Letter to the Editor

Detection of rotavirus from faeces by reversed passive haemagglutination method

Rotavirus particles in faeces with gastroenteritis can be detected by electron microscopy (EM) (Bishop *et al.*, 1974), counter-immunoelectrophoresis (CEP) (Middleton *et al.*, 1976), or radioimmunoassay (RIA) (Middleton *et al.*, 1977). However, EM requires time and expensive equipment, while the CEP method is slightly less sensitive than EM. Although the sensitivity of RIA seems the best of all, it also requires elaborate procedures.

We have developed a simple reversed passive haemagglutination (RPHA) method for the detection of human rotavirus using the Nebraska calf diarrhoea virus (NCDV), which is antigenically related to human strains (Kapikian *et al.*, 1974; Matsuno *et al.*, 1977) and can be propagated in cell cultures. The presence of rotavirus in faecal extracts of 94 infants with acute gastroenteritis was sought by both the EM and RPHA methods and results were compared.

Preparation of anti-NCDV IgG sensitised cells was performed as follows. Sheep erythrocytes fixed with 5% glutaraldehyde followed by treatment with tannic acid were coated with guinea-pig anti-NCDV IgG which was purified by affinity chromatography linked with NCDV. Each faecal specimen was prepared for testing as follows: 10% faecal suspensions in phosphate buffered saline (pH 7.2 PBS) were thoroughly mixed using a Teflon homogenizer¹. The homogenate was centrifuged at 1500 g for 20 minutes at 4°C. To eliminate non-specific reactions, sheep erythrocytes fixed with glutaraldehyde (25% volume of the supernatant) were added to the supernatant. The mixture was incubated at 37°C for 1 hour, then centrifuged at 1500 g for 10 minutes. The supernatant was used for examination.

The RPHA test was carried out by a microtitration method. Permanent trays with 120 V-shaped wells were used. Serial twofold dilutions of the specimens were made in duplicate using 25 µl loops and a diluent consisting of PBS containing 2%

normal rabbit serum and 1% sheep erythrocyte stroma. In one dilution series, 25 µl of PBS was added to each well, and in the other the same amount of anti-NCDV (CF titre 1:160) was added to each well. The mixture was incubated for 1 hour at 37°C, and then a 25 µl suspension (0.6%) of erythrocytes coated with anti-NCDV IgG was added to each well. After shaking, the tray was covered and kept at room temperature, and the pattern of agglutination was observed after 1 hour. The EM method was previously described by Bishop *et al.* (1974). The results are shown in the Table.

Detection of rotavirus from faecal extracts of infants with acute gastroenteritis by reversed passive haemagglutination (RPHA) and electron microscopic (EM) methods

	RPHA method	EM method
Positive	58*	45
Negative	36	49
Total	94	94

*The presence of 75% or more specific reduction of haemagglutination titre by blocking test was regarded as positive.

Although the numbers examined were small, the RPHA method was found to be useful and practical for the detection of rotavirus in faeces. Since human rotavirus and NCDV are not identical viruses, it is possible that some antigenically deviated rotavirus may not be detected by the cross-reaction method with anti-NCDV. However, no false-negative cases with the RPHA method were found out of 45 cases determined to be rotavirus-positive by the EM method. The advantages of the RPHA method are its simplicity, higher sensitivity compared with the EM method, and the short time taken.

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¹Potter-Eluehjem Teflon tissue grinder (cat. no. 1978).