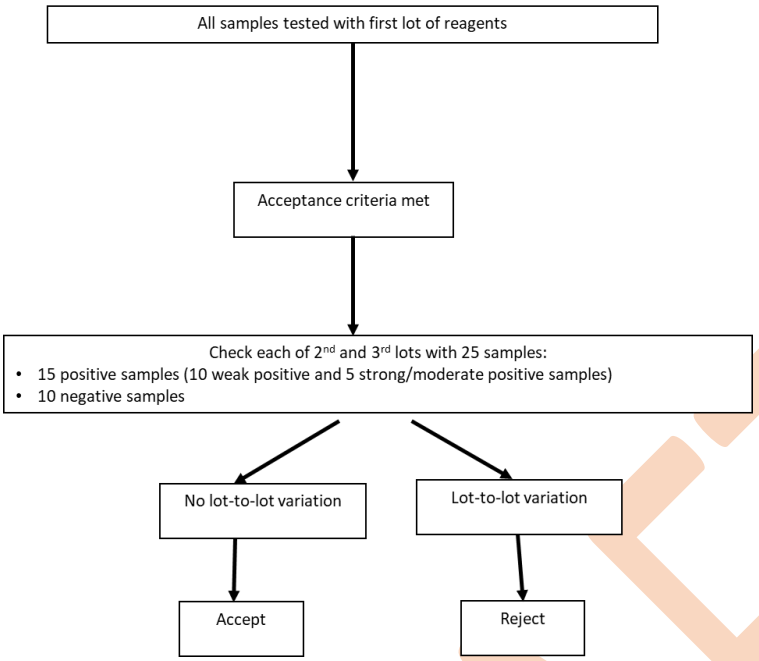
Fig.1: Sample size for Lot-to-lot reproducibility



B. Sample size for reader-to-reader reproducibility

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

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

12. Acceptance Criteria

Expected sensitivity: ≥80%

Expected specificity: ≥95%

Cross-reactivity with other flavivirus antigens: Nil

Invalid test rate: ≤5%

13. Publication Rights:

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

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

DRAFT

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, 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.
2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
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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)	

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Performance evaluation protocol for Dengue NS1 ELISA kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Dengue NS1 ELISA kits received for performance evaluation (Verification/Storage/Unpacking etc).

DRAFT

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

3. Preparation of Dengue NS1 ELISA IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required*	*Sample Panel Composition*

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		[Sample size rounded off]	
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

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

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

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

Note:

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.

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).

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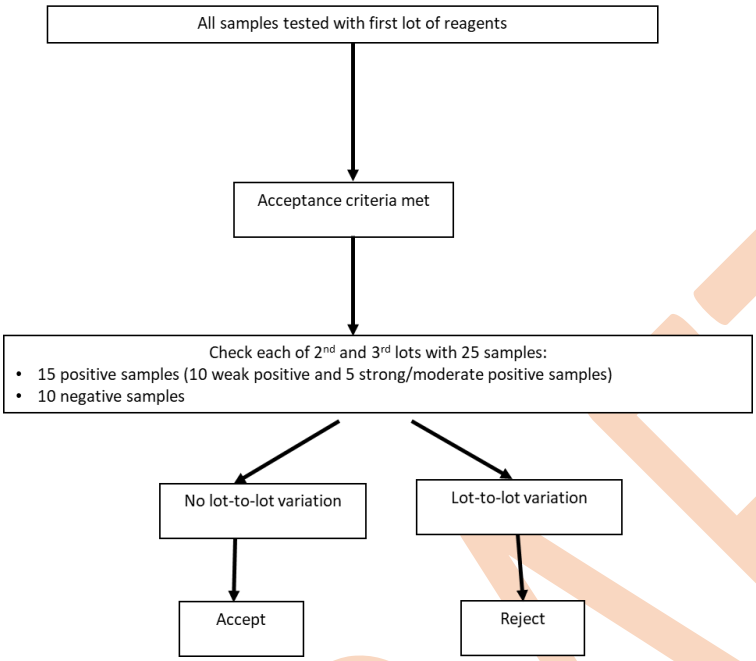
Refer the flowchart below (Fig. 1):

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



7. Acceptance Criteria

Expected sensitivity: ≥90%

Expected specificity: ≥95%

Cross reactivity with other flavivirus antigens: Nil

9. Publication Rights:

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

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

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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, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoS Negl Trop Dis. 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoS Negl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
3. Central Drugs Standard Control Organization. In-Vitro Diagnostic (IVD) Medical Devices Frequently Asked Questions. 2022. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/IVD/FAQs/CDSCO-IVD-FAQ-03-2022-.pdf
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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 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)	

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

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Field evaluation protocol for Dengue NS1 ELISA kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approval:

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

V. Procedure:

1. **Study design/type**: Cross-sectional study
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

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3. Sample size for performance evaluation:

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

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

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

Sensitivity	*Calculated sample size*	*No. of individuals* [Sample size rounded off]*
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

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

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

Specificity	*Calculated sample size*	*No. of individuals* [Sample size rounded off]*
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

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

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

4. Inclusion criteria:

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

5. Exclusion criteria

Individuals with already known positive history for other pathogens

6. Reference assay:

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

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

7. *Study implementation*:

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

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

8. Positive samples:

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

9. Negative samples:

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

A. Cross reactivity:

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

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

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

Note:

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

10. Statistical analysis:

Sensitivity and specificity will be calculated.

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

11. 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):

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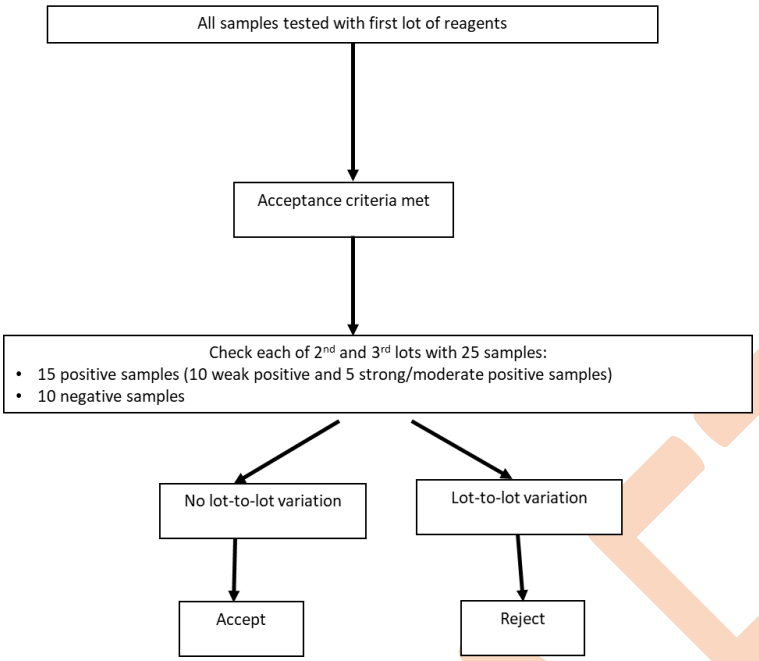
Fig.1: Sample size for Lot-to-lot reproducibility



12. Acceptance Criteria

Expected sensitivity: ≥90%

Expected specificity: ≥95%

Cross-reactivity with other flavivirus antigens: Nil

13. Publication Rights:

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

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

VI. References:

1. 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

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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.

2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
3. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic review and meta-analysis. PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi: 10.1371/journal.pntd.0006618.

4. Castro-Jorge LA, Machado PR, Fávero CA, Borges MC, Passos LM, de Oliveira RM, Fonseca BA. Clinical evaluation of the NS1 antigen-capture ELISA for early diagnosis of Dengue virus infection in Brazil. J Med Virol. 2010 Aug;82(8):1400-5. doi: 10.1002/jmv.21814.

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

VII. Performance evaluation report format

DRAFT

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 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)	

Results

		Reference assay …….…… (name)		
		Positive	Negative	Total
Name of Dengue NS1 based ELISA kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

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

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Performance evaluation protocol for Dengue IgM RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

b) Evaluation sites/laboratories (With required equipment)

c) Reference test kits

d) Characterised Evaluation panel

e) Laboratory supplies

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/ leftover clinical samples
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Dengue IgM Rapid IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Dengue IgM Rapid IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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)			

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

*Note: 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.

6. Test reproducibility

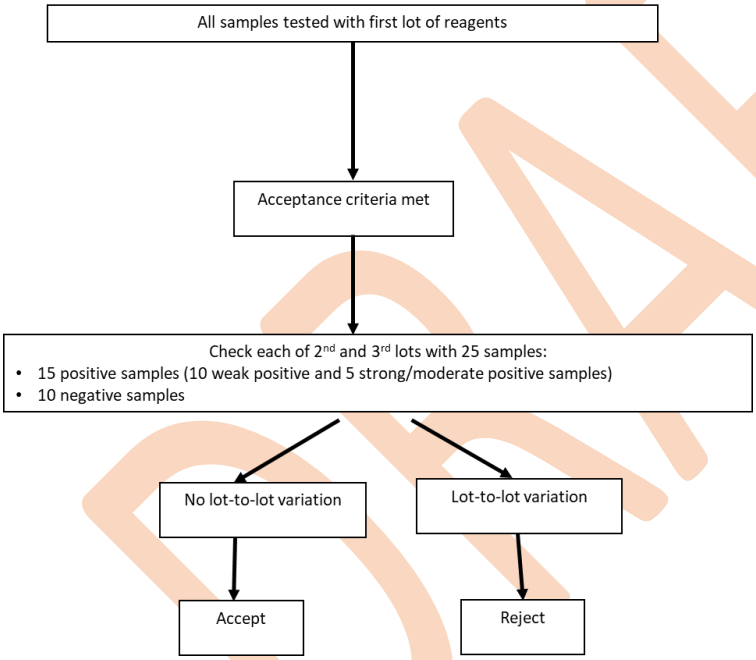
A. Sample size for lot-to-lot reproducibility

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



B. Sample size for reader-to-reader reproducibility

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

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

7. Acceptance Criteria

Expected sensitivity: ≥80%

Expected specificity: ≥90%

Invalid test rate: ≤5%

8. Publication Rights:

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

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

VI. References:

1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vázquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo Lic NR, Bessoff KE, Kliks S, Peeling RW. Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. Emerg Infect Dis. 2009 Mar;15(3):436-40. doi: 10.3201/eid1503.080923.
2. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoS Negl Trop Dis. 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
3. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.**
4. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro Diagnostic Medical Devices. 2018. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical device/guidanceperformanceivd.pdf
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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.**

VII. Performance evaluation report format

DRAFT

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 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)	

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

Conclusions:

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

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Performance evaluation protocol for Dengue IgM ELISA kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approval:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Dengue IgM ELISA IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Dengue IgM ELISA IVD kit evaluation panel

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

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

4. Reference assay:

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

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

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

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

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

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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)			

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

*Note: 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.

6. Test reproducibility

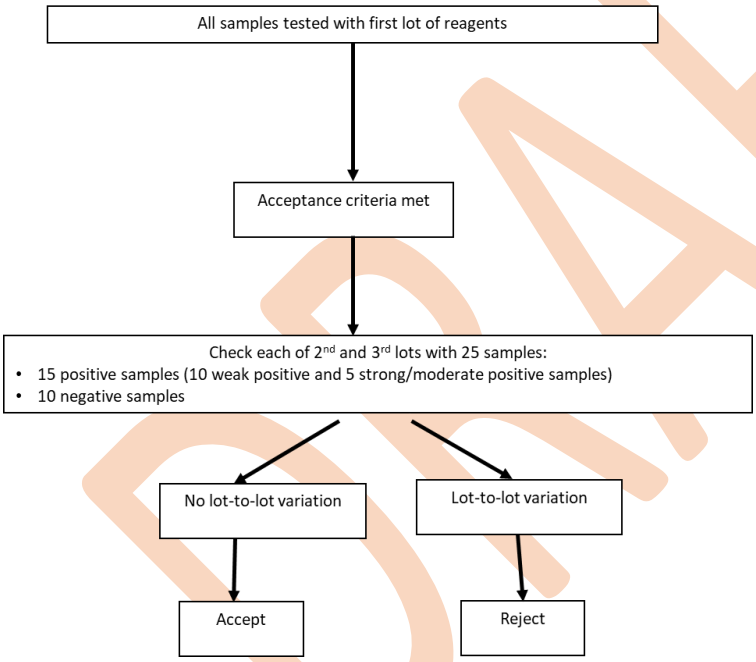
A. Sample size for lot-to-lot reproducibility

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



7. Acceptance criteria

Expected sensitivity: ≥90%

Expected specificity: ≥95%

8. Publication Rights:

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

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

VI. References:

1. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Pelegrino JL, Vázquez S, Artsob H, Drebot M, Gubler DJ, Halstead SB, Guzmán MG, Margolis HS, Nathanson CM, Rizzo Lic NR, Bessoff KE, Kliks S, Peeling RW. Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. Emerg Infect Dis. 2009 Mar;15(3):436-40. doi: 10.3201/eid1503.080923.
2. Hunsperger EA, Yoksan S, Buchy P, Nguyen VC, Sekaran SD, Enria DA, Vazquez S, Cartozian E, Pelegrino JL, Artsob H, Guzman MG, Olliaro P, Zwang J, Guillerm M, Kliks S, Halstead S, Peeling RW, Margolis HS. Evaluation of commercially available diagnostic tests for the detection of Dengue virus NS1 antigen and anti-Dengue virus IgM antibody. PLoSNegl Trop Dis. 2014 Oct 16;8(10):e3171. doi: 10.1371/journal.pntd.0003171.
3. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.**
4. Central Drugs Standard Control Organization. Guidance on Performance Evaluation of In-vitro Diagnostic Medical Devices. 2018. Available at: https://cdsco.gov.in/opencms/export/sites/CDSCO_WEB/Pdf-documents/medical device/guidanceperformanceivd.pdf
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7. World Health Organization. Technical Guidance Series (TGS) for WHO Prequalification – Diagnostic Assessment TGS-3. 2017. Available at: https://iris.who.int/bitstream/handle/10665/258985/WHO-EMP-RHT-PQT-TGS3-2017.03-eng.pdf;sequence=1

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

VII. Performance evaluation report format

PERFORMANCE EVALUATION REPORT FOR DENGUE IgM ELISA KIT

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

Results:

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

Conclusions:

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

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Performance evaluation protocol for Dengue NS1/IgM combo RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/leftover clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

- Preparation & characterization of kit evaluation panel
- Handling of Dengue NS1/IgM combo IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).

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

3. Preparation of Dengue RDT IVD kit evaluation panel

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

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

4. Reference assay:

Anti-DENV IgM detection ELISA US-FDA approved kit

AND/OR

DENV NS1 ELISA US-FDA approved kit

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off for balanced allocation]*	*Sample Panel Composition*
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

*** all 4 serotypes shall be represented**

Note:

In the absence of natural samples, spiked samples may be used as per details provided below:

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.

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

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off for balanced allocation]*	*Sample Panel Composition*
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.*			

5. 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 with adequate representation of NS1 and IgM, and 10 negative samples).
- Third lot of the assay: should be tested on 25 samples (15 positive samples comprising 10 low positive **AND** 5 moderate/high positive samples, and 10 negative samples).

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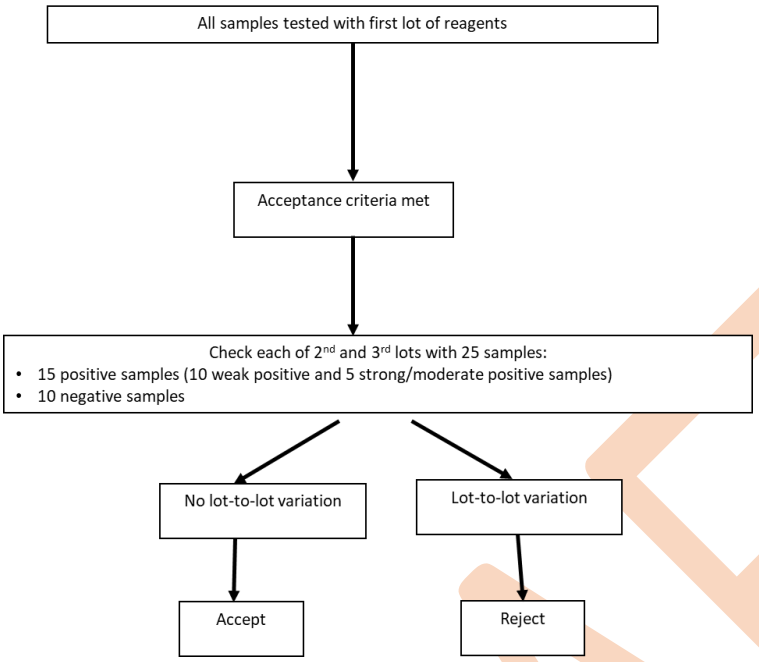
Refer the flowchart below (Fig. 1):

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



B. Sample size for reader-to-reader reproducibility

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

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 combo format, concordance has to be calculated separately for NS1 and IgM, and the overall sensitivity and specificity have to be calculated based on the combined results of 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 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

+	-	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

6. Acceptance criteria:

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

Cross reactivity with other flavivirus antigens: Nil

Invalid test rate: ≤5%

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

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

9. Publication Rights:

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

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

VI. References:

1. 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.
2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
3. Yow KS, Aik J, Tan EY, Ng LC, Lai YL. Rapid diagnostic tests for the detection of recent Dengue infections: An evaluation of six kits on clinical specimens. PLoS One. 2021 Apr 1;16(4):e0249602. doi: 10.1371/journal.pone.0249602.

4. 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
5. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.**

VII. Performance evaluation report format

DRAFT

PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 and IgM COMBO 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 (provide details: strong, moderate, weak)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel)	

Results:

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

	Estimate (%)	95% CI
Combined Sensitivity		
Combined Specificity		

- Details of cross reactivity with other flavivirus NS1 antigens:
- **Conclusions:**

- Concordance for NS1, Concordance for IgM
- Sensitivity, specificity
- Performance: **Satisfactory / Not satisfactory**

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Field evaluation protocol for Dengue NS1 and IgM combo RDT kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approval:

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

V. Procedure:

1. **Study design/type**: Cross-sectional study
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

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3. Sample size for performance evaluation:

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

Sample size has to be calculated based on both the sensitivity and the specificity. The final sample size will be the maximum of the two. For example, at 95% sensitivity and 95% specificity, the sample size required will be 260 (maximum of 260 and 110). It is desirable to cover at least one Dengue season so that adequate samples are available for evaluation.

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

Sensitivity	*Calculated sample size*	*No. of individuals* [Sample size rounded off]*
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		

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

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

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

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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		

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

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

4. **Inclusion criteria**:

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

5. **Exclusion criteria:**

Individuals with already known positive history for other pathogens

6. **Reference assay**:

Anti-DENV IgM detection ELISA US-FDA approved kit

AND/OR

DENV NS1 ELISA US-FDA approved kit

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

7. **Study implementation**:

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

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

8. **Positive samples**:

Samples which are positive for IgM or NS1 or both by the reference assays will be considered as true positive samples.

9. **Negative samples**:

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

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

A.1 NS1:

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

i. Japanese Encephalitis PCR/antigen positive: 5 samples*
ii. West Nile Virus PCR/antigen: 5 samples*
iii. Zika Virus PCR/antigen: 5 samples*

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

Note:

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

A.2 IgM:

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

i. Japanese Encephalitis IgM positive: 5 samples
ii. West Nile Virus IgM positive: 5 samples
iii. Zika Virus IgM positive: 5 samples

Note: 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.

10. Statistical analysis:

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

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

11. Test reproducibility

A. Sample size for lot-to-lot reproducibility

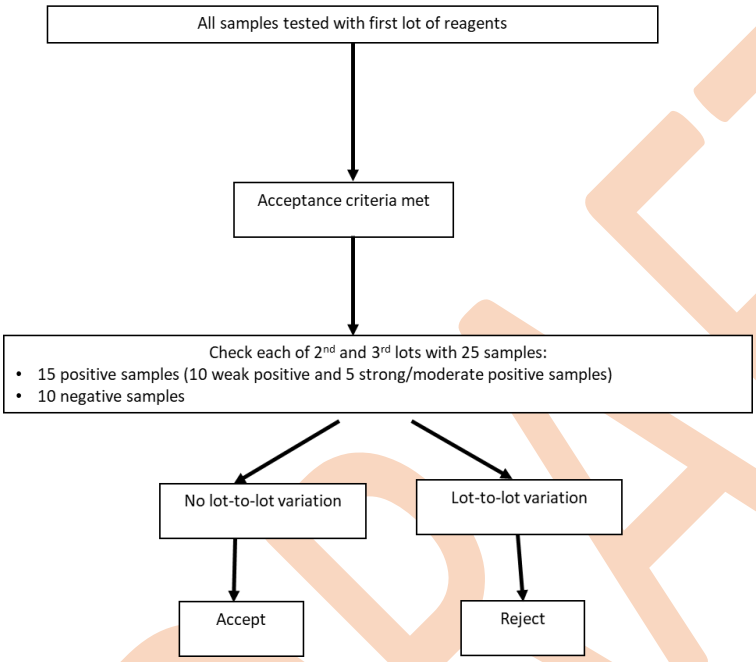
Three lots of an assay shall be evaluated. Sample size for lot-to-lot reproducibility should be as follows:

- 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



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).

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

12. Acceptance criteria:

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

Cross reactivity with other flavivirus antigens: Nil

Invalid test rate: ≤5%

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

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

13. Publication Rights:

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

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

VI. References:

1. 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.

2. Hermann LL, Thaisomboonsuk B, Poolpanichupatam Y, Jarman RG, Kalayanarooj S, Nisalak A, Yoon IK, Fernandez S. Evaluation of a Dengue NS1 antigen detection assay sensitivity and specificity for the diagnosis of acute Dengue virus infection. PLoSNegl Trop Dis. 2014 Oct 2;8(10):e3193. doi: 10.1371/journal.pntd.0003193.
3. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic review and meta-analysis. PLoSNegl Trop Dis. 2018 Jul 16;12(7):e0006618. doi: 10.1371/journal.pntd.0006618.
4. 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
5. **WHO, Evaluation of commercially available anti-Dengue virus immunoglobulin M tests. (Diagnostics evaluation series, 3). ISBN 978 92 4 159775 3.**

VII. Performance evaluation report format

DRAFT

PERFORMANCE EVALUATION REPORT FOR DENGUE NS1 and IgM COMBO 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)	

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Details of cross reactivity with other flavivirus NS1 antigens:
- Conclusions:
    - Sensitivity, specificity

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- Performance: **Satisfactory / Not satisfactory**

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Performance evaluation protocol for Dengue real-time PCR kit

I. Background:

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

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using archived/ leftover/ spiked clinical samples.
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

3. Preparation of Dengue RNA evaluation panel

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

4. RNA extraction

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

5. Real-Time PCR System

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

6. Internal control/Extraction control

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

7. Reference assay:

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

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

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

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

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

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

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

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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
Note: If clinical samples positive for a particular serotype is not available, tissue culture fluid (5-10 different isolates with a plaque forming unit of 10^{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, 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.			

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

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*
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

			[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) [*] Note: If PCR positive samples for cross reactive flaviviruses not available, commercially available RNA panels should be used to test cross reactivity.			

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

9. Evaluation method:

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

10. Test reproducibility

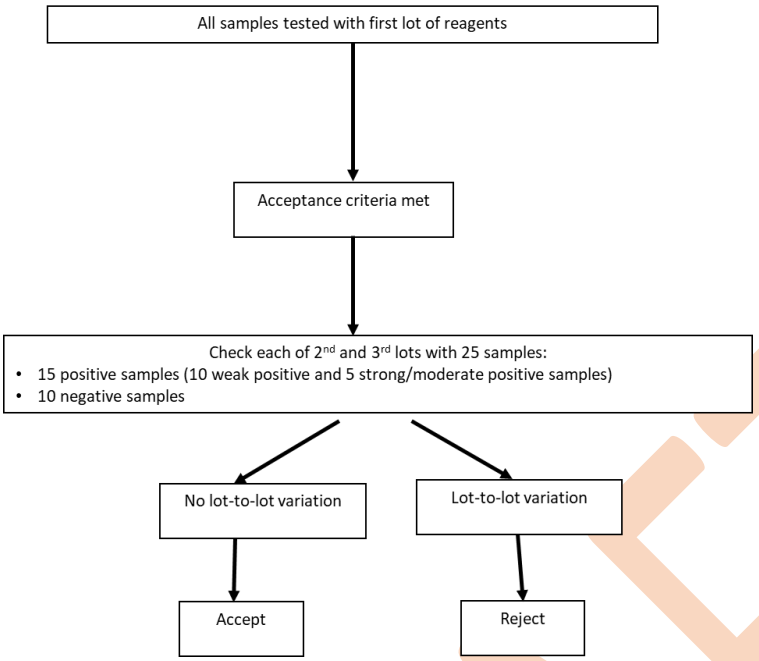
A. Sample size for lot-to-lot reproducibility

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



11. Acceptance Criteria

Expected sensitivity: ≥95%

Expected specificity: ≥98%

Cross reactivity with other flavivirus: Nil

Invalid test rate: ≤5%

13. Publication Rights:

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

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

VI. References:

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

VII. Performance evaluation report format

DRAFT

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 CDSCO/State licensing Authority		
Lot No / Batch No.:		
Product Reference No/ Catalogue No		
Type of Assay		
Kit components		
Manufacturing Date		
Expiry Date		
Pack size (Number of tests per kit)		
Intended Use		
Number of Tests Received		
Regulatory Approval: Import license / Manufacturing license/ Test license License Number:Issue date: Valid Up to:		
Application No.		
Sample Panel	Positive samples (provide details: clinical/ spiked, strong, moderate, weak)	
	Negative samples (provide details clinical/ spiked, including cross reactivity panel)	

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Details of cross reactivity with other flaviviruses:
- **Conclusions:**
  - Sensitivity, specificity

- Performance: **Satisfactory / Not satisfactory**

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

Field evaluation protocol for Dengue real-time PCR kits

I. Background:

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

II. Purpose:

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

III. Requirements:

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

IV. Ethical approvals:

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

V. Procedure:

1. **Study design/type**: Cross-sectional study
2. **Preparation of Evaluation sites/laboratories:**

Identified IVD kit evaluation laboratories should establish their proficiency through

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

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

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

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3. Sample size for performance evaluation:

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

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

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

Sensitivity	*Calculated sample size*	*No. of individuals* [Sample size rounded off]*
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		

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

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

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

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

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

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

4. Inclusion criteria:

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

5. Exclusion criteria:

Individuals with already known positive history for other pathogens

6. RNA extraction

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

7. Real-Time PCR System

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

8. Internal control/Extraction control

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

9. Reference assay:

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

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

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

10. Study implementation:

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

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

11. Positive samples:

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

12. Negative samples:

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

A. Cross reactivity:

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

a. Japanese Encephalitis PCR positive: 5 samples
b. West Nile Virus PCR positive: 5 samples
c. Zika Virus PCR positive: 5 samples

Alternatively, tissue culture fluid of cross reactive flaviviruses (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 negative samples can be used for evaluation.

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

13. Statistical analysis:

Sensitivity and specificity will be calculated.

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

14. 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):

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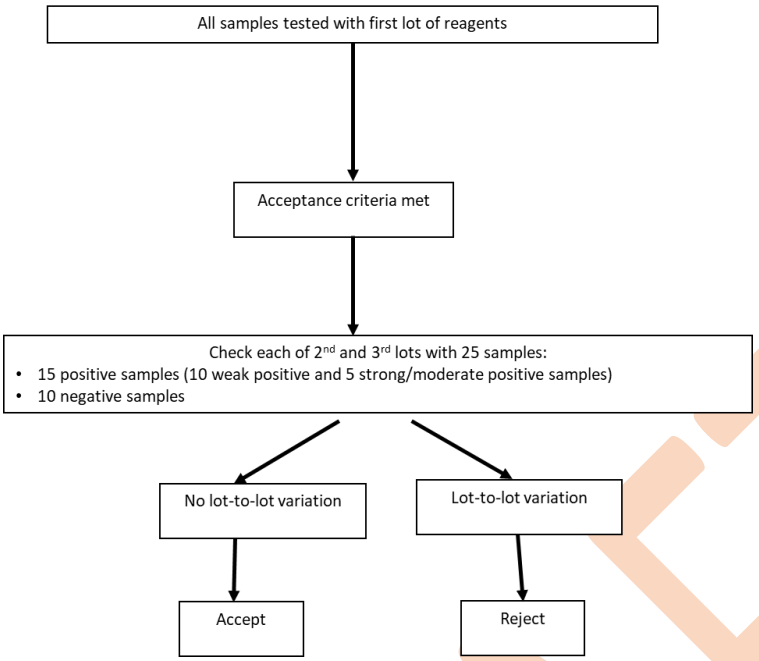
Fig.1: Sample size for Lot-to-lot reproducibility



15. Acceptance Criteria

Sensitivity: ≥95%

Specificity: ≥98%

Cross reactivity with other flavivirus: Nil

Invalid test rate: ≤5%

16. Publication Rights:

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

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

VI. References:

1. Santiago, G.A., Vázquez, J., Courtney, S. et al. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun 9, 1391(2018). https://doi.org/10.1038/s41467-018-03772-1
2. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A, John D, Mehendale SM. Dengue infection in India: A systematic

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

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

VII. Performance evaluation report format

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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 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)	

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Details of cross reactivity with other flaviviruses:
- **Conclusions:**
  - Sensitivity, specificity

- Performance: **Satisfactory / Not satisfactory**

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

DRAFT

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

I. Background:

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

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

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

III. Requirements:

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

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

V. Procedure:

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

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

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

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

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

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

4. Preparation of Zika reference evaluation panel

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

- Frozen samples (≤-70°C) may be used, if stored appropriately and analytical data demonstrate that accuracy of test results is not affected.
- Samples that previously tested positive by FDA approved PCR and/or CDC/NIV approved protocols may be used.
- In the absence of natural samples, spiked clinical samples may be used.

5. RNA extraction

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

6. Real-Time PCR System

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

7. Internal control/Extraction control

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

8. Reference assay:

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

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

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

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

Positive Samples:

- Clinical positive samples: Sample tested positive by Zika virus molecular reference assay from clinically suspect cases.
- 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 per the note below:

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

Sensitivity	*Calculated sample size*	*No. of Positive Samples required [Sample size rounded off]*	*Sample Panel Composition*
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 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 $10^{5\text{-}6}$/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.

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

Specificity	*Calculated sample size*	*No. of Negative Samples required [Sample size rounded off]*	*Sample Panel Composition*

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

Note:
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.

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

10. Evaluation method:

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

11. Test reproducibility

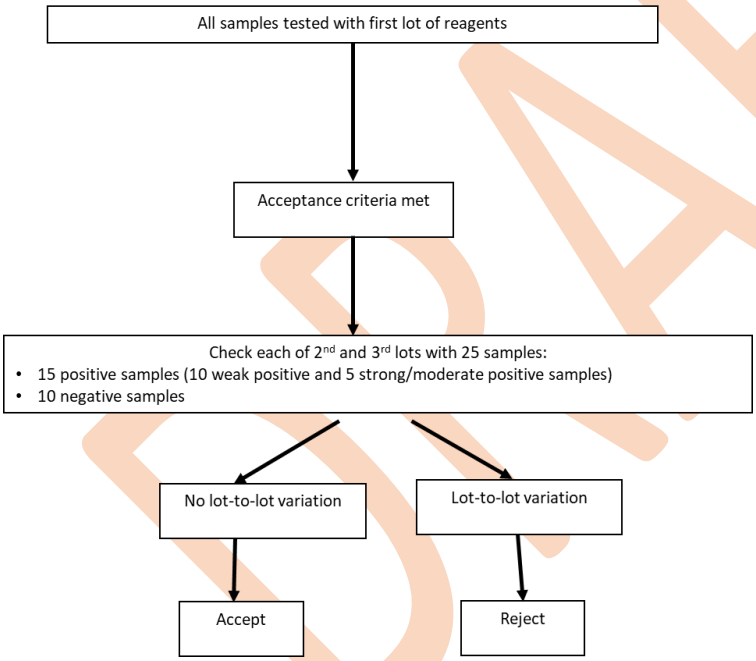
A. Sample size for lot-to-lot reproducibility

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



12. Acceptance criteria

Sensitivity: ≥95%
Specificity: ≥98%
Cross reactivity with other pathogens: Nil
Invalid test rate: ≤5%

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

14. Publication Rights:

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

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

VI. References:

1. 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

2. Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, Butler AE, Roulo R, Bowzard J, Villanueva JM, Muñoz-Jordan JL. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, Dengue, and Chikungunya viruses. Nat Commun. 2018 Apr 11;9(1):1391. doi: 10.1038/s41467-018-03772-1.

3. Stone M, Bakkour S, Grebe E, Emperador DM, Escadafal C, Deng X, Dave H, Kelly-Cirino C, Lackritz E, Rojas DP, Simmons G, Rabe IB, Busch MP. Standardized evaluation of Zika nucleic acid tests used in clinical settings and blood screening. PLoS Negl Trop Dis. 2023 Mar 17;17(3):e0011157.

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 CDSCO/State licensing Authority		
Lot No / Batch No.:		
Product Reference No/ Catalogue No		
Type of Assay		
Kit components		
Manufacturing Date		
Expiry Date		
Pack size (Number of tests per kit)		
Intended Use		
Number of Tests Received		
Regulatory Approval: Import license / Manufacturing license/ Test license License Number:Issue date: Valid Up to:		
Application No.		
Sample Panel	Positive samples (provide details: clinical/spiked, strong, moderate, weak)	
	Negative samples (provide details clinical/spiked, including cross reactivity panel)	

Results

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

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Details of cross reactivity with other flaviviruses:

FINAL CONCLUSION

Performance: Satisfactory / Not satisfactory

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

Disclaimers

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

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

Evaluation Done on ……

Evaluation Done by …….

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

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

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

The manufacturer should provide the following details about the IVD:

1. Instructions for Use

2. Scope of the IVD: to diagnose Dengue and/or/Chikungunya and/or Zika virus

3. Intended Use Statement

4. Principle of the assay

5. Intended testing population(cases of acute febrile illness/suspected cases of Dengue and/or Chikungunya and/or Zika virus infection)

6. Intended user(laboratory professional and/or health care worker at point-of-care)

7. Detailed test protocol

8. Lot/batch No.

9. Date of manufacture

10. Date of Expiry

11. Information on operational Characteristics

i. Configuration of the kit/device

ii. Requirement of any additional equipment, device

iii. Requirement of any additional reagents

iv. Operation conditions

v. Storage and stability before and after opening

vi. Internal control provided or not

vii. Quality control and batch testing data

viii. Biosafety aspects- waste disposal requirements

10. Information on Test Performance Characteristics

i. Type of sample-serum/plasma/whole blood/other specimen (specify)

ii. Volume of sample

iii. Sample rejection criteria (if any)

iv. Any additional sample processing required

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

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vi. Name of analyte to be detected

vii. Pathogens targeted by the kit

viii. Time taken for testing

ix. Time for result reading and interpretation

x. Manual or automated(equipment)reading

xi. Limit of detection

xii. Diagnostic sensitivity

xiii. Diagnostic specificity

xiv. Stability and reproducibility (including data)

xv. Training required for testing (if any)

xvi. If yes, duration

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

xviii. Details of cross reactivity, if any

xix. Interpretation of invalid and indeterminate results to be provided

xx. It is recommended to provide data demonstrating the precision

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

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

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