Comparison of six different commercial IgG-ELISA kits for the detection of TBEV-antibodies

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Abstract

Background: Tick-borne encephalitis virus (TBEV) is a pathogenic human flavivirus endemic in some parts of Europe and Asia. Commercial enzyme immunoassays (EIA) for the detection of IgG antibodies are often used in TBEV-seroprevalence studies, as well as for the confirmation of a successful vaccination against TBEV. However, the detection of TBEV-specific antibodies can be biased by the cross-reactivity between different flavivirus genera.

Objectives: To compare different EIA test systems for the detection of TBEV-IgG antibodies.

Study design: Six commercial EIA kits for the detection of TBEV-specific antibodies are compared, using serum panels (n = 139) of subjects with a documented clinical history (109 sera from TBEV infected patients, 30 sera from people vaccinated against TBEV). For the analysis of possible cross-reactivities, 24 sera from yellow fever vaccinated people and 13 sera positive for Dengue virus-specific antibodies were also included.

Results: The sensitivity of the different TBEV test systems ranges from 73 to 99%. However, when testing the yellow fever and Dengue virus positive specimens, problems with the flavivirus cross-reactivity become obvious, resulting in specificities between 14 and 81%.

Conclusions: This study shows the necessity of further improvement of the existing TBEV test systems regarding both sensitivity and specificity. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cross-reactivity; Enzyme immuno-assay; Tick-borne encephalitis virus

1. Introduction

Tick-borne encephalitis virus (TBEV) belongs to the genus flavivirus within the family Flaviviridae. Like other flaviviruses, e.g. yellow fever or Dengue virus, it is an important human pathogen. There are two subtypes, the western and the far eastern subtype, both transmitted by ticks (Ixodes spp). Transmission of TBEV can cause febrile
illness lasting for 4–10 days in the infected individual, followed by meningitis or meningoencephalitis (Heinz, 1993). TBEV is endemic in some parts of Europe and Asia. Control of TBEV can be achieved by elimination of ticks; for endemic areas and people at risk, a vaccine is available. Diagnosis of TBEV can be mainly performed using, for example, hemagglutination, PCR, or detection of TBEV-specific IgM antibodies by enzyme immuno-assays (EIA) or blotting techniques. In TBEV-seroprevalence studies, easy-to-use EIA kits are often used for the detection of IgG antibodies, as well as for the confirmation of a successful vaccination against TBEV (Klokkmann, 1991; Gassmann, 1997; Kreil, 1998). However, the close antigenic relationship between different flavivirus species can lead to false positive results with sera from individuals after infection with, or vaccination against, yellow fever or Dengue virus, for example (Dobler, 1996).

In this study, six commercial ELISA kits for the detection of TBEV-specific antibodies are compared, using a panel of 139 seropositive for TBEV. Because cross-reactivity is described for several flavivirus genera, sera positive for yellow fever virus and Dengue virus are also included.

2. Material and methods

2.1. Specimens

Human serum samples (n = 139) were collected, submitted to our laboratory, and stored at −70°C: 109 serum samples from patients with TBEV infection were collected at the Center for Communicable Diseases, Prevention and Control, Vilnius, Lithuania; TBEV infection was confirmed by a hemagglutination test (titre > 1:10). Thirty serum samples from TBEV vaccinees were collected at the Chiron Behring Marburg GmbH, Marburg, Germany; the presence of IgG antibodies was confirmed by neutralization assay.

In addition, a total of 37 sera from yellow fever-vaccinated individuals (n = 24) and from patients with known and documented clinical history for Dengue virus (n = 13) were included to examine the specificity of the different test systems; all these sera were negative for specific TBEV antibodies.

2.2. EIA diagnostic kits

The following enzyme immunoassay (EIA) diagnostic kits for the detection of TBEV IgG antibodies were used according to the manufacturer’s directions: (1) Serion classic (Virion-Serion, Würzburg); (2) Vir-Elisa (Mast-Diagnostika, Reinfall); (3) Immunozym (Immuno GmbH, Heidelberg); (4) FSME-Elisa (Virotech GrnbH, Rüsselsheim); (5) Enzygnost (Dade Behring Marburg GrnbH, Marburg); (6) anti-FSME Elisa (Euroimmun, Gross Grönau).

2.3. Specimen preparation for EIA methods

All EIA kits required untreated plasma, 1:10 diluted in 0.1 M PBS.

2.4. Test performance

Unless indicated otherwise, EIA diagnostic kit procedures were followed, and the results were examined and interpreted according to the manufacturers’ directions. Sera with false positive or negative results in at least three different test systems were sent to all manufacturers for further evaluation (n = 33; 12 sera from TBEV-infected individuals, 10 sera from TBE vaccinees, and 11 sera positive for Dengue virus).

3. Results

The qualitative results from the evaluated test systems for the different serum panels are given in Table 1; no specific quantification is used or reported with the results. Table 2 displays the sensitivity and specificity for the different test systems, as derived from the results of our study.

There was a good correlation between all test systems for most of the specimens. For the serum panel sent to the manufacturers for re-evaluation, the average number of correct results with TBEV decreased slightly from 11 to 10; the number of false positive results with the Dengue virus-posi-
Table 1

<table>
<thead>
<tr>
<th>Test system</th>
<th>TBEV+(Inf) [n = 109]</th>
<th>TBEV+(vac) [n = 30]</th>
<th>YF+ [n = 24]</th>
<th>Dengue+ [n = 13]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serion classic</td>
<td>100 (92%)</td>
<td>28 (93%)</td>
<td>19 (79%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Vir-ELISA</td>
<td>94 (86%)</td>
<td>8 (27%)</td>
<td>1 (4%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>Immunozym</td>
<td>94 (86%)</td>
<td>21 (70%)</td>
<td>10 (42%)</td>
<td>9 (69%)</td>
</tr>
<tr>
<td>FSME-ELISA</td>
<td>107 (98%)</td>
<td>30 (100%)</td>
<td>7 (29%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Enzygnost</td>
<td>88 (81%)</td>
<td>29 (97%)</td>
<td>1 (4%)</td>
<td>7 (54%)</td>
</tr>
<tr>
<td>Anti-FSME ELISA</td>
<td>86 (79%)</td>
<td>23 (77%)</td>
<td>16 (67%)</td>
<td>6 (46%)</td>
</tr>
</tbody>
</table>

*TBE+: sera positive for anti-TBE IgG antibodies after TBE infection (inf) or vaccination (vac); YF+: sera positive for anti-yellow fever-virus antibodies after vaccination; Dengue+: sera positive for anti-Dengue antibodies after Dengue infection; total number of tested sera; (), percentage of positive results for TBEV-specific IgG antibodies.

The results of this study show the necessity for a further improvement of the existing TBEV test systems regarding both sensitivity and specificity. In addition, information on the risk of false positive results through cross-reacting antibodies should be included in the test manuals, and patients tested for TBEV must be asked for prior vaccination against, or infection with, yellow fever and Dengue virus. To increase the reliability of the test results, specimens should be measured in duplicates; for some applications, it might be useful to confirm the results obtained by ELISA using a different test system, e.g. immuno-blot. It should be noted that some of the evaluated test systems can also detect IgM antibodies, which are of major importance for the detection of acute infection. The evaluation of test systems for the detection of IgM antibodies is an important task for the future (Roggendorf, 1981).

Table 2

<table>
<thead>
<tr>
<th>Test system</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serion classic</td>
<td>92</td>
<td>14</td>
</tr>
<tr>
<td>Vir-ELISA</td>
<td>73</td>
<td>81</td>
</tr>
<tr>
<td>Immunozym</td>
<td>83</td>
<td>49</td>
</tr>
<tr>
<td>FSME-ELISA</td>
<td>99</td>
<td>46</td>
</tr>
<tr>
<td>Enzygnost</td>
<td>84</td>
<td>78</td>
</tr>
<tr>
<td>Anti-FSME ELISA</td>
<td>78</td>
<td>41</td>
</tr>
</tbody>
</table>

Positive results for the detection of TBEV-specific IgG antibodies, as determined with the different test systems. The sensitivity and specificity for the different test systems were calculated with our serum panel.
References

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