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Evaluation of two commercially available ELISAs for the diagnosis of Japanese encephalitis applied to field samples

Penny Lewthwaite[1], M. Veera Shankar[2], Phaik-Hooi Tio[3], Janet Daly[1], Anna Last[1], R. Ravikumar[2], Anita Desai[4], V. Ravi[4], Jane M. Cardosa[3] and Tom Solomon[1,5]

1 *Brain Infections Group, University of Liverpool, Liverpool, UK*
2 *Vijayanagar Institute of Medical Sciences, Bellary, India*
3 *Institute of Health and Community Medicine, Universiti Malaysia, Kota Samarahan, Malaysia*
4 *National Institute of Mental Health and Neurological Sciences, Bangalore, India*
5 *Division of Neurological Science, University of Liverpool, Walton Centre for Neurology and Neurosurgery, Liverpool, UK*

Summary

OBJECTIVE To compare two commercially available kits, Japanese Encephalitis-Dengue IgM Combo ELISA (Panbio Diagnostics) and JEV-CheX IgM capture ELISA (XCyton Diagnostics Limited), to a reference standard (Universiti Malaysia Sarawak – Venture Technologies VT ELISA).

METHODS Samples were obtained from 172/192 children presenting to a site in rural India with acute encephalitis syndrome.

RESULTS Using the reference VT ELISA, infection with Japanese encephalitis virus (JEV) was confirmed in 44 (26%) patients, with central nervous system infection confirmed in 27 of these; seven patients were dengue seropositive. Of the 121 remaining patients, 37 (31%) were JEV negative and 84 (69%) were JEV unknown because timing of the last sample tested was <10 day of illness or unknown. For patient classification with XCyton, using cerebrospinal fluid alone (the recommended sample), sensitivity was 77.8% (59.2–89.4) with specificity of 97.3% (90.6–99.2). For Panbio ELISA, using serum alone (the recommended sample), sensitivity was 72.5% (57.2–83.9) with specificity of 97.5% (92.8–99.1). Using all available samples for patient classification, sensitivity and specificity were 63.6% (95% CI: 48.9–76.2) and 98.4% (94.5–99.6), respectively, for XCyton ELISA and 75.0% (59.3–85.4) and 97.7% (93.3–99.2) for Panbio ELISA.

CONCLUSION The two commercially available ELISAs had reasonable sensitivities and excellent specificities for diagnosing JEV.

keywords Japanese encephalitis virus, diagnostics, ELISA, India

Introduction

Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus; it is the leading cause of encephalitis in South-east Asia. Over 3 billion people live in areas where Japanese encephalitis (JE) is endemic; it causes an estimated 20 000–175 000 cases annually with 6000–15 000 deaths (Tsai 2000; Halstead & Tsai 2004; Ghosh & Basu 2009). The virus continues to spread into new areas and often co-circulates with the related flavivirus, dengue.

Affordable vaccines are now becoming available, but implementation programmes are hampered because the epidemiological data and surveillance for JE are poor; this is largely because of lack of standardised diagnostics. JE is endemic in resource-poor areas and in these settings; patients often have a clinical diagnosis only. Serological tests are the gold standard for diagnosis of JEV as the period of viraemia is short, making methods to detect genomic material or viral antigen unreliable (WHO 2006). The timing of sample collection and type of sample collected impact on the ability to confirm the diagnosis of JEV. Cerebrospinal fluid (CSF) is the sample of choice, as detection of antibodies in the CSF confirms viral infection of the central nervous system (CNS). Serum samples demonstrate recent peripheral infection only. Even demonstrating a rising serum IgM is insufficient for confirmation of JEV as the causative agent for an acute encephalitis syndrome (AES), as this does not distinguish peripheral from CNS infection. Studies from Thailand suggest that high titres of JEV IgM may be found in sera of asymptomatic individuals (Grossman *et al.* 1973). Burke *et al.* (1985) found that by day 7 of illness, 100% of patients

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