Relevance of Commercial Diagnostic Tests to Detection of Enteric Adenovirus Infections in South Africa

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The prevalence of enteric adenoviruses detected by an in-house enzyme-linked immunosorbent assay (the RIVM-ELISA) ranged from 13 to 38%, and subgroup F adenoviruses comprised 86%. All subgroup F adenoviruses reacted with both RIVM anti-adenovirus type 40 (Ad40) and anti-adenovirus type 41 (Ad41) monoclonal antibodies but were not detected by Adenoclone Type 40/41 enzyme immunoassay (EIA). The correlation between the Biotrin EIA and RIVM-ELISA results was low (26%). Immunospecific tests suggest that a significant proportion of enteric adenoviruses, possibly comprising previously unidentified or emerging types, are not detected by commercial diagnostic tests in South Africa.

Pediatric diarrheal disease is an important cause of childhood morbidity and mortality in Africa. Adenoviruses, particularly those comprising subgroup F (types 40 and 41), have gained acceptance as important causes of childhood gastrointestinal illness worldwide (15). Adenoviruses have been associated with 3.1 to 13.5% of cases of pediatric diarrhea in studies from Europe, Asia, and North and South America; types 40 and 41 reportedly comprise between 37.5 and 100% of these adenoviruses (1, 3, 4, 12, 15). Epidemiological studies in South Africa have shown an enteric adenoviral prevalence similar to that reported worldwide (6, 13).

Diagnosis of gastroenteritis-associated adenoviruses in South Africa is widely reliant on the use of commercial kits such as the Adenoclone Type 40/41 enzyme immunoassay (EIA) (Cambridge Biotech) and the Biotrin Adenovirus Antigen EIA (Biotrin International). The development of these kits has facilitated diagnosis of adenoviruses elsewhere in the world (9), but their efficacy in the South African diagnostic setting remains unknown. This study investigated the prevalence of enteric adenoviruses in South Africa and the sensitivity of commercial diagnostic kits for diagnosis of enteric adenoviruses in that country.

Pediatric stool specimens were obtained during 1997 from a private pathology laboratory in Johannesburg (n = 87) and from pediatric wards of two hospitals in metropolitan Johannesburg, Coronation (n = 150) and Chris Hani Baragwanath (n = 150). Specimens from the private pathology laboratory were collected from children with a clinical diagnosis of diarrheal disease, while those from hospitals were generally from children hospitalized for reasons unrelated to gastroenteritis. Approximately 10% (wt/vol) suspensions of stool specimens in phosphate-buffered saline, pH 7.2, were prepared and stored at −20°C until used for further testing. The ages of patients shedding adenoviruses are shown in Table 1.

Stool suspensions were thawed once and variously tested by the Biotrin Adenovirus Antigen EIA, the Adenoclone Type 40/41 EIA, and an in-house enzyme-linked immunosorbent assay (ELISA) (the RIVM-ELISA) based on antibodies kindly supplied by J. C. de Jong (Rijksinstituut voor Volksgezondheids en Milieuhygiëne, Bilthoven, The Netherlands). Commercial ELISAs were performed according to the manufacturers’ instructions. The Biotrin EIA reportedly detects all adenoviruses regardless of type. The Adenoclone Type 40/41 EIA detects only adenovirus type 40 (Ad40) and adenovirus type 41 (Ad41).

The RIVM-ELISA utilizes the following antibodies: MA5-8, monoclonal immunoglobulin A (IgA) type-specific anti-Ad40 (RIVM anti-Ad40); MA5-15, monoclonal IgG2a type-specific anti-Ad41 (RIVM anti-Ad41); MA66-23, monoclonal IgG1 genus-specific antiadenovirus (RIVM anti-Ad); and polyclonal horse anti-Ad10 hyperimmune serum (coating antibody). The RIVM-ELISA can be used to detect all adenoviruses (by using RIVM anti-Ad), specifically Ad40 (by using RIVM anti-Ad40), or specifically Ad41 (by using RIVM anti-Ad41). RIVM-ELISAs were performed according to the method of de Jong et al. (2), using horseradish peroxidase-conjugated sheep antimouse IgG and IgA, with tetramethyl benzidine as a substrate.

Specimens were considered positive when the absorbance (at 450 nm) exceeded 2.1 times the mean negative control value. Adenovirus type strains supplied by C. T. Tiemessen (National Institute for Virology, Johannesburg, South Africa) served as positive controls.

Considerable differences in adenovirus prevalence were detected, with values ranging from 13 to 38% (Table 2). Specimens were further screened for Ad40 and Ad41 by using separate antibodies (RIVM anti-Ad40 and anti-Ad41 monoclonal antibodies [MAbs], respectively), with Ad40 and Ad41 type strains being employed as positive controls. Subgroup F isolates were found to comprise between 73 and 93% of the adenoviruses detected (Table 2). All specimens found to react with RIVM anti-Ad40 also reacted with RIVM anti-Ad41. This was unexpected since these MAbs reportedly show strict type specificity (2). Of further interest was the finding that none of these specimens (positive by both RIVM anti-Ad40 and RIVM anti-Ad41) was positive by Adenoclone Type 40/41 EIA, a commercial ELISA specific for subgroup F adenoviruses.

A total of 272 of the above-described specimens, representing all three groups, were further screened for adenoviruses by using the commercial Biotrin EIA to compare the RIVM-ELISA and the Biotrin EIA. We found only a 26% concordance in positivity between the Biotrin EIA and the RIVM-ELISA. Of those specimens that were negative by the Biotrin
EIA, 23% were positive for adenoviruses by the RIVM-ELISA. The presence of adenoviruses in 6 (of 25 tested) representative specimens of this Biotrin-negative group, for which a complete ELISA profile had been obtained, was confirmed by PCR of the VA RNA gene (7). Of these six specimens, three were negative by RIVM-ELISA with RIVM anti-Ad40 and anti-Ad41 while three reacted with both RIVM anti-Ad40 and anti-Ad41; four of the six specimens were propagated in PLC/PRF/5 cells, with growth confirmed by electron microscopy of infected cell supernatants and by restriction fragment length polymorphism analysis with SmalI performed as previously described (8).

The prevalence of adenoviruses as determined by the Biotrin EIA was, with the exception of specimens obtained from the private pathology laboratory, considerably lower than that suggested by the RIVM-ELISA (Fig. 1). In addition, despite the relatively high adenovirus prevalence determined regardless of the test used, the number of specimens positive by both of these tests was low (1.1% to 6.5% of the specimens screened).

Further investigation of the RIVM-ELISA involved performing radioimmunoprecipitation as described by Tiemessen (14), using Ad2-infected PLC/PRF/5 cell lysate, and showed that the RIVM anti-Ad MAb reacts with a single protein of approximately 120 kDa (corresponding to the hexon polypeptide). Six stool specimens were resolved by electrophoresis on 12% polyacrylamide gels (10). Western blotting was then performed with the RIVM anti-Ad MAb diluted 1:500 in blocking solution (phosphate-buffered saline containing 5% nonfat milk and 0.01% antifoam A) and horseradish peroxidase-labeled sheep anti-mouse serum diluted 1:500 in blocking solution (10). Enhanced chemiluminescence (Amerham) studies detected no binding other than to a protein corresponding in size to the adenovirus hexon. The RIVM-ELISA positivity is therefore not simply due to an artifactual interaction with unrelated components in adenovirus-containing stools.

Our results show a considerable lack of concordance in South Africa between results obtained with different diagnostic kits reported to detect all human adenoviruses. They suggest that 23% of enteric adenoviruses in South Africa are currently undetectable with the Biotrin EIA diagnostic kit.

The prevalence of adenoviruses in South Africa may also have been underreported in previous surveys. Although the prevalence of adenoviruses as detected by the Biotrin EIA conforms to previous reports, their prevalence indicated by screening with the RIVM-ELISA is considerably higher than that previously reported in South Africa or elsewhere in the world.

It is interesting to speculate on the possibility that the prevalence of adenoviruses is linked to socioeconomic factors. Chris Hani Baragwanath hospital receives patients from predominantly low socioeconomic areas, while Coronation Hospital serves a relatively higher socioeconomic bracket. Specimens from the private pathology laboratory generally come from individuals able to afford private medical assistance, i.e., individuals of higher socioeconomic status. The prevalence of adenoviruses as detected by the RIVM-ELISA shows a decline with increasing socioeconomic status.

This trend is not maintained when screening is performed with the Biotrin EIA. The correlation in this case may be with the severity of gastrointestinal illness. Most specimens collected from hospitals came from children hospitalized for non-gastroenteritis-associated reasons. However, specimens obtained from the private pathology laboratory came from children presenting with pronounced diarrhea. It is therefore possible that the Biotrin EIA specifically detects adenoviruses that are, in South Africa, most associated with gastroenteritis. Nevertheless, although the etiological significance is unknown, the fact that over 30% of the specimens from Baragwanath Hospital contained adenoviruses that were not detected by the Biotrin EIA is of concern.

The lack of concordance between results obtained by the RIVM-ELISA and by the Biotrin EIA suggests the possibility of either previously unidentified adenoviruses or newly emerging adenovirus types circulating in South Africa. This is further suggested by the existence of a group of adenoviruses that react with both the RIVM anti-Ad40 and the RIVM anti-Ad41 (reportedly mutually exclusive antibodies) and yet are not detected with the Adenoclone Type 40/41 kit. The Adenoclone Type 40/41 EIA utilizes MAbs directed against type-specific epitopes on the adenovirus hexon (5). The emergence of hexon variants has previously been shown to compromise the sensitivity of the Adenoclone kit, with several strains of Ad41 having been shown to escape detection by an early version of the kit. Although the modified kit detects the Ad41 strains prevalent in North America (11), the potential for hexon variants to escape detection still exists. This is, to our knowledge, the first

### TABLE 1. Biotrin- and/or RIVM-ELISA-positive stool specimens classified by patient age

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>&lt;10 yrs (pediatric)</th>
<th>&gt;10 yrs</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baragwanath</td>
<td>72</td>
<td>57 (79)</td>
<td>5 (7)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Coronation</td>
<td>33</td>
<td>32 (97)*</td>
<td>0 (0)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Pvt. Path.</td>
<td>36</td>
<td>32 (89)*</td>
<td>2 (5.5)</td>
<td>2 (5.5)</td>
</tr>
</tbody>
</table>

* >95% of pediatric specimens were from children younger than 5 years.

### TABLE 2. Results of pediatric stool specimen screening by RIVM-ELISA

<table>
<thead>
<tr>
<th>Location</th>
<th>n</th>
<th>Any adenovirus</th>
<th>Ad40/41</th>
<th>% of total adenovirus positive that were Ad40/41 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baragwanath</td>
<td>150</td>
<td>57 (38)</td>
<td>53 (35)</td>
<td>93</td>
</tr>
<tr>
<td>Coronation</td>
<td>150</td>
<td>31 (21)</td>
<td>24 (16)</td>
<td>77</td>
</tr>
<tr>
<td>Pvt. Path.</td>
<td>87</td>
<td>11 (13)</td>
<td>8 (9)</td>
<td>73</td>
</tr>
<tr>
<td>Total</td>
<td>387</td>
<td>99 (26)</td>
<td>85 (22)</td>
<td>86</td>
</tr>
</tbody>
</table>
report of the existence of adenoviruses reactive with both RIVM anti-Ad40 and anti-Ad41 MAbs.

This research suggests that the locally circulating adenoviruses in South Africa differ, in both serological characteristics and prevalence, from those described thus far in the literature. These results have significant implications for the detection of gastroenteritis-associated adenoviruses in South Africa.

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REFERENCES