A comparison of lyphogel, ammonium sulphate, and ultracentrifugation in the concentration of stool viruses for electron microscopy

Recently we described a method for concentrating viruses in faecal suspensions and urines with polyacrylamide hydrogel (lyphogel) before diagnostic electron microscopy and compared this with the widely used technique of ultracentrifugation.¹ The lyphogel method detected the same range of virus types as did ultracentrifugation and had a similar sensitivity and advantages, such as simplicity, preservation of both specimen and virus morphology, and no need for specialised equipment. Others have concentrated virus particles by immune electron microscopy (IEM), density gradient formation, low-speed centrifugation,² and ammonium sulphate precipitation.³ The techniques of IEM and density gradient formation, from their complex nature, are not easily applied in a diagnostic laboratory, and the sensitivity of low-speed centrifugation alone may be questioned.⁴

We have examined 100 stool specimens for gastroenteritis-associated viruses by negative stain electron microscopy after concentration with lyphogel, by ultra-

centrifugation, and after ammonium sulphate precipitation. The sensitivities of the methods were compared by end-point dilution studies, as previously described,¹ and their relative merits were assessed.

The numbers of particles and the range of virus types were detected with about equal frequency, irrespective of the mode of concentration used (Table 1), the sensitivity of each method being similar (Table 2). However, the lyphogel method seemed better than the other two procedures: it required no specialised equipment; it used very small sample volumes; it involved fewest manipulations, being technically the simplest of the three methods; and it was adaptable to variations in the number of specimens handled. In terms of cost, though slightly more expensive than ammonium sulphate precipitation, it was obviously less expensive than ultracentrifugation. After lyphogel treatment the amount of debris present was small, specimen background staining was low, and virus ultrastructure was well preserved. From ultracentrifuged deposits disrupted virus particles were common, and with ammonium sulphate precipitation careful washing of EM grids, possibly resulting in loss of virus particles, was required to remove crystalline deposits

which otherwise obscured virus morphology.

Based on this comparative study of 100 specimens, and its use on more than 1000 others in the course of diagnostic investigations over 18 months, during which results were similar to those of previous years, we consider that the lyphogel method is the best available for concentrating virus particles in faecal specimens before electron microscopic examination.

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Table 1 Comparison of concentration methods on 100 faecal specimens

Virus	Positive by*				Total
	All 3 methods	Lyphogel only	Ammonium sulphate only	Ultracentrifugation only	
Rotavirus	31	2	0	0	33
Adenovirus	6	1	1	0	8
Coronavirus	12	0	0	0	12
Calicivirus	2	0	0	0	2
Astrovirus	1	1	0	0	2
SRV†	8	0	0	1	9
Total	60	4	1	1	66

*34 negative by all methods.

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

Table 2 Comparison of the sensitivity of concentration methods

Virus	Lyphogel concentration (0.55 ml)	Ammonium sulphate precipitation (4 ml)	Ultracentrifugation	
			(0.55 ml)	(5 ml)
Rotavirus	200*	100	<10	100
Adenovirus	100	100	10	100
Coronavirus	10	10	<10	10
Calicivirus	100	100	<10	100
Astrovirus	100	200	<10	100
SRV	200	200	<10	100

*After treatment by each method the concentrates were diluted 1:10, 1:50, 1:100, 1:200, and 1:400 in distilled water. Titres indicate the reciprocal of highest dilution showing virus particles.