TRUSTwell®

Scrub Typhus IgM ELISA Kit

REF

AE0620

IVD

- 96-well ELISA kit for the qualitative determination of the Scrub Typhus IgM antibodies in human serum or plasma
- Store at 2-8°C upon receipt

INTENDED USE

The TRUSTwell Scrub Typhus IgM ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of Scrub Typhus IgM antibodies in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with Scrub Typhus. Any reactive specimen with the TRUSTwell Scrub Typhus IgM ELISA Test must be confirmed with alternative testing method(s) and clinical findings.

INTRODUCTION

TRUSTwell Scrub Typhus(ST) is caused by infection with the obligate, intracellular, gram-negative bacterium Orientia tsutsugamushi. Globally, over one billion people are at risk for Scrub Typhus and an estimated one million cases occur annually. Karp strain is predominant in India other than that Kato, Gilliam, Boryong, Kawasaki and other 30 are the most habitual serotypes of O.tsutsugamushi. The World Health Organization has dubbed Scrub Typhus is one of the world's most underdiagnosed diseases that often requires hospitalization highlighting the necessity for a better understanding of the vectors, outbreaks, and pathogenesis associated with this potentially fatal organism that has been linked to human cases both within and beyond its previously recognized region of endemicity.

In India, Scrub Typhus is a major public health threat and it has been reported from Tamilnadu, Andhra Pradesh, Karnataka, and Kerala in the South, Himachal Pradesh, Uttaranchal, Jammu, and Kashmir in the North, Meghalaya, Assam, and Nagaland in the North-East, West Bengal and Bihar in the East, and Maharashtra and Rajasthan in the West (Devasagayam et al., 2021). While Scrub Typhus is confined geographically to the Asia Pacific region, and mortality rates for Scrub Typhus range from < 1% to 50% it may increase depends upon the age. It also depends on proper antibiotic treatment, status of the individual infected, and the strain of O. tsutsugamushi encountered (kala et al., 2020). In addition, few cases have been tested positive for IgM antibodies for scrub typhus from Sikkim, Darjeeling, Manipur.

ELISA detects IgM antibodies against the 56kDA antigen, the major immunodominant protein located on its cell envelope. (Martina L.Jones et al., 2007) Sera from 95–99% of patients with a history of scrub Typhus react strongly with this protein, which reflects the abundance of the molecule on the cell surface and its high immunogenicity. (Sheng Ni et al., 2005) After the onset of symptoms, the IgM antibody titres increased gradually over 2–3 weeks, peaked at about 4 weeks. The onset of disease is characterized by fever, headache, myalgia, cough, and gastrointestinal symptoms.

The TRUSTwell Scrub Typhus IgM utilizes recombinant Scrub Typhus antigens for the detection of IgM antibodies associated with the Scrub Typhus

TEST PRINCIPLE

TRUSTwell Scrub Typhus IgM ELISA test is a solid phase enzyme linked immunosorbent assay based on the principle of Indirect immunoassay technique for the detection of IgM antibodies of scrub Typhus in human serum/plasma.

The TRUSTwell Scrub Typhus IgM ELISA is composed of two key components:

1. Solid microwells are precoated with recombinant Scrub Typhus antigens
2. Liquid and conjugate composed of Antihuman IgM Antibody and with horseradish peroxidase (HRP- Anti- Human IgM conjugates);

During the assay, the test specimen is first incubated with the coated microwells, If IgM antibodies present in the specimen, binds to antigen coated microwell plate. In the second incubation with the HRP antihuman IgM conjugates,Scrub Typhus IgM antibodies absorbed on the surface of microwell reacts to conjugates forming a complexed conjugate. Unbound conjugates are removed by washing. The presence of the complexed conjugate is shown by a blue colour upon addition of TMB substrate. The reaction is stopped with stop solution and absorbance are read using a spectrophotometer at 450/620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit:

Item	Description	Quantity	Catalog
1	Microwells coated with recombinant Scrub Typhus Antigens	8 wells x 12 strips	AE0620W
2	ST IgM Positive control	0.75 mL	AE0620P
3	ST IgM Negative control	0.75 mL	AE0620N
4	Sample Diluent	60 mL	AE0620SD
5	HRP-antihuman IgM Conjugate	12 mL	AE0620H
6	Wash Buffer (30x Concentrate)	20 mL	AWE3000
7	TMB Substrate A	6 mL	ATME2000A
8	TMB Substrate B	6 mL	ATME2000B
9	Stop Solution	12 mL	ASE1000
10	ELISA working Sheet	2 Nos	AE0001ES
11	Product Insert	1 No	PI-AE0620
12	Sealant	3 Nos	N/A
13	Dessicant	3 Nos	N/A

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 10 µL, 50 µL and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable
3. Absorbent paper for blotting the microplate wells.
4. Parafilm or other adhesive film sealant for sealing plate.
5. Timer.
6. Distilled or de-ionized water.

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before opening. After removing the desired number of wells, place unused wells in the resealable plastic bag provided with desiccant and return to 2-8°C. All reagents are stable through the expiration date printed on the label if not opened. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the expiration of the label.

WARNING AND PRECAUTIONS

For in Vitro Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired devices.
3. Bring all reagents to room temperature (18°C-28°C) before use.
4. Do not use the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
13. The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16. Avoid strong light during color development.

SPECIMEN COLLECTION AND PREPARATION

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with serum or plasma specimen without additives only.
3. If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
4. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
5. Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (20 - 25 °C).
   Preparation of Scrub Typhus IgM specimens:
2. Dilute the patient specimens 100 fold with Sample Diluent: (**e.g. to 5 µL of specimen, add 500 µL Sample Diluent.)**
3. Dilute concentrated Wash Buffer 30 fold with water as following:

Plate	DI Water	30x Wash Buffer	Final Volume
Full Plate	580mL	20mL	600mL
Half Plate	290mL	10mL	300mL
A Quarter Plate	145ml	5mL	150mL

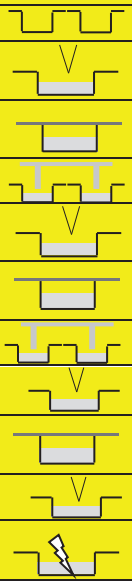
   Warm up the concentrated Wash Buffer at 37˚C to dissolve the precipitant if it appears.
4. Mix each reagent before adding to the test wells.
5. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microwell frame. Reseal un-used strips.
2. Add specimens according to the designation on the ELISA Working Sheet
   2.1 Blank well: Leave the blank well alone. Don't add any reagents.
   2.2 Control wells: Add 100 µL of Positive, Negative Control into the designated control wells, respectively.
   2.3 Test wells: Add **100 µL of diluted patient specimen(e.g. to 5 µL of specimen, add 500 µL Sample Diluent.)** to all the test wells
   To ensure better precision, use pipette to handle solution.
3. Gently rock the plate wells for 20 seconds, then cover the plate with sealant.
4. Incubate the wells at room temperature(20 - 25 °C) for 30 minutes.
5. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer (350µL per well) and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6. Add 100 µL of HRP- anti-human IgM conjugates into each well except the blank well, cover the plate, and incubate at room temperature(20 - 25 °C) for 30 minutes.

7. Wash the plate 5 times as described in step 5.
8. Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the blank well.
9. Incubate at room temperature (20-25°C) in dark for 10 minutes.
10. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20-30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Set the microplate reader wavelength at 450nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620 - 690nm can be used as a reference wavelength to optimize the assay result.

Flow chart of assay procedure

		
1.	Secure strips in microwell frame	Number of strips
2.	Add controls and diluted specimens. Gently rock	100 µL
3.	Incubate	RT(20-25°C), 30 minutes
4.	Wash: manual or automatic	5 times, 350 µL /well
5.	Add HRP conjugate	100 µL
6.	Incubate	RT(20-25°C), 30 minutes
7.	Wash: manual or automatic	5 times, 350 µL /well
8.	Add TMB Substrate A and B Gently rock	50 µL + 50 µL
9.	Incubate in dark	RT (20-25°C) 10 minutes
10.	Add Stop Solution. Gently rock	100 µL
11.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A.Set up the cut-off value

The cut-off value = 0.20 + NC

NC: Mean OD of the Negative Control.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off value}}$$

C. Assay Validation

The mean OD value of the positive controls should be ≥ 0.50

The mean OD value of the negative controls should be ≤ 0.10

D. Interpretation of the results

Specimen OD ratio

Negative < 1.0

Positive ≥ 1.0

If above specification are not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

1.A negative result indicates that there is no detectable Scrub Typhus IgM antibody in the specimen.

2. Results just below the cut-off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).

3.Specimens with OD ratio ≥ 1.0 are initially considered to be positive by the TRUSTwell Scrub Typhus IgM ELISA Kit. They should be retested in duplicate before a final interpretation is made.

If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with theTRUSTwell Scrub Typhus IgM ELISA Kit.
Non-repeatable reactions are often caused by:

- Inadequate microwell washing,
- Contamination of negative specimens by serum or plasma with a high antibody titer,
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.),
- Contamination of the Stop Solution.

If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the TRUSTwell Scrub Typhus IgM ELISA Kit, subject to the limitations of the procedure, described below.

PERFORMANCE CHARACTERISTICS

1) Clinical Performance

A total of 1232 patient specimens from susceptible subjects were tested by the TRUSTwell Scrub Typhus IgM ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell Scrub Typhus IgM ELISA Kit		
Reference ELISA	**Positive**	**Negative**	**Total**
Positive	48	0	48
Negative	5	1179	1184
Total	53	1179	1232

Relative Sensitivity:100%, Relative Specificity:99.57 %, Overall Agreement:99.59 %

2) Precision

Intra-assay precision was determined by assaying 16 replicates of three weak positives and three strong positives. The mean, SD & CV determined. The results are shown in the following table.

Specimens	Runs	Mean OD	SD	CV %
Strong Positives	16	2.039	0.071	3
Weak Positives	16	0.670	0.035	5

Inter assay precision was determined by assaying 3 patients specimens in 16 seperate run of weak positives and strong positives. The mean, SD & CV determined. The results are shown in the following table.

Specimens	Runs	Mean OD	SD	CV %
Strong Positives	16	2.003	0.060	3
Weak Positives	16	0.697	0.060	9

3) Cross-reactivity

No Cross-reactivity Scrub Typhus IgM ELISA test results were observed on positives specimens from each of the following disease states or special conditions, respectively:
Malaria / Dengue IgM / Covid IgG / Typhoid / HBsAg / HCV / HIV / Syphilis

4) Interference

Common substances (such as pain and fever medication and blood components) may affect the performance of the TRUSTwell Scrub Typhus IgM ELISA Kit. Interference was studied by spiking these substances into 3 clinical specimens: negative, Scrub Typhus low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TRUSTwell Scrub Typhus IgM ELISA Kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34 mmol/L
2. Sodium citrate 1.3 %
3. Creatinine 442 µmol/L
4. EDTA 3.4 µmol/L
5. Glucose 55 mmol/L
6. Heparin 3,000 U/L
7. Bilirubin 10 mg/dL

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of Scrub Typhus IgM antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell Scrub Typhus IgM ELISA Kit is limited to the qualitative detection of Scrub Typhus IgM antibodies in human serum or plasma. The intensity of the color does not have a linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates the absence of detectable Scrub Typhus. However, a negative test result does not preclude the possibility of infection with Scrub Typhus.
4. A negative result can occur if the quantity of Scrub Typhus present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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3. Devasagayam, E., Dayanand, D., Kundu, D., Kamath, M. S., Kirubakaran, R., & Varghese, G. M. (2021). The burden of scrub Typhus in India: A systematic review. PLoS neglected tropical diseases, 15(7), e0009619.
4. Kala, D., Gupta, S., Nagraik, R., Verma, V., Thakur, A., & Kaushal, A. (2020). Diagnosis of scrub Typhus: recent advancements and challenges. 3 Biotech, 10(9), 396.

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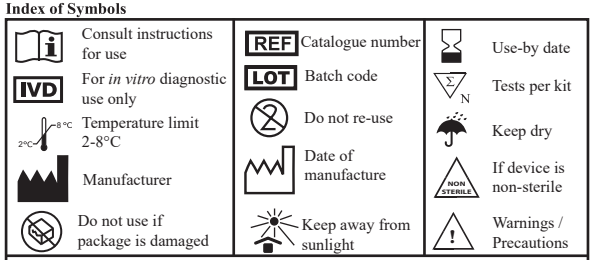

ATHENESE-Dx
Athenese-Dx Pvt. Ltd.
Module No. 407 & 408,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

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