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# Closing the Diagnostic Gap" - Utility of Paired Serum Samples in the Interpretation of Scrub Typhus ELISA

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## ABSTRACT

**Background** Scrub typhus is the re-emerging acute febrile illness caused by *Orientia tsutsugamushi*. The spectrum of the disease varies from self-limiting, mild to fatal illness. The disease starts with fever, headache, myalgia, cough, gastrointestinal symptoms, non-specific symptoms resembling other causes of pyrexia. Only few presents with eschar. Though IFA is considered as the gold standard technique for diagnosing scrub typhus, requires paired samples testing and costly equipment. Hence, in resource poor settings, ELISA can be done. Since there is no standard cutoff value, paired serum sample testing is meaningful in eliminating the false positivity and confirming the diagnosis.
**Materials & Methods** This is a retrospective study conducted with 90 samples. Scrub typhus ELISA was done for the samples collected from suspected Scrub typhus patients. Optical Density (OD) < 0.5 was considered as Negative. Paired samples (repeat sample after 3 days) were collected for patients with OD value > 0.5 and tested for Scrub typhus ELISA.
**Results** In the paired serum sample testing, out of 9 samples which were initially between OD 0.5-<1.0, 4 samples turned negative (OD <0.5). Remaining 5 samples showed increased OD (>1.0); all the samples which initially showed OD >1.0 were also positive (OD>1.0) in the repeat sample.
**Conclusion** Paired serum samples have a major role in the accurate determination of cut-off values for scrub typhus IgM ELISA and can be used as an alternative to the gold standard assay in resource poor settings.

***Keywords****: paired sample, Scrub typhus, ELISA, cut off.*

## INTRODUCTION

Scrub typhus is the re-emerging acute febrile illness caused by the gram-negative intracellular rickettsial organism, *Orientia tsutsugamushi*. The bite of the chiggers (larva of trombiculid mites) transmits the infection[1]. The spectrum of the disease varies from self-limiting, mild to fatal illness. The disease starts with fever, headache, myalgia, cough and gastrointestinal symptoms. Fewer than 40% presents with the classical black eschar. Also associated with non-specific signs and symptoms resembling other causes of pyrexia such as enteric fever, dengue, malaria and leptospirosis[2]. Scrub typhus is one of the causes of acute undifferentiated febrile illness in southern India including Tamilnadu[3]. Various serological diagnostic techniques are available for the diagnosis of scrub typhus such as Weil-Felix test, Enzyme linked immunosorbent assay (ELISA), Immunochromatographic test (ICT),Immunofluorescence assay (IFA), Indirect immunoperoxidase assay. IFA is considered as the gold standard technique for diagnosing scrub typhus; however, it requires paired samples testing and costly equipment[4,5]. Hence, in resource poor settings, ELISA can be done but there is no standard cutoff value for the prompt diagnosis of scrub typhus. Thus, paired serum sample testing is meaningful in eliminating the false positivity and confirming the diagnosis.

### Aims & objectives

1. To assess the utility of paired serum samples in the diagnosis of Scrub typhus by ELISA
2. To determine the cut off values for the interpretation of ELISA for our geographical location.

**Study design**
This is a retrospective study carried out from the serum samples which were tested during the period October 2022 to March 2023 at Department of Microbiology, Government Medical College Virudhunagar. Clinical data were retrieved from the medical records department.

**Sample size** – 90 serum samples

**Inclusion criteria** – Serum samples received for Scrub typhus suspected cases according to DHR-ICMR criteria[6]. Fever for more than 5 days with any of the following – eschar, rash, headache, lymphadenopathy.

**Exclusion criteria** – Serum Samples with other established cause of pyrexia were excluded.

## MATERIALS & METHODS

Blood was collected aseptically in a clot vial and serum separation was done by centrifugation at 3000 rpm for 8 min. 90 Scrub typhus suspected serum samples were subjected to Scrub typhus IgM ELISA (InBios kit), Dengue IgM ELISA, Leptospirosis IgM ELISA and Widal tube agglutination test.
For Scrub Typhus IgM ELISA, Scrub typhus Detect TM IgM ELISA kit (InBios International, Seattle, USA) was used. The ELISA plates were coated with recombinant antigens of *O. tsutsugamushi*, detects antibodies to the 56kDa antigen. The procedure was performed in strict compliance with the manufacturer's instructions. According to the manufacturer's instructions, no cut-off is provided as cut-off value differs in each geographical location depending upon the disease prevalence. Hence, cut-off value must be calculated for each geographical location.
In this study, Scrub typhus Optical Density (OD) < 0.5 was considered as Negative. Paired samples (repeat sample after 3 days) were collected for patients with OD value > 0.5 and tested for Scrub typhus ELISA.
Statistical analysis was done by SPSS software using Chi square test.

## RESULTS

Among the study population (n =90), 71 were adults and 19 were children.

**Table 1: Cause of fever among suspected Scrub typhus**

| Cause of fever among suspected Scrub typhus | No. of samples positive (n=90) |
|---|---|
| Scrub typhus | 55 (61.1%) |
| Dengue | 25 (27.7%) |
| Widal | 1 (1.1%) |
| Scrub coinfection with other fever | 5 (5.5%) |
| NIL | 4 (4.4%) |

**Table 2: Presenting feature of Scrub typhus**

| Presenting feature | Frequency (n=60) |
|---|---|
| Fever 5 - 7 days | 53 (88.3) |
| Fever > 7 days | 7 (11.6) |
| Thrombocytopenia | 19 (31.6) |
| Rash | 15 (25) |
| Abdominal pain | 5 (8.3) |
| Eschar | 3 (5) |
| Seizure | 1 (1.6) |

**Figure 1: Scrub positivity among suspected Scrub typhus Children**

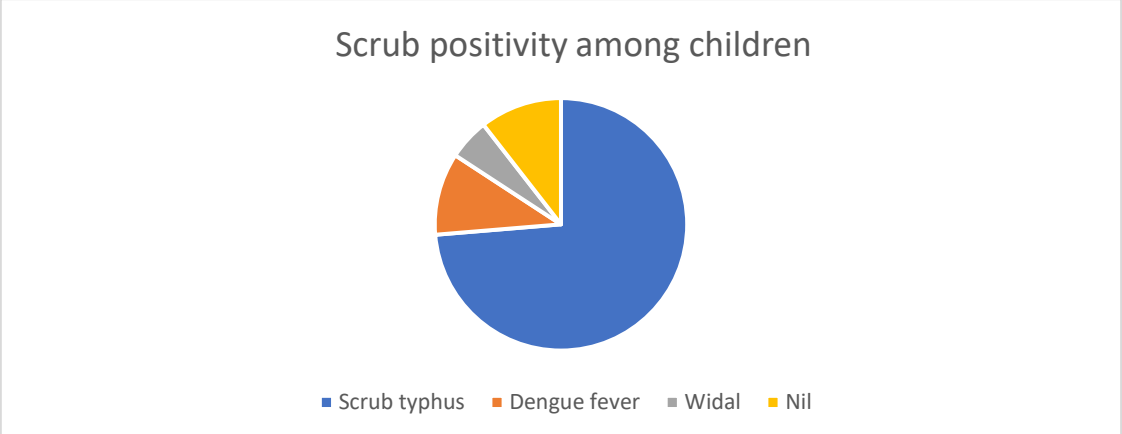

**Figure 2: Scrub positivity among suspected Scrub typhus adults**

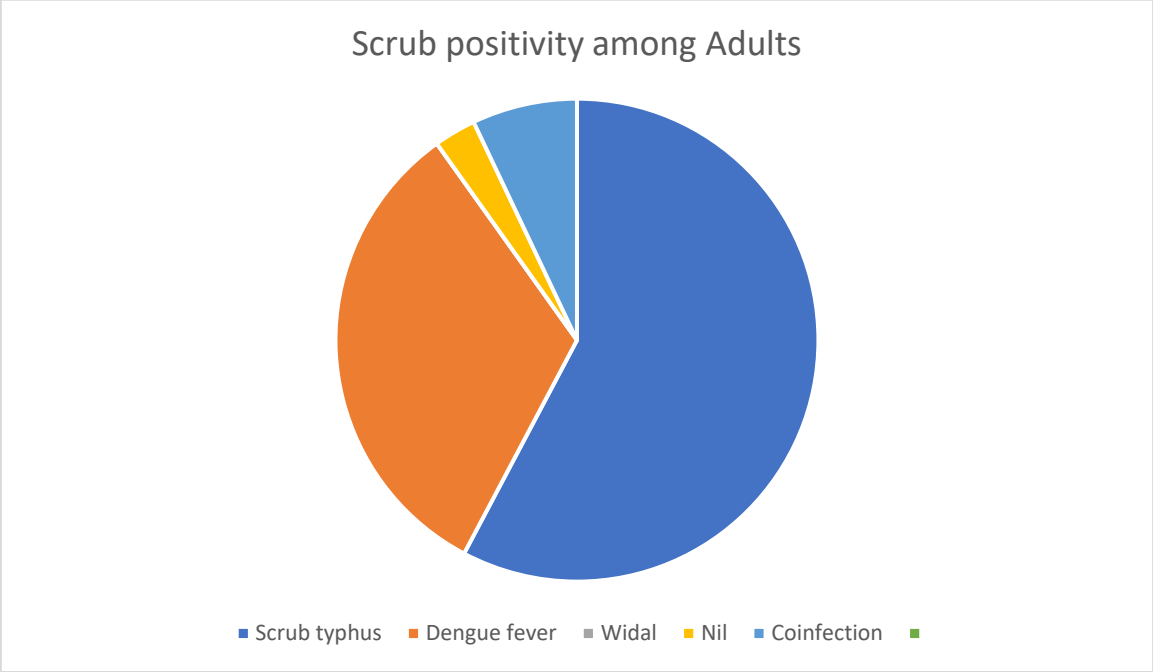


**Table 3: Scrub typhus OD value of the first sample**

| Scrub typhus IgM ELISA OD value | No. of samples (n=90) |
|---|---|
| **OD < 0.5** | 26 (28.9) |
| **OD > 0.5** | 64 (71.1) |

Samples with OD<0.5 (n= 26) was considered as Negative for Scrub typhus
For samples with OD>0.5 (n=64), repeat specimen was collected after 3 days and tested for Scrub typhus ELISA

**Table 4: Comparison of the OD values of paired serum samples (n=64)**

| Scrub typhus OD value | First sample | Second sample |
|---|---|---|
| < 0.5 | 0 | 4 |
| 0.5 - < 1.0 | 9 | 0 |
| ≥1.0 | 55 | 60 |

In the paired serum sample testing, out of 9 samples which were initially between OD 0.5-<1.0, 4 samples turned negative (OD <0.5). Remaining 5 samples showed increased OD (>1.0); all the samples which initially showed OD >1.0 were also positive (OD>1.0) in the repeat sample.

Statistical analysis was done by using Chi-square test; p< 0.001 – significant

## DISCUSSION

As discussed by Gupta et al[7], the clinical manifestations in Scrub typhus are varied, non-specific and overlapping with other causes of pyrexia warranting laboratory confirmation. In our study, similar presentation is seen among scrub suspected patients. Fever was present in all, but the pathognomonic Eschar is seen only in 5% of the patients. 28.9% of the scrub suspected patients were negative for Scrub typhus by ELISA.

The specific gold standard confirmatory tests like indirect immunoperoxidase and IFA are costly, requires dedicated equipment, trained personnels and not easily available in resource poor settings. ELISA is a comparatively cheaper and easier alternative to perform even on large number of patients. ELISA technique is highly sensitive and reproducible for IgM antibodies and can be used as a specific test for diagnosis of Scrub typhus.[5,8]

Based on our study findings, for our geographical location, in Scrub typhus ELISA OD ≥1.0 can be considered as Positive; if OD falls between 0.5 and <1.0, then paired serum sample can be collected after 3 days to rule out false positivity due to baseline antibody titre in endemic areas and cross reactivity. OD value <0.5 can be considered as negative.

## CONCLUSION

Paired serum samples have a major role in the accurate determination of cut-off values for scrub typhus IgM ELISA, by overcoming the limitations of cross-reactivity and background IgM levels in endemic areas. Hence, this can be used as

an alternative to the gold standard assay (IFA-Indirect Immunofluorescence Assay) in resource poor settings to confirm the diagnosis.

**Limitations**

Other causes of fever such as Chikungunya, Malaria were not tested.
Comparison with molecular methods like Polymerase chain reaction was not done in this study.
Gold standard assay, Indirect immunofluorescence (IFA) was not done. It would have imparted more value to the study findings.

**Conflicts of interest statement** - Nil

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