Evaluation of a Latex Test for Rotavirus Detection

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A latex agglutination (LA) test (Slidex Rota-Kit; bioMérieux, Marcy-l’Etoile, France) was a rapid, easily
used method for detecting rotavirus (RV) in pediatric fecal specimens. With 45 RV-positive and 50 RV-negative
diarrhea specimens, the sensitivity of the LA test was 82%, and the specificity was 100%. Six other specimens
produced indeterminate results. The frequency of positive LA tests appeared to be proportional to the
concentration of virions in the stool.

Rotaviruses (RVs) are recognized as among the most
common causes of acute pediatric gastroenteritis, responsi-
ble for one-third or more of diarrheal illness in hospitalized
infants and young children (1, 3, 9, 10). RV disease often
occurs in epidemic prevalence during the winter. Since the
feces of patients suffering from acute viral gastroenteritis
often contain 107 to 109 virions per g (5), direct electron
microscopy (EM) can be used quite efficiently to detect the
presence of RV (and other viruses) in unconcentrated fecal
samples within minutes after sample collection (2, 10).
However, access to a laboratory that is equipped to perform
EM is often limited. An enzyme-linked immunosorbent
assay (ELISA) is generally more sensitive than EM for
detection of RV in stools (2, 8, 19, 20) but typically requires
several hours to perform, may not be ideal for the testing of
single specimens, and at times may produce nonspecific
results (2, 15). Thus, a need exists in the clinical laboratory
for a diagnostic technique which is sensitive, specific, sim-
ple, and rapid.

Sanekata et al. (17) first described a latex agglutination
(LA) method for the detection of RV in stools in 1981. Since
then, a number of LA procedures for RV detection have
been reported (6–8, 11–16, 18).

The purpose of this investigation was to compare the
Slidex Rota-Kit (bioMérieux, Marcy-l’Etoile, France) with
direct EM supplemented with a confirmatory ELISA proce-
dure for the detection of RV.

Fecal samples were collected from the first available stool
from 101 pediatric inpatients and outpatients (age range, 1 to
228 months; mean, 25 months; median, 10 months), all of
whom had acute diarrhea.

EM. EM was performed essentially as previously de-
scribed (2, 4). All of the EM preparations were read by the
same experienced microscopist, who set a clock timer for 6
min and then scanned portions of each specimen grid for that
time or until a virus had been recognized and the approxi-
mate virion count per minute of viewing had been deter-
mined.

Confirmatory ELISA. A confirmatory ELISA was per-
formed as previously described (2) using the same reagents
or reagents very similar to those originally described by
Yolken et al. (19, 20). All tests were done with RV-negative
(preimmunization) and RV-positive (postimmunization) cap-
ture antibody from the same goat. Positive specimens had an
endpoint optical density with the RV-positive serum of at
least twice that seen with the RV-negative serum.

LA. The LA tests were done on randomly assigned, coded
fecal specimens that had tested positive or negative for RV
by EM and ELISA and that were then stored frozen at
−60°C. Patient data and previous laboratory findings were
not revealed until the study was completed. The LA kit
consisted of an RV rabbit antibody-coated latex suspension
(R1), a negative control latex suspension (R2), a positive
antigen (inactivated rotavirus) control (R3), a buffer (pH 7.2)
(R4), plastic stirring sticks, and a reusable glass slide. A 10%
(vol/vol) suspension of each specimen was prepared in a
15-ml screw-cap centrifuge tube by vortex mixing approxi-
mately 0.2 g of stool with 2.0 ml of buffer. The suspension
was allowed to stand for 10 min at room temperature and
then was centrifuged for 10 min at 800 × g. One drop of R1
reagent was added to one circle on the glass slide, and one
drop of R2 reagent was added to another circle. One drop of
the stool supernatant was added to each circle and was
mixed with the reagents using plastic or wooden stirring
sticks. The glass slide was then rotated for 2 min at room
temperature on a Macro-Vue Card Test Rotator (BBL
Microbiology Systems, Cockeysville, Md.). Agglutination pat-
terns were then read under the existing fluorescent light in
the laboratory. Most patterns were also checked using a
low-power magnifying glass. Positive agglutination was
rated on a scale ranging from ± (slightly grainy appearance)
to 4+ (maximum clumping). Negative agglutination was
recorded when a smooth milky suspension was present after
2 min. More distinct agglutination patterns without any
false-positive reactions were seen after one additional
minute of rocking the slide by hand. Tests were considered
indeterminate if there was agglutination only in the R2
suspension or in both the R1 and R2 suspensions. Specimens
exhibiting indeterminate reactions were recentrifuged one or
more times for 10 min each at 2,300 × g, and the LA
procedure was repeated. The positive antigen control rea-
gent and the buffer (negative control) were used for quality
control of the procedure before each batch of specimens was
tested.

The results comparing the LA test with EM and ELISA
are shown in Table 1. Since there was complete agreement
between EM and ELISA, these results are combined. Of the
101 specimens tested, 94 gave appropriately negative LA
reactions with the control reagent, R2. One additional specimen produced a trace of agglutination with R2 but was negative for RV by EM and ELISA. With these specimens, the overall sensitivity of the LA test was 82% (37 of 45) with eight false-negative reactions. There were no false-positive reactions, giving a specificity of 100% (50 of 50). Initially, 13 specimens (12.9%) produced indeterminate results because of agglutination with both the R1 and R2 suspensions. The LA result with six specimens (five RV positive and one RV negative) remained indeterminate, even after one or more recentrifugation steps. However, with seven other samples, agglutination in R2 was eliminated after recentrifugation.

A comparison was made of the virion concentration (number of virions counted per minute of EM viewing) and the frequency of positive LA results (Table 2). The percent agreement of LA test results decreased dramatically as the virion count decreased.

**Discussion.** The simplicity, high specificity, and speed of LA tests, such as the Slidex Rota-Kit, make them useful for rapidly screening symptomatic patients. This test requires only about 25 min to produce results and would be advantageous over complex, slower methods, such as an ELISA, for field studies and when small numbers of specimens are being tested. Compared with some commercially available LA tests, the Slidex Rota-Kit offers the advantage of requiring only 0.2 g of stool. The sensitivity of the LA test in this study was comparable to that reported previously for this test, as well as other LA tests (6, 12, 16). However, a recent report found a sensitivity of 96% with this test compared with that of EM (18). In our study, the lower sensitivity of this LA test compared with EM or ELISA was most evident in samples from patients with low RV virion counts (≤2 RV virions per min).

**TABLE 1. Presence of rotaviruses in fecal specimens as determined by Slidex Rota-Kit versus EM and ELISA**

<table>
<thead>
<tr>
<th>Slides result</th>
<th>EM/ELISA result (no.)</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>37</td>
<td>0</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>8*</td>
<td>50*</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>5*</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>51</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

* One specimen was positive on two successive retests of the same suspension, done after the suspension had been at room temperature for an additional 2 h.

* The R2 control for one specimen showed a trace of granularity even though the R1 test was completely negative. One additional specimen required four centrifugation steps to remove turbidity that confused the R1 and R2 readings.

* With three specimens, the R1 test result was clearly more positive than the R2 control result, suggesting that rotavirus was actually present.

**TABLE 2. Relationship of LA sensitivity to virion concentration, as determined by the number of rotavirus virions detected by EM**

<table>
<thead>
<tr>
<th>EM result (mean no. of virions seen per min)</th>
<th>No. of specimens tested*</th>
<th>No. (%) showing agreement by LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50</td>
<td>11</td>
<td>11 (100)</td>
</tr>
<tr>
<td>~10</td>
<td>16</td>
<td>15 (93.8)</td>
</tr>
<tr>
<td>~2</td>
<td>12</td>
<td>9 (75)</td>
</tr>
<tr>
<td>~0.5</td>
<td>6</td>
<td>2 (33)</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>87 (91.6)</td>
</tr>
</tbody>
</table>

* Six specimens with indeterminate LA results were excluded.


