RESEARCH NOTE

Evaluation of two commercial enzyme immunoassays for the detection of norovirus in faecal samples from hospitalised children with sporadic acute gastroenteritis

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ABSTRACT

Two commercially available enzyme immunoassays (EIAs), IDEIA and Ridascreen, for norovirus antigen detection were evaluated with 117 faecal samples from hospitalised children with acute gastroenteritis. Eighteen of 39 samples positive by RT-PCR were characterised by sequence analysis, and 17 of these were related to norovirus genogroup II. When compared with RT-PCR, the sensitivity and specificity values were 76.9% and 85.9%, respectively, for the IDEIA assay, and 59.0% and 73.1%, respectively, for the Ridascreen assay. The sensitivity and specificity of both EIA tests require improvement, but they could both eventually be of use in the diagnosis of norovirus diarrhoea in clinical laboratories.

Keywords Diagnosis, diarrhoea, enzyme immunoassay, norovirus, RT-PCR, viral gastroenteritis

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The family Caliciviridae contains two viruses that infect humans: norovirus (NoV) and sapovirus (SV). Norovirus is the leading cause of epidemic non-bacterial outbreaks of gastroenteritis [1], and is also an important cause of infantile sporadic acute gastroenteritis [2–4]. Norovirus genogroups I and II, comprising at least 29 different genetic clusters, contain most of the strains that infect humans [5]. Currently, RT-PCR is the main diagnostic tool for human Caliciviridae infections and is considered to be the diagnostic standard [6]. Commercial enzyme immunoassays (EIAs) have recently been developed, but limited data concerning their performance are available. In previous studies, the IDEIA NVL assay (DakoCytomation, Ely, UK) was reported to have a sensitivity of 39–55.5% and a specificity of 98.3–100% in comparison with RT-PCR [7,8]. The aim of the present study was to evaluate the IDEIA NVL assay together with a second assay, Ridascreen NLV (R-BioPharm, Darmstadt, Germany), for their ability to detect NoV antigen in faecal samples.

The study was performed with children aged <5 years with a clinical diagnosis of acute gastroenteritis, hospitalised in Severo Ochoa Hospital, Madrid, Spain between 1 October 2002 and 1 April 2004. Stool specimens were collected within 24–48 h of admission and were stored at 4°C. All samples were screened for bacterial pathogens, rotaviruses, adenoviruses and astroviruses. A subset of 117 samples negative for
these pathogens was tested for Caliciviridae by RT-PCR and the IDEIA NVL and Ridascreen NLV EIA s. Both EIA s use monoclonal antibodies specific for the capsid antigens of NoV genotypes I and II; in addition, the IDEIA assay differentiates the two genogroups.

For the RT-PCR assay (One-Step RT-PCR kit; QIAGEN, Valencia, CA, USA), RNA was first extracted using the guanidine thiocyanate method, as described previously [9], and the RNAid Spin Kit (Qbiogen, Inc., Irvine, CA, USA). Samples were tested initially with the JV12y-JV13i primer pair, which detects the majority of known strains of the NoV genus [10]. Samples that tested negative were then analysed with the p289-p290 primer pair, which detects NoV and SV [11]. PCR products were analysed by electrophoresis in agarose 2% w/v Tris-borate-EDTA gels, and were visualised by UV illumination after staining with ethidium bromide. In order to detect possible inhibitors, RNA of a known NoV-positive sample was added to the RT-PCR mixture. A tube containing the RT-PCR mixture without an RNA sample was used as a negative control.

Eighteen RT-PCR-positive specimens were characterised genetically, following purification of the PCR products with a QIAquick PCR Purification Kit (QIAGEN), by sequencing the partial polymerase gene sequences with an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3700 automated sequencer (Applied Biosystems). A multiple-sequence alignment was generated from the consensus sequence of each of the isolates and ten reference strains (X86557, U46500, U07611, AX348232, U22498, X81879, U97072, U04469, M87661 and L07418) by the CLUSTALX 1.8 method. The partial polymerase gene sequences were analysed further with the MEGA 3 analytical package using the neighbour-joining [12] and Kimura 2p algorithm methods [13] to assess phylogenetic relationships in comparison with the NoV reference strains. Statistical comparisons were calculated using a standard formula [14], with RT-PCR as the reference method. The kappa coefficient was also calculated, with values of ≥0.6 denoting that agreement between assays was not random [15].

In total, 642 faecal samples from hospitalised children were included in the study. No pathogens were detected in 169 (25.7%) specimens. Of these, 117 samples were tested with the RT-PCR, IDEIA NVL and Ridascreen NLV assays. Thirty-nine samples were positive by RT-PCR (33 with the JV12y-JV13i and six with the p289-p290 primer pairs). Overall, 77 (65.8%) samples yielded concordant results with the three methods, whereas 40 (34.2%) samples yielded discordant results (Table 1). The sensitivity, specificity, positive and negative predictive values, agreement and kappa index values are shown in Table 2. Genotyping and sequence analysis were performed on 18 of 39 RT-PCR-positive samples for which there was an adequate amount of material. Of these 18 samples, one contained SV and 17 contained NoV (13 Lorsdale (12 nGII.4 and one Camberwell), one Leeds and three GGIIb). The IDEIA assay failed to detect one of the 17 NoV strains (the sample containing the Leeds strain), whereas the Ridascreen assay failed to detect five samples (one Leeds, two GGIIb and two Lorsdale strains).

Overall, the sensitivity (76.9%) of the IDEIA assay in the present study, compared with RT-PCR, was higher than in previous studies [7,8], but the specificity was lower (85.9% vs. 98.3% [7] and 100% [8]). The Ridascreen assay had lower sensitivity (59.0%) and specificity (73.0%) than in previous studies [16, 17]. The 12 samples positive for NoV were genotyped and sequenced. The IDEIA assay failed to detect one of the 117 samples (the sample containing the Leeds strain), whereas the Ridascreen assay failed to detect five samples (one Leeds, two GGIIb and two Lorsdale strains).

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<table>
<thead>
<tr>
<th>IDEIA*</th>
<th>Ridascreen</th>
<th>RT-PCR</th>
<th>n</th>
<th>(%)</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>22</td>
<td>(18.8)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>9</td>
<td>(7.7)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>8</td>
<td>(6.8)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td>(0.8)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>8</td>
<td>(6.8)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>12</td>
<td>(10.2)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>(1.7)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>55</td>
<td>(47.0)</td>
</tr>
</tbody>
</table>

*All positive IDEIA results corresponded with norovirus genogroup II.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test method</th>
<th>IDEIA</th>
<th>Ridascreen</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>76.9%</td>
<td>59.0%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>85.9%</td>
<td>73.1%</td>
<td></td>
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<tr>
<td>PPV</td>
<td>73.2%</td>
<td>52.3%</td>
<td></td>
</tr>
<tr>
<td>NPV</td>
<td>88.2%</td>
<td>78.1%</td>
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</tr>
<tr>
<td>Agreement</td>
<td>82.9%</td>
<td>68.4%</td>
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</tr>
<tr>
<td>Kappa index</td>
<td>0.6203</td>
<td>0.3103</td>
<td></td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value.
only by the Ridascreen assay showed absorbance units at least 1.5-fold greater than the cut-off value. Previous studies have also encountered similar false-positive results with this EIA method [16]. The nine samples that were negative by RT-PCR, but positive by both EIAs, also exhibited high absorbance values. The possibility of false-negative RT-PCR results for these samples cannot be excluded, and may be associated with factors that might affect the sensitivity of the RT-PCR assay, e.g., the instability of viral RNA, the presence of PCR inhibitors in faecal samples, and the genetic diversity of the NoV genomes [7].

In conclusion, neither of the EIA assays analysed in this study was sufficiently sensitive to be useful as a sole diagnostic method for sporadic cases of NoV-associated acute gastroenteritis, and neither can substitute for the RT-PCR method. The lack of a simple test has limited the routine diagnosis of NoV diarrhoea, and RT-PCR is unavailable in many clinical laboratories [7]. Although the EIA method shows some promising results, its sensitivity must be increased for these assays to become useful as a primary tool for the detection of NoV diarrhoea in clinical laboratories.

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REFERENCES