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Quantitative Estimation of Infantile Gastroenteritis Virus Antigens in Stools by Immune Adherence Hemagglutination Test

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Infantile gastroenteritis virus antigens in stools were titrated by the immune adherence hemagglutination test. A good correlation was observed between immune adherence hemagglutination titers and electron microscope counts.

In 1973 a reovirus-like agent was first detected by electron microscopy (EM) in fecal specimens from infants with acute gastroenteritis (3). The virus has since been shown to be a major etiological agent of acute gastroenteritis in infants and young children (1, 2, 4, 8). Since this agent, called infantile gastroenteritis virus, does not grow well in cell cultures, direct visualization of the virus in feces by EM has been the standard procedure for etiological diagnosis. However, simpler and more rapid methods are needed for screening large numbers of stool specimens. As we showed previously, the immune adherence hemagglutination (IAHA) test is sensitive in the detection of rotavirus antigens (6). In the present study we compared the IAHA test with the EM technique for determining the concentration of virus antigens in human stools.

Stool specimens were suspended in 10 volumes of phosphate-buffered saline and homogenized in a Potter-Elvehjem glass homogenizer. Thorough mixing at this step was essential for efficient recovery of viral antigen and for removal of nonspecific hemagglutinating activity. The homogenate was clarified by centrifugation at 800 × *g* for 30 min, mixed with an equal volume of fluorocarbon, and homogenized as above. The aqueous phase, separated by light centrifugation, was then tested for IAHA. The IAHA test was performed by the method described by Mayumi et al. (7). Four antibody units of hyperimmune serum to infantile gastroenteritis virus, prepared in guinea pigs, were used for the test. Nonspecific agglutination in the absence of antiserum was also tested. Anticomplementary activity of fluorocarbon-treated specimens was determined by the complement fixation test using five 50% hemolytic units of complement.

For electron microscopy, 4 ml of fluorocarbon-treated specimens was placed on a 1-ml cushion of 40% sucrose and centrifuged for 70 min at 100,000 × *g* in a Beckman SW50.1 rotor. The pellet was suspended in 0.2 ml of 0.002 M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.0). The suspension (10 μl) was mixed with 10 μl of a polyethylene latex beads suspension containing 1.42×10^{12} particles (Dow Chemical Co., Midland, Mich.), 60 μl of 3% ammonium

TABLE 1. *EM counts and IAHA titers of fluorocarbon-treated stool specimens*

Specimen no.	Particle count (× 10^7/ml)	IAHA titer	Nonspecific agglutination titer	Anticomplementary titer
1	10,600	1,024	<2	2
2	7,980	1,024	<2	4
3	2,030	256	<2	<2
4	160	8	4	<2
5	1,480	512	16	4
6	980	32	<2	<2
7	300	16	<2	2
8	0	<2	<2	<2
9	0	<2	<2	<2
10	35	<2	<2	<2
11	80	<2	<2	<2
12	240	32	8	2
13	2,210	128	32	4
14	30	<2	<2	2
15	2,070	128	<2	4
16	260	16	<2	2
17	580	64	<2	<2
18	1,890	128	16	4
19	700	64	<2	<2
20	0	<2	<2	<2
21	430	16	<2	<2
22	920	32	<2	<2
23	0	<2	<2	<2
24	0	<2	<2	<2
25	900	2	2	<2
26	830	16	<2	<2
27	510	2	2	<2
28	40,610	512	8	<2
29	1,510	128	<2	<2
30	1,060	32	4	<2

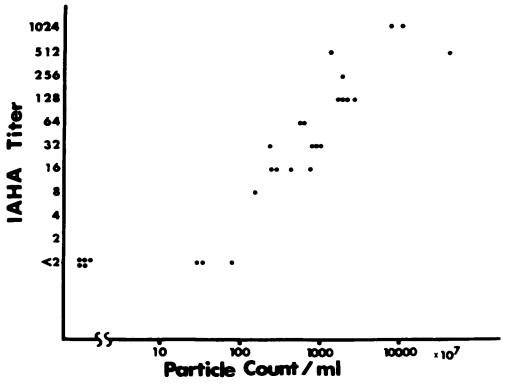

FIG. 1. *Relationship between EM counts and IAHA titers.*

molybdate, and 20 μl of 1% bovine serum albumin. The mixture was sprayed onto carbon-coated grids as described (5, 9, 10). Droplets were photographed in an Hitachi HU-11D microscope.

The results of the IAHA tests and EM with 30 specimens are shown in Table 1. IAHA titers were considered positive when they exceeded titers of nonspecific agglutination and anticomplementary activity. IAHA titers were plotted against EM counts (Fig. 1). It is seen that the IAHA test was usually positive when EM counts exceeded 1×10^9 particles per ml, and there was a roughly linear relationship between particle counts and IAHA titer.

Although the IAHA test was slightly less sensitive than the EM test for detection of IGV, the former is much simpler and better suited for examination of large numbers of specimens.

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