Rapid Detection of Human Viruses in Faeces by a Simple and Routine Immune Electron Microscopy Technique

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SUMMARY

An immune electron microscopy technique modified from the Anderson & Doane (1973) method by using commercial pools of human gamma-globulins has been successfully used for the morphological identification of several different viruses from clarified faeces. This technique is simple, rapid and numerous samples can be easily processed.

Faeces were prepared for electron microscopy as a 20% (w/v) suspension made in phosphate-buffered saline (PBS). The suspension was first centrifuged at 1000 g for 5 min and then at 10000 g for 20 min and the supernatant used directly. In some instances, supernatants of infected cell cultures showing a clear cytopathic effect (c.p.e.) were used but without clarification.

For the preparation of agar containing human gamma-globulins, a molten aqueous solution of 4% Noble agar (Difco) cooled to 56 °C was mixed with an equal volume of human gamma-globulins (Connaught Laboratories, Willowdale, Ont., Canada) diluted 1:12.5 in PBS and previously brought to 56 °C. Merthiolate was added at a final concn. of 0.01% and 0.3 ml of the mixture was then distributed in each well of disposable flexible vinyl microtitre plates (Cooke Engineering, Alexandria, Va., U.S.A.). Once the agar had solidified, each microtitre plate was sealed with a pre-cut adhesion acetate tape (Dynatech Laboratories, Alexandria, Va., U.S.A.) and the plates were kept at 4 °C until use. Each well thus contained 2% agar with 1:25 human gamma-globulins. When needed, individual wells were cut with scissors and held upright by placing them into wells of an empty microtitre plate. The sample (25 μl) was deposited on to the surface of the agar and a carbon–Formvar-coated copper grid was placed, face downward, on top of the drop. After almost complete diffusion of the aqueous phase (15 to 30 min), the grid was picked up, stained with 3% phosphotungstic acid (PTA) pH 6.8 and examined with a Philips EM 300 electron microscope.

Most of the faeces examined by electron microscopy were obtained from cases of infantile gastroenteritis. In those instances, the human gamma-globulins-in-agar method permitted the rapid detection of rotaviruses in about a third of the samples (Table 1). Rotaviruses were present in the form of aggregates of different sizes and rarely appeared as isolated virions.
Table 1. **Immune electron microscopy of infantile gastroenteritis faeces***

<table>
<thead>
<tr>
<th>Virus observed</th>
<th>No.</th>
<th>%</th>
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<tbody>
<tr>
<td>Rotavirus</td>
<td>50</td>
<td>31.3</td>
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<tr>
<td>Picornavirus-like</td>
<td>5</td>
<td>3.1</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>4</td>
<td>2.5</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>62.5</td>
</tr>
<tr>
<td>Total no. of positive samples</td>
<td>60</td>
<td>37.5</td>
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* Samples were provided during a 1 year period by the department of microbiology of Ste-Justine Hospital, Montréal, Québec, Canada.

The aggregates contained various morphological forms of rotaviruses such as intact or penetrated, single- or double-shelled particles together with fragmented virions. Even when the number of rotaviruses was low, small aggregates were readily seen on the grid and easily identified among the debris. In some specimens, a few highly altered rotaviruses or just fragments of capsids were the only aggregated structures observed but were sufficient to diagnose a positive sample. In the latter cases detection of rotaviruses would have been much more difficult without agglutination of the viral material. An aggregate of rotaviruses in a sample prepared using the IEM technique is shown in Fig. 1 (a).

This technique also permitted the detection of other viruses less frequently encountered in infantile gastroenteritis faeces. Small enterovirus-like particles, without distinctive morphological features and which can be easily mistaken as debris when seen in isolation, were also aggregated and rapidly detected by the human gamma-globulins-in-agar technique as shown in Fig. 1 (b). Astroviruses were also specifically aggregated by this approach thus facilitating their detection. These viruses were positively classified as astroviruses by the visualization of the 5-pointed star on some particles. Such an example is presented in Fig. 1 (c) which shows a fairly large aggregate of mostly intact astroviruses. Adenoviruses were also observed in some samples. Being clumped and because of their characteristic size and morphology, their identification was fairly easy. Here again, intact and broken adenoviruses were seen in the aggregates as in Fig. 1 (d).

Supernatants of infected cell cultures were also examined by IEM with specimens that developed a clear c.p.e. on passage in tissue cultures. Although cell debris was concentrated on the surface of agar, this material did not hinder the formation of aggregates of small or large virus particles as shown in Fig. 1 (e, f). Faeces obtained from cases other than infantile gastroenteritis also showed by IEM a wide range of aggregated virus particles such as rotaviruses and adenoviruses, picornavirus-, parvovirus- and coronavirus-like particles. Although morphologically similar, in our experience parvovirus-like particles appeared much smaller (20 to 22 nm) than picornavirus-like particles (26 to 30 nm). In faeces from patients suspected of having viral hepatitis, picornavirus-like particles in aggregates were visualized (Fig. 1 g) and were later identified as hepatitis A virus by using a specific antiserum. This technique has also been tested in cases of mixed infections. Since samples with more than one virus were not available, we mixed samples of infantile gastroenteritis faeces containing rotaviruses and astroviruses or rotaviruses or rotavirus and adenoviruses. In both instances, specific aggregates of the two viruses involved were rapidly detected by IEM as shown in Fig. 1 (h).

The electron microscope has become an invaluable tool in the diagnosis of viral infections (Kjeldsberg, 1980; Doane, 1980; Almeida, 1980). This is especially true in the examination of faeces where several non-cultivable viruses can be present. Using agar containing human gamma-globulins, we have been able to rapidly detect rotaviruses, adenoviruses, astroviruses, picornavirus-, parvovirus- and coronavirus-like particles in aggregates from faeces obtained from various clinical cases. In agreement with numerous previous reports, a study of 160
Fig. 1. Viruses detected by immune electron microscopy using a human gamma-globulins-in-agar method. (a) Rotaviruses, (b) picornavirus-like particles, (c) astroviruses, (d) adenoviruses, (e, f) cell-cultured picornaviruses and adenoviruses, (g) hepatitis A viruses, (h) aggregated rotaviruses and astroviruses (a to d, g, h from clarified faeces). Sections were stained with 3% phosphotungstic acid pH 6-8. Bar marker represents 100 nm.
faeces showed that rotaviruses were the most common viral agent seen in cases of infantile gastroenteritis.

This diagnostic IEM technique is simple, rapid and has been successfully used with either clarified faeces or supernatants of infected cell cultures. In the latter case, large fragments of cellular debris did not interfere with electron microscopy, a fact which has been previously reported (Almeida, 1980). The serum/agar preparations can also be kept for several months at 4 °C in microtitre plates, thus eliminating the need for repeating a more lengthy classical IEM procedure for each sample. The use of agar has several advantages: it removes interfering salts, concentrates viruses at the surface (Kelen & McLeod, 1974), while diffusing antibodies aggregate viral particles leading to an improved sensitivity over direct electron microscopy (Anderson & Doane, 1973).

Very recently, the use of grids with protein A-fixed specific antibodies was successful in rotavirus detection from infantile gastroenteritis faeces but failed to reveal the presence of coronaviruses and other 27 nm virus-like particles otherwise seen by direct electron microscopy in the same samples (Nicolaieff et al., 1980). A similar modification of this technique using pooled sera may presumably improve this approach in order to detect a wider range of viruses. The use of a specific antiserum in classical IEM is also of limited value since unexpected viruses will escape immune agglutination. Direct electron microscopy of faeces is a standard approach widely used. However, Paver et al. (1973) have reported that parvovirus-like particles are hardly distinguished from other small cellular debris examined by direct EM unless aggregated by immune serum or collected inside a membrane. Also, small icosahedral viruses may be distinguished from each other on close examination (Kjeldsberg, 1977; Madeley, 1979), but such viruses, or viruses with uncharacterized morphology, are more easily detected when aggregated, especially when the viral concentration is low (Kjeldsberg, 1980; Almeida, 1980). Accordingly, the use of the human gamma-globulins-in-agar method would appear more profitable since it allows the formation of a wide range of viral immune complexes because it contains antibodies to a variety of viruses. The method should also detect more than one virus in the cases of double-infection which are occasionally seen in animals (McFerran et al., 1971) and humans (Blaskovic et al., 1980). In view of the results presented here and the associated advantages, this technique represents an efficient tool in modern diagnostic procedures.

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REFERENCES


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