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COMPARISON OF INDIRECT DOUBLE ANTIBODY AND DOUBLE ANTIBODY SANDWICH ELISA TECHNIQUES WITH LATEX AGGLUTINATION TEST FOR THE DIAGNOSIS OF HUMAN ROTAVIRUS INFECTION

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Summary. — 94 faecal samples from infants and children suffering of acute gastroenteritis were investigated for rotavirus by indirect double antibody sandwich ELISA kit (WHO, Geneva), Rotavirus ELISA kit (DAKOPATTS A/S, Copenhagen) and Rotalex(R) latex-agglutination kit (Orion Diagnostica, Helsinki). The ELISA techniques gave almost identical results and seemed to be of same sensitivity and specificity. Rotalex(R) agglutination had an overal agreement of 88% with ELISA. It is concluded that strongly positive reactions found by Rotalex(R) may be regarded as true positive reactions, whereas samples producing weakly positive and/or negative reactions should be retested in a more specific and sensitive assay, such as enzyme linked immunosorbent-assay (ELISA).

Key words: ELISA; *latex-agglutination*; *diagnosis*; *human rotavirus*

Introduction

Since the discovery of rotavirus as a common cause of acute gastroenteritis in infants and children, several diagnostic methods for detection of rotavirus infection have been developed (Grauballe *et al.*, 1981). In the present paper we have compared the sensitivity and specificity of two commercially available kits for the diagnosis of rotavirus infections with the recently launched ELISA-kit from WHO, Geneva.

Materials and Methods

Faecal samples. 56 and 38 faecal samples originated from infants and children admitted to Copenhagen and Warsaw hospitals respectively, during the rotavirus seasons of 1980 and 1981. All patients suffered from acute gastroenteritis. The Danish samples had previously been investigated by double antibody sandwich ELISA (Grauballe *et al.*, 1981) using the Rotavirus ELISA kit of DAKOPATTS A/S (Copenhagen, Denmark), (DAKOPATTS kit). The Polish samples were originally tested by the WHO indirect double antibody sandwich ELISA kit

(WHO kit). Before being tested all samples, meanwhile stored at −20 °C, were re-coded and then investigated simultaneously by two different ELISA techniques and by the latex-agglutination test (Rotalex(R), Orion Diagnostica, Helsinki, Finland).

ELISA. The ELISA techniques were performed according to the manuals attached to the kits. Briefly, the procedures were as follows: The solid phase was identical in both assays and consisted of polystyrene microwell plates (NUNC A/S, Denmark).

The principles of the WHO kit were identical with those described by Yolken *et al.* (1977) as modified by Brandt *et al.* (1981). Screening test was followed by a blocking test performed on all samples found positive by the screening test. In the screening test, hyperimmune rabbit anti-rotavirus serum was coated onto the solid phase. Faecal samples were tested in duplicates at a dilution of 1:4 and incubated in the microwell plate overnight. The detecting antibody was a hyperimmune guinea pig anti-rotavirus serum and the final layer was a goat anti-guinea pig serum conjugated to alkaline phosphatase. In our investigation, the screening test was regarded as positive when the OD value of the enzymatic end-product in the wells was $E_{405nm}{}^{1cm} \geq 0.3$.

All faecal samples positive by the screening test were tested in duplicates in the confirmatory blocking test. The steps in this test are the same as in the screening test, except that before being tested each faecal sample was divided into two portions. One was mixed with preimmune goat serum, the other with postimmune goat anti-rotavirus serum. The confirmatory test was regarded positive when the enzymatic end-product in wells incubated with samples mixed with postimmune serum showed at least a 50% lower OD value than the enzymatic endproduct in wells incubated with samples mixed with preimmune serum. Strongly positive samples, not blocked when tested at a dilution of 1:4, were retested in the blocking assay at a dilution of 1:40, as recommended in the manual. Thus, up to 3 days may pass before the final results have been obtained for certain samples.

The DAKOPATTS ELISA is a modification of the assay described by Grauballe *et al.* (1981). In the DAKOPATTS kit the solid phase was coated with the immunoglobulin fraction of rabbit antiserum to human rotavirus (2 wells/sample) and immunoglobulin fraction of normal rabbit serum (2 other wells/sample). Samples were tested at a dilution of 1:10 and incubated in the microwell plate for 1 hr. Detecting antibody was a rabbit anti-human rotavirus coupled to horseradisch peroxidase. Before the detecting antibody was added, ,,nonspecific binding'' had been blocked by incubation with normal rabbit immunoglobulin. The blocking of ,,nonspecific binding'' is a part of the kit procedure. When blocking was ommited, about 10% of faecal samples showed an increase in background staining. In the DAKOPATTS ELISA a result is considered positive when the mean OD value ($E_{495nm}{}^{1cm}$) of test wells less the mean OD value of control wellsexceeds 0.1 and, moreover, if the first of these values is at least 6 times greater than the secondone.

The latex-agglutination test, originally described by Sanekata *et al.* (1981) for rotavirus, was performed according to the manufacturer's recommendations. In short, each faecal specimen was tested undiluted (compared to the ELISA techniques) against latex beads coated with rotavirus antiserum and against control beads. Two separate drops of faecal supernatant were mixed with one drop of each of the reagents on a microscope slide. Positive control was performed with the positive control antigen supplied. Agglutination reactions were graded from + to ++++, as read by eye.

Results

The results are given in Table 1. When the 57 samples positive in the WHO test were tested in the blocking assay diluted 1 : 4 as stated in the manual, only 38 samples could be blocked. The other 19 positive samples were blocked only when diluted 1 : 40 and retested in the blocking assay. These 19 samples were all characterized by high OD values in the screening test. When the code was broken, it was found that 4 out of 5 samples negative in the DAKOPATTS ELISA but positive in the WHO ELISA had been scored positive by the DAKOPATTS ELISA when tested one or two years earlier. We know from experience that the antigens of rotavirus deteriorate

Table 1. Comparison of indirect ELISA (WHO kit), direct ELISA (DAKOPATTS kit) and latex-agglutination kit (Rotalex(R)) applied to 94 faecal samples for the diagnosis of human rotavirus

WHO-kit	DAKO-kit		Rotalex(R)		
	+	−	+	−	unspecific
+57	56*	1	48	8	1
−37	0	37	2	35	0
	co-positivity**		co-negativity**		overall agreement**
DAKOPATTS kit/ WHO kit	56/57 = 98%		37/37 = 100%		93/94 = 99%
Rotalex(R) kit/ WHO kit	38/57 = 84%		35/37 = 95%		83/94 = 88%

* 4 samples were positive only when tested diluted 1 : 4, as for the WHO kit.
** According to Buck and Gart (1966).

during storage of faecal material at −20 °C, therefore the DAKOPATTS ELISA was repeated with these 5 samples at a dilution identical to that used for the WHO ELISA. When this was done, the 4 samples originally positive in the DAKOPATTS ELISA became positive again.

In order to evaluate the relative sensitivity of two ELISA techniques serial dilutions were made of 3 samples with high OD values in both assays. Representative results of one of these dilution series are given in Fig. 1. It appears that the sensitivity is virtually the same for both WHO and DAKOPATTS assays when the cut-off values mentioned in Materials and Methods are used to evaluate the sensitivity of the ELISA techniques. It was more correct to use 0.3 than 0.1, as cut-off value in the WHO ELISA, as appeared from the fact that the 57 positive samples in this assay had median OD value of 1.07 (range 0.36—1.57), whereas the 37 negative samples had a value of 0.14 (range 0.10—0.21). The negative control material supplied in the kit had OD values in the same range as the negative faecal samples. The reason for these relatively high background levels could be that we used an optical path (not well defined in the WHO ELISA manual) of 1 cm when measuring OD values. A comparison of OD values of positive samples in two ELISA techniques by Spearman's rank correlation coefficient test showed a strongly positive correlation ($N = 56$, $r = 0.65$, $p < 0.001$).The median and range of OD values found for samples tested in the DAKOPATTS ELISA were similar to those previously described (Grauballe *et al.*, 1981).

It appears from Table 1 that 50 samples were positive by Rotalex(R). Five of these 50 samples were only weakly positive (score +). When the code was broken it was found that 2 false positive results by Rotalex(R) (Table 1) fell in that group. Samples that with Rotalex(R) scored results of 2+ to 4+ were all positive in the ELISA. Of the 8 false negative results by Rotalex(R) 5 samples had high and 3 had low OD values.

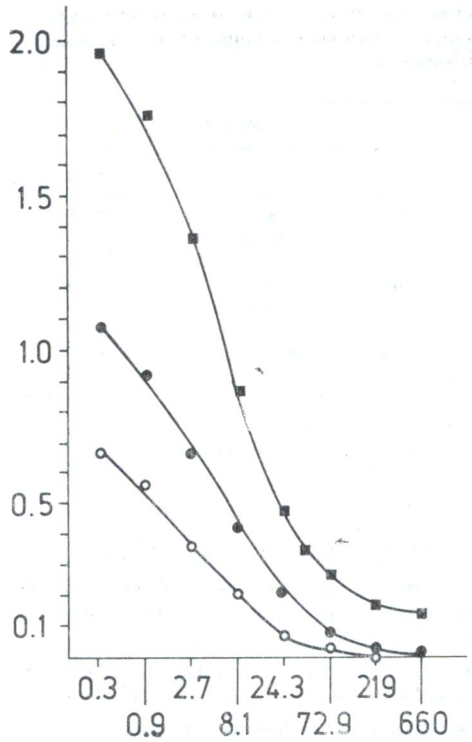

Fig. 1.

Comparison of sensitivity of double antibody sandwich ELISA (DAKOPATTS kit) and indirect double antibody sandwich ELISA (WHO kit)
○ Faecal samples incubated for 1 hr
● Faecal samples incubated overnight (Dakopatts kit)
▪ Faecal samples incubated overnight (WHO kit)
Abscissa: dilutions of the faecal sample ($\times 10^{-2}$)
Ordinate: OD values measured as $E_{495\,nm}^{1cm}$ (Dakopatts kit) or as E_{405nm}^{1cm} (WHO kit)

By testing dilutions of a positive faecal sample diluted in a negative faecal material we found that the DAKOPATTS ELISA kit was approximately 100 times as sensitive as Rotalex(R) in detecting rotavirus antigen.

Discussion

Indirect double antibody sandwich as well as double antibody sandwich ELISA techniques are very sensitive methods for the detection of rotavirus in faecal samples as concluded recently by Brandt *et al.* (1981) and Grauballe *et al.* (1981). In the present study we found that two different ELISA techniques gave almost identical results and possessed comparable sensitivities. The two ELISA kits also seem to measure the same rotavirus antigens, as a strongly positive correlation was found between OD values of either assay used. The DAKOPATTS ELISA kit allows a rapid viral diagnosis to be made, as results are obtained in 4—5 hr, whereas with the WHO ELISA results are obtained only at the third or fourth laboratory working day. The time necessary for the WHO ELISA could be reduced to 3 days, if strongly positive samples (samples giving OD values > 1.0 in the screening test) were tested directly at a dilution of 1 : 40 instead of 1 : 4 as recommended in the manual for the blocking test.

We found 4 positive samples that had OD values less than 0.1 (cut-off value), when tested diluted 1 : 10 in the DAKOPATTS ELISA. When the test was repeated with these samples diluted 1 : 4, the samples were classified as positive. It may, therefore, be concluded that the manual of this kit should recommend a repeated test with less diluted faecal material, when a faecal sample produced an OD value in the range from 0.04 to 0.1. On the other hand, in our experience with this kit such low values are found very rarely when fresh, unstored material is investigated. On fresh material, results can be read very easily with the naked eye (Grauballe *et al.*, 1981), whereas in the WHO kit it is difficult to read the results of blocking test by eye. Thus, for the WHO ELISA kit a spectrophotometer is needed.

The latex-agglutination method (Rotalex(R)) was less sensitive than ELISA and gave a co-positivity of 84%. However, this co-positivity was achieved only by reading weakly positive reactions as positive ones. This gave rise to the two false positive reactions found by Rotalex(R). We have previously experienced (unpublished results) that this problem becomes even greater when a material with a smaller number of positive samples than in the present material is tested. Although the Rotalex(R) method is very rapid as compared with DAKOPATTS ELISA, this advantage is lost when many samples are to be tested.

Thus, it might be concluded that the Rotalex(R) latex-agglutination test can be recommended as a screening test when few samples are to be tested. Strongly positive reactions may be regarded as truly positive, whereas samples giving weakly positive reactions or negative results should be tested in more specific and sensitive tests.

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