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Detection of adenoviruses in faeces and the confirmation of isolates by latex agglutination

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Summary

An adenovirus (AV) group-specific latex agglutination test (LA) was assessed for its ability to detect AVs in faeces and to confirm the isolation of AVs in cell culture. It detected all of 62 strains belonging to subgenus F (AdF) but only 4/40 non-AdF serotypes. This is possibly due to the concentration of AdF strains in faeces being greater than non-AdF strains. No false positive reactions were found with 55 AV-negative faeces, 42 of which contained other viruses frequently found in faeces. Only one of the 157 specimens examined gave a non-specific reaction, i.e. test and control latexes both agglutinated. All of 14 AV isolates but none of the 12 isolates of other viruses were LA positive. It is concluded that LA would be useful for detecting AdF strain in faeces and for confirmation of AV isolates, especially if electron microscopic facilities were not available.

Key words: Adenoviruses, latex agglutination, faeces

Introduction

Adenoviruses (AV) are frequently present in children's faeces. Low numbered serotypes, such as AV 1, 2 and 5, are often isolated in cell culture but may not be of aetiological significance. Of greater importance are those belonging to subgenus F (AdF), i.e. types 40 and 41, which account for about 10% of cases of juvenile gastroenteritis¹.

Diagnosis is usually made by electron microscopy (EM) of faeces but about a third of those detected may be non-AdF strains². However AdF strains tend to be present in significantly greater numbers than non-AdF serotypes. Enzyme immunoassays (EIA) which may be group (Gp) or AdF specific have been developed^{3–5} but the AdF variant is preferable as it detects only types 40 and 41, whereas the Gp specific EIA may be positive with samples containing other serotypes³. Latex agglutination (LA) tests, which are frequently used for

rotavirus diagnosis⁶ as they are rapid and ideal for examining small numbers of samples, although not as sensitive as EIA, have also been developed for detecting AV. Grandien and colleagues⁷ considered one test to be 100% specific with sensitivities of 100% and 95% compared with EIA and EM respectively. Approximately three-quarters of the positive samples in their study were AdF serotypes but they did not state specifically the numbers detected by EM and EIA or jointly by both tests. They also found that 13% of the total samples examined gave non-specific reactions, i.e. test and control latexes both agglutinated.

An LA test, specific for AV 41, was developed by Sanekata et al.⁸ but while they considered it to be four times more sensitive than EM it was evaluated on only a very small number of samples. It is therefore evident that, despite its obvious potential, LA for AV diagnosis requires further investigation. The isolation of AVs in cell culture may be confirmed by the Gp EIA and, as the concentration of virus in such isolates is usually high, it is evident that LA could be as sensitive as, and considerably quicker than EIA for this purpose.

This paper reports further studies of the detection of AVs, including AdF and non-AdF strains, in faeces by LA and its ability to identify AV isolates.

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Materials and methods

Faecal samples

One hundred and fifty-seven faeces, which had been stored at +4° C for up to 12 months post-collection, were examined; 102 were known to be AV positive. Of those, 99 were EM positive and had been classified as AdF and non-AdF serotypes, of which there were 62 and 37 respectively, by Gp and AdF EIAs (Adenoclone, Croft Biotech Ltd, Wetherby, UK) and isolation in cell culture followed by specific neutralization during previous studies². The other three AV-positive samples were negative by EM but had been found to contain AV 1 or 5 by isolation; one was also weakly positive by Gp EIA. The remaining 55 samples of the cohort were considered to be AV negative by EM, Gp EIA and isolation in cell culture. Forty-two were known to contain other viruses; 19 were rotavirus positive, nine enterovirus, six small round structured viruses, including calicivirus and Norwalk-like agents, three coronavirus and three astrovirus.

Cell culture isolates

Twenty-six were examined. Fourteen were AV positive (five type 1, two type 2, one type 3, two type 5, two type 10, and two untyped) and isolated from the EM-positive, AdF EIA-negative, faeces referred to above. The remaining 12 contained other viruses, viz various enteroviruses (7), herpes simplex virus (2), parainfluenzavirus 1 (1), influenzavirus A (1), and cytomegalovirus (1), which had been isolated from clinical samples. The AV isolates were tested when the cytopathic effect (cpe) had freshly developed or after a few days storage at +4° C. The other isolates had been stored at -70° C for up to 12 months.

Latex agglutination test

The reagents were prepared by Ani Biotech Oy, Helsinki, Finland, and supplied by Mercia Diagnostics, Guildford, UK. The test latex was coated with immunoglobulins to the AV hexon antigen and the control latex with normal immunoglobulin; the test was therefore group specific in principle. The method recommended by the supplier was followed for examining faeces. Dilution buffer was prepared by dissolving one of the buffer tablets supplied (constituents not stated) in 100 ml distilled H₂O. An approximate 10% suspension of each faeces was prepared by thoroughly mixing about 0.1 g sample with 1 ml buffer in clean plastic 75 × 12 mm tubes. The suspensions were clarified by centrifugation at 2000 rpm for 5 min. One drop (approximately 50 µl) of supernatant was placed in each of two circles on a glass agglutination plate: to one a drop of test latex was added and a drop of control latex to the other. These were mixed with the tip of a wooden swab stick and the plate gently rocked. The time taken for any agglutination to occur was noted. A sample was deemed

positive if definite agglutination of the test latex, but not control latex, occurred with 3 min rocking. The strength of reaction was graded from + to +++; a ± reaction in which there was slight granularity of the latex, but not unequivocal agglutination, was considered to be negative. If both test and control latexes clumped the reaction was classified as non-specific. Cell culture isolates were tested as described above except that undiluted culture fluid was used in place of the faecal extract.

Results

Faecal samples

The LA findings for the 157 faeces examined are presented in Table 1. All 62 AdF strains but only four of the 36 non-AdF strains were positive. None of the 55 AV-negative samples, of which 42 contained other viruses, was LA positive but one gave non-specific agglutination of test and control latexes. The overall non-specificity rate was therefore less than 1% (1/157).

The 66 LA positive samples were analysed by strength and speed of reaction (Table 2). Strong (+++) agglutination was found with 56 (85%) samples, of which 45 (68%) reacted within 30 s. Overall, moderate to strong (++ and +++) reactions which developed within 120 s were produced by 94% of all LA positive samples. Of the four non-AdF strains which were LA positive, two (an Ad2 and an untyped strain) gave a strong (+++) reaction within 60 s, one (Ad2) a moderate (++) reaction within 120 s and the fourth one (also Ad2) only weak (+) agglutination which took 150 s to develop.

Table 1. LA results of the 157 faeces examined

Virus	No.	Latex Agglutination		
		+	-	NS*
AdF strains	62	62	0	0
Non-AdF strains	40	4	36	0
AV negative	55†	0	54	1

* NS = non-specific reaction; † Forty-two contained other viruses.

Table 2. Analysis of positive samples by strength and speed of reaction

Strength of LA reaction	n	Time			
		<30 s	31-60 s	61-120 s	121-180 s
+++	56	45	9*	2	0
++	6	0	1	5	0
+	4	0	0	2†	2
Total	66	45	10	9	2†

* Including an AV2 and an untyped strain; † Including an AV2 strain each.

Cell culture isolates

All 14 AV isolates, 13 of which reacted strongly within 40 s, but none of the 12 isolates of other viruses were LA positive. The fourteenth AV isolate was found to be LA negative on initial testing when the cpe was 75% but after a further 24 h incubation the cpe had increased to 100% and moderate (++) agglutination of the latex developed within 90 s.

Discussion

These results show that LA, even if based on a Gp-specific principle, would be useful in detecting AdF strains in faeces. It has been shown previously² that clinically-significant AdF strains in faeces are often excreted in large numbers ($>10^7$ ml⁻¹); non-AdF serotypes are usually excreted in smaller numbers and are of doubtful clinical significance. It is clear that the LA test described here requires the presence of large numbers of virus particles to be reliable. This is confirmed by the reactions obtained with cell culture AV isolates which usually contain in excess of 10^7 particles per ml. Unlike Grandien et al.'s⁷ study which reported LA being positive with 9/12 (75%) of faeces shown to contain non-AdF serotypes, only 10% (4/40) were LA positive in the present one. This suggests that their test was more sensitive than the one used in this study, but no conclusion may be drawn specifically as comparisons of this nature need to be done on the same cohort of specimens. It is possible that a greater proportion of our non-AdF positive samples contained low concentrations of particles, although 37/40 were EM positive suggesting 10^6 virions ml⁻¹ at least.

The present study found the non-specificity rate, when examining faeces, to be <1%, compared with 13% by Grandien et al., and no cross reaction with other faecal viruses was found. The present study also shows that LA would be useful for confirming AV isolates rapidly as all but one of 14 examined reacted strongly. The likely explanation for the isolate, positive only after re-incubation, is that there were insufficient extracellular particles in the medium when first examined with virtually all the progeny being cell-associated at that stage. During re-incubation the particle concentration in the medium clearly increased so that a positive result was obtained on re-testing. This suggests that physical disruption of infected cells to

release progeny virus, by freeze-thawing or sonication, would be advantageous.

Although only isolates from faeces were examined, similar results could be expected from AV isolates from other sites, for example eyes, where AV may be pathogenically important. Further research into the use of LA for AV diagnosis is required, perhaps with the objective of developing an AdF-specific version, possibly similar to that of Sanekata et al.⁸, because of the importance of AV 40 and 41 in juvenile gastroenteritis.

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