A SIMPLE AND CHEAPER IN HOUSE VARICELLA ZOSTER VIRUS ANTIBODY INDIRECT ELISA

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SUMMARY

We have developed a cheaper an simple in house indirect ELISA that uses the live attenuated VZV vaccine as a coating antigen.

The alternative ELISA had an agreement of 94% when compared with a commercial VZV ELISA kit. Moreover, our ELISA proved to be more reliable than the kit when assessing true negative samples.

By adding a standard serum, we were able to produce results in international units per millilitre. Also, the addition of an extra step with 8M urea allowed the assessment of VZV IgG avidity without excessive costs.

The cost per sample to test VZV IgG was 2.7 times cheaper with our ELISA, allowing the testing of many samples without the burden of production of VZV antigen in the laboratory.

KEYWORDS: Varicella; ELISA; Antibody avidity.

INTRODUCTION

Assessment of varicella zoster virus (VZV) antibodies is performed routinely by enzyme immunoassays (ELISA) with commercially available kits. A cheaper and as accurate alternative is the use of “in house” ELISAs. However, that requires the production of VZV antigen in tissue culture and its subsequent extraction from VZV-infected cells. Moreover, most in house ELISAs use a control for nonspecific antibody binding, a similarly prepared extract of uninfected cells14.

Production of VZV antigen is usually performed in cell lines derived from human fibroblasts (e.g., MRC5 cell line), known to be time consuming and very often not so easy to handle21.

The increasing use of a VZV vaccine in different populations has added to the necessity of measuring anti-VZV antibodies as an indicator of prior or recurrent infection, to predict susceptibility to disease, and to evaluate immune response to vaccination3,5,10,12,16,17,18,22,23.

We have developed a cheaper and simple alternative indirect ELISA that uses the live attenuated VZV vaccine as a coating antigen. The use of a standard reference serum allowed us to produce results in international units per millilitre (IU/mL). Finally, with the addition of an extra step with 8M urea, we could also assess VZV IgG avidity.

MATERIAL AND METHODS

Serum samples: Two hundred and thirty-nine serum samples were tested: 221 were from healthy adults, 122 (55%) of them who referred having had varicella in the past and 99 (45%) who denied previous clinical VZV infection; the remaining 18 sera were drawn from 12-month-old infants without clinical or serologic evidence of previous VZV infection.

Serum samples were collected to investigate VZV seroprevalence in different populations. All studies were approved by the Ethics Committee of the Federal University of São Paulo, in São Paulo, Brazil.

VZV ELISA kit: A Hemagen indirect ELISA kit to detect IgG VZV antibodies (Columbia, USA) was used according to the manufacturer’s instructions.

High, medium and low calibrators were added together with serum samples diluted 1 in 41, all in duplicates. A standard curve with a corresponding linear regression curve fit equation was obtained and results were calculated by interpolation of mean optical density (OD) values onto the curve fit.

Results were considered positive if they were higher than 20 arbitrary units per millilitre (AU/mL), as suggested by the kit’s manufacturer.
**RESULTS**

**Comparison of “in house” ELISA with commercial ELISA kit:** In house ELISA and commercial ELISA kit showed an agreement of 94%. When compared with commercial kit, our in house ELISA showed a sensitivity of 95% and specificity of 87%. Only 13 out of 239 samples tested produced discordant results when assessed by both ELISAs (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Commercial Kit of indirect ELISA</th>
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<tbody>
<tr>
<td>In house ELISA</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>177</td>
</tr>
</tbody>
</table>

Sensitivity: 97%; Specificity: 87%; Agreement: 94%

**True positive and true negative samples assessed by “in house” ELISA and commercial ELISA kit:** As described in the Materials and Methods session, we selected among the samples tested by both methods those that were supposedly positive (adults who referred previous clinical VZV infection) and those that were supposedly negative (12-month infants without clinical and laboratory evidence of VZV infection).

While the commercial ELISA kit detected 98% (120 in 122) supposedly positive samples and 89% (16 in 18) supposedly negative samples, our in house ELISA detected 98% (120 in 122) and 100% (18 in 18) of the same serum samples (Table 2).

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>In house ELISA</th>
<th>Commercial Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supposedly positive</td>
<td>120/122 (98%)</td>
<td>120/122 (98%)</td>
</tr>
<tr>
<td>(previous varicella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>infection)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supposedly negative</td>
<td>18/18 (100%)</td>
<td>16/18 (89%)</td>
</tr>
<tr>
<td>(12 months of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without history of</td>
<td></td>
<td></td>
</tr>
<tr>
<td>varicella infection)</td>
<td></td>
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**Assessment of VZV ELISA specificity:** Among the 5 samples from VZV IgG seronegative adults, 4 were positive from CMV IgG and one was negative. All 5 sera were also positive for VCA IgG antibodies.

Among the 3 samples from VZV IgG seropositive children, 2 were seronegative for CMV IgG and one was positive; 2 were seronegative for VCA IgG and one was weakly positive (Table 3).

**Cost assessment of “in house” ELISA and commercial ELISA kit:** Our in house ELISA proved to be cheaper than the commercial kit, with a cost per sample of US$0.99 and US$2.70, respectively (Table 4).

IgG VZV antibody avidity: By the addition of an extra 8M urea step, we were able to assess IgG VZV antibody avidity using the in house ELISA. As shown on Table 5, individuals with past primary VZV infection had high (above 60%) or intermediate IgG avidity (between 30% and 60%). Those with recent or concurrent infection showed low IgG avidity (below 30%).

Table 5
IgG VZV antibody avidity assessed in different patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>VZV antibodies (IU/mL)</th>
<th>VZV IgG avidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult A with VZV infection in childhood</td>
<td>1.4</td>
<td>62</td>
</tr>
<tr>
<td>Adult B with VZV infection in childhood</td>
<td>1.1</td>
<td>48</td>
</tr>
<tr>
<td>Adult C with concurrent herpes zoster infection</td>
<td>1.1</td>
<td>62</td>
</tr>
<tr>
<td>Child A with concurrent VZV infection</td>
<td>11.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Child B 2 months after infection VZV</td>
<td>3.2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

**DISCUSSION**

We have developed a modified indirect ELISA that proved to be simple, cheap and easy to perform. Most “in house” ELISAs that detect antibodies against viruses require the production and extraction of virus antigen. Many laboratories do not have tissue culture facilities, precluding the use of such techniques.

However, previous immunoassays well accepted in the literature have used alternatives for less accessible reagents. In special, toxoid vaccines have been routinely used in ELISAs to detect tetanus and diphtheria antibodies.

Live attenuated vaccines against measles and mumps have also been used as antigens for “in vitro” T cell proliferation assays, with good results.

Our modified ELISA had a good agreement when compared with a commercial ELISA kit and did not show cross-reactivity with antibodies from other Herpesviruses. Moreover, it proved to be superior than the kit when true negative samples were tested. As suggested by others, we used sera from 12 month-old infants without evidence of previous VZV infection.

Another advantage of the test we have developed was the use of a reference serum, calibrating antibodies measured in IU/mL. As it is well known, this allows the comparison of results obtained in different countries.

Finally, introducing an extra step allowed the assessment of VZV IgG avidity without excessive costs.

In sum, we have developed a cheap and accurate alternative ELISA to measure VZV antibodies that proved suitable for most laboratories without the cumbersome needs of tissue culture, that produced results in IU/mL and permitted the assess of IgG avidity.

**RESUMO**

Desenvolvimento de ELISA indireto simples e de baixo custo para detecção de anticorpos anti-varicela zoster

Desenvolvemos um ensaio imunoenzimático (ELISA) indireto simples e econômico para detecção de anticorpos contra o vírus da varicela zoster (VZV) que utiliza a vacina contendo o vírus vivo atenuado como antígeno para recobrir a placa.

Este ELISA mostrou uma concordância de 94% quando comparado com um kit de ELISA comercial para anticorpos contra varicela. Além disso, nosso ELISA mostrou ser mais confiável que o kit quando amostras comprovadamente negativas foram testadas.

O uso de um soro padrão de referência, calibrado em unidades internacionais por mililitro, possibilitou também que os resultados pudessem ser comparados com outros estudos. O acréscimo de uma etapa extra com solução de ureia 8M permitiu avaliação de avidez de IgG para VZV sem custos excessivos.

O custo por amostra para testar IgG contra VVZ com nosso ELISA foi 2,7 vezes mais barato quando comparado com o kit comercial.
REFERENCES


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