STANDARD PERFORMANCE EVALUATION PROTOCOLS

SCRUB TYPHUS IN-VITRO DIAGNOSTICS

JULY, 2026

New Delhi, India

Scrub Typhus IVD Performance Evaluation Protocols

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Performance evaluation protocol for scrub typhus IgM RDT kit

I. Background:

CDSCO/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 in-vitro diagnostic kit (IVD) performance.

II. Purpose:

To evaluate the performance of *Orientia tsutsugamushi* IgM RDT kits in the diagnosis of Scrub typhus infection using irreversibly de-identified leftover archived/ spiked clinical samples

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. The accessories including UPS are needed to be provided to the testing institute for the duration of the evaluation.
2. Evaluation sites/laboratories (With required equipment)
3. Reference test kits
4. Characterised evaluation panel
5. Laboratory supplies including the necessary labware and plastic ware.

IV. Ethical approvals:

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

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

V. Procedure:

1. **Study design/type**: Diagnostic accuracy study using irreversibly de-identified leftover clinical/spiked 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 systems (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 *Orientia tsutsugamushi* IgM RDT IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).
- Testing, interpreting, recording of results & reporting
- Data handling, data safety & confidentiality

3. Preparation of *Orientia tsutsugamushi* IgM RDT IVD kit evaluation panel

Well characterised *Orientia tsutsugamushi* IgM RDT IVD kit evaluation panel is critical requirement for performance evaluation of IVD kits. Hence statistically significant number of Serum/ plasma samples should be collected from *Orientia tsutsugamushi* IgM confirmed cases.

Further characterised for *Orientia tsutsugamushi* IgM positivity by using approved reference kits having high sensitivity and specificity *Orientia tsutsugamushi* 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 standard:

- Microimmunofluorescence (MIF) IgM assay (IVD kit) and/or Scrub Typhus Detect™ IgM ELISA (InBios International) should be used as the reference standard. US FDA-authorized/ PMDA Japan-approved/ ATAGI Australia-recommended, or WHO prequalified *Orientia tsutsugamushi* IgM ELISA/IFA/MIF may also be used as the reference standard, if available.
- Serostatus shall be determined using the designated reference standard.
- Semi-quantification of scrub typhus IgM concentration (mandatory for identifying weakly reactive samples) should be carried out using MIF/well-characterized scrub typhus IFA test.
- Representation of samples positive for all four major *Orientia tsutsugamushi* serotypes (e.g., Karp, Kato, Gilliam, and TA763) is desirable but not mandatory and is subject to sample availability.

5. Sample size & Sample panel composition:

- **Sample size** is calculated with 90% sensitivity and 95% specificity of the index test, 95% confidence interval, absolute precision of 5% and invalid test rate ≤5%. Formula used are as follows:

$$\square_{\square\square} \geq \frac{\square^2 \square \square_{\square} (1 - \square_{\square})}{\square^2 \square (1 - \square\square)}$$

$$\square_{\square\square} \geq \frac{\square^2 \square \square_{\square}(1-\square_{\square})}{\square^2 \square (1-\square\square)}$$

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

A minimum of 146 *Orientia tsutsugamushi* IgM positive samples and a minimum of 77 negative samples are calculated. Rounding the sample size, a **minimum** of 150 *Orientia tsutsugamushi* positive samples and a **minimum** of 80 negative samples are required for evaluation.

However, to adequately represent various subcategories within the negative sample panel for robust performance evaluation of scrub typhus IgM RDTs, 255 negative samples are recommended. The minimum and recommended number of negative samples have been calculated using the Wilson score interval, wherein it has been found that **255 negative samples significantly enhances statistical robustness and precision due to narrower interval estimates (10.20% vs 5.48%)**. ***

** With **a specificity assumption of 95%, a 95% CI, 5% precision and an invalid test rate of 5%**, the narrower interval for negative samples n = 255 (Interval estimate for True Specificity: [0.916, 0.971]) compared to n = 80 (Interval estimate for True Specificity: [0.878, 0.980]) for the interval estimate of True Specificity were obtained. It reflects greater precision and reliability in estimating the true proportion of negative results. **The choice of n = 80 as the minimum negative sample size ensures baseline statistical validity**, allowing for the fulfilment of assumed specificity, CI, precision, while compensating for potential data losses from assumed level of invalid test rate. However, **to secure adequate representation for different categories of negative samples, increasing the negative sample size to n = 255 is found to be preferable**, as it would safeguard against fluctuations in test validity, ensuring robustness in results.*

- **Seropositive panel** shall constitute samples reactive for *Orientia tsutsugamushi* IgM. The seropositive panel should be characterized as follows:

1. Serology reactive by reference standard in strong clinical suspicion of scrub typhus (e.g.: presence of eschar)

OR

2. Sample testing positive by *Orientia tsutsugamushi* PCR (47 kDa/ 56 kDa protein gene) & seroreactive by reference assay

OR

3. Serum sample collected from *Orientia tsutsugamushi* PCR confirmed cases of scrub typhus 10-14 days after PCR test.

The positive panel should have a minimum of 35% weakly reactive samples (MIF titers: 64 to 256)

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

Sensitivity	*Calculated sample size*	*Minimum no. of positive samples required (sample size rounded off) #*	*Sample panel composition*
99%	16	20	Strong/Moderate Positive: 13 Weak Positive: 7
95%	77	80	Strong/Moderate Positive: 52 Weak Positive: 28
90%	145	150	Strong/Moderate Positive: 95 Weak Positive: 55
85%	206	210	Strong/Moderate Positive: 135 Weak Positive: 75
80%	258	260	Strong/Moderate Positive: 165 Weak Positive: 95
The samples need to be classified as strong, moderate and weak positive based on ELISA units of the reference assay. *#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.*			

- **Seronegative panel (Recommended n=255, Minimum n= 80)**: The seronegative panel should be non-reactive for *Orientia tsutsugamushi* by the reference assay. The minimum number of seronegative samples required for testing is 80, however, 255 seronegative samples are recommended to adequately assess cross-reactivity and calculate the specificity of scrub typhus IVDs. The seronegative panel should constitute the following categories of samples:

1. *Acute Febrile illness cases negative for common etiologies of fever:* Samples from acutely febrile patients that are negative for etiologies such as Dengue,

Chikungunya, Malaria, Leptospira (**Recommended n=60, Minimum n= 25**): *Refer Table 1 for details*

2. Samples non-reactive for Scrub typhus IgM but positive for other common etiologies of acute fever in India (**Recommended n=155, Minimum n= 30):** *Refer Table 1 for details.* It is recommended to use as many as a number of types of cross-reactive samples as feasible.
3. Samples from healthy subjects from endemic regions: Serum/plasma samples testing seronegative for *Orientia tsutsugamushi* by reference test, IgM serology by Dengue, Chikungunya (**Recommended n=40, Minimum n= 25).** *Refer Table 2 for details*.

Table 2: Composition of *Orientia tsutsugamushi* seronegative panel

S. No.	Agent	Recommended no. of samples	Minimum no. of samples required
B.1 Acute febrile illness cases negative for common etiologies			
	Acute febrile illness cases negative for Dengue/ Chikungunya/ Malaria/ Leptospira	60	25
B.2 Samples non-reactive for Scrub typhus IgM but positive for other etiologies			
B 2.1	Chikungunya IgM	15	5
B 2.2	Malaria antigen (with representation from Pv and Pf)	25	10
B 2.3	Leptospira IgM	20	5
B 2.4	Blood sample from Pulmonary tuberculosis at the time of diagnosis	20	0
B 2.5	Spotted fever IgM	15	0
B 2.6	Dengue NS1	20	5
B 2.7	Dengue IgM	15	5
B 2.8	JE IgM	15	0
B 2.9	West Nile virus IgM	10	0
B.3 Samples from healthy subjects			

	Samples from healthy subjects from endemic regions (Serum/plasma samples testing seronegative for *Orientia tsutsugamushi* by Reference test, IgM serology by Dengue, Chikungunya may be used)	40	25
GRAND TOTAL		**255**	**80**

6. Evaluation method:

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

7. Interpretation of results:

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

8. Resolution of discrepant results:

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

9. Repeatability and Reproducibility Assessment:

A. Repeatability Assessment

This should be done to assess the repeatability of the detection of target analyte using the kit under evaluation.

3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs.
Concordance should be 100% based on positive and negative test results(qualitative).

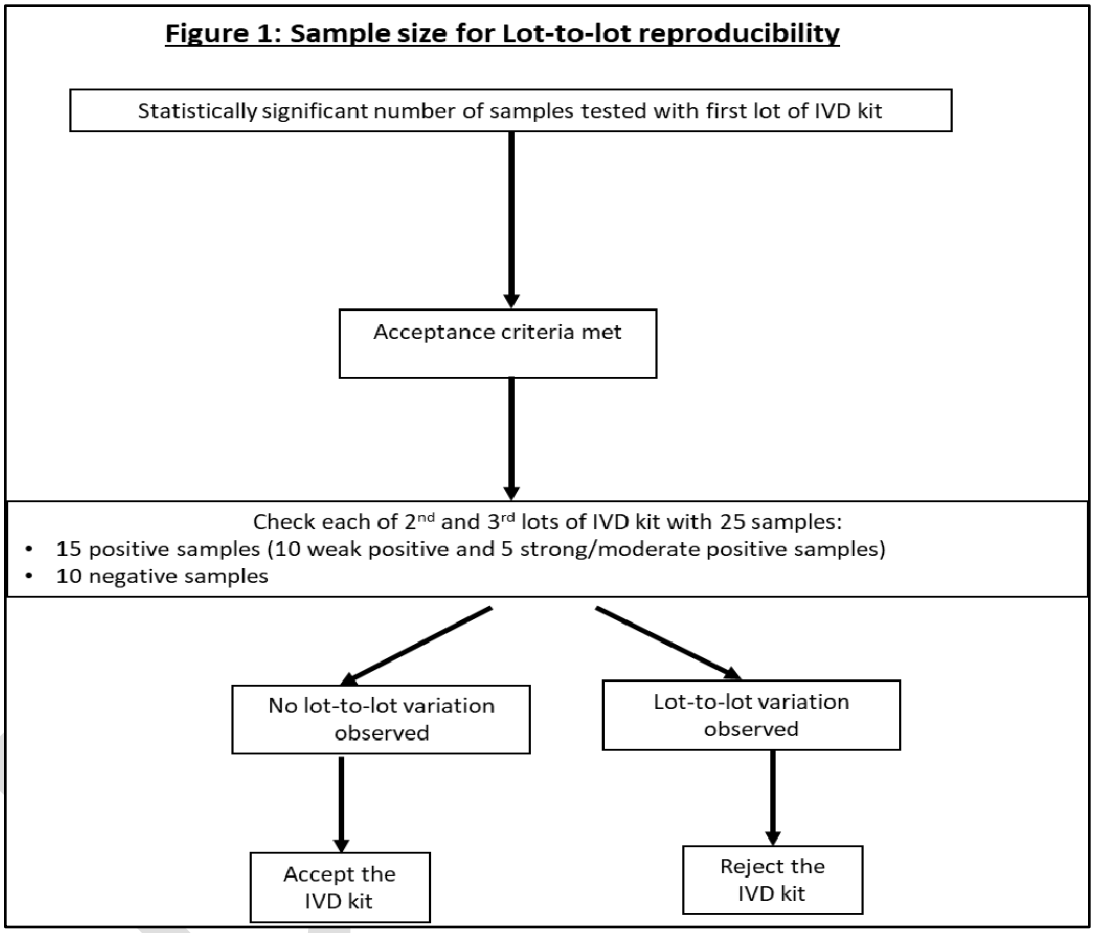
B. Reproducibility Assessment

Reproducibility testing should include the following:

a) Lot-to-lot reproducibility

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

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



- Second lot of the kit: should be tested on 25 samples (15 positive samples comprising 10 low positive AND 5 moderate/strong positive samples, and 10 negative samples).
- Third lot of the kit: should be tested on 25 samples (15 positive samples comprising 10 low positive AND 5 moderate/strong positive samples, and 10 negative samples).
- There should be no lot-to-lot variability (qualitative). Refer Figure 1.

b) Inter-Operator variability: Testing should be conducted by two different operators, keeping all other testing parameters undisturbed. Within-run and between run imprecision (if applicable) should be measured. 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test results (qualitative).

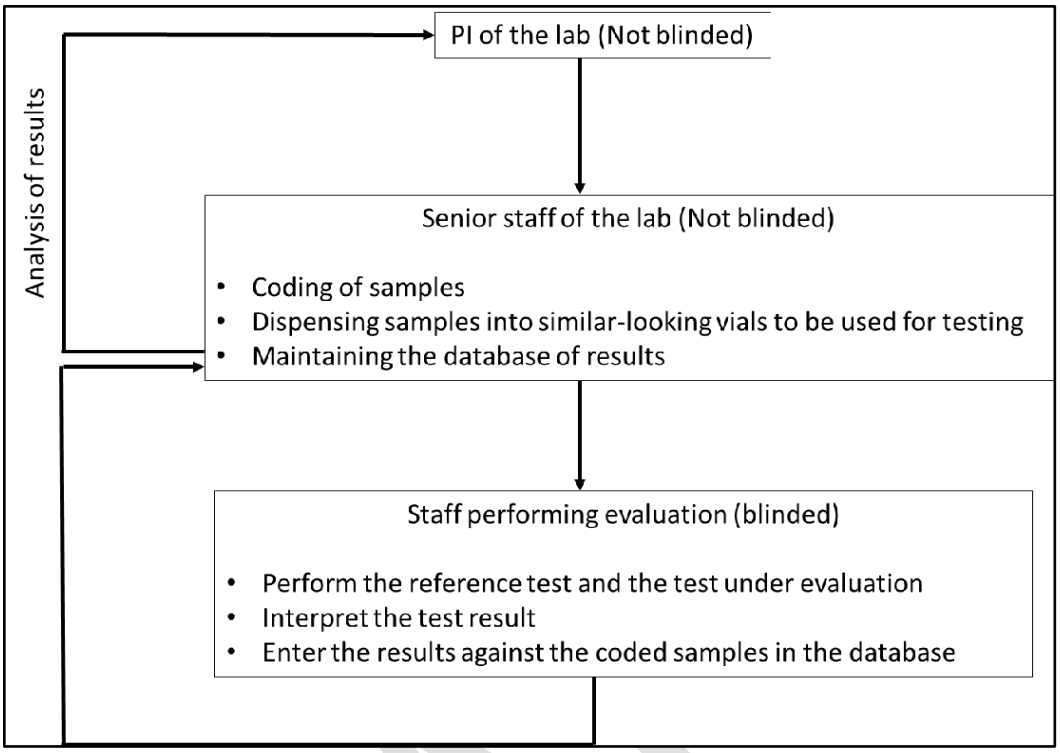
c) Day-to-day variability: Testing should be performed on at least two non-consecutive days. 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test result(qualitative).

d) Machine-to-machine variability: It is desirable (not mandatory) to evaluate the IVD Kit using two different manufacturer recommended platforms (if applicable). 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test results(qualitative).

10. Blinding of laboratory staff:

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

Figure 2: Blinding in evaluation exercise



11. Acceptance Criteria:

Sensitivity: ≥90%

Specificity: ≥95%

Cross-reactivity with other pathogens listed in the negative sample panel: Minimal

Invalid test rate: ≤5%

To achieve at least the performance characteristics outlined in the acceptance criteria, a minimum of 150 positive samples and minimum 80 negative samples should be used for evaluation.

12. Publication Rights:

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

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 for re-validation from the same manufacturer for the same test type will only be entertained after a minimum of 3 months and only if a high-level technical summary of

modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.

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

VI. References:

1. Kannan, Kavitha et al. "Performance of molecular and serologic tests for the diagnosis of scrub typhus." *PLoS neglected tropical diseases* vol. 14,11 e0008747. 12 Nov. 2020, doi:10.1371/journal.pntd.0008747
2. Kim, Young-Jin et al. "Clinical Evaluation of Rapid Diagnostic Test Kit for Scrub Typhus with Improved Performance." *Journal of Korean medical science* vol. 31,8 (2016): 1190-6. doi:10.3346/jkms.2016.31.8.1190
3. Kingston, Hugh W F et al. "Comparative Accuracy of the InBios Scrub Typhus Detect IgM Rapid Test for the Detection of IgM Antibodies by Using Conventional Serology." *Clinical and vaccine immunology : CVI* vol. 22,10 (2015): 1130-2. doi:10.1128/CVI.00390-15
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

VII. Performance Evaluation Report Format

PERFORMANCE EVALUATION REPORT FOR SCRUB TYPHUS 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	Sample type	
	Positive samples (provide details: strong, moderate, weak/simulated samples)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel/simulated samples)	

Results:

		Reference assay ……..…… (name)		
		Positive	Negative	Total
Name of Scrub typhus IgM RDT kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Cross-reactivity:
- Invalid test rate:

- Conclusion
    - Sensitivity, Specificity
    - Performance: **Satisfactory / Not Satisfactory**

(Sensitivity and specificity have been assessed in controlled lab setting on serum samples only, using kits provided by the manufacturer from the batch mentioned above. Results should not be extrapolated for any other sample type.)

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 ……..

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

Performance evaluation protocol for scrub typhus IgM ELISA Kits

I. Background:

CDSCO/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 in-vitro diagnostic kit (IVD) performance.

II. Purpose:

To evaluate the performance of *Orientia tsutsugamushi* IgM ELISA kits in the diagnosis of Scrub typhus infection using irreversibly de-identified leftover archived/ spiked clinical samples.

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. The accessories, including UPS are needed to be provided to the testing institute for the duration of the evaluation.
2. Evaluation sites/laboratories (With required equipment)
3. Reference test kits
4. Characterized evaluation panel
5. Laboratory supplies include the necessary labware and plasticware.

IV. Ethical approvals:

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

V. Procedure:

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

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

   Identified IVD kit evaluation laboratories should establish their proficiency through:

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

   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 *Orientia tsutsugamushi* IgM ELISA IVD kits received for performance evaluation (Verification/Storage/Unpacking etc).
- Testing, interpreting, recording of results & reporting
- Data handling, data safety & confidentiality

3. Preparation of *Orientia tsutsugamushi* IgM ELISA IVD kit evaluation panel

Well characterized *Orientia tsutsugamushi* IgM ELISA IVD kit evaluation panel is a critical requirement for performance evaluation of IVD kits. Hence statistically significant numbers of serum/ plasma samples should be collected from *Orientia tsutsugamushi* IgM confirmed cases. Further characterised for *Orientia tsutsugamushi* IgM positivity by using approved reference kits having high sensitivity and specificity.

Orientia tsutsugamushi IgM performance evaluation panel needs 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:

- Microimmunofluorescence (MIF) IgM assay (IVD kit) and/or Scrub Typhus Detect™ IgM ELISA (InBios International) should be used as the reference standard. US FDA-authorized/ PMDA Japan-approved/ ATAGI Australia-recommended, or WHO prequalified *Orientia tsutsugamushi* IgM ELISA/IFA/MIF may also be used as the reference standard, if available.
- Serostatus shall be determined by using the designated reference standard.
- Semi-quantification of scrub typhus IgM concentration (mandatory for identifying weakly reactive samples) should be carried out using MIF/well-characterized scrub typhus IFA test.
- Representation of samples positive for all four major *Orientia tsutsugamushi* serotypes (e.g., Karp, Kato, Gilliam, and TA763) is desirable but not mandatory, and is subject to sample availability.

5. Sample size for & Sample panel composition:

- **Sample size** is calculated with 95% sensitivity and 95% specificity of the index test, 95% confidence interval, absolute precision of 5%. Formulae used are as follows:

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

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

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

A minimum of 73 *Orientia tsutsugamushi* IgM positive samples and a minimum of 73 negative samples are calculated. Rounding the sample size, a total of 80 *Orientia tsutsugamushi* positive samples and a **minimum** of 80 negative samples are required for evaluation.

However, to adequately represent various subcategories within the negative sample panel for robust performance evaluation of scrub typhus IgM ELISA, 255 negative samples are recommended. The minimum and recommended number of negative samples have been calculated using the Wilson score interval, wherein it has been found that **255 negative samples significantly enhance statistical robustness and precision due to narrower interval estimates (10.20% vs 5.48%)**. *****

***** *With **a specificity assumption of 95%, a 95% CI, 5% precision,** the narrower interval for negative samples n = 255 (Interval estimate for True Specificity: [0.916, 0.971]) compared to n = 80(Interval estimate for True Specificity: [0.878, 0.980]) for the interval estimate of True Specificity were obtained. It reflects greater precision and reliability in estimating the true proportion of negative results. **The choice of n = 80 as the minimum negative sample size ensures baseline statistical validity**, allowing for the fulfilment of assumed specificity, CI, precision, while compensating for potential data losses from assumed level of invalid test rate. However, **to secure adequate representation for different categories of negative samples, increasing the negative sample size to n = 255 is found to be preferable**, as it would safeguard against fluctuations in test validity, ensuring robustness in results*

- **Seropositive panel** shall constitute samples reactive for *Orientia tsutsugamushi* IgM. The seropositive panel should be characterized as follows:
  1. Serology reactive by reference standard in strong clinical suspicion of scrub typhus (e.g.: presence of eschar)

     OR
  2. Sample testing positive by *Orientia tsutsugamushi* PCR (47 kDa/ 56 kDa protein gene) & seroreactivity by reference assay

     OR
  3. Serum sample collected from *Orientia tsutsugamushi* PCR confirmed cases of scrub typhus 10-14 days after PCR test.

The positive panel should have a minimum of 35% weakly reactive samples (MIF titers: 64 to 256)

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

Sensitivity	*Calculated sample size*	*Minimum no. of positive samples required (sample size rounded off) #*	*Sample panel composition*
99%	15	20	Strong/Moderate Positive: 13 Weak Positive: 7
95%	73	80	Strong/Moderate Positive: 52 Weak Positive: 28
90%	138	140	Strong/Moderate Positive: 95 Weak Positive: 55
85%	196	200	Strong/Moderate Positive: 135 Weak Positive: 75
80%	246	250	Strong/Moderate Positive: 165 Weak Positive: 95
The samples need to be classified as strong, moderate and weak positive based on ELISA units of the reference assay. *#It is recommended to calculate the sample size as per manufacturer's claims of sensitivity and specificity; however, a higher sample size is suggested to ensure adequate power of the study in case the kit falls short of claimed performance characteristics.*			

- **Seronegative panel (Recommended n=255, Minimum n= 80)**: The seronegative panel should be non-reactive for *Orientia tsutsugamushi* by the reference assay. The minimum number of seronegative samples required for testing is 80, however, 255 seronegative samples are recommended to adequately assess cross-reactivity and calculate the specificity of scrub typhus IVDs. The seronegative panel should constitute the following categories of samples:

1. *Acute Febrile illness cases negative for common etiologies of fever:* Samples from acutely febrile patients that are negative for etiologies such as Dengue, Chikungunya, Malaria, Leptospira (**Recommended n=60, Minimum n= 25**): *Refer Table 1 for details*
2. Samples non-reactive for Scrub typhus IgM but positive for other common etiologies of acute fever in India (**Recommended n=155, Minimum n= 30):** *Refer*

Table 1 for details. It is recommended to use as many numbers and types of cross-reactive samples as feasible.

3. Samples from healthy subjects from endemic regions: Serum/plasma samples testing seronegative for *Orientia tsutsugamushi* by reference test, IgM serology by Dengue, Chikungunya (**Recommended n=40, Minimum n= 25).** *Refer Table 2 for details.*

Table 2: Composition of *Orientia tsutsugamushi* seronegative panel

S. No.	Agent	Recommended no. of samples	Minimum no. of samples required
B.1 Acute febrile illness cases negative for common etiologies			
	Acute febrile illness cases negative for Dengue/ Chikungunya/ Malaria/ Leptospira	60	25
B.2 Samples non-reactive for Scrub typhus IgM but positive for other etiologies			
B 2.1	Chikungunya IgM	15	5
B 2.2	Malaria antigen (with representation from Pv and Pf)	25	10
B 2.3	Leptospira IgM	20	5
B 2.4	Blood sample from Pulmonary tuberculosis at the time of diagnosis	20	0
B 2.5	Spotted fever IgM	15	0
B 2.6	Dengue NS1	20	5
B 2.7	Dengue IgM	15	5
B 2.8	JE IgM	15	0
B 2.9	West Nile virus IgM	10	0
B.3 Samples from healthy subjects			
	Samples from healthy subjects from endemic regions (Serum/plasma		

	samples testing seronegative for *Orientia tsutsugamushi* by Reference test, IgM serology by Dengue, Chikungunya may be used)	40	25
GRAND TOTAL		**255**	**80**

6. Evaluation method:

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

7. Interpretation of results:

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

8. Resolution of discrepant results:

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

9. Repeatability and Reproducibility Assessment:

A. Repeatability Assessment

This should be done to assess the repeatability of the detection of target analyte using the kit under evaluation.

3 positive samples (strong, moderate and weak positive samples) and 3 negatives samples should be tested 5 times in independent runs.
Concordance should be 100% based on positive and negative test results(qualitative).

B. Reproducibility Assessment

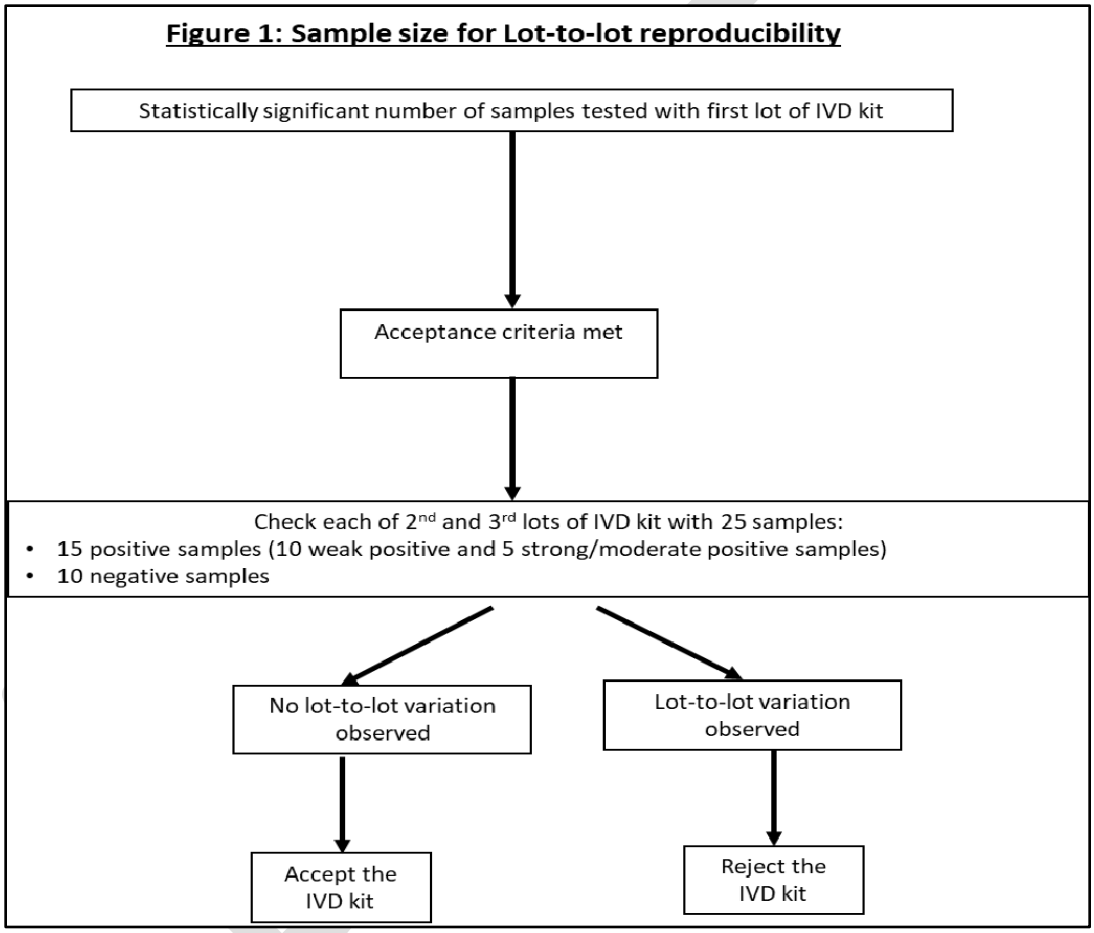
Reproducibility testing should include the following:

a. Lot-to-lot reproducibility

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

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

Figure 1: Sample size for Lot-to-lot reproducibility



- Third lot of the kit: should be tested on 25 samples (15 positive samples comprising 10 low positive AND 5 moderate/strong positive samples, and 10 negative samples).
- There should be no lot-to-lot variability (qualitative). Refer Figure 1.

b. Inter-Operator variability: Testing should be conducted by two different operators, keeping all other testing parameters undisturbed. Within-run and between run imprecision (if applicable) should be measured. 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test results (qualitative).

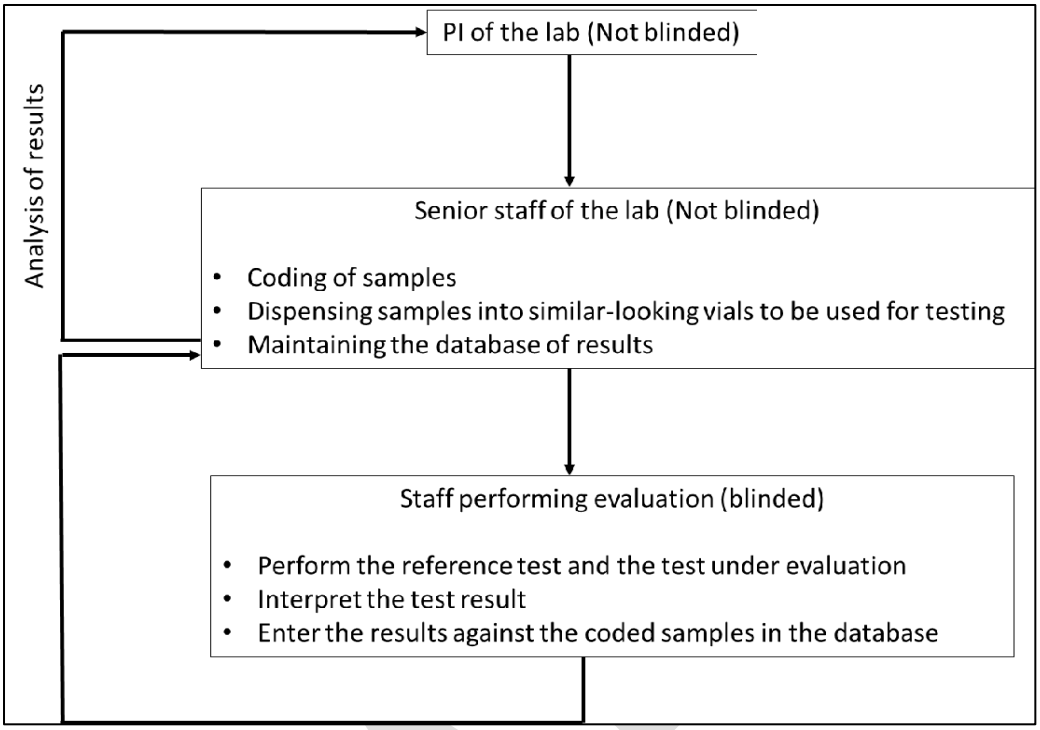
c. Day-to-day variability: Testing should be performed on at least two non- consecutive days. 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test result(qualitative).

d. Machine-to-machine variability: It is desirable (not mandatory) to evaluate the IVD Kit using two different manufacturer recommended platforms (if applicable). 3 positive samples (strong, moderate and weak positive samples) and 3 negative samples should be tested 5 times in independent runs. Concordance should be 100% based on positive and negative test results(qualitative).

10. Blinding of laboratory staff

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

Figure.2: Blinding in evaluation exercise



11. Acceptance Criteria:

Sensitivity: ≥ 95%
Specificity: ≥ 95%
Cross-reactivity with other pathogens listed in the negative sample panel: Minimal
Invalid test rate: ≤5%

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

12. Publication Rights:

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

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 for re-validation from the same manufacturer for the same test type will only be entertained after a minimum of 3 months and only if a high-level technical

summary of modifications or functional improvements to the kit design is submitted, without explicit disclosure of proprietary information.

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

VI. References:

1. Kannan, Kavitha et al. "Performance of molecular and serologic tests for the diagnosis of scrub typhus." *PLoS neglected tropical diseases* vol. 14,11 e0008747. 12 Nov. 2020, doi:10.1371/journal.pntd.0008747
2. Kim, Young-Jin et al. "Clinical Evaluation of Rapid Diagnostic Test Kit for Scrub Typhus with Improved Performance." *Journal of Korean medical science* vol. 31,8 (2016): 1190-6. doi:10.3346/jkms.2016.31.8.1190
3. Kingston, Hugh W F et al. "Comparative Accuracy of the InBios Scrub Typhus Detect IgM Rapid Test for the Detection of IgM Antibodies by Using Conventional Serology." *Clinical and vaccine immunology: CVI* vol. 22,10 (2015): 1130-2. doi:10.1128/CVI.00390-15
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

VII. Performance Evaluation Report Format

PERFORMANCE EVALUATION REPORT FOR SCRUB TYPHUS 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	Sample type	
	Positive samples (provide details: strong, moderate, weak/simulated samples)	
	Negative samples (provide details: clinical/spiked, including cross reactivity panel/simulated samples)	

Results:

		Reference assay ……..…… (name)		
		Positive	Negative	Total
Name of Scrub typhus IgM ELISA kit	Positive			
	Negative			
	Total			

	Estimate (%)	95% CI
Sensitivity		
Specificity		

- Cross-reactivity:

- Invalid test rate:

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

(Sensitivity and specificity have been assessed in controlled lab setting on serum samples only, using kits provided by the manufacturer from the batch mentioned above. Results should not be extrapolated for any other sample type)

Disclaimers

1. ICMR's validation process does not approve / disapprove the kit design
2. ICMR's 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 ……

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

Annexure-1: Information on Operational and Test Performance Characteristics Required from Manufacturers for Scrub Typhus IVD

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

1. Instructions for Use

2.Scope of the IVD

3. Intended Use Statement

4. Principle of the assay

5. Intended testing population

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

11. 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

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.