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Comparison of three commercial IgG and IgM ELISA kits for the detection of tick-borne encephalitis virus antibodies

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ABSTRACT

Tick-borne encephalitis (TBE) is endemic in many parts of Europe and Asia. The diagnosis of this disease is essentially based on the demonstration of specific antibodies. For reasons of simplicity, automatization and quick availability of test results, enzyme-linked immunosorbent assays (ELISAs) are the method of choice for serological diagnosis of TBE. Here, we evaluated three commercially available anti-TBEV IgG and IgM ELISAs using 251 serum samples: the SERION ELISA *classic* FSME Virus/TBE Virus IgG and IgM kit (Virion\Serion), the RIDASCREEN® FSME/TBE IgG and IgM kit (R-Biopharm), and the anti-FSME/TBE virus ELISA "Vienna" IgG/anti-FSME/TBE virus ELISA IgM kit (Euroimmun). In total, discrepant test results for IgG and/or IgM were observed for 37/251 (14.7 %) of tested samples; differences were statistically significant. Reference values defined by serum neutralization test (SNT, n = 25) or results provided by EQA organizers (n = 2) were established for a subset of samples. In relation to these values, false-positive results were observed mainly for Euroimmun Vienna IgG and RIDASCREEN IgG, whereas false-negative results were primarily observed for Virion\Serion IgG and RIDASCREEN IgM kits. In routine diagnostics, specificity problems are of major relevance and may be addressed by analyzing the respective samples using SNT.

1. Introduction

Tick-borne encephalitis virus (TBEV) is the most important tick-borne arbovirus infecting humans in Europe and Asia. The TBEV species belongs to the mammalian tick-borne flavivirus group in the genus *Flavivirus*, family *Flaviviridae*. Based on antigenetic properties, it is subdivided into a Far Eastern, a European and a Siberian subtype. These subtypes correspond to the major TBEV genotypes 1, 2, and 3, respectively (Demina et al., 2010; Lindquist, 2014). TBEV is typically transmitted through bites of infected ticks, wherefore its distribution correlates with the presence of ixodid vectors. In Central Europe, TBEV is principally transmitted by *Ixodes ricinus* (Lindquist, 2014), although its presence in other tick species as well as the transmission via infected milk products has also been documented (Balogh et al., 2010; Holzmann et al., 2009; Mierzejewska et al., 2015).

Infections with TBEV are asymptomatic in 70–95% of cases. Symptomatic disease is typically biphasic when caused by European subtype viruses, including a viremic stage with flu-like symptoms starting about 8 days (4–28 days) after the tick bite, an asymptomatic interval of about one week (range 1–33 days), and a second stage with neurological manifestations ranging from mild meningitis to severe encephalitis with or without myelitis and spinal paralysis (Lindquist, 2014; Lindquist and Vapalahti, 2008). In contrast, Far Eastern and Siberian viruses most often induce monophasic diseases. Chronical forms may be observed in association with the Siberian subtype (Gritsun et al., 2003). Case fatality rates range between 0 and 1.4 % and increase with age for European subtype viruses. For infections with the Siberian and Far Eastern subtypes, mortality ranges between 2 and 3 % and about 35 %, respectively. This latter high rate, however, might be due to the lack of detection and reporting of mild cases (Charrel et al., 2004; Gritsun et al., 2003; Kaiser, 1999; Lindquist, 2014).

In biphasic tick-borne encephalitis (TBE), the virus can be detected by polymerase chain reaction (PCR) in blood during the viremic phase of illness. However, patients typically seek medical care only during the second phase of illness, when neurological symptoms occur. During this phase, direct detection of the virus is rarely successful, wherefore

Abbreviations: CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assays; EQA, external quality assessments; Ig (G/M), immunoglobulin type G/M; PCR, polymerase chain reaction; SNT, serum neutralization test; TBE, tick-borne encephalitis; TBEV, tick-borne encephalitis virus

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Table 1
Summary of the tested commercial anti-TBEV IgG and IgM ELISA kits[a].

Name	TBEV strain	Sample dilution	Processing	OD measurement	Standards/controls	Unit	Cut-offs	Test evaluation	Inter-assay variation (vc)	Intra-assay variation (vc)
IgG										
SERION ELISA classic FSME/TBE Virus IgG (Virion \Serion)	MoscowB4	1:101	sample incubation 60 min. 37 °C; wash 4x; conjugate incubation 30 min. 37 °C; wash 4x; substrate incubation 30 min. 37 °C; stop	405 nm/650 nm	2 standard sera: standard control, negative control	U/ml	negative < 100 U/ml; equivocal 100–150 U/ml; positive > 150 U/ml	quantitative, 4-parameter logistic equation function, lot-specific parameters A-D provided by the manufacturer	8.8–13.4 %	4.3–8.3%
RIDASCREEN® FSME/TBE IgG (R-Biopharm)	Neudörfl	1:100	sample incubation 30 min. 37 °C; wash 4x; conjugate incubation 30 min. 37 °C; wash 4x; substrate incubation 30 min. 37 °C; stop	450 nm/620 nm	2 standard sera: standard control, negative control	U/ml	negative < 100 U/ml; equivocal 100–126 U/ml; positive > 126 U/ml	quantitative, 4-parameter logistic equation function, lot-specific parameters A-D provided by the manufacturer	11.5–22.7 %	6.9–9.1%
Anti-FSME/TBE Virus ELISA Vienna (Euroimmun)	K 23	1:101	sample incubation 60 min. RT; wash 3x; conjugate incubation 60 min. RT; wash 3x; substrate incubation: 30 min. RT; stop	450 nm/620 nm	4 calibration sera: 1′000 VIEU/ml, 300 VIEU/ml, 150 VIEU/ml (cut-off), 15 VIEU/ml	VIEU/ml	negative < 120 VIEU/ml; equivocal 120 − < 165 U/ml; positive ≥165 U/ml	quantitative, 4-parameter logistic equation function, standard curve based on measured values of calibration sera calculated by user	3.9–10.5%	2.7–7.9%
IgM										
SERION ELISA classic FSME/TBE Virus IgM (Virion \Serion)	MoscowB4	1:101	IgG/RF absorption 15 min. RT; sample incubation: 60 min. 37 °C; wash 4x; conjugate incubation 30 min. 37 °C; wash 4x; substrate incubation 30 min. 37 °C; stop	405 nm/650 nm	2 standard sera: standard control, negative control	U/ml	negative < 10 U/ml; equivocal 10–15 U/ml; positive > 15 U/ml	quantitative, 4-parameter logistic equation function, lot-specific parameters A-D provided by the manufacturer	3.7–7.0 %	3.1–7.9%
RIDASCREEN® FSME/TBE IgM (R-Biopharm)	Neudörfl	1:100	IgG/RF absorption: 15 min. RT; sample incubation: 30 min. 37 °C; wash 4x; conjugate incubation 30 min. 37 °C; wash 4x; substrate incubation: 30 min. 37 °C; stop	450 nm/620 nm	2 standard sera: standard control, negative control	U/ml	negative < 100 U/ml; equivocal 100–126 U/ml; positive > 126 U/ml	quantitative, 4-parameter logistic equation function, lot-specific parameters A-D provided by the manufacturer	18.5–24.2 %	11.4–14.7 %
Anti-FSME/TBE Virus ELISA IgM (Euroimmun)	K 23	1:101	IgG/RF absorption: 10 min. RT; sample incubation: 30 min. RT; wash 3x; conjugate incubation 30 min. RT; wash 3x; substrate incubation: 15 min. RT; stop	450 nm/620 nm	2 standard sera: positive control, negative control	Ratio	negative ratio < 0.8; equivocal ratio ≥0.8 − < 1.1; positive ratio ≥1.1	semi-quantitative, extinction sample/extinction calibrator = ratio	3.9–5.7 %	3.8–5.5 %

[a] ELISA, Enzyme-linked immunosorbent assay; RF, rheuma factor; RT, room temperature; TBE, tick-borne encephalitis; TBEV, tick-borne encephalitis virus; U, Units; vc, variation coefficiant; VIEU, Vienna Units. All information is given as specified by the manufacturers.