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Measurement of Rotavirus Antibody by an Enzyme-Linked Immunosorbent Assay Blocking Assay

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A new method for the measurement of rotavirus antibody is described, utilizing the system of enzyme-linked immunosorbent assay (ELISA). In this method, serum is incubated with a fixed amount of rotavirus antigen, and the amount of antibody is determined by measuring the amount of unneutralized antigen. Such an assay system proved to be as efficient as the other available rotaviral antibody systems. The ELISA blocking assay also has the advantages of not requiring purified or gnotobiotic antigen and of being able to measure rotaviral antibody in all animal species.

Rotavirus has been shown to be an important cause of human infantile gastroenteritis in many parts of the world (2, 3, 7). Because the human virus does not grow efficiently in tissue culture (13), conventional neutralization techniques cannot be used to measure antibody response. Although a number of assays, including immune electron microscopy, complement fixation (CF), fluorescent antibody (FA), and neutralization of antigenically related, tissue-culture-adapted animal viruses, have been developed (5, 6, 8), none is ideal for field work.

We have recently described an enzyme-linked immunosorbent assay (ELISA) binding assay for the measurement of antibody to human rotavirus (16). Whereas this test is highly efficient and uses very small amounts of serum, it requires antigen obtained from infected gnotobiotic animals, a reagent not widely available. In addition, a colorimeter is required for maximal efficiency. Because of these problems we have developed an ELISA blocking assay which can not only use human rotavirus obtained from human stools, but also can be read easily by eye.

MATERIALS AND METHODS

Sera. Paired sera were obtained from children with gastroenteritis who shed rotavirus, as determined by immune electron microscopy and ELISA assay. Sera were also obtained from children who had either gastroenteritis not found to be associated with rotavirus or respiratory illness (7). Paired sera were also available from adult contacts of the children with gastroenteritis. Adult contacts were either parents of patients with gastroenteritis or members of the medical staff who attended such patients. This population was described previously (4).

Antigen. Rotavirus antigen was obtained from the following sources. USA-D was a 2% stool filtrate from a gnotobiotic calf experimentally infected with a rotavirus-containing stool obtained from an ill child (16). USA-L was a 2% stool filtrate from a child with rotavirus gastroenteritis (13a). Bangladesh antigen was prepared by centrifuging diarrheal stool from a 2-year-old child from Bangladesh with rotavirus gastroenteritis, at 3,000 × *g* for 30 min. The supernatant fluid was diluted approximately 1:10 with phosphate-buffered saline (pH 7.4) and stored at −70°C (this antigen was kindly supplied by Michael Merson, Cholera Research Laboratory, Dacca, Bangladesh). An additional source of antigen was a 20% suspension of a stool (diluted in phosphate-buffered saline, pH 7.4) from a child with rotavirus gastroenteritis (specimen kindly supplied by Graham Barnes, Ruth Bishop, and William Robson, Children's Hospital, Melbourne, Australia). The Nebraska calf diarrhea virus (NCDV) used was a 2% stool filtrate derived from a gnotobiotic calf infected with NCDV, Cody strain (10). Each of the antigens was titrated in an ELISA antigen assay, and a dilution 10 times greater than the end point (i.e., 10 U of antigen) was used in the blocking assay.

Performance of the ELISA blocking assay. The ELISA blocking assay was performed as previously described (13a, 14, 15). Briefly, the sera to be tested were diluted in twofold steps, starting at 1:10, in an uncoated microtiter plate, using a multichannel pipette (Titertek). To minimize nonspecific reactivity, a diluent containing phosphate-buffered saline with 0.05% polysorbate (Tween 20), 1% fetal calf serum, and 0.5% normal goat serum was used, as previously described (15). An equal volume of rotavirus antigen (10 U) was added to each serum dilution, and the plate was incubated for 2 h at 37°C. A 100-μl amount of each virus-serum mixture was transferred to a microtiter plate precoated with hyperimmune goat anti-human rotavirus serum, and the unneutralized virus was measured by the ELISA antigen assay as de-

scribed previously. A 1:2,000 dilution of commercial human immune serum globulin was also incubated with rotavirus antigen as above and served as a positive control for the blocking procedure. In addition, diluent was incubated with or without rotavirus as above as controls for the antigen-diluent system.

The results of the assay were either read with a colorimeter which was capable of measuring optical density through the bottom of the microtiter plate, as described previously (1, 14), or read by eye. When colorimeter readings were used, a dilution of serum was considered to contain antibody when there was at least a 50% reduction of the optical density when compared to the wells containing virus-diluent control mixture as described above. When visual readings were used, a dilution was considered positive if it had less color than the control wells containing the virus-diluent mixture. Readings were made by an observer who did not have knowledge of the source of the sera or the positivity or negativity of the sera in other tests.

Other antibody assays. The ELISA binding assay was performed with alkaline phosphatase-labeled goat anti-human immunoglobulin G (IgG) as described previously (16). The results are expressed as end point titrations, using twofold dilutions (16). The CF test, using O agent and NCDV as the antigens (5), and the FA test, using frozen sections of calf intestine infected with human virus, were performed as described previously (11). Correlations were determined by the Spearman rank method (9).

RESULTS

The correlation between the ELISA blocking assay and the other assays is presented in Fig. 1. Table 1 presents the Spearman rank coefficients for these comparisons. The correlation of ELISA blocking antibody titers with those measured by the other techniques was significant at the 0.001 level. The correlation coefficient between the ELISA blocking assay and CF was lower than that with ELISA binding or FA because the CF test did not detect antibody in some adult contacts who had antibody measurable by the other assays. The ELISA blocking assay was efficient in detecting a seroresponse when children infected with rotavirus and their adult contacts were studied (Table 2). The various assay systems were equally efficient for detection of a seroresponse in infants and children. However, the ELISA blocking assay and the ELISA binding assay detected an antibody response in some adult contacts who did not have a response by

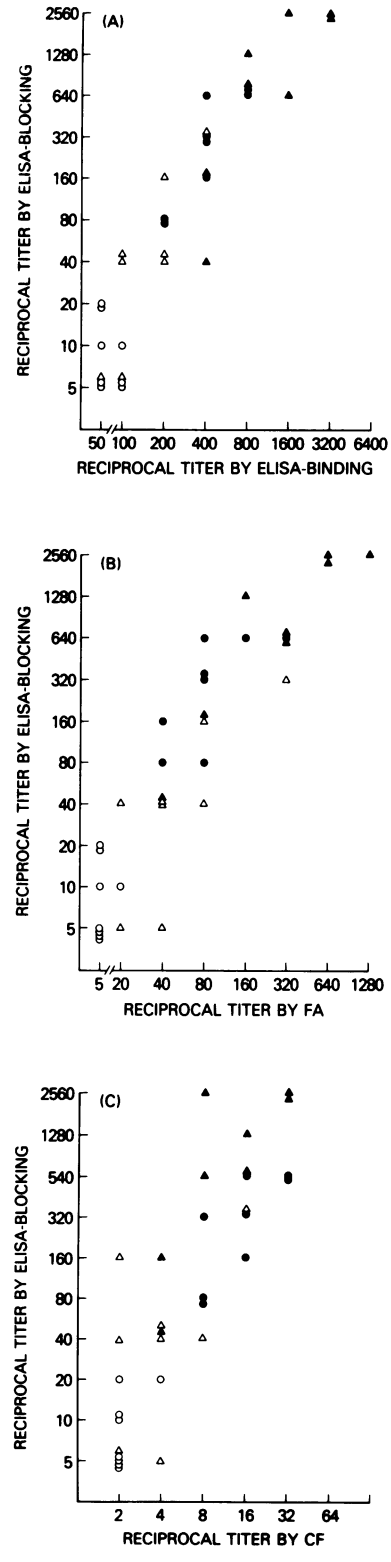

FIG. 1. *Comparison of titers of sera from rotavirus infected-patients as measured by the ELISA blocking assay and (A) ELISA binding, (B) FA, and (C) CF. Symbols:* (○) *acute sera from children from rotavirus gastroenteritis;* (●) *convalescent sera from children with rotavirus gastroenteritis;* (△) *acute sera from adult contacts;* (▲) *convalescent sera from adult contacts.*

FA or CF. Figure 2 shows that the ELISA blocking assay also detected antibody in the sera of cows and piglets experimentally infected with human rotavirus.

Whereas all of the above data were obtained using as antigen stool suspensions derived from a gnotobiotic calf infected with the human rotavirus, Table 3 demonstrates that rotavirus-positive stools obtained from humans with gastroenteritis can also be used as the source of antigen for the assay. However, NCDV, although demonstrating some cross-reactivity, was not as efficient as human rotavirus in detecting an antibody response in humans.

All of the above data are presented in terms of colorimeter readings. In addition, the ELISA blocking assay was quite easy to read by eye, and, with one exception, seroresponses were also detected visually.

Control sera. None of 15 paired sera obtained from children with nonrotavirus gastroenteritis or respiratory illness showed an antibody rise by the ELISA blocking test. In addition, three pairs of sera were tested from adults infected with each of the following agents: respiratory syncytial virus, influenza A, Norwalk virus, or enterotoxigenic *Escherichia coli*. None demonstrated an antibody response to rotavirus by the ELISA blocking method.

Reproducibility. ELISA blocking assays were performed on 10 sera on 5 consecutive days. There was no variation in ELISA blocking titer for seven of the sera and a twofold variation in the other three.

TABLE 1. *Spearman rank coefficients of rotavirus antibody assays*[a]

Assay	ELISA binding	FA	CF
ELISA blocking	0.930	0.919	0.850
ELISA binding		0.943	0.868
FA			0.833

[a] The significance of each rho value is <0.001.

DISCUSSION

The ELISA blocking test is an efficient assay for the detection of an antibody response to human rotavirus. In addition to being sensitive,

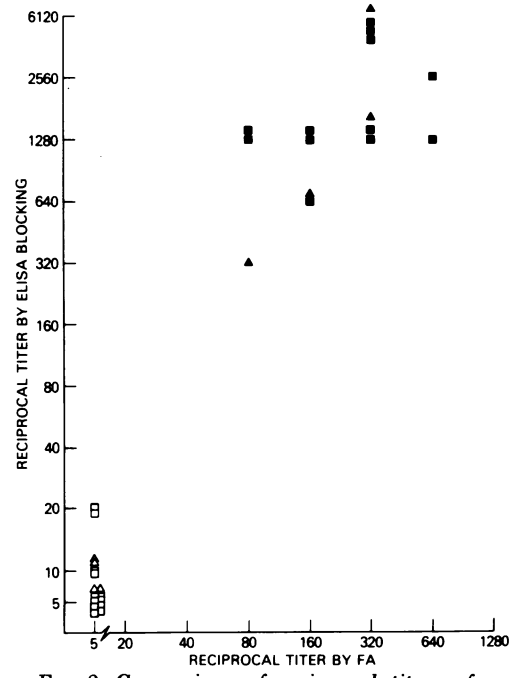

FIG. 2. *Comparison of reciprocal titers of sera from animals experimentally infected with human rotavirus as measured by the ELISA binding and FA methods. Symbols: (□) acute sera from calves infected with human rotavirus; (■) convalescent sera from calves infected with human rotavirus; (△) acute sera from piglets infected with human rotavirus; (▲) convalescent sera from piglets infected with human rotavirus.*

TABLE 2. *Comparison of methods of measuring rotavirus antibody*

Subject	ELISA blocking		ELISA binding (IgG)		CF		FA	
	ΔGMT[a]	No. of rises/no. tested	ΔGMT	No. of rises/no. tested	ΔGMT	No. of rises/no. tested	ΔGMT	No. of rises/no. tested
Rotavirus-positive children	14.7	14/15[b]	8.3	14/15	4.6	12/15	9.6	14/15
Adult contacts	10.0	15/20	7.2	15/20	2.2	7/20	6.6	12/20
Rotavirus-negative children	1.1	0/15	1.1	0/15	1.2	0/15	1.3	0/15

[a] ΔGMT, Geometric mean titer (reciprocal) of convalescent serum/geometric mean titer (reciprocal) of acute sera.

[b] One paired serum did not show a rise by any of the tests.

TABLE 3. *Comparison of rotavirus antigens for detection of seroresponse by the ELISA blocking assay*

Host species	Antigen[a]	Geometric mean titer (reciprocal)		No. of rises[b]/no. tested
		Acute	Convalescent	
Human	USA-D	26	507	12/12
	USA-L	32	562	12/12
	Bangladesh	27	485	12/12
	Australia	28	540	12/12
Bovine	NCDV	16	63[c]	8/12

[a] For source of antigens, see Materials and Methods.

[b] Paired sera from infants and young children who shed rotavirus in their stools during the course of a gastroenteric illness.

[c] Significantly different from human rotavirus antigens, $P < 0.01$. Differences among human rotavirus antigens were not significant.

it is quite simple to perform, and, when visual readings are used, expensive equipment is not required. In addition, since the reagents used are identical to those used in the ELISA antigen assay, laboratories need only have one set of reagents to detect both the presence of rotavirus in stools and antibody response to that virus. Since virus obtained from human stool can be used for the blocking assay, there is no need for a gnotobiotic animal source of the human rotavirus.

Results of the ELISA blocking assay correlated well with those of the FA and ELISA binding tests. The FA assay is an efficient assay system, but the requirement for microscopic examination of frozen sections of rotavirus-infected calf intestine make it difficult to use in large-scale studies. The ELISA binding assay has the advantage of being somewhat more sensitive and able to measure immunoglobulin subclasses such as IgM and IgA but the disadvantage of requiring either purified antigen or antigen derived from a gnotobiotic animal. In addition, the ELISA binding assay and the FA assay require a separate set of labeled reagents for each animal species tested, whereas the ELISA blocking assay can be used to study the response of any animal infected with human rotavirus. Thus, where the major need is the measurement of total rotavirus antibody, the simplicity of the ELISA blocking technique and the more ready availability of antigens used in this assay suggest that this method may be readily applicable for use in the field.

The results of the ELISA blocking assay correlated well with those of the CF assay when sera from rotavirus-positive children were studied. However, the CF assay was not efficient in detecting a seroresponse in their adult contacts (4).

Whereas four different sources of human rotavirus were equally efficient as antigens for the ELISA blocking assay, NCDV, a bovine rotavirus, was not as efficient. This lower efficiency of NCDV is similar to that found in the CF system (5). This result is also in keeping with previous findings which indicated that rotaviruses derived from different animal species could be differentiated by the ELISA blocking technique (13a).

In conclusion, the ELISA blocking assay is a simple, efficient technique to study the antibody response to rotavirus in humans and animals.

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ADDENDUM

Recently, the existence of distinct serotypes of human rotavirus has been reported (17). The panel of 12 sera used in Table 3 was tested with type 1 and type 2 rotaviruses (kindly supplied by George Zissis) as the antigen. Although there was variation in the geometric mean titers, all 12 showed at least fourfold rises to both antigens. Further experiments will be necessary to determine if a single source of antigen can be used to determine immunological response to all serotypes of human rotavirus.

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