Fig. Application indicated by arrows.
From L to R: 1 normal plasma; 2 normal saliva; 3 plasma from a case of parotitis; 4 pancreatic homogenate; 5 plasma from a case of pancreatitis; 6 normal urine.

The findings agree closely with those of Hobbs and Aw (1968).

The isoamylase patterns of normal plasma and urine are similar, though the activity in urine is greater. Three bands are usually found in normal urine, the slower, and usually more distinct, band being the pancreatic component, and the faster those derived from salivary gland. The activities of these isoenzymes in plasma appear to be equal.

In cases of pancreatitis, the slower band becomes much more distinct, and often further activity can be detected between this and the salivary bands. This is a similar pattern to that found in pancreatic extract.

Saliva shows three bands, all faster than those of pancreatic origin, and a similar pattern is seen in the plasma in parotitis, with an increase in the fastest band.

These appearances can all be clearly seen in the accompanying photograph.

I wish to acknowledge the advice of Dr J. Kohn in preparing this paper, the help of the Department of Medical Photography for preparing the photograph, and to thank Pharmacia (GB) Ltd for providing the Phadebas tablets.

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A screen test for rubella haemagglutination inhibition antibodies

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When live rubella vaccine became available, it was apparent that there was need for a pre-vaccination screen test to assess the susceptibility of the subject before vaccination. Such a test should be specific and be capable of being applied to large numbers of sera without calling for great increases of staff. In this laboratory all antenatal sera are now screened by the modification of a standard haemagglutination inhibition test described below.

Materials

The test is based on the method of Stewart, Parkman, Hopps, Hope, Douglas, Hamilton, and Meyer (1967). Sera are absorbed in disposable plastic tubes and reagents are added by Eppendorf¹ pipettes delivering 0.1, 0.2, 0.6, and 0.9 ml. Microtitre equipment and rigid plastic U-plates² are employed. Twenty-five percent acid-washed kaolin in borate saline for absorptions and borate saline with 0.4% bovine albumin pH 9 for diluent are as described by Clarke and Casals (1958). Red cell diluent is the Clarke and Casals phosphate-buffered borate saline with 0.2% bovine albumin pH 6.2 with 0.0078% CaCl added as advised by Auletta, Gitnick, Whitmire, and Sever (1968). Commercial rubella haemagglutination antigen is used. Each new vial of lyophilized antigen is titrated and 4 HA units are used in the test. A 20% suspension of washed day-old chick cells is used for absorptions of sera when necessary, and from this the 0.16% suspension for the screen test and titrations is prepared. The chick cells used are never more than five days old and all unused diluted suspensions are discarded at the end of a day's run. Unheated sera are tested since inactivation of sera has been found unnecessary.

Method

THE SCREEN TEST

Serum, 0.2 ml, is placed in a disposable tube and 0.6

¹From Anderson and Co Ltd, 87-95 Tooley Street, London, SE1.

²From Flow Laboratories, Heatherhouse Road, Irvine, Scotland.

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ml kaolin suspension is added. The tubes are hand shaken and after refrigeration for two and a half hours the kaolin is packed in a centrifuge fitted with multitube carriers enabling 96 tubes to be accommodated at the same time. A 1 in 32 dilution of absorbed serum is prepared by adding 0.1 ml of the supernatant, which represents a serum dilution of 1 in 3.25, to 0.9 ml diluent in a second tube. Racks of both sets of tubes are covered with plastic or foil and are returned to the refrigerator till tested.

Two wells are used for each serum. One drop (0.025 ml) of diluent is added to each well in the rear row, the serum control row of wells. One drop (0.025 ml) of serum dilution is added to each well of the pair and a similar drop of antigen in each well of the front row. In this part of the test, any suitable glass dropper can be used instead of microtitre pipettes. The plates are shaken, covered and incubated, unstacked, in the refrigerator for two hours. One drop from a large microtitre pipette (0.05 ml) of 0.16% chick cell suspension is added to each well. If a glass dropper is used for the screen 2 drops of the erythrocyte suspension must be added. The plates are sealed and after shaking are returned to the refrigerator for a minimum of an hour and a half till the tests are read.

On reading, various results are possible. First neither well shows agglutination of the chick cells. Such sera are reported as 'rubella H.I. antibodies $\geq 1:32$ '. The patient is assumed to be immune and the absorbed serum and its dilution are discarded. Secondly there is complete or partial agglutination of the cells in either the test well or both wells. Here the absorbate needs further absorption and full titration, while the dilution is discarded.

TITRATION OF SERA

To those tubes retained from the screen 0.1 ml 20% chick erythrocyte suspension is added. The tubes are shaken to suspend the cells evenly and absorption takes place in the refrigerator for two hours or, as is usually more convenient, overnight. A gentle spin is sufficient to settle the cells on top of the packed kaolin and the supernatant, which now represents a 1 in 4 dilution of serum, is transferred to a third disposable tube. The packed kaolin and erythrocytes are discarded. The serial dilution of this fully absorbed serum is made as described by Sever (1962) with doubling dilutions from 1 in 8 to 1 in 256. The addition of antigen and erythrocyte suspension and conditions of incubation are as in the screen test. For each serum there is a control well containing 1 in 8 fully absorbed serum but diluent instead of antigen. Each batch of tests contains a known positive and negative control serum treated at the same time, and a back titration of antigen. The end-

point of the titration is taken as the highest initial dilution of serum giving a pattern of partially agglutinated red cells.

The report on these sera reads 'rubella haemagglutination inhibition antibody titre $\leq 1:8$ ' (or whatever the titre is), and it is recommended that those patients with no antibodies should be vaccinated against rubella immediately after the baby is born.

All sera are retained deep frozen for at least six weeks in case a repeat test with a second serum is necessary. Absorbates are discarded.

Results

Three hundred and twenty-four routine antenatal sera were divided into two portions. The first portion was fully absorbed by kaolin and fowl erythrocytes and the standard titration of haemagglutination inhibition antibody was performed. The second portion was screened as described above. The results are set out in Table I.

Titre of Serum ¹	No Agglutination in Control Well Agglutination in Test Well			Agglutination in Control Well
	None	Partial	Full	
8	0	0	58	0
16	1	1	6	0
32	7	9	5	2
64	57	4	1	2
128	67	0	0	0
256	102	0	0	2

Table I Haemagglutination inhibition antibody titres of 324 sera compared with readings of screen test

¹The figure in the titre column is the reciprocal of the serum titre.

The complete screen test with titrations of the fully absorbed sera, where necessary, was carried out on 630 consecutive sera under routine conditions. One hundred and eighty-eight of these showed a degree of haemagglutination in one or both wells of

Titre of Serum ¹	No Agglutination in Control Well but Agglutination in Test Well		Agglutination in Control and Test Wells
	Full	Partial	
8	96	12	4
16	9	4	0
32	10	36	1
64	0	11	1
128	0	0	3
256	0	0	1

Table II Titres of 188 sera showing haemagglutination in the screen test out of 630 sera submitted to the screen

¹The figure in the titre column is the reciprocal of the serum titre.

the screen. The titres of these sera are shown in Table II.

Comment

Absorption of non-specific inhibitors from sera by kaolin was chosen in preference to the newer methods described by Cooper, Matters, Rosenblum, and Krugman (1969) or Liebhaver (1970) because kaolin was specific in our hands, highly reproducible, and did not have disadvantages the other methods showed.

In this laboratory, serological tests for syphilis are made on about 140 antenatal sera each week. To perform an additional screen test on these sera involves no extra labour in bleeding patients and separating clots. The rubella screen test is carried out twice a week, which is not an undue delay for this type of investigation, but an immediate titration of serum can always be made when indicated.

Sera are dealt with in batches of about 70, titrations being performed at the same time as the next batch of screens. It is found that the total technician time involved is less than five technician hours a week for screens, while the further treatment and titration of sera takes an additional two hours a week. The work load has been absorbed by fragmentation of the procedure into stages which can be fitted into re-organized schedules without disruption. A few routines which experience shows no longer produce results commensurate with the effort involved have been discarded, but this is a constant feature of any laboratory with an expanding work load.

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A rapid staining technique for *Pneumocystis carinii*

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Pneumocystis carinii pneumonia is an acute, severe, rapidly progressive and generalized pulmonary infection occurring almost exclusively in patients receiving immunosuppressive therapy for organ transplantation, leukaemia, or other malignancies and in premature or debilitated infants. Early therapy with pentamidine isethionate may be life-saving. However, diagnosis requires the demonstration of *P. carinii* in lung tissue or fluid obtained by biopsy or percutaneous needle aspiration. The organism can be clearly demonstrated by the methenamine silver nitrate impregnation technique of Gomori. Although the preparations by this method are satisfactory, there are practical disadvantages to the technique. The Gomori method is unduly long, requiring two to three hours to complete and the skill of an experienced technician. Clinically, such a delay in diagnosis hours may impede successful treatment. If patients present during off-duty hours for laboratory personnel, more prolonged delays can be expected.

These considerations were the basis for the development of a technique described below, which is both rapid and simplified, can be performed in the routine clinical laboratory, and retains the basic principles and specificity of Gomori's method. The procedure can be completed in five minutes.

Method

The technique for aspiration of pulmonary fluid has been described (Johnson and Johnson, 1970). Usually 0.1 ml or less of the aspirate is dropped onto a clean slide and allowed to dry.

SOLUTIONS REQUIRED

Five percent chromic acid, 1% sodium bisulphite, working methenamine silver nitrate (Grocott), dimethyl sulphoxide (DMSO), working light green (Grocott), 95% ethyl alcohol, absolute ethyl alcohol, and xylene

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