The abilities of hybridization probes to detect all human adenovirus types and to identify enteric adenovirus types were evaluated. The efficiency of hybridization was compared to other tests currently in routine laboratory use on clinical specimens from young children with gastroenteritis. Probes were derived from various regions of the adenovirus types 2 and 41 genomes, and were evaluated by hybridization with a series of DNA quantities from 1 ng to 10 pg of one adenovirus type from each human subgenus, lambda phage, and HEp 2 cells. The sensitivity of hybridization with the HPll probe (92.7%), containing the conserved hexon gene, compared well with EM (54.6%), culture and neutralization (45.5%), and enzyme immunoassay (61.8%). The sensitivity of detection of enteric adenovirus isolates by the cloned BglII D fragment probe (92.9%) and by a synthetic probe (85.7%), manufactured from type-specific sequences of the Ad41 hexon gene were comparable to Ad40/Ad41 specific enzyme immunoassay (84.6%). Hybridization was found to be a sensitive method of adenovirus detection in comparison to traditional methods of laboratory diagnosis. Synthetic oligonucleotides enable specific detection of individual enteric adenovirus types. Hybridization had additional advantages over other tests in identifying cases of infection with more than one adenovirus type and in allowing an estimate of the concentration of adenovirus in the specimen.

KEY WORDS: hybridization, sensitivity, specificity, virus concentration

INTRODUCTION

Despite the specific detection of adenoviruses in 5–17% of young children with gastroenteritis [Christiansen, 1989], determination of the involvement of adenoviruses in the aetiology of gastroenteritis has been difficult. Adenoviruses have been found consistently in the stools of apparently healthy children during surveillance programs [Fox et al., 1977; Rodriguez et al., 1985]. The ubiquity of adenoviruses makes it difficult to establish criteria to define adenoviral agents of gastroenteritis and prevents unequivocal substantiation of adenoviral causation of diarrhoea [Madeley, 1983]. The problem is compounded by differences in pathogenicity and cultivability between adenovirus types. The readily growing, lower numbered adenovirus types can be carried asymptptomatically [Fox et al., 1977; Kidd et al., 1982; Rodriguez et al., 1985], while isolates of the enteric adenovirus types 40 and 41 that cause the majority of clinical disease [Uhnoo et al., 1984; Wigand et al., 1983] have fastidious growth characteristics and tend to escape identification. Additionally, enteric adenoviruses present in dual infections are frequently not observed [Brown, 1985]. Adenoviruses of a different type or viruses of most other groups tend to overgrow enteric adenoviruses in culture, even when present at a lower concentration [Brown, 1990], and fastidious adenoviruses are underdiagnosed.

Adenoviruses are found as the only pathogen present in a high proportion of stools of sick children [Christiansen, 1989] and should be included in a diagnostic protocol for pediatric gastroenteritis. An adenovirus test should operate directly on the initial specimen, to avoid difficulties with culture. The capacity of the test to distinguish the enteric adenovirus types, which have a specific association with gastroenteritis, would be advantageous. In this study we examined cloned and synthesized sequences for the detection of all adenovirus types and for the specific identification of enteric types by hybridization. The sensitivity and specificity of the hybridization probes were evaluated in comparison to other conventional methods of adenovirus detection on clinical specimens from children with gastroenteritis.

MATERIALS AND METHODS

Virus and Probe Preparation

Cell lines HEp 2, A549, and 293; and adenovirus types Ad31 strain 1315; Ad7 strain Gomen; Ad2 strain Adenoid 6; Ad8 strain Trim; Ad4 strain RI-67; Ad40

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Address reprint requests to Gregory W. Hammond, Cadham Provincial Laboratory, P.O. Box 8540, 750 William Avenue, Winnipeg, Manitoba R3C 3Y1.
strain Dugan, and Ad41 strain Tak from each subgenus A to F were obtained from American Type Culture Collection (ATCC, 12301 Parklawn Drive, Rockville, MD). Viruses were cultured, purified on CsCl density gradients, and extracted as previously described [Scott-Taylor and Hammond, 1992]. Fragments of the adenovirus genome Ad41 strain Tak were amplified in competent E. coli strain JM109 in plasmid pGem 3Z (Promega Biotech, Mississauga, Ontario). EcoRI fragments A, B, and C were cloned in plasmids p41EAC (containing both A and C fragments), p41EA, p41EB, and p41EC. Ad41 hexon gene sequences were electroeluted from p41EA after digestion with Sall and HindIII. An attempt was also made to isolate the type-specific hexon gene sequences by cloning HindIII fragments of the p41EA plasmid in the blunt-ended SMAI site in the pGEM 3Z vector. Useful transformants were identified by colony hybridization with the Sall fragment of the Ad41 genome. The BglIID fragment probe was kindly donated by Howard Takiff in the form of an insert in Ad41 genome. The BglIID fragment probe was kindly provided by Howard Takiff in the form of an insert in lambda phage, and HEp 2 cells, spotted at 10 pg to 100 pg per ml. DNA preparations were denatured with the addition of 0.1 volume of 3 M NaOH, neutralized after 30 min incubation with 0.1 volume of 3 M ammonium acetate, and equilibrated to physiological salt conditions with the addition of 0.3 volumes of 20× SSC (1× SSC = 0.15 M NaCl/0.015 M Na citrate). Then 150 µl of each dilution was applied by a slot blot apparatus (Schleicher and Schuell, no. 03431, Keene, NH) under very low vacuum to nylon membrane prewetted in 6× SSC.

Preliminary investigation demonstrated that the protein extraction of the clarified stool suspension improved the clarity and completeness of hybridized spots, as noted by Kidd et al. [1982]. Stool suspensions in 450 µl aliquots were incubated with 50 µl of 10% SDS and 250 µg of proteinase K for 30 min at 37°C before extraction with phenol and chloroform. Extracted samples were then boiled and cooled in ice water before 150 µl of sample were applied to prewetted nylon membranes with the slot blot manifold under low vacuum. Membranes were washed twice in 6× SSC, air dried, and baked at 80°C for 2 h prior to hybridization. Hybridization was carried out at 68°C as previously described [Scott-Taylor et al., 1992a] with at least 107 cpm/ml random prime labelled probe (Boehringer Mannheim, kit no. 1004 760). The melting temperatures of some hybridizations were lowered by the addition of formamide to the hybridization solution, according to the estimate that 1% formamide lowers the melting temperature by 0.72°C [McConaughy et al., 1969].

RESULTS

Evaluation of Sequences of the Adenovirus Species 41 Genome for Specific Detection of Enteric Adenovirus

Ad41 EcoRI fragments A, B, and C, together comprising 84% of the genome [Scott-Taylor et al., 1992], were used to evaluate the specificity of Ad41 sequences for the detection of enteric adenoviruses. The EcoRI fragments, cloned in plasmid vectors, were hybridized with a series of adenovirus DNA preparations of each subgroup. The reaction of the DNA dilutions with a genomic Ad41 DNA probe is shown in the first panel of Figure 1 as the standard to which other probe reactions were compared. The reaction of plasmid p41EA with the subgroup DNAs is shown in panel ii. This large plasmid, containing over 50% of the Ad41 genome from 8 to 61 map units, reacted more strongly with the DNA.
of other subgroups than the whole Ad41 genomic probe, detecting lower amounts of heterologous subgroup DNA relative to the quantity of Ad41 DNA detected in the same autoradiographic time interval. Various \textit{PvuI} fragments were electroeluted from the p41EA plasmid to assess the specificity of isolated central sequences of the Ad41 genome as probes. \textit{PvuI} fragments B, D, E, and F, extending from map units 26 to 48, 48 to 59, 17 to 21, and 21 to 26, respectively (Fig. 1, RE map), all demonstrate reactions (Fig. 1, panels iii, iv, v, and vi) comparable to the parent plasmid. Isolation of segments of the \textit{EcoRI} A fragment by cleavage with \textit{PvuI} did not demarcate any area able to better distinguish between the DNA of Ad41 and other types. The \textit{PvuI} F fragment shown in panel vi may be useful as a subgenus F specific probe. This fragment detected Ad41 DNA within one log dilution of the reaction with Ad41 DNA and distinguished between other subgroups by at least 3 log dilutions. \textit{PvuI} F fragment probe has an approximately equal reactivity with enteric types and would not detect other adenovirus types unless present at 1,000 times the concentration of Ad41 virions.

### Strategies to Isolate an Ad41 Type-Specific Probe

A number of fragments from different areas of the Ad41 genome were examined for their ability to differentiate between enteric adenovirus types. The reactions of these various probes are shown in a succession of panels in Figure 2 as tested against both enteric adenovirus DNA preparations on membrane spotted with DNA of species of each subgroup. In comparison to the whole Ad41 DNA probe in the uppermost panel, the \textit{EcoRI} B and C fragments inserted in pGEM 3Z vector have a relatively insensitive reaction with the DNA of other subgroups (panels ii and iv), corresponding with their position at the nonconserved right-hand end of the genome. The \textit{BglII} D fragment, derived from the portion of the \textit{EcoRI} B fragment nearer the right terminus of the Ad41 genome, hybridized with greater relative intensity with homologous Ad41 DNA than the parent plasmid. The reaction of the \textit{BglII} D fragment with Ad40 DNA in panel iii of Figure 2 is highly equivalent in sensitivity to the homologous DNA reaction. The difference in sensitivity for Ad41 and Ad40 DNA, apparently only 2 to 4 fold, is the least of any of the Ad41 fragments tested, and the \textit{BglII} D fragment was the best prospect for use as an enteric adenovirus specific probe defined.

Further attempts to distinguish an Ad41 specific probe were made by testing small restriction fragments from within the hexon gene that code for the type-specific epitopes of the capsid [Roberts et al., 1986]. The reaction of electroeluted \textit{SalI} D and \textit{HindIII} I fragment probes (Fig. 2, panels v and vi) do not adequately distinguish Ad41 DNA from DNA of other types for use as specific probes. The reaction of a cloned \textit{HindII} hexon fragment probe, called plasmid p41HH in panel vii of Figure 2, varies with the different subgroup DNA preparations. No Ad41 specific probe was isolated by cloning or electroelution of Ad41 DNA fragments.

### Ad41 Synthetic Probes

Published Ad41 sequences were compared to Ad2 and Ad40 genes to determine exact sequences unique to Ad41. The longest stretches of unique Ad41 sequence in the available sequences were found in the hexon gene. The L1 surface epitope sequence [Roberts et al., 1986] was divided into 4 sequences of variation of 30 base pairs or more that could serve as diagnostic probes. The most suitable sequence, however, was a fifth unique stretch of 84 nucleotides from residues 1225 to 1308 of the Ad41 hexon sequence [Toogood and Hay, 1988] forming the L2 epitopic loop. This sequence was synthesized as two 40 base oligomers, designated Hex5A and Hex5B, signifying the fifth unique hexon region, with the following sequences:

\begin{verbatim}
Hex5A: GCAGCTACAGACACGTACTCTGGCATAAAGGCCAATGGCCAT
       AACCTGGACTGCAGACGACAATTATGC
Hex5B: AACCTGGACTGCAGACGACAATTATGCGACAGAGGGCGCA.
\end{verbatim}

The sensitivity of the two probes was tested empirically by hybridization with dilutions of adenovirus, \lambda phage, and HEp 2 DNA in conditions of increasing stringency as shown in a series of panels in Figure 3. Hex5A has a greater content of guanine and cytosine residues and could be used in conditions of greater stringency (Fig. 3A). Hex5B sequence is unique to Ad41. This is reflected in the loss of reactivity with Ad40 and nonhomologous DNA in reactions carried out above 35°C (Fig. 3B). The Hex5B probe was used in evaluation of the hybridization test with clinical samples.

### Diagnosis of Adenovirus in Clinical Samples by Various Tests

Electron microscopy, EIA, and viral culture of stool specimens were performed routinely through the period of study. Adenovirus was detected by at least one conventional test in 55 specimens of the 1,071 stool samples examined. Isolates were identified by neutralization or restriction analysis. All identified enteric isolates had restriction patterns of the Ad41A strain [Scott-Taylor et al., 1990]. The positive samples were spotted in random order among 200 samples on a nylon membrane for hybridization. Suspensions used in prior evaluations of 9 adenovirus positive specimens were spotted to determine whether virus in original suspensions had deteriorated. Samples 81 and 88 were from a single specimen, spotted twice to ensure reproducibility. The 200 spotted samples consisted, therefore, of 55 unique adenovirus positive specimens, 28 of which were assessed as enteric isolates, 10 duplicates, and 135 adenovirus negative specimens. Twelve of the adenovirus negative samples contained rotavirus, 8 grew enteroviruses, and small round virus particles were seen by electron microscopy in 6 and coronavirus in 1 more. The specimens reacting with the various diagnostic
Fig. 1. Adenovirus subgenera DNA hybridized with plasmid p41EA and electroeluted PvuI fragments.

Then 100 μl aliquots of a phage, cellular and adenovirus DNA of one species from each human subgenus, spotted in tenfold dilutions, were hybridized at 68°C with genomic Ad41 DNA (i), plasmid p41EA (ii), and the electroeluted PvuI fragments B (iii), PvuI D (iv), PvuI E (v), and PvuI fragment F (vi). The PvuI map of the p41EA plasmid is shown at the bottom. Autoradiographs were developed after 48 hr.
Fig. 2. Hybridization of Ad41 EcoRI and hexon gene fragments with the DNA of each subgenus. Log dilutions of DNA of Ad40 and one species from each adenovirus subgenus were hybridized at 68°C with genomic Ad41 DNA (i), EcoRI fragment B plasmid p41EB (ii); BglII D fragment containing plasmid (iii); EcoRI C fragment containing plasmid p41EC (iv); fragment SacI D (v); fragment HindIII I (vi); and a HindII hexon gene fragment containing plasmid (vii).
Fig. 3. Hybridization of the Hex5A and Hex5B probes with subgenera DNA at various stringencies. The synthetic probes were hybridized with membranes at 25°C, 35°C, and 55°C with either 0% or 14% formamide in the hybridization solution. Each membrane was enclosed with film for 48 hr.

tests are compiled in Table I in the order spotted on the hybridization membrane. A total of 60 of the samples, including 51 of the unique specimens, reacted with the HPII probe in the first panel of Figure 4. The BgII D fragment and synthetic Hex5B probes in the two panels below reacted with 26 and 24 specimens, respectively. The amount of viral DNA in the specimen could be estimated from the dilution series of control DNA below the specimens. The Ad41 genomic DNA probe and plasmid p41EC, containing the Ad41 EcoRI C fragment, hybridized with specimens containing adenoviruses other than enteric types (reactions not shown). Cloned probes p41EC and BgII D reacted with negative specimens 122, 124, and 166. Specimens 27 and 199 reacted strongly with all Ad41 DNA probes, although types Ad2 and Ad5 emerged from culture. These specimens were subsequently tested with the subgenus F-specific EIA and were found to harbour a conventional type as well as an enteric adenovirus in concurrent infection. A sample of 5 of the 9 uncultivable specimens not identified to type was tested in the blocking assay. Preincubation with the Ad41 monoclonal antibody reduced binding to the capture antibody by between 12% and 37%. The Ad40 antibody had no effect and for the purposes of calculation of test performance all the uncultivable specimens were presumed to contain Ad41. The sensitivity and specificity of the diagnostic tests are compared in Table II. The 92.7% sensitivity of the HPII probe in hybridization, detecting 51 of 55 unique specimens, compares favourably with conventional diagnostic tests. The specificities of the genomic Ad41 DNA, p41EC, and BgII D probes were evaluated on the detection of enteric adenovirus types and were reduced by reactions with unrelated adenovirus types or negative specimens. The synthetic DNA probe specificity was evaluated for Ad41 specimens alone. No false positives were attributed to HPII or Hex5B probes. The predictive values of the tests (Table II) indicate that most hybridization probes had greater reliability in reporting a positive or negative test result than the conventional diagnostic methods.

**DISCUSSION**

Hybridization demonstrated a higher sensitivity than the methods of adenovirus detection currently employed. Results indicate that hybridization could improve the efficiency of diagnosis of adenovirus infection in gastroenteritis by one and a half times or more over individual methods in routine use. The technique has great flexibility and can utilize cloned or synthetic sequences to diagnose groups or individual types of virus. Oligomeric probes can evidently form effective means
TABLE I. Detection of Adenovirus in 200 Clinical Samples by Various Tests*

| Sample number | Electron microscopy | Group EIA | Culture neutraliz. | Enteric EIA | Restriction analysis | Hybridization probe |
|---------------|---------------------|-----------|--------------------|-------------|----------------------|--------------------|
| 2             | -                   | Ad2       | nd                 | nd          | ±                    | 1                  |
| 19            | -                   | Ad4       | nd                 | nd          | ±                    | 1                  |
| 20A           | -                   | Uld       | nd                 | Ad2         | +                    | 1                  |
| 21            | -                   | NG        | +                  | NG          | 3+                   | 1                  |
| 22            | +                   | Uld       | + Ad4lv            | ±           | ± ± ± + ±            | 1                  |
| 27            | +                   | Ad2       | nd                 | 5+          | ± ± ± + +            | 1                  |
| 28            | +                   | Ad2       | nd                 | +           | ± ± ± + +            | 1                  |
| 34            | -                   | Ad2       | nd                 | +           | ± ± ± ± ±            | 1                  |
| 39            | +                   | Ad4       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 43A           | -                   | Ad2       | nd                 | 2+          | ± ± ± ± ±            | 1                  |
| 44            | +                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 45I           | +                   | Uld       | + Ad4lv            | 4+          | ± ± ± ± + +          | 1                  |
| 51            | +                   | Uld       | Ad2v               | +           | ± ± ± ± ±            | 1                  |
| 53            | +                   | Uld       | Ad3v               | +           | ± ± ± ± ±            | 1                  |
| 57            | -                   | Uld       | Ad1v               | ±           | ± ± ± ± ±            | 1                  |
| 61            | +                   | Ad2       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 63            | -                   | Ad5       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 68            | +                   | Uld       | + Ad4lv            | 5+          | 4+ ± ± ± + +         | 1                  |
| 72B           | +                   | Ad4       | nd                 | +           | ± ± ± ± ±            | 1                  |
| 73            | +                   | Uld       | + Ad4lv            | 4+          | ± ± ± ± + +          | 1                  |
| 77            | +                   | Ad6       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 76            | +                   | Ad2       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 79            | +                   | Uld       | Ad2v               | +           | ± ± ± ± ±            | 1                  |
| 811           | +                   | Uld       | Ad4lv              | +           | ± ± ± ± ±            | 1                  |
| 86            | +                   | Uld       | Ad4lv              | ± ± ± ± + + | 1                  |
| 87D           | +                   | Ad5       | nd                 | ± ± ± ± + + | 1                  |
| 88C           | +                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 91E           | +                   | NG        | + NG               | 2+          | ± ± ± ± ±            | 1                  |
| 94            | +                   | Uld       | + Ad4lv            | 5+          | ± ± ± ± ±            | 1                  |
| 95E           | +                   | NG        | + NG               | 2+          | ± ± ± ± ±            | 1                  |
| 97F           | +                   | Ad3       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 104G          | -                   | Ad2       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 107           | -                   | Uld       | Ad2v               | ±           | ± ± ± ± ±            | 1                  |
| 108B          | +                   | Ad4       | nd                 | ±           | ± ± ± ± ±            | 1                  |
| 112           | +                   | Uld       | + Ad4lv            | 2+          | ± ± ± ± + +          | 1                  |
| 116           | +                   | Uld       | + Ad4lv            | 4+          | ± ± ± ± + +          | 1                  |
| 119           | -                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 122           | -                   | NG        | nd                 | ± ± ± ± + + | 1                  |
| 124           | -                   | NG        | nd                 | ± ± ± ± + + | 1                  |
| 128           | +                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 129           | +                   | Ad2       | nd                 | ± ± ± ± + + | 1                  |
| 133H          | -                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 134G          | +                   | Uld       | Ad2v               | ± ± ± ± + + | 1                  |
| 137D          | -                   | Ad5       | nd                 | ± ± ± ± + + | 1                  |
| 139           | +                   | Uld       | nd                 | ± ± ± ± + + | 1                  |
| 141           | +                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 142           | +                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 155           | +                   | Ad1       | nd                 | ± ± ± ± + + | 1                  |
| 159           | -                   | NG        | nd                 | ± ± ± ± + + | 1                  |
| 160           | +                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 164I          | +                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 166           | -                   | NG        | nd                 | ± ± ± ± + + | 1                  |
| 167           | -                   | NG        | nd                 | ± ± ± ± + + | 1                  |
| 169           | +                   | Uld       | Ad4lv              | ± ± ± ± + + | 1                  |
| 170H          | +                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 175           | +                   | NG        | + NG               | ± ± ± ± + + | 1                  |
| 176           | -                   | Ad2       | nd                 | ± ± ± ± + + | 1                  |
| 182B          | +                   | Ad4       | nd                 | ± ± ± ± + + | 1                  |
| 184I          | +                   | Uld       | + Ad4lv            | ± ± ± ± + + | 1                  |
| 186           | -                   | Ad2       | nd                 | ± ± ± ± + + | 1                  |
| 187F          | -                   | Ad1       | nd                 | ± ± ± ± + + | 1                  |
| 191F          | +                   | Ad3       | nd                 | ± ± ± ± + + | 1                  |
| 193           | +                   | Ad2       | nd                 | ± ± ± ± + + | 1                  |
| 196           | -                   | Ad2       | nd                 | ± ± ± ± + + | 1                  |
| 199           | +                   | Ad5       | nd                 | ± ± ± ± + + | 1                  |
| 200           | +                   | Uld       | + Ad4lv            | 5+          | ± ± ± ± + +          | 1                  |

*A to I = duplicate samples from same patient/stool; NG = no growth; Uld = unidentified; nd = not done; v = variant strain. Hybridization probe 1 = HPII, 2 = Ad41, 3 = p41EC, 4 = BglII D, 5 = HIX5B.*
Fig. 4. Hybridization of 200 clinical samples with an HPII fragment probe (upper panel); BglII D fragment probe (middle panel), and Hex5B synthetic probe (lower panel). The 10% stool suspensions, deproteinated and boiled, were spotted on membrane, baked, and hybridized with the probes at 68°C. The film enclosed with the membranes were developed for 48–60 hr.
of diagnosis, and selection of the appropriate shared or unique sequence can enable differentiation of groups or individual adenovirus types according to the degree of specificity required. The type-specific hexon sequences provide a means to differentiate between closely related adenoviruses by hybridization with DNA probes or neutralisation with antipeptide sera [Toogood et al., 1986; Brown, 1985; Kidd et al., 1982; Wigand et al., 1983]. The lack of false-positive reactions of vector DNA with plasmids derived from alimentary flora has previously been observed [Huang and Deibel, 1988; Takiff et al., 1985]. The loss of specificity of some hybridization probes was probably due to reaction with plasmid DNA from bacterial flora, as several false-positive specimens reacted only with probes amplified in bacteria. The reaction of vector DNA with plasmids derived from alimentary flora has previously been observed [Huang and Deibel, 1988; Takiff et al., 1985]. The lack of false-positive reaction of the HPII probe, however, demonstrates that extensive electroelution can render probes sufficiently free of plasmid DNA contamination for use with faecal specimens. It may be advisable to saturate the probe with unlabelled plasmid DNA to completely eliminate this source of false results in hybridization. The uncultivable specimens tested with the blocking assay were reduced in binding by less than the 50% critical value that identifies an adenovirus type by the test methods. The low reduction in binding may signify that either these specimens were grossly denatured or that commercially available Ad41 monoclonal antibodies [Herrmann et al., 1987; Wood et al., 1989] are not able to react efficiently with local variant strains of Ad41 specific to Manitoba. The prevalent Ad41 strain in Manitoba was not detected by the first commercial

| Diagnostic method | Sensitivity | Specificity | Positive | Predictive value |
|-------------------|-------------|------------|----------|-----------------|
| Electron microscopy | 54.6% | 100% | 100% | 84.4% |
| Culture & neutralization | 45.5% | 100% | 100% | 81.8% |
| Group enzyme immunoassay | 61.8% | 100% | 100% | 87.1% |
| Enteric enzyme immunoassay | 94.6% | 100% | 100% | 97.1% |
| HP11 probe | 92.7% | 100% | 100% | 97.1% |
| Y probe | 11 | 135/135 | 51/51 | 135/135 + 4 |
| B probe | 85.7% | 94.9% | 72.7% | 97.7% |
| R Ad41 probe | 101.4 | 170/170 + 9 | 20/24 + 9 | 170/170 + 4 |
| D probe | 64.3% | 93.9% | 62.1% | 94.4% |
| I probe | 18/28 | 170/170 + 11 | 18/18 + 11 | 170/170 + 10 |
| Z probe | 92.9% | 98.8% | 92.9% | 98.8% |
| T probe | 26/28 | 170/170 + 2 | 26/26 + 2 | 170/170 + 2 |
| O probe | 85.7% | 100% | 100% | 97.7% |
| N probe | 24/24 | 170/170 | 24/24 | 170/170 + 4 |
Adenovirus Hybridization

enzyme immunoassay [Scott-Taylor et al., 1990] marketed with monoclonal antibodies developed to the prototype strain Tak of Ad41 [Herrmann et al., 1987]. These observations suggest that DNA hybridization tests, less affected by the variation found in circulating strains of adenovirus types, may have more long-term efficacy than highly specific serological detection methods.

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