



DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



				96 tests		8406-25
--	--	--	--	----------	--	---------

Scrub Typhus IgG ELISA

8406-25

For Research Use Only

Test	Scrub Typhus IgG 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
Shelf Life	12 Months from the manufacturing date

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

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

Intended Use

DAI Scrub Typhus IgG ELISA test for exposure to *Orientia tsutsugamushi* (OT; formerly *Rickettsia*) is an ELISA assay system for the detection of IgG antibodies in human plasma/serum to OT derived recombinant antigen (1-10). It is not intended to screen blood or blood components, and is for research use only. Not for use in diagnostic procedures.

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 IgG Test is a qualitative, membrane-based immunoassay for the detection of IgG antibodies to *O. tsutsugamushi* (OT) in serum, and plasma. Wells of each plate have been coated with unique recombinant antigen mix. During testing, the serum sample is 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 IgG enzyme conjugate (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 IgG 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

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.

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

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 IgG Positive Control:** One vial, 50 µL. The positive control will aid in monitoring the integrity of the kit as well. Stable at -2-8°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 as well. 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 IgG:** 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. This is 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°C~25°C) and mixed thoroughly by gentle inversion prior to use.

Materials required but not supplied

- Microtiter plate reader capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- 37°C Incubator without CO₂ supply or humidification
- Plate washer
- Multi-channel pipettors
- 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 material used in the preparation of controls has been tested using FDA-approved methods for antibody to Human Immunodeficiency Virus 1 & 2 (HIV 1&2), Hepatitis C (HCV) as well as Hepatitis B surface antigen and found to be negative. However no test

method can offer complete assurance and all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.

2. This test must be performed with 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. 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:
 - 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.
 - Avoid prolonged exposure to direct light.
 - Some detergents may interfere with the performance of the Liquid TMB Substrate.
 - 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 with components from another kit lot.
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. The serum 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 to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

Test Procedure

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:

- 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 four months

Note: Discard the 1X Wash Buffer if you see any microbial growth.

- 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, all sera cannot be repeatedly thawed and frozen. Sera should be further aliquoted in a smaller volume and stored at -20 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. You can do the dilution either in tubes or in ELISA type of plastic wells (untreated plastics; not provided)

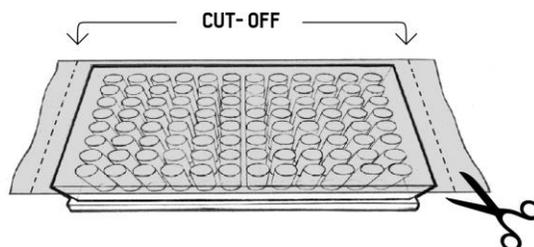
Example for Sera Application, 1/100 Diluted Samples, 100µLWell

	1	2	3	4	5	6	7	8	9	10	11	12
A	Negative Control	Negative Control	S# 13	S# 21	S# 29	S# 37	S# 45	S# 53	S# 61	S# 69	S# 77	S# 85
B	Positive Control	Positive Control	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	S# 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 4 µl of serum plus 396 µl of Sample dilution Buffer for SCRUB TYPHUS). Mix well.

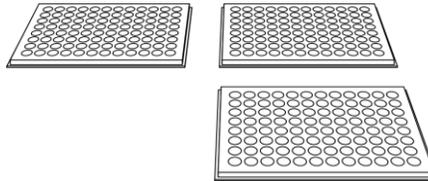
Note: Do not use less than 4µl of serum and controls.

4. Apply 100 µl per well of the 1/100 diluted test sera and controls to marked antigen-coated 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 an 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 CO₂, or any other gases used for tissue culture



CORRECT METHOD

6. After the incubation is complete wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer. Use 300 µl per well of 1X Wash Buffer in each wash cycle for all plate washing.
7. Add 100 µl per well of Ready to Use Enzyme Conjugate-HRP 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 an incubator.
10. After the incubation, wash the plate 6 times with automatic plate washer using 1X wash buffer (300µl per well).
11. Add 150µl per well of Wash Solution into all wells by a multi-channel pipettor.
12. Incubate the plate at room temperature (20-25°C) for 5 minutes without any cover on the plate.
13. After the incubation, wash the plate 6 times with automatic plate washer using 1X wash buffer.
14. Add 100 µl per well of Liquid TMB substrate into all wells by multi-channel pipettor.
15. Incubate the plate at room temperature (20-25°C) in a dark place (or container) for 10 minutes without any cover on the plate.
16. After the incubation, add 50 µl per well of Stop Solution into all wells by multi-channel pipettor and incubate at room temperature (20-25°C) for 1 minute without any cover on the plate.
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 blank background.

Results

Cut off Calculation and Interpretation of Results:

Calculation of Cut-off value:

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

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.

The following cut-off values were calculated using a limited number of endemic control sera from India.

Cut-off: 0.370

Note:

Cut off values have not been determined using a larger 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.

Summary

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) OD	< 0.200
Positive Control (PC) OD	> 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 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.

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. Immun.* 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.
6. 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.* 31:2057-2060.
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 tsutsugamushi and its use in enzyme-linked immunosorbent assays. *Clin. Diagn. Lab Immunol.* 5: 519:526.
9. 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.
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.

Date Adopted	2018-05-24
REF 8406-25	DA- Scrub Typhus IgG
EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu



DIAGNOSTIC AUTOMATION, INC.

21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA

Tel: (818) 591-3030 Fax: (818) 591-8383

ISO 13485-2003



Revision Date: 2018-05-01