Detection of virus particles by electron microscopy with polyacrylamide hydrogel

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SUMMARY The use of lyphogel to concentrate the number of virus particles in specimens for electron microscopic examination was studied in parallel with ultracentrifugation. One hundred faecal and urine samples were compared. Both methods had a similar sensitivity. Lyphogel was economical, simple, and rapid in use; in contrast to ultracentrifugation, it required relatively little material. The procedure could be done within a safety cabinet, and virus particles were morphologically undamaged by the process.

Viruses excreted in the course of enteric infections often do not grow in cell culture, nor, without specific antiserum, are immunofluorescent methods satisfactory. For such viruses, electron microscopy is an effective approach. It can detect any virus group present by its morphological appearance. The drawback is a relative insensitivity. Because of this, techniques for concentrating virus particles before examination have been studied. For virus-induced gastroenteritis, ultracentrifugation of faecal extracts has been widely practised.^{1,2} Immune electron microscopy,³ density gradient formation,⁴ and ammonium sulphate precipitation⁵ have also been used successfully, leading to the implication of several species of viruses in this syndrome.⁶ Similarly, papova and cytomegaloviruses have been associated with prolonged infection in immunosuppressed renal transplant patients and in congenital abnormalities.^{7,8}

This report compares the ability of lyphogel*, a selectively absorbent polyacrylamide hydrogel, to concentrate virus particles in faecal extracts and urine samples before electron microscopy with the alternative method of ultracentrifugation.

Material and methods

Of 100 specimens examined by negative stain electron microscopy for virus particles, 72 were stool samples from patients with infantile gastroenteritis and 28 were urine samples from patients with renal

transplants or suspected congenital abnormalities. Ten per cent faecal suspensions in Hanks' balanced salt solution (HBSS) were prepared by vortex mixing on an MSE whirlimixer and then clarified by centrifugation at 4000 rpm for 20 minutes at +4°C in an MSE Coolspin centrifuge. Of the 100 specimens, both lyphogel concentration and ultracentrifugation were used on 81, but for 18 urine samples and one stool extract from neonates only lyphogel could be used due to shortage of material.

GRID PREPARATION: LYPHOGEL METHOD

Clarified faecal suspension or uncentrifuged urine (0.55 ml) was added to a serum vial containing 0.1 g of lyphogel and left for a minimum of 4 hours at room temperature to allow fluid absorption to occur. The residual fluid was removed by pipette and applied to a formvar-carbon-coated 400 mesh electron microscope grid. Excess was drained off with filter paper, and grids were washed with 1-2 drops distilled water, blotted, and negatively stained with 3% phosphotungstic acid pH 6.5 (PTA). After inactivation of infectious material on the grids by exposure to short-wave ultraviolet irradiation for 5 minutes per side, each grid was examined for a minimum of 20 minutes in an AEI Corinth 500 or Jeol 100C electron microscope.

GRID PREPARATION: ULTRACENTRIFUGATION METHOD

Approximately 5 ml of each clarified faecal suspension was centrifuged at 8000 rpm for 45 minutes, and the supernates from these, and the urine samples, were centrifuged at 40 000 rpm for 1 hour at +4°C in a Beckman L5-65 ultracentrifuge. The

*Gelman Hawksley Limited

pellets were resuspended in 2 drops distilled water, mixed with an equal volume of 3% PTA, and added to electron microscope grids for examination.

SENSITIVITY

The comparison included stool specimens positive for rotavirus, adenovirus, coronavirus, or caliciviruses. Samples were diluted until virus was just detectable by direct examination of clarified suspensions. These were then concentrated from 5 ml and 0.55 ml samples by ultracentrifugation and from 0.55 ml volumes by lyphogel. The concentrates from each of the three methods were diluted 1:10, 1:50, 1:100, and 1:200 in distilled water and were prepared by negative staining for electron microscopy.

Results

Virus particles were seen with equal ease in samples

concentrated by either method (Table 1). In the lyphogel preparations, however, the amount of background debris on grids was small, facilitating the recognition of virus particles (Figure).

Table 1 81 stool and urine specimens examined in parallel*

Virus	Positive			Total
	By both methods	By lyphogel only	By ultracentrifugation only	
Rotavirus	25	1	0	26
Adenovirus	5	1	0	6
Coronavirus	9	0	0	9
Calicivirus	1	0	0	1
SRV†	5	0	1	6
Total	45	2	1	48

*33 were negative by both methods.

†Small, round, isometric virus particles, approximately 25 nm in diameter.

Viruses in faecal extracts concentrated by lyphogel: (a) smooth rotavirus particle; (b) single adenovirus; (c) coronavirus; (d) group of antibody-coated small round virus particles (SRV). All negatively stained with 3% PTA $\times 150\,000$.

The ability to detect viruses in stool extracts concentrated by either method was similar if 10 times the volume used for lyphogel treatment was ultracentrifuged. When equal volumes of extract were used in both methods, the lyphogel technique was more sensitive (Table 2). In addition, washed extracts of the lyphogel used to concentrate virus positive specimens were negative for viruses by electron microscopy, indicating that virus particles were not adsorbed to the hydrogel, and therefore did not affect the sensitivity of the test.

Table 2 Sensitivity of concentration method for detection of viruses by electron microscopy

Virus	Lyphogel (0.55 ml)	Ultracentrifugation	
		0.55 ml	5 ml
Rotavirus	100*	<10	50
Adenovirus	50	10	50
Coronavirus	10	<10	10
Calicivirus	100	<10	100

*Reciprocal of highest dilution showing virus particles.

Of the 18 urine and one stool specimens from neonates concentrated by lyphogel, only one urine contained small round virus (SRV) particles. In later studies, 135 specimens have been concentrated for electron microscopy by lyphogel only, with no apparent deterioration of results.

Discussion

The lyphogel technique for concentrating viruses in specimens for electron microscopy is simple, reliable, and comparatively safe. An ultracentrifuge is not required. When large numbers of faecal extracts have to be examined, the use of lyphogel is quicker and less laborious. It also leaves the centrifuge free for other procedures. Its cost, at approximately 3p per test, compares favourably with that of ultracentrifugation. The concentration factor was similar for both, but, with the smaller volumes needed for lyphogel, valuable material could be retained, such as specimens from neonates.

With lyphogel, specimens can be prepared individually as they are received in the laboratory or accumulated till the end of the day, then treated and left overnight at +4°C to concentrate, so that they can be ready for immediate examination next morning with reports available by mid-day.

Lyphogel has been used to concentrate hepatitis B surface antigen (HBsAg) in patients' sera to improve the sensitivity of serological tests.⁹ However, numerous small lipoprotein-like molecules resembling HBsAg¹⁰ were observed in sera examined

by electron microscopy after lyphogel concentration, and these made interpretation of results, in this instance, difficult. In aqueous extracts, however, due to the non-selectivity of lyphogel for low molecular weight substances, the pH and molarity of the concentrates remained unchanged during the fluid absorption process so that virus particles were not affected by changing ionic concentrations. Absorption of water, salts, and small molecules of less than 20 mega-daltons did not denature proteins and had no detrimental effect on antibody-coated virus particles. Without the need for ultracentrifugation, the lyphogel method maintained the integrity of virus particles. Capsids were free from distortion, disruption, or removal of the outer projections from, for example, coronaviruses. These effects are known to occur in ultracentrifugation. In addition, the use of lyphogel to concentrate viruses facilitates the examination of pathogens under safe conditions by reducing the amount of specimen handling required, and it can all be done in a safety cabinet.

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