Abstract 1.1

Development of Multiplex Real-time PCR for Diagnosis of Scrub Typhus

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Background: Diagnosis of scrub typhus is usually based on indirect immunofluorescence assay (IFA), but this technique is sometimes difficult to interpret and not sensitive for early diagnosis. Therefore, real-time PCR is required to help diagnose scrub typhus.

Methods: Primers and hydrolysis (Taqman) probes, labeled with different fluorochromes, were newly designed from Orientia tsutsugamushi (OT) 47 kDa, groEL and human IFNB (served as an internal control) genes. PCR amplification efficiency was calculated by standard curve method. Diagnosis of scrub typhus in patients with acute undifferentiated febrile illness (AUFI) was confirmed by a 4-fold increase in IgG and/or IgM titer determined by IFA. Genomic DNA was extracted from EDTA-buffy coats.

Results: The PCR amplification efficiency of OT 47 kDa and groEL was 94% and 98%, respectively. The lower limit of detection of the multiplex PCR assay is 5 copies per reaction. Among patients whose paired sera were collected, PCR was positive in 42/46 (91%) of patients with confirmed scrub typhus, and 25/41 (61%) of patients with inconclusive IFA results. PCR was negative in all 138 non scrub typhus patients. Among patients whose only single sera were obtained, PCR was positive in 22/29 (76%) of patients with positive IgM titer (≥ 1:400), and 13/204 (6%) of patients with negative IgM.

Conclusion: We demonstrated a sensitive, specific and reliable multiplex-real time PCR assay for scrub typhus diagnosis. Real-time PCR together with IFA provides us with the more accurate prevalence of scrub typhus.