

## Intended Use

For the qualitative screening of serum IgG antibodies to Scrub Typhus using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.

## Principle of Procedure

The micro test wells are coated with Scrub Typhus 56 kDa recombinant antigen. During the first incubation with the diluted patients' sera, any antibodies that are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) and a substrate (hydrogen peroxide) are added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

## Reagents

ITEM	DESCRIPTION	SYMBOL
<b>Test Strips</b>	Microwells containing a 56 kDa recombinant Scrub Typhus antigens – 96 test wells in a test strip holder.	<b>MT PLATE</b>
<b>Enzyme Conjugate</b>	One (1) bottle containing 11ml of Protein A conjugated to peroxidase.	<b>CONJ</b>
<b>Positive Control</b>	One (1) vial containing 2 ml of a surrogate positive.	<b>CONTROL +</b>
<b>Negative Control</b>	One (1) vial containing 2 ml of diluted negative human serum.	<b>CONTROL –</b>
<b>Chromogen</b>	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).	<b>SUBS TMB</b>
<b>Wash Concentrate (20X)</b>	Two (2) bottles containing 25 ml of concentrated buffer and surfactant.	<b>WASH BUF</b>
<b>Dilution Buffer</b>	Two (2) bottles containing 30 ml of buffered protein solution.	<b>SPECM DIL</b>
<b>Stop Solution</b>	One (1) bottle containing 11 ml of 1 M phosphoric acid.	<b>SOLN</b>

## Precautions

Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2-8 °C. Crystallization will disappear after dilution to working strength.

Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.

Treat all sera as if capable of being infectious. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

Do not add azide to the samples or any of the reagents.

## Storage Conditions

Reagents, strips and bottled components:

Store between 2 – 8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

### **Preparation**

Wash Buffer - Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling. Avoid generating bubbles in the wells during the washing steps.

### **Collection and Preparation of Serum**

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately. Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:100 dilution of patient's sera using the dilution buffer (e.g. 5 µl sera and 495 µl dilution buffer).

### **Procedure**

#### **Materials Required But Not Provided**

- Pipettes
- Squeeze bottle for washing strips (narrow tip is recommended)
- Reagent grade water and graduated cylinder
- Tubes for sample dilution
- Absorbent paper

#### **Suggested Materials**

ELISA plate reader with a 450 nm and a 650 to 620 nm filter (optional if results are read visually)

### **Performance of Test**

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1:100) test samples to the remaining wells.

Note: Negative and positive controls are supplied prediluted. Do not dilute further.

3. Incubate at room temperature (15 to 25 °C) for **30 minutes**.
4. Shake out contents and wash 3 times with the diluted wash buffer. Slap wells against paper towels to remove all the wash buffer.
5. Add 100 µl of Enzyme Conjugate to each well.
6. Incubate at room temperature for **10 minutes**.
7. Shake out contents and wash 3 times with wash buffer. Slap wells against paper towels to remove all of the wash buffer.
8. Add 100 µl of the Chromogen to every well.
9. Incubate at room temperature for **10 minutes**.
10. Add 100 µl of the Stop Solution and mix by tapping strip holder.

**\* Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five.**

### **Reading of Results**

Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.  
ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

### **Test Limitations**

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

### **Quality Control**

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range. Expected values for the controls are:

**Negative** - 0.0 to 0.09 OD units  
**Positive** - 0.3 OD units and above

## **Troubleshooting**

Negative control has excessive color after development.

**Reason:** inadequate washings.

**Correction:** wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

## **Interpretation of the Test – ELISA Reader**

1 – Calculate the average extinction value by taking the average OD value of the Negative Control.

2 – Add 0.200 to this average extinction value. This value is the cut-off value used in the Sample Index Calculation.

Example:

Negative Control 1 OD = 0.084

Negative Control 2 OD = 0.100

Average is  $0.084 + 0.100 = 0.184 / 2 = 0.092 = \text{Average Extinction Value}$

Cut-off value is the Average Extinction Value + 0.200 (in this example  $0.092 + 0.200 = 0.292$ )

3 – Determine the Sample Index by dividing the patients OD value by the Cut-off value.

Example:

Patient OD value of 1.225

Cut-off value of 0.292

$1.225 / 0.292 = 4.19$

4 – Evaluate the Sample Index.

Negative = less than 1.0 Sample Index

Equivocal = 1.0 to 1.5

Positive = greater than 1.5

## **Limitations of The Procedure**

Diagnosis of infection should not be made solely based on results of the ELISA test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.

Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

## **Expected Values**

The number of antibody positive subjects in a population depends on two factors: disease prevalence and clinical criteria used to select the tested population. Because very few positives should be seen in a randomly screened population in a non-endemic area, most serology tests are not specific enough to screen non-endemic populations. Even in an endemic region, serology screening often yields many false positives if used to randomly screen patients. Serology tests are useful to test patients in an endemic region with signs and symptoms consistent with the disease.

## **References**

1. CDC Website June 2023
2. WHO Website June 2023
3. Kala, D. et. Al. Diagnosis of scrub typhus: recent advances and challenges. 3 Biotech (2020) 10:396
4. Singh, O. B. and Panda, P. K., Scrub Typhus, NCBI Bookshelf, StatPearls Publishing, Jan 2023



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