Comparison of Premier™ Rotaclone®, ProSpecT™, and RIDASCREEN® Rotavirus Enzyme Immunoassay Kits for Detection of Rotavirus Antigen in Stool Specimens

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Abstract

Background—Rotaviruses are the major cause of severe dehydrating diarrhea in children throughout the world. Enzyme immunoassays (EIA) have been the standard method for detection of rotavirus in stool specimens since the 1980s. The World Health Organization (WHO) Rotavirus Surveillance Network has proposed including three EIA kits in the WHO-GSM (Global Management System/Système Mondial de Gestion) catalogue for easy procurement of EIA kits by participating rotavirus surveillance network laboratories.

Objectives—In this study, we conducted a comparative analysis of 3 commercially available enzyme immunoassay kits: Premier™ Rotaclone® (Meridian Bioscience, Inc.), ProSpecT™ (Oxoid, Ltd.) and RIDASCREEN® (R-biopharm AG) for rotavirus diagnostics.

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WHO, CDC

Please state whether Ethical Approval was given, by whom and the relevant Judgement’s reference number

Not applicable.

If you are submitting a Randomized Controlled Trial, please state the International Standard Randomised Controlled Trial Number (ISRCTN)

Not applicable.
Study design—Using reverse-transcriptase-PCR (RT-PCR) as the gold standard, the 3 EIA kits were evaluated by testing a stool panel consisting of 56 rotavirus-positive and 54 rotavirus negative samples.

Results—The sensitivities of the Premier™ Rotaclone®, ProSpecT™ and RIDASCREEN® kits were 76.8%, 75% and 82.1% respectively, but did not differ significantly. The specificity of all the 3 kits was 100%. The use of RT-PCR as a gold standard lowered the observed sensitivity of all 3 EIA kits but helps to reduce equivocal results that can be seen when another EIA or other non-molecular methods are used as the reference assay in comparison studies.

Conclusion—Our study found that all three kits are suitable for use by rotavirus surveillance programs.

Keywords
rotavirus; EIA; comparison; RT-PCR; sensitivity; specificity

Background

Group A rotaviruses are the leading cause of acute gastroenteritis (AGE) in infants and young children worldwide\(^1,2\). Enzyme immunoassays (EIAs) offer a simple, rapid, and sensitive method for routine laboratory detection of rotavirus antigen in stool specimens\(^3\). Commercial EIA kits have been available since the 1980s and evaluations of these kits have been performed\(^3-5\), but a comparison of current generation EIA kits has not been done. The Rotavirus Surveillance Laboratory, Centers for Disease Control and Prevention, Atlanta, Georgia, USA is the Global Reference Laboratory for the World Health Organization (WHO) Rotavirus Surveillance Network.

Objectives

To assist the WHO in recommending the best commercially available kits for rotavirus detection to laboratories that participate in the global network, we conducted a comparative analysis study of 3 EIA kits in order to determine if these 3 kits meet performance criteria required for inclusion in the WHO-GSM (Global Management System/Système Mondial de Gestion) catalogue to facilitate reagent procurement by network surveillance laboratories. The EIA kits included in this study were: 1) the Premier™ Rotaclone® (Meridian Bioscience, Inc., Cincinnati OH, USA); 2) the ProSpecT™ Rotavirus Microplate Assay (Oxoid, Ltd., Basingstoke, Hampshire, UK); and the 3) RIDASCREEN® Rotavirus (R-Biopharm AG, Darmstadt, Germany). All 3 kits use a solid-phase sandwich EIA format. The Premier™ Rotaclone® kit is the only multi-well EIA kit approved by the U.S. Food and Drug Administration (FDA) for in vitro diagnostic (IVD) use. It uses monoclonal antibodies raised against rotavirus structural protein VP6. The ProSpecT™ Rotavirus Microplate Assay EIA kit is a replacement kit for the widely-used rotavirus IDEIA™ Rotavirus EIA (Dako Diagnostics Ltd., Ely UK), which was discontinued in March 2009. It uses polyclonal capture and detector antibodies raised against rotavirus structural proteins. The RIDASCREEN® Rotavirus EIA kit uses monoclonal antibodies raised against rotavirus structural protein VP6. In 2010, RIDASCREEN® was reformulated to incorporate a
biotinylated detector antibody and streptavidin-conjugated peroxidase. The analytical performance of these kits has not been compared directly.

**Study Design**

Stool samples from AGE cases were selected from domestic and international surveillance samples received by the CDC for genotyping of rotavirus strains. All the samples selected for this study were tested for the presence of rotavirus VP4 and VP7 and/or VP6 genes using reverse transcription-PCR (RT-PCR)\(^6\)-\(^8\). Fifty-six rotavirus-positive samples and 54 rotavirus-negative samples were selected for this study. All 110 samples were tested for rotavirus antigen according to manufacturers’ instructions for each kit. Three operators performed all tests, for a total of 3 replicates per sample. EIA plates were read on an MRXe ELISA plate reader (Dynex Technologies, Chantilly, VA USA). A sample was considered to test positive by a kit if the optical density (OD) values for 2 or 3 replicates were above the calculated cut-off value for that kit. The analytical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each kit. Statistical analyses were performed by using Prism Version 5.02 Software for Windows (GraphPad Software, Inc., La Jolla, CA). Testing results were analyzed by chi-square test. OD values were compared by Kruskal-Wallis test, and pairwise comparisons mean OD values from each kit were performed using Dunn’s Multiple Comparison test.

**Results**

The results of testing 110 samples in triplicate by each kit are shown (Table 1). For each of the 3 kits, all EIA-positive samples had tested as rotavirus-positive by RT-PCR for VP4 and VP7 or VP6 and all EIA-negative samples had tested negative by RT-PCR. However, some RT-PCR positive samples tested negative by EIA, ranging from 10 for RIDASCREEN® Rotavirus to 14 for ProSpecT™. Using RT-PCR as the gold standard, the performance characteristics of the kits were: Premier™ Rotaclone® EIA, 76.8% sensitivity, 100% specificity, PPV = 100%, NPV = 80.6%; ProSpecT™ EIA, 75% sensitivity, 100% specificity, PPV = 100%, NPV = 79.4%; and, RIDASCREEN® Rotavirus, 82.1% sensitivity, 100% specificity, PPV = 100%, NPV = 84.4%. When the sample testing results of the 3 kits, expressed as positives and negatives, were analyzed by chi-square test (Table 1), the results obtained by each kit were not found to differ significantly. Distribution plots of OD values for the 3 kits (n = 330; Figure 1) showed that the distribution for the Premier™ Rotaclone® and ProSpecT™ Rotavirus kits were similar, with each plot skewed to the right. For both assays, numerous data points lay within 0.05 OD units on either side of the cut-off value (Rotaclone®, n=23; ProSpecT™, n=20). In contrast, for the RIDASCREEN® kit, OD values were bimodally distributed, with one large peak on the left side of the graph containing all the negative values, and a broad peak on the right side containing the majority of the positive OD values. Only 1 data point lay within 0.05 OD units on either side of the cut-off value. The OD values from the 3 kits were found to differ significantly, and this difference was observed when all data points were analyzed (p = 0.0131), when positive OD values only were analyzed (p < 0.001), and when negative OD values only were analyzed (p < 0.001). Pairwise comparisons showed that the OD values from the RIDASCREEN® kit differed significantly (p < 0.05) from those of the ProSpecT™
kit but did not differ significantly from those of the Rotaclone® kit. The OD values
generated by the Premier™ Rotaclone® and ProSpecTM kits did not differ significantly.
We found the Premier™ Rotaclone® EIA to have 76.8% sensitivity and 100% specificity
compared with 100% sensitivity and 92% specificity as reported by its manufacturer,
Meridian Bioscience, Inc. and 100% sensitivity and 99-100% specificity as reported in
published studies.3,5. The ProSpecTM Rotavirus EIA exhibited 75% sensitivity and
100% specificity in this study as compared with 100% sensitivity and 99.2% specificity as
reported by Oxoid, Ltd. RIDASCREEN® EIA showed 82.1% sensitivity and 100%
specificity as compared to 98.4% sensitivity and 100% specificity as reported by R-
biopharm AG. The differences between the sensitivity and specificity values that we found
and those reported by the manufacturers and others results from using different gold
standard methods. We used RT-PCR as a gold standard whereas Meridian Bioscience
compared Premier™ Rotaclone® EIA results to electron microscopy (EM) and other studies
used a reference EIAs3,5. Oxoid compared ProSpecTM Rotavirus EIA results to EM and a
commercial EIA kit, and R-Biopharm compared RIDASCREEN® EIA results to other
certified EIA kit results. The use of the more sensitive RT-PCR technique9 for establishing
the gold-standard lowers the observed sensitivity of all 3 EIA kits but helps to reduce
equivocal results that can be seen when another EIA or other methods are used as the
reference assay in comparison studies 3,5. In May 2010, Oxoid Ltd. changed the
method used to calculate the cut-off value of ProSpecTM Rotavirus kit by specifying that
cut-off limit should be calculated by adding 0.2 absorbance units to the negative control
value; previously 0.1 absorbance units were added. When the ProSpecTM Rotavirus results
from this study were reinterpreted using a cut-off value calculated using the original method,
the sensitivity increased to from 75 to 87.5%, surpassing sensitivity attained by the
RIDASCREEN® kit. Oxoid Ltd. changed the method of calculating the cut-off limit for the
ProSpecTM kit so that its performance would be equivalent to that of the IDEIA™ kit,
which it replaced. However, this change appears to have reduced the sensitivity of the
ProSpecTM Rotavirus kit.

Conclusions
In this study, we evaluated and compared 3 EIA kits, Premier™ Rotaclone®, ProSpecTM
Rotavirus, and RIDASCREEN® Rotavirus for detection of rotavirus antigen in stool
samples. Testing of the kits against a stringent gold standard showed them to have
comparable sensitivity and specificity, thus, all 3 kits are suitable for use in rotavirus
surveillance programs worldwide.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AGE</td>
<td>acute gastroenteritis</td>
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<tr>
<td>EIA</td>
<td>Enzyme immunoassay</td>
</tr>
</tbody>
</table>

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ELISA  Enzyme-linked Immunosorbent Assay
FDA  Food and Drug Administration
GSM  Global Management System/Système Mondial de Gestion
IVD  in vitro diagnostic
NPV  negative predictive value
OD  optical density
PPV  positive predictive value
WHO  World Health Organization

References

Figure 1.
Frequency distributions of OD values for the Premier™ Rotaclone®, ProSpecT™ Rotavirus, and RIDACREEn® Rotavirus EIA kits. Bins (logical ranges) were set at every 0.05 OD units. The vertical dashed line on each graph indicates the assay cut-off value. The y-axis of the bottom graph has been condensed between 100 and 180 to maintain the visibility of the shorter data bars.
Table 1
Comparison of Rotavirus Detection Results by Premiere™ Rotaclone®, ProSpecT™ Rotavirus and RIDASCREEN® Rotavirus EIA kits using RT-PCR as the Gold Standard.

<table>
<thead>
<tr>
<th>EIA Kit</th>
<th>RT-PCR</th>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>Rotaclone</td>
<td>43</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>Positive</td>
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<tr>
<td>Total</td>
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<td>54</td>
<td>110</td>
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<tr>
<td>ProSpecT</td>
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<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
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<td>68</td>
</tr>
<tr>
<td>Total</td>
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<td>54</td>
<td>110</td>
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<tr>
<td>RIDASCREEN</td>
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<tr>
<td>Positive</td>
<td>10</td>
<td>54</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>56</td>
<td>54</td>
<td>110</td>
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</table>

Results obtained by each kit were not found to differ significantly (p = 0.8483, Chi-square = 0.3291, df = 2)