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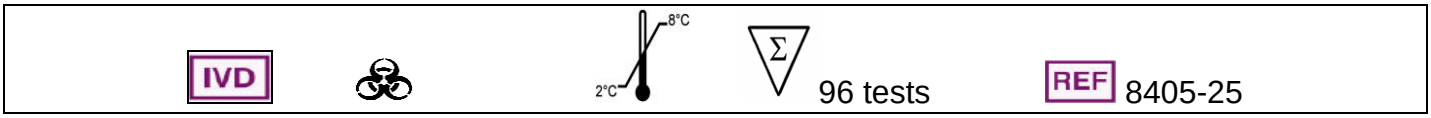


Scrub Typhus IgM ELISA

REF 8405-25

For Research Use Only

Test	Scrub Typhus IgM ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Membrane-based immunoassay
Detection Range	Qualitative: Positive & Negative Control
Sample	100µL
Specificity	Not Determined
Sensitivity	Not Determined
Incubation Time	~75 min

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

Intended Use

The Scrub Typhus *IgM* ELISA test is an ELISA assay system for the detection of IgM antibodies in human serum to *Orientia tsutsugamushi* (OT; formerly *Rickettsia*) derived recombinant antigen (1-10). This test is to aid in the diagnosis of human exposure to OT species. It is not intended to screen blood or blood components. For research use only.

Summary and Explanation of the Test

Scrub Typhus is an infectious disease that is caused by Orientia tsutsugamushi (formerly Rickettsia), a tiny parasite about the size of bacteria that belongs to the family Rickettsiaceae. A bite from the larval trombiculid mite, a parasite of rodents, will transmit the disease. An ulcer of the skin is characteristic of a bite from a trombiculid mite, followed by symptoms including fever, a spotted rash on the torso, and swelling of the lymph glands. Scrub typhus generally occurs after exposure to areas with secondary (scrub) vegetation, which is where its name is derived from. However, the disease can also be prevalent in sandy, mountainous, and tropical areas. Scrub Typhus is a world wide illness, but particular to South East Asia and the Western Pacific. It accounts for approximately 20% of fever in some regions in South East Asia, where it is endemic. Illness lasts for a period of 10 to 12 days after the initial bite. With therapy, the fever will break within 36 hours, but if left untreated, complications or death may occur.

Principle of the Test

The Scrub Typhus ELISA system for IgM Test is a qualitative ELISA for the detection of IgM antibodies to O. tsutsugamushi (OT) in serum. Wells of each plate have been coated with unique recombinant antigen mix. During testing, the serum samples are diluted in DAI sample diluent and applied to each well. See "Example for Sera Application" below. After incubation and washing, the wells are treated with polyclonal Goat anti-human IgM antibodies labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450nm. The absorbance measured is directly proportional to the concentration of IgM antibodies to OT present. A set of positive and negative controls are provided as internal controls. These are provided to monitor the integrity of the kit components.

Materials Supplied

The Scrub Typhus ELISA system for IgM (1 x 96 Wells) contains sufficient reagents for 96 wells. Each kit contains the following reagents:

Warning:

- Do not use any reagents where damage to the packaging has occurred.
- Controls(*) must be centrifuged for 10 seconds at high speed prior to opening the vial to avoid loss of contents.

1. **Scrub Typhus ELISA Plate:**
   One strip holder in ziplock foil, containing 96 polystyrene microtiter wells coated with OT derived recombinant antigens in each well. Stable at 2-8°C until the expiration date.
2. **Sample Dilution Buffer for Scrub Typhus:**
   Two bottles, 25 mL each, to be used for preparing sample dilutions. A slight precipitate may form. Mix gently before use. Stable at 2-8°C until the expiration date.
3. **Scrub Typhus IgM Positive Control:**
   One vial, 50 µL. The positive control will aid in monitoring the integrity of the kit as well. Stable at 2°C - 8°C until the expiration date. Before use, quickly centrifuge the vial so that contents can be collected at the bottom.
4. **Scrub Typhus Negative Control:**
   One vial, 50 µL. The negative control will aid in monitoring the integrity of the kit. This is stable at 2-8°C until the expiration date. Before use, quickly centrifuge the vial so that

contents can be collected at the bottom.

5. **Ready to Use Enzyme Conjugate-HRP for Scrub Typhus IgM:**
   One bottle, 12 mL of a pre-diluted conjugate to be used as is in the procedure below. Stable at 2-8°C until the expiration date.
   Note: The conjugate should be kept in a light-protected bottle at all times as provided.
6. **10X Wash Buffer:**
   One bottle, 120 mL of 10X concentrate Wash Buffer to be diluted and used in all the washing steps of this procedure. Stable at 2-8°C until the expiration date.
   Note: See Preparation of Reagents in Test Procedure section to prepare 1X Wash Buffer.
7. **Wash Solution:** One bottle, 20 mL of Wash Solution to be used following the post enzyme conjugate-HRP washes but prior to the addition of liquid TMB Stable at 2-8°C until the expiration date.
8. **Liquid TMB Substrate:**
   One bottle, 12 mL of liquid substrate to be used in this procedure. Stable at 2-8°C until the expiration date.
   Note: The substrate should be kept in a light -protected bottle at all times.
9. **Stop Solution:**
   One bottle, 6 mL to be used to stop the reaction. Stable at 2-8°C until the expiration date.
   Caution: strong acid, wear protective gloves, lab coat and safety goggles. Dispose of all materials according to safety rules and regulations.

NOTE: *All reagents and controls must be allowed to reach room temperature (20℃~25℃) and mixed thoroughly by gentle inversion prior to use.*

Materials required but not supplied

1. Microtiter plate reader capable of absorbance measurement at 450 nm
2. Biological or High-Grade Water
3. 37°C Humidified Incubator without CO_2 supply.
   Note: Humidification can be achieved using a water tray at the bottom of incubator.
4. Plate washer
5. Multi-channel pipettors
6. Timer

Precautions

General Precautions

- A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.
- Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
- Cover working area with disposable absorbent paper.

Sample Precautions

1. All human source materials used in the preparation of controls have tested using FDA approved methods for antibody to HIV 1&2, Hepatitis C and Hepatitis B surface antigen and found to be negative. However, no test method can offer complte assurance and all human controls and antigen should be handled as potentially infectious material. The Center for Disease Control and the National Institute of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.

2. This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established.
3. It is advised that icteric or lipaemic sera, or sera exhibiting hemolysis or microbial growth not be used.
4. Do not heat inactivate sera.
5. Dispense reagents directly from bottles using clean pipette tips. Transferring reagents may result in contamination.
6. To avoid cross contamination a new pipette tip must be used for dispensing each control and test sera.

Kit Reagents Precautions

1. All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
2. Dispense reagents directly from bottles using clean pipette tips. Transferring reagents may result in contamination.
3. Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do this may cause erroneous results.
4. Substrate System:
   (a) As the Liquid TMB Substrate is susceptible to contamination from metal ions, do not allow the substrate system to come into contact with metal surfaces.
   (b) Avoid prolonged exposure to direct light.
   (c) Some detergents may interfere with the performance of the Liquid TMB Substrate.
   (d) The Liquid TMB Substrate may have a faint blue color. This will not affect the activity of the substrate or the results of the assay.
5. Do not mix lots of any kit component within an individual assay microtiter plate.
6. Do not use any component beyond the expiration date shown on its label.
7. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
8. Some reagents may form a slight precipitate, mix gently before use.
9. Incomplete washing will adversely affect the outcome and assay precision.
10. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the Liquid TMB Substrate solution.
11. Avoid microbial contamination of reagents, especially of the Ready to use Enzyme Conjugate-HRP. Avoid contamination of the Liquid TMB Substrate Solution with the Ready-to-Use Enzyme Conjugate-HRP.
12. Do not use a humidified chamber for 37°C incubations, as this may affect assay performance.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD:

Material Safety Data Sheets (MSDS) are available for all components of this kit. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

Specimen Collection and Preparation

1. Human serum must be used with this assay. Whole blood or plasma cannot be tested directly.

2. Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
3. Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
4. Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 48 hours or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
5. Do not use hemolyzed or lipemic samples.
6. Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
7. If sera are shipped, pack in compliance with Federal Regulations covering transportation of infectious agent.

Caution: This kit has not been optimized by DAI for use with any particular automated ELISA processing system. Use with automated ELISA processing will require proper validation to ensure results are equivalent to the expectations described in this package insert. Modifications to the protocol of these systems and/or different volumes may be required.

Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion.

PREPARATION OF REAGENTS

1. 1X Wash Buffer
   Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water (Mix the provided 120mL of 10X Wash Buffer with 1080mL of Biological or High-Grade Water). After diluted to 1X, store at room temperature for a maximum of six months.
   Note: Discard the 1X Wash Buffer if any microbial growth is observed.
2. Microtiter Wells
   Select the number of coated wells required for the assay. The remaining unused wells should be placed back into the pouch, sealed with desiccant, and stored at 2-8°C until ready to use or expiration.

Note: For long-term storage, sample sera cannot be repeatedly thawed and frozen. Sample sera should be further aliquoted in a smaller volume and stored at –20°C to -70°C.

Assay Procedure

Allow all reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Positive, negative controls and unknowns should be assayed in duplicate. Test samples may be assayed in singlet.

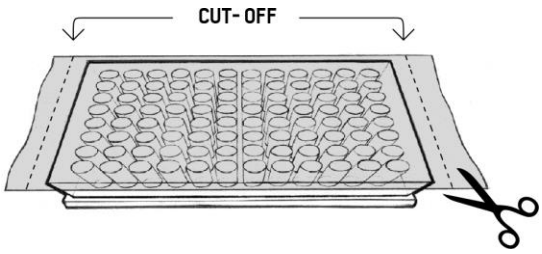
1. Determine number of sera to be tested.
2. Organize sera according to the “Example for Sera Application” provided below or any preferred arrangement. Dilution can be made either in tubes or in ELISA type plastic wells (Untreated plastics; not provided).

Example for Sera Application, 1/100 Diluted Samples, 100 µl /Well

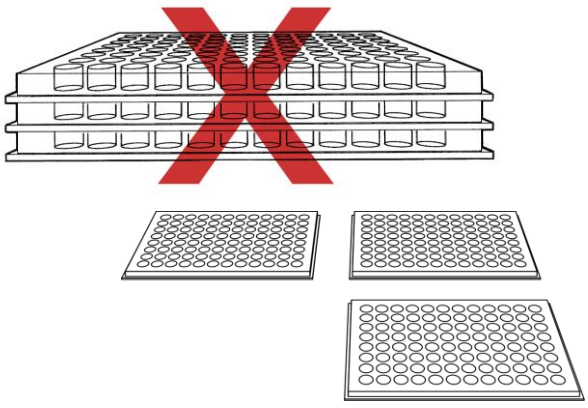
Example for Serum Sample Application												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**Neg. Con**	**Neg Con**	**S# 13**	**S# 21**	**S# 29**	**S# 37**	**S# 45**	**S# 53**	**S# 61**	S# 69	S# 77	**S# 85**

B	**Pos. Con.**	**Pos. Con.**	**S# 14**	**S# 22**	**S# 30**	**S# 38**	**S# 46**	**S# 54**	**S# 62**	**S# 70**	**S# 78**	**S# 86**
C	**S# 1**	**S# 7**	**S# 15**	**S# 23**	**S# 31**	**S# 39**	**S# 47**	**S# 55**	**S# 63**	**S# 71**	**S# 79**	**S# 87**
D	**S# 2**	**S# 8**	**S# 16**	**S# 24**	**S# 32**	**S# 40**	**S# 48**	**S# 56**	**S# 64**	**S# 72**	S# 80	**S# 88**
E	**S# 3**	**S# 9**	**S# 17**	**S# 25**	**S# 33**	**S# 41**	**S# 49**	**S# 57**	**S# 65**	**S# 73**	**S# 81**	**S# 89**
F	**S# 4**	**S# 10**	**S# 18**	**S# 26**	**S# 34**	**S# 42**	**S# 50**	**S# 58**	**S# 66**	**74**	**S# 82**	**S# 90**
G	**S# 5**	**S# 11**	**S# 19**	**S# 27**	**S# 35**	**S# 43**	**S# 51**	**S# 59**	**S# 67**	**S# 75**	**S# 83**	**S# 91**
H	**S# 6**	**S# 12**	**S# 20**	**S# 28**	**S# 36**	**S# 44**	**S# 52**	**S# 60**	**S# 68**	**S# 76**	**S# 84**	**S# 92**

3. Dilute test sera to 1/100 by using the provided Sample dilution Buffer for Scrub Typhus (you can use the proportion such as 4µl of serum plus 396 µl of Sample dilution Buffer for Scrub Typhus). Mix well.
   Note: *Do not use less than 4µl of serum and controls.*
4. Apply 100µl per well of the 1/100 diluted test sera and controls to marked Scrub Typhus ELISA plate.
5. Cover the plate with parafilm or plate covers just on the well opening surface, so the bottom of the plate is not covered. (Please read the important note below.) Incubate the plate at 37°C for 30 minutes in a incubator.



Note: *This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm should be cut-off once the top is sealed to block evaporation.*
Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO_2, or any other gases used for tissue culture*



6. After the incubation is complete wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer. Use 300µl per well of 1X Wash Buffer in each wash cycle for all plate washing.
7. Add 100µl per well of Ready to Use Enzyme-HRP Conjugate for Scrub Typhus IgM into all wells by multi-channel pipettor.
8. Cover the plate with parafilm just on the well opening surface, so the bottom of the plate is not covered. (*as described in step 5)*
9. Incubate the plate at 37°C for 30 minutes in a incubator.
10. After the incubation, wash the plate 6 times with automatic plate washer using 1X wash buffer, 300 µl per well.
11. Add 150µl per well of Wash Solution into all wells by a multi-channel pipette.
12. Incubate the plate at room temperature (20-25°C) for 5 minutes.
13. After the incubation, wash the plate 6 times with automatic plate washer using 1X wash buffer, 300 µl per well.
14. Add 100 µl per well of Liquid TMB substrate into all wells using a multi-channel pipettor.
15. Incubate the uncovered plate at room temperature (20-25°C) in a dark place (or container) for 10 minutes .
16. After the incubation, add 50 µl per well of Stop Solution into all wells by multi-channel pipettor and Incubate the plate uncovered at room temperature (20-25°C) for 1 minute .

Note: Care should be taken to apply Stop Solution at the same speed and order as Liquid TMB Substrate for accurate results.

17. After the incubation, read the OD 450nm value with a Microtiter plate reader.

Note: Do not subtract any background.

Results

Calculation of Cut-off value:

Calculation of the cut-off value requires determining the average of OD plus three times of the Standard Deviation (SD) of normal human serum and/or human sera with unrelated infections.

Note: Cut off values have not been determined using a large population. Therefore, it is preferred that the end users calculate their cut-off using geographically relevant serum samples.

Interpretation of the results:

1. Samples with spectrophotometric readings > Cut off are considered to be “Reactive” and samples below this criterion are considered to be “Non-Reactive”.
2. Any “Reactive” sample must be repeated to verify the result. Values near the Cut off are considered to be doubtful and the assay must be repeated in triplicate or more.

Ensuring Assay Performance:

The results on the table below must be obtained using provided positive and negative control to calculate discrimination capacity of the assay: Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor	Tolerance
Negative Control(NC)	< 0.200
Positive Control(PC)	> 0.500
Discrimination Capacity ($R_{PC/NC}$)	≥ 5.0

Limitations

1. Serological cross-reactivity across the mycobacterium group may be present.
2. The reagents supplied in this kit are optimized to measure OT-derived antigen reactive antibody levels in serum.
3. Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided. Do not freeze the liquid TMB substrate.
4. Hemolyzed and lipemic specimens may give false values and should not be used.
5. The assay performance characteristics have not been established for visual result determination.

6.Serum and Plasma Comparisons: The assay described here has been optimized with serum. Care should be taken on the quality of sample. Particulate, lipemic, and aged samples should not be used. Use of freshly drawn sample is preferred.

References

1. Kelly, D. J., A. L. Richards, J. Temenak, D. Strickman, G. A. Dasch. 2001. The present and past challenge of rickettsial disease to military medicine and public health. Clin Infect Dis 2002;34(suppl 4):s145-s169.
2. Weddle, J. R., T. C. Chan, K. Thompson, H. Paxton, D. J. Kelly, G. Dasch, and D. Strickman. 1995. Effectiveness of a dot-blot immunoassay of anti-Rickettsia tsutsugamushi antibodies for serologic analysis of scrub typhus. Am. J. Trop. Med. Hyg. 53:43-46, 12.
3. Kelly, D. J., D. Marana, C. Stover, E. Oaks, and M. Carl. 1990. Detection of Rickettsia tsutsugamushi by gene amplification using polymerase chain reaction techniques. Ann. N. Y. Acad. Sci. 590:564-571.
4. Tamura, A., H. Sakurami, K. Takahashi, and M. Oyanagi. 1985 Analysis of polypeptide composition and antigenic components of Rickettsia tsutsugamushi by polyacrylamide gel electrophoresis and immunoblotting. Infect. Immum. 48:671-675
5. Ohashi, N., A. Tamura, M. Ohta, and K. Hayashi. 1989. Purification and partial characterization of a type-specific antigen of Rickettsia tsutsugamushi. Infect. Immun. 57:1427-1431. 20.Eisemann, C. S., and J. V. Osterman. 1981.
6. 21.Kim, I-S., S-Y. Seong, S-G. Woo, M-S. Choi, and W-H. Chang. 1993. High-level expression of a 56-kilodalton protein gene (bor56) of Rickettsia tsutsugamushi Boryong and its application to enzyme-linked immunosorbent assays. J. Clin. Microbiol. 31:598-605.
7. Kim, I-S., S-Y. Seong, S-G. Woo, M-S. Choi, and W-H. Chang. 1993. Rapid diagnosis of scrub typhus by a passive hemagglutination assay using recombinant 56-kilodalton polypeptide. J. Clin. Microbiol. 31:2057-20606.
8. Ching, W-M., H. Wang, C. Eamsila, D. Kelly, and G. A. Dasch. 1998.Expression and refolding of truncated recombinant major outer membrane protein antigen (r56) of Orientia 25.Land MV, Ching WM, Dasch GA, Zhang Z, Kelly DJ, Graves SR, Devine PL (2000). Evaluation of a Commercially-Available Recombinant Protein ELISA for Detection of Antibodies Produced in Scrub Typhus Rickettsial Infections. J. Clin. Micro. 38:2701-2705
9. Ching, WM. D. Rowland, Z. Zhang, A. L. Bourgeois, D. Kelly, G. A. Dasch, and P. L. Devine. 2000. Early Diagnosis of Scrub Typhus with a Rapid Flow Assay Using Recombinant Major Outer Membrane

Protein Antigen (r56) of Orientia tsutsugamushi. Clin. Diag. Lab. Immunol. 8:409-414.
10. Ohashi, N., H. Nashimoto, H. Ikeda, and A. Tamura. 1992. Diversity of immunodominant 56-kDa type-specific antigen (TSA) of Rickettsia tsutsugamushi. Sequence and comparative analyses of the genes encoding TSA homologues from four antigenic variants. J. Biol. Chem. 267:12728-12735

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