A Solid-Phase System (SPACE) for the Detection and Quantification of Rotavirus in Faeces

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(Accepted 16 February 1979)

SUMMARY

This report describes the development of a solid-phase haemadsorption system using chromic chloride-linked, antibody coated erythrocytes. It is proposed to call this technique solid phase aggregation of coupled erythrocytes (SPACE). The system is suitable for the detection of virus antigens, such as from rotavirus infections, which are present in 'dirty' or 'mixed' preparations such as faeces, urine or exudates. The test uses microtitre U-form plates coated with specific antiviral antibody; faecal suspensions are added and virus or antigen allowed to adsorb. The plates are then washed and adsorbed antigens are detected by the addition of virus-specific IgG-coated erythrocytes. The resultant settling pattern is read in the same manner as a conventional haemagglutination test. The system is compared with electron microscopy and fluorescent antibody techniques.

INTRODUCTION

The rotavirus group are ubiquitously associated with gastroenteritis in a variety of animals. In common with many other recently discovered viruses associated with diarrhoeal conditions, they are often difficult to isolate in tissue culture (Banatvala et al. 1975; Bryden et al. 1977). With only a few exceptions (Spence et al. 1976; Kalica et al. 1978) they do not haemagglutinate and rather time-consuming tests such as immune electron microscopy (IEM) or immunofluorescence (Bishop et al. 1974; Grauballe et al. 1977; Yolken et al. 1977) have been used to reveal their presence in faeces. More recently it has been shown that much of the virus antigen in the faeces is not assembled into virus particles (Mathan et al. 1977) and it is therefore possible to apply serological tests that make use of this antigen. These include immunodiffusion (Mathan et al. 1977), counterelectrophoresis (Middleton et al. 1975) and the more sensitive tests of solid phase radioimmunoassay (SPRIA), enzyme-linked immunosorbent assay (ELISA; Middleton et al. 1977; Yolken et al. 1978), and immune adherence haemagglutination (IAHA; Matsuno & Nagayoshi, 1978).

The test described in this paper has several features in common with solid phase ELISA and radioimmunoassay (RIA). However, instead of using an enzyme or radioisotope-labelled antibody, the test uses erythrocytes which are coated with specific anti-rotavirus antibody. The technique has been termed solid phase aggregation of coupled erythrocytes (SPACE). To assess the sensitivity of the SPACE test it was compared with the results
obtained by electron microscopy and immunofluorescence as well as a direct reversed passive haemagglutination system.

**METHODS**

**Production and purification of rotavirus.** A local isolate of bovine rotavirus (WRV) was cultivated in either primary calf kidney culture or in the BSC-1 African green monkey cell line. In both cases cells were washed free of foetal calf serum (from the growth medium) and the virus was adsorbed in the presence of 10 μg/ml of trypsin. The virus was then cultivated in maintenance medium containing 10 μg/ml trypsin as previously described (Almeida et al. 1978). Cells were harvested at 48 h, frozen and thawed. Because much virus remains cell-associated, the cell debris was removed by low speed centrifugation, resuspended in 1/20th vol. of distilled water and sonicated for 1 min (MSE Sonicator). This homogenate was recombined with the original culture medium and the whole was concentrated 100-fold on a 'Pellicon' Concentrator (Millipore) using an XM100 filter with a mol. wt. cut-off of 100,000. The resulting concentrate was sonicated, and layered on to a 1 m column of Sepharose 6B gel. The material which eluted first in the void-volume, as monitored by its absorbance of u.v. light at 280 nm, was collected and concentrated. Material which eluted later from the column was also pooled and concentrated. The void-volume concentrate was layered on to a cushion of caesium chloride/sucrose of density 1.28 g/ml and was centrifuged for 18 h at 60,000 g. The resulting pellet was centrifuged to equilibrium on a pre-formed caesium chloride gradient and fractions between densities of 1.30 to 1.40 g/ml were pooled, dialysed against normal saline and stored at −20 °C. Electron microscopic examination of this material revealed an abundance of rotavirus particles, estimated at 10^9/ml, with complete (double shelled) forms constituting about 60% of the total.

**Production of antisera.** Antibody to the purified rotavirus was raised in a calf and four guinea-pigs. Sera from all animals were checked by immunodiffusion (ID) and immune electron microscopy for pre-immunization antibody to rotavirus. Whereas the guinea-pigs were negative for antibody at the start, the calf (from a local dairy herd of Jersey cattle) was not. However, rotavirus antibody fell from 1/8 (by ID) 2 days after birth to 1/2 at the time of inoculation and almost certainly represented maternal antibody in colostrum. In all animals the first injection of purified virus was given in Freund's incomplete adjuvant and three subsequent injections in aluminium hydroxide colloid were given at approximately monthly intervals. All injections were given by the intramuscular route.

**Erythrocytes** were obtained from turkeys, from Jersey steers, or from adult goats. The blood was diluted with an equal vol. of Alsever's solution and processed within 24 h. None of these species of erythrocytes was ever found to be directly agglutinated by purified bovine rotavirus.

**Trypsin treatment of erythrocytes.** The method used was a modification of a previously reported technique (Coombs et al. 1977). Erythrocytes were washed five times with phosphate-buffered saline (PBS) with centrifugation for 10 min at 600 g between washes. Cells were resuspended to 10% in Earle's BSS containing 0.025% crystallized trypsin (Armour) and left for 30 min at 37 °C. After incubation the cells were washed and centrifuged twice more in PBS (5 min at 150 g) and 0.02% trypsin inhibitor (Sigma) in PBS was added to the deposited cells. After 10 min the cells were washed twice more in normal saline (0.85% NaCl) as it is necessary to remove phosphates before adding the chromic chloride reagent. Trypsin-treated cells could be stored for up to a week at +4 °C before further use.

**Preparation of globulin fractions.** Antibody was prepared as a general Ig fraction using caprylic acid for the precipitation of other serum proteins (Steinbuch & Audran, 1969). The globulin solution was dialysed extensively against normal saline and diluted to 3 mg/ml for use. Globulin preparations were made from both the guinea-pig and bovine antisera.
**Rotavirus solid-phase haemadsorption test**

**Chromic chloride coupling.** A 1% solution of chromic chloride was made in distilled water and periodically adjusted to pH 5-2 with 0·2 M-sodium hydroxide, over a period of 2 months. The stock solution was stored at room temperature. The stock solution was diluted 1/50 in saline 1 h before use. For coupling, equal vol. of trypsin-treated packed cells and bovine antibody solution (at 3 mg/ml) were mixed in unit vol. of 0.5 ml or less. Whilst mixing vigorously, diluted (0·02%) chromic chloride was added, using a Compupet diluter to add 10 or 20 μl samples at 1 s intervals. The same vol. of chromic chloride as that of the erythrocytes and globulin solution combined was eventually added (i.e. RBC : IgG : CrCl₄ = 1 : 1 : 2). The mixing was done in a plastic centrifuge tube and the mixture was then put on a slowly rotating platform held at an angle of 60° to the vertical, for 1 h at room temperature. The mixture was then diluted 10-fold in PBS, to stop the action of chromic chloride, and the cells were washed three times, with gentle centrifugation between each washing step. Finally, cells were resuspended to 5% in PBS containing 0·01% bovine serum albumin (Armour) plus 0·01% sodium azide and stored at 4°C. Control cells coated with immunoglobulin unreactive to rotavirus, but of the same species as the specific antibody, were also produced.

**Faecal specimens.** Thirty-eight diarrhoeal stool specimens from a wide age range of humans were examined. In addition, 14 diarrhoeal specimens from both calves and piglets were screened for the presence of rotavirus. The human specimens were available as a 10% clarified suspension and were delivered under a sealed code, having previously been examined by electron microscopy. Negative samples were included. Most of the human samples had been tested for their ability to produce rotavirus-specific fluorescence, in an indirect test, in either BSC-1 or LLCMK₂ monkey kidney cells, using the bovine antiserum described above, under conditions previously reported (Gardner & McQuillin, 1974). The animal specimens had been examined for rotavirus by electron microscopy (Moosai et al. 1979) and were also tested under code.

**SPACE test.** For coating polyvinyl U-form plates (Cooke), guinea-pig anti-rotavirus globulin was diluted in a 1:10 dilution of a stock 0·1 M-carbonate/bicarbonate buffer, pH 9·5, containing an additional 0·001% phenol red and 0·01% sodium azide. The optimal dilution in coating buffer for this γ-globulin preparation was found to be 1/5000 (final concentration = 10 μg/ml); 100 μl was placed in each well and the plates were then incubated for 2 h at 45 °C in a humidified box; afterwards they were washed twice with washing buffer (50 ml of 0·5 M-sodium phosphate, pH 7·2, and 5 ml Tween 20 to 10 l distilled water) and blotted dry. Washing buffer was stored at room temperature for not more than 1 month. Subsequently the faecal or tissue culture antigens were titrated in a buffer consisting of 0·1% bovine serum albumin (Armour) in PBS to which 0·01% sodium azide had been added (BSA/PBS), 100 μl vol. were dispensed in the wells and held overnight at 4°C. Plates were than washed twice more with washing buffer. After this the plates were washed once with PBS and were blotted dry over an absorbent surface such as a paper towel. Standard safety precautions were used when handling the faecal suspensions or subsequent plate washings. Fifty μl of coupled erythrocyte suspension at 0·4% was added and the plates were left undisturbed at room temperature for 1 h for the cells to settle. The results were read in the same manner as any haemagglutination test: a button of red cells representing a negative result and a carpet of cells being positive.

**Reverse passive haemagglutination (RPH).** This was performed in rigid U-form polystyrene plates (Sterilin) with the tissue culture or faecal antigens being diluted in twofold or approx. threefold (4-log) dilutions in BSA/PBS Buffer. Coupled erythrocyte suspension at 0·8% and in 25 μl drop-volumes was then added. The settling patterns of the erythrocytes were read after 2 h at room temperature.

**Fluorescent antibody technique (FAT) and electron microscopy.** These were performed
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Table 1. *A comparison of RPH and SPACE techniques on various rotavirus antigens derived from faeces and tissue culture*

<table>
<thead>
<tr>
<th>No.</th>
<th>Nature of antigen</th>
<th>Effective concentration with respect to starting material</th>
<th>RPH</th>
<th>SPACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Concentrated uninfected tissue culture fluid</td>
<td>5</td>
<td>8</td>
<td>&lt;3</td>
</tr>
<tr>
<td>2</td>
<td>Fluid from bovine rotavirus-infected tissue culture</td>
<td>1</td>
<td>16</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>100-fold concentrate of (2) after dia-filtration</td>
<td>100</td>
<td>1000</td>
<td>4000</td>
</tr>
<tr>
<td>4</td>
<td>Gel filtration of (3) on Sepharose 6B - excluded volume</td>
<td>50</td>
<td>4000</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>Gel filtration of (3) on Sepharose 6B - non-excluded volume</td>
<td>50</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>6</td>
<td>Isopycnic centrifugation of (4) - virus band</td>
<td>500</td>
<td>4000</td>
<td>1000</td>
</tr>
<tr>
<td>7</td>
<td>Human rotavirus - positive faeces</td>
<td>1/10</td>
<td>1000</td>
<td>1500</td>
</tr>
<tr>
<td>8</td>
<td>Porcine faeces - rota-virus positive</td>
<td>1/10</td>
<td>3000</td>
<td>100</td>
</tr>
</tbody>
</table>

* Added erythrocytes were lysed up to a dilution of 1/100 of the 10% faecal suspension in the RPH test.

using standardized techniques (Moosai et al. 1979). The bovine anti-rotavirus antiserum described in this report was employed at a dilution of 1 in 200, followed by FITC conjugated rabbit anti-bovine Ig antiserum (Wellcome Reagents).

RESULTS

Reverse passive haemagglutination (RPH)

With purified rotavirus antigens the coated erythrocytes gave clear, reproducible patterns when tested in microtitre U-form plates. Unconcentrated tissue culture fluids had reciprocal titres ranging between 2 and 150; concentrated antigens such as those used for agar gel-diffusion tests had reciprocal titres of 256 to 5000. It was also noted that antigens pretreated so as to destroy the whole virus, but still able to react with antibody in agar gel diffusion, reacted well in this test.

When faecal samples were tested against the coupled cells in RPH there was often lysis, sometimes as far as 1/100 dilution of the 10% suspension, or there was spontaneous agglutination. The latter was detected by the observation that erythrocytes coated with non-rotavirus-specific antibodies were also agglutinated. The spurious reactions were attributed to bacterial exo-enzymes and possibly agglutinins, as they were not removed even by centrifugation of the faecal suspension at 6000 g for 20 min. Because of this it was decided that simple RPH was not a suitable test for faecal samples.

SPACE test

Table 1 shows the results that were obtained with this test on the purified, tissue culture-grown rotavirus antigen and compares them with those obtained by the direct RPH technique. Both techniques yielded titres of greater than 1:1000 on the purified material and
as can be seen from the Table, RPH was slightly more sensitive than the SPACE test for this material. Examination of 52 faecal specimens by the SPACE test showed that 44 were positive by electron microscopy and 42 were positive by both SPACE and microscopy. Of the 52 specimens, 38 were of human origin and 14 were from domestic animals. More extensive analysis of 20 human faecal samples by electron microscopy, fluorescent antibody technique and SPACE yielded the results shown in Table 2.

**Development of the SPACE test**

Variations in all the parameters set out in the Methods section were investigated during the development of the SPACE test.

**Well geometry**

The settling of the erythrocytes in various types of microtitre well geometry was investigated. Although the V-shaped well format gave a more rapid result, there was sometimes incomplete settling of erythrocytes in negative control wells; in this respect the parabolic U-shape and smooth surface of polyvinyl plates gave the most satisfactory settling.

**Coating antibody**

The pre-coating of the microtitre wells with immune serum or IgG was necessary since a 10000-fold difference in antigen titre was observed between plates coated with pre-immunization serum, as against hyperimmune antiserum. Coating of the plastic surface with total immunoglobulin concentrations more than double that indicated (10 μg/ml) did not give greater sensitivity, and eventually produced a deterioration in both the sensitivity and the settling patterns. Because of this fairly critical immunoglobulin concentration, the more potent the antiserum used, the better the result.

**Incubation periods and temperatures**

The minimum period required for coating the microtitre wells with IgG was 30 min at 45 °C. At room temperature the optimal degree of coating was reached after 10 h. It was found that the antigen adsorption step could be shortened to 2 h at 37 °C, but with some loss of sensitivity.

**Species, concentration and volume of coupled erythrocytes**

Turkey erythrocytes settle very rapidly as they are nucleated; however, the chromic chloride coupling often produced an excessively sticky product where cells were spontaneously agglutinated to varying degrees. Decreasing chromic chloride concentrations, or increasing IgG concentrations, produced less sticky, but less sensitive coupled cells. Bovine erythrocytes were usually satisfactory, but there was considerable variation between steers (Uhlenbruck et al. 1967). These cells were also liable to give unstable agglutination patterns. Caprine cells, although taking three times as long as turkey cells to settle, gave very stable patterns and became the cell type of choice.

A cell concentration of more than 0.4% for turkey cells and 0.6% for bovine or caprine cells resulted (in a vol. of 50 μl) in buttons of cells being present even in strongly positive antigen samples. This was due to an excess of erythrocytes over that needed to cover the bottoms of the microtitre wells. Cell concentrations of less than 0.25% were very difficult to read, particularly when erythrocytes became aged and settled less well. No advantage was found in increasing the vol. of erythrocytes to more than 100 μl and vol. of less than 40 μl did not reproducibly cover the coated surface of the microtitre wells.
Table 2. Comparison of the SPACE technique with fluorescent antibody (FAT) and electron microscopy (EM) in the detection of rotavirus in a series of 20 human faecal specimens*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rating by fluorescent antibody technique</th>
<th>Detection by electron microscopy</th>
<th>Titre of specimen by SPACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+++</td>
<td>+</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>B</td>
<td>+++</td>
<td>+</td>
<td>&gt; 1000</td>
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<tr>
<td>C</td>
<td>+++</td>
<td>+</td>
<td>1000</td>
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<td>D</td>
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<td>+</td>
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<td>+++</td>
<td>+</td>
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<td>F</td>
<td>+</td>
<td>+</td>
<td>&gt; 1000</td>
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<td>G</td>
<td>+</td>
<td>+</td>
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<td>H</td>
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<td>+</td>
<td>&gt; 1000</td>
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<td>+</td>
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<td>M</td>
<td>-</td>
<td>+</td>
<td>300</td>
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<tr>
<td>N</td>
<td>+</td>
<td>+</td>
<td>&lt; 3</td>
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<td>-</td>
<td>-</td>
<td>&lt; 3</td>
</tr>
<tr>
<td>T</td>
<td>-</td>
<td>-</td>
<td>&lt; 3</td>
</tr>
</tbody>
</table>

* Samples are arranged in descending order of positivity, by FAT and EM.

**Electron microscopy and fluorescent antibody technique**

All 52 samples of faeces both human and animal were examined by electron microscopy, of these, 44 contained recognizable rotavirus particles. If care is taken to avoid cross-contamination, there should be no false positives by electron microscopy, also the sensitivity of the electron microscope compares well with other serological methods. It was therefore decided to use the findings obtained by electron microscopy as a base line with which other results were compared.

Immunofluorescence was carried out on 20 of the 38 human faecal samples and results are shown in Table 2. The results from the fluorescent antibody method differed from that of electron microscopy for only one specimen (M) which was positive by EM but negative by FAT.

**DISCUSSION**

The SPACE test differs from reverse passive haemagglutination (RPH), which is a test for virus (antigen) in a fluid phase, in that it measures virus which has been first adsorbed specifically to a solid phase. In this respect it is more closely related to tests such as ELISA and RIA but differs from them inasmuch as it employs a red cell marker rather than an enzyme or a radioisotope. The test developed as an extension of the mixed reverse passive antiglobulin haemagglutination [MRPAH] technique of Coombs et al. (1978) which uses antigen-coated microtitre plates for the detection of the immunoglobulin classes involved in antibody reactions with bacterial cells. (Thornley & Coombs, 1979).

The principle of haemagglutination or haemadsorption to a solid phase is not new and it has been applied to most haemagglutinating viruses when grown in tissue culture. Virus-infected cells grown on glass surfaces will cause red cells to haemadsorb. Again, in the so-called mixed haemadsorption test of Fagraeus & Espmark (1961), antibody which has been allowed to react with virus-infected cells growing on glass is revealed by adherence of red cells carrying antiglobulin. This is essentially a mixed antiglobulin reaction (Coombs &
Rotavirus solid-phase haemadsorption test

In the SPACE test, virus adhering specifically to the antibody-coated wells is revealed by adherence of erythrocytes coupled with anti-virus antibody.

Although the test described here is applied particularly to the detection of rotavirus antigen, the method can obviously be adapted for the detection of other antigens or antibodies. For antibody detection there are several methods which can be considered. First, virus antigen itself can be attached to the plastic wells followed by the patient's antibody and a MRPAH reaction performed as a plate test as is presently being investigated with gonococcal antigen (Thornley & Coombs, 1979). Alternatively, the virus can be specifically attached to the walls of the wells by antibody as in the present SPACE test. The test serum is added and virus-specific antibody will adsorb. This can subsequently be detected by the addition of erythrocytes coated with anti-globulin antibody. In this case the antibody attaching the virus to the solid phase and the anti-globulin antibodies linked to the red cells must be produced in the same animal species to avoid cross-reaction. Alternatively, for antibody detection, a straight inhibition of the SPACE reaction may be possible. After the specific capture of attachment of virus antigen to the solid phase the sample to be screened for antibody is added and tested for its ability to inhibit the haemadsorption of red cells linked with anti-virus antibody. Such a test only has an advantage over inhibition of reversed passive haemagglutination if the specimen of antibody under test included interfering substances. Such specimens are typified by colostrum samples in which specific antibodies are being sought.

Specimens which contain virus or antigens but which also contain interfering substances and a high proportion of non-virus material are a frequent problem in clinical virology. Problems also exist in research if material such as caesium chloride, sucrose or detergents are present in antigen-containing fractions. To this end, the solid-phase modifications of ELISA and RIA systems have been successful as only specific antigen is adsorbed on to a solid phase and other substances are then removed by subsequent washing. However, radioimmunoassay has the disadvantage of requiring specific counting apparatus as well as the inherent dangers of radioisotopes. ELISA tests suffer in similar respects; the chromogenic substrates used for enzyme detection are often unstable, and may be hazardous, even carcinogenic. A major advantage of the SPACE test is that it employs a red cell marker that has no biological risks attached to it. The disadvantage of conventional haemagglutination tests is that they are not suitable for use with biologically 'dirty' specimens and the reverse passive haemagglutination and IAHD tests that have been developed for rotavirus (Matsuno & Nagayoshi, 1978) have required that the faecal samples be pre-treated with agents such as fluoro-carbon to remove bacterial agglutinins and other lytic factors. Since the SPACE test employs a solid phase, such contaminants are simply removed during the washing step. This special ability of the SPACE test to deal with 'dirty' specimens is shown in the different results obtained in the series comparing RPH and SPACE. When untreated and purified tissue culture preparations were used the RPH test was slightly more sensitive than the SPACE test. However, in a screen of diarrhoeal stools, RPH was unusable as most specimens lysed the erythrocytes below a dilution of 1 in 100 of the 10% faecal suspension.

In a consideration of tests that are available for the detection of rotavirus it becomes apparent that different parameters are being examined. Immunofluorescence detects only viable virus; electron microscopy relies on the presence of morphologically recognizable virus particles; ELISA, SPRIA, RPH and SPACE are all able to detect both intact virus and virus components. In spite of this, we have found little overall variation in the number of positives recognized by these techniques although individual specimens will vary in their degree of positivity when examined by different methods. In the present series, we have compared the SPACE test with FAT and EM. The latter test remains the most satisfactory method for establishing the status of a specimen because of the direct visualization of virus
particles. Table 2 shows the results that were obtained and illustrates that some specimens were low grade positives by FAT but high titre positives by SPACE. In the series of faecal samples tested there were no false positives. Two specimens (M and N in Table 2) gave anomalous results. M gave positive results by EM and SPACE but was negative by FAT, presumably the specimen contained virus antigen and recognizable capsids but lacked viable virus. The other discrepancy, N, was positive by EM and fluorescence but negative by SPACE. This specimen was from a 14 year old patient with diarrhoea and the electron microscope examination had revealed that the virus was complexed with antibody. This antibody would have complexed subviral components and would prevent absorption of virus particles on to the basic antibody coating the plate thus yielding a false negative result.

As mentioned previously, much of the sensitivity of the SPACE test could be reliant on the presence of antigenic subcomponents, and in this instance they would have been blocked by the antibody present in the faecal sample. Conversely, a consignment of eight human faecal samples, which had been delayed for one week in transit, had lost all reactivity by fluorescent microscopy. Because of the deterioration of the specimens, EM examination was not possible, and yet seven of the eight were still positive in the SPACE test.

As with most immunological tests, the most critical aspect of the SPACE system is the antisera that are employed. These must be of high titre and have been raised against purified virus preparations so that they are as near monospecific as possible. If there is any possibility of the test material containing antiglobulins (as sometimes are found in serum specimens), then it is preferable to use antisera from different species for the coating of the microtitre plates and the erythrocytes respectively. By these means, the possibility of false positives caused by an antiglobulin are obviated.

In conclusion, we describe a solid phase test that employs the red cell as a marker for the presence of virus or antigen. We have employed it to detect the presence of rotavirus in faecal specimens but it could be adapted to many other virus systems. In particular, the SPACE test offers advantages for clinical specimens where there is a high background of extraneous or interfering substances.

The authors wish to acknowledge the invaluable technical assistance given by Miss A. Shersby, Mrs A. Wilson and Mr B. Gurner, and would like to thank Mr P. Weston of Wellcome Reagents Ltd for technical expertise rendered. Professor J. E. Banatvala (St Thomas’ Hospital, London) and Mr G. Craig (Wellcome Veterinary Biological Services) are acknowledged for the collection and supply of some of the faecal specimens used in this study.

REFERENCES


Rotavirus solid-phase haemadsorption test


*(Received 12 December 1978)*