for a probability matrix from which identification scores are derived by multiplication of the entries for each taxon, it is important that the entries should be complete. It was therefore necessary for our purposes to determine the reaction, and this paper serves to report our findings.

We would like to thank Professor P. H. A. Sneath of the MRC Microbial Systematics Unit for providing cultures of *Chromobacterium lividum*.

References

- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature (Lond.)*, **178**, 703.
- Lapage, S. P., Bascomb, S., Willcox, W. R., and Curtis, M. A. (1970). Computer identification of bacteria. In *Automation, Mechanization and Data Handling in Microbiology*, edited by A. Baillie and R. J. Gilbert, pp. 1-22. Society for Applied Bacteriology Technical Series no. 4. Academic Press, London.

A screening test for IgG Deficiency

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Immunodeficiency syndromes with hypogammaglobulinaemia are rare (Medical Research Council, 1971). However, there are well defined groups of patients in whom the incidence of IgG deficiency is likely to be higher than in the population as a whole, for example, patients admitted to hospital with infections; children who fail to thrive; relatives of immunodeficient patients. This paper describes a method whereby these 'at risk' patients can be screened for IgG deficiency. The method fulfils several important provisos for a screening procedure. The diagnosis benefits the patient (early treatment) and his family (genetic counselling); the initial screening procedure is rapid and relatively inexpensive; and abnormal results can be confirmed or refuted by formal measurement of serum immunoglobulins.

Method

The screening technique, as described here, has been modelled on the Guthrie test for phenylketonuria screening (Guthrie and Susi, 1963; Newman and Starr, 1971).

COLLECTION AND TRANSPORT OF SPECIMENS

Wards and clinics are supplied with printed sample papers (see fig.), polythene bags, envelopes printed with the address of the Ig screening laboratory, and a printed instruction card. Schleicher and Schull

Fig. Printed filter paper which is used for collection and transport of dried blood spots

filter paper 903 is used for the sample papers; sheets of filter paper were printed by the University of Glasgow printing department. Blood is obtained by

Received for publication 12 December 1972.

heel prick (or from the syringe if venepuncture is required for other tests). Two drops of blood each of 1 cm diameter are collected on the sample paper; the paper is allowed to dry in air and batches of papers (in polythene bags) are sent to the screening laboratory, by post, or by the hospital delivery services.

IMMUNODIFFUSION METHOD

Ten cm square disposable petri dishes are used (Sterilin). Into each plate is poured 20 ml of 0.85% ion agar no. 2 in barbitone buffer pH 8.2, containing antihuman IgG antiserum. Several commercial anti-IgG preparations have been tested, also several antisera prepared in this department. All have proved suitable but for each antiserum it was necessary to define the best concentration of antiserum in agar, and optimal duration of incubation. Two types of plate are prepared.

Plate A is a screening plate to give visible rings of precipitate (up to 10 mm diameter) around the test specimens after overnight incubation. In the work described below I used plates with 0.5 ml Meloy sheep antihuman IgG (batch A101-5) in 19.5 ml agar, and with 0.2 ml locally prepared sheep antihuman IgG (Professor R. G. White) in 19.5 ml agar.

Plate B is for confirmation of low IgG levels to give 5 to 15 mm diameter precipitin rings (after staining) with standards prepared from serum, of IgG content 100 to 400 mg per 100 ml: 0.5 ml Nordic swine antihuman IgG (batch 8-570) in 19.5 ml agar was used.

Standards

Standards used are dried spots of serum of known IgG concentration. When blood and serum or plasma spots are mixed, a correction factor must be applied to the results because experiments with ^{125}I -globulin have shown that dried blood spots contain more globulin than dried plasma spots of the same diameter (see below).

Samples for immunodiffusion are punched from the centre of dried blood or serum spots with a Maun ticket punch (3 mm size). The 3 mm discs are placed on the surface of the agar. Seventy-five test samples and six standards are put on each 10 × 10 cm screening plate. For confirmation of low levels, six standards and up to 19 test samples are put on each 'low IgG' plate.

Screening plates are incubated overnight, at room temperature, in a moist chamber. Next morning the paper discs are washed off by immersing the plate in saline for a few minutes and precipitin ring diameters measured with a simple paper scale. The low IgG plates are incubated for two days, washed in saline

for 24 hours, stained with chlorazol black and washed in tap water. The diameter of precipitin rings is measured accurately, with a special ruler.

A routine procedure has now been established whereby all specimens are screened within a week of delivery to the laboratory; with the concentration of antiserum used, ring diameter of less than 4 mm corresponds to an IgG content of under 300 mg per 100 ml. Thus, in all specimens giving precipitin rings of 4 mm or less the IgG content of a sample disc is measured accurately in the low IgG plate.

Evaluation of the Method

Several experiments have been carried out to allow evaluation of the different stages of the procedure.

1 GLOBULIN CONTENT OF BLOOD DISCS

^{125}I Rabbit globulin, 0.1 mg, was added to 10 ml heparinized rabbit blood. After mixing, the blood was divided into two 5 ml aliquots; plasma was separated from one aliquot and spotted on to filter paper and the other aliquot used to obtain spots of whole blood.

The paper was allowed to dry, 3 mm discs were punched out, and then the ^{125}I content was measured in an automatic well gamma counter. Twenty blood spots and 20 plasma spots were measured. Results (table I) showed that there was little variation in the globulin content of discs within each group of samples, but in spots of whole blood the filter paper retained 28% more globulin than when the spots were of plasma.

Counts per 10 Minutes				
No. of Specimens		Mean	SD	Range
Blood	20	1919	118	1773-2118
Plasma	20	1491	81	1306-1642

Table I ^{125}I globulin content of 3mm discs of dried blood and plasma

2 GLOBULIN CONTENT OF 3 MM DISCS RELATED TO SIZE OF BLOOD SPOT

Rabbit blood with ^{125}I -globulin was prepared as in (1) above. Whole blood was spotted onto filter

Diameter of Blood Spot (cm)	Number of Specimens	Counts per 10 Minutes	
		Mean	Range
0.5	5	758	711-814
0.75	5	830	787-890
1.0	5	840	773-886
1.5	5	871	835-916
2.0	5	886	828-939

Table II ^{125}I -globulin content of 3 mm discs punched from the centres of blood spots of different diameters

paper; diameters of spots ranged from 0.5 to 2.0 cm. A 3 mm disc was punched from the centre of each spot and the ^{125}I content was measured in an automatic well gamma counter. Results (table II) show that the globulin content was higher in discs punched from the larger blood spots.

3 PRECIPITIN RING DIAMETERS RELATED TO IgG CONTENT OF SAMPLE

Ten dilutions of a standard human serum were prepared (bovine serum albumin, 30 mg per ml, was used as diluting fluid). These were spotted onto filter paper and for each dilution 5 spots were placed on an IgG screening plate and incubated overnight. Table III shows the mean values and ranges of precipitin ring diameters for each of the 10 dilutions.

Normal Human Serum (%)	No. of Specimens	Precipitin Ring Diameter (mm)	
		Mean	Range
10	5	4.78	4.6-4.9
20	5	5.12	5.0-5.2
30	5	5.58	5.4-5.7
40	5	6.52	6.3-6.7
50	5	6.92	6.8-7.0
60	5	7.60	7.5-7.7
70	5	8.02	7.9-8.2
80	5	8.54	8.4-8.7
90	5	8.64	8.4-8.8
100	5	8.94	8.9-9.0

Table III IgG screening test applied to 10 dilutions of normal human serum

4 EFFECTS OF STORAGE OF SAMPLES

Fifty serum specimens were spotted onto filter paper, dried, and stored in a dark cupboard at room temperature. The sera were stored at -20°C ; four months later they were thawed and 50 fresh dried spots were prepared. Both groups were tested for IgG content and the mean percentage fall in precipitin ring diameter was calculated. There was, on average, 26% reduction in precipitin ring diameter after storage at room temperature for four months.

No IgG could be detected in a group of dried blood specimens which had been stored for a year, at room temperature, in the Phenylketonuria Screening Laboratory, Stobhill Hospital, Glasgow.

5 PILOT STUDIES

This IgG screening test has been applied to 522 human serum samples, previously submitted to this department for immunological tests, and stored at -20°C or -70°C for up to two years. The IgG screening test detected nine specimens with IgG content lower than 200 mg per 100 ml. All were from patients known to have hypogammaglobulinaemia.

Six hundred and fifty inpatient and outpatient

filter paper specimens have been screened, but, to date, no patients with hypogammaglobulinaemia have been detected.

Comment

The collection of blood spots on paper has proved completely acceptable to nursing and medical staff, probably because most people know of the routine phenylketonuria screening of all 6-week-old Scottish babies. The laboratory procedures require no elaborate equipment and the only significant costs are for antisera and staff. Even with the most expensive antiserum used (Melo, £7 for 2 ml), 150 specimens were screened per ml of antiserum. However, by using locally prepared antiserum for the screening plates, the final cost for materials can be reduced to around one penny per patient. The technician who does these IgG screening tests takes about 40 min to set up a plate (75 specimens) and about 10 min to read the results. The only major limitation of the technique would appear to be that the presence of maternal IgG in infant serum makes it impossible to diagnose IgG deficiency before the age of 4 to 6 months, thus IgG screening of very young infants is of little value.

We have found that dried spots of blood or serum can be used for a variety of immunochemical studies, for example, for the detection of specific antibody by double diffusion in agar, passive haemagglutination, reverse immunodiffusion, and radioimmuno-electrophoresis. Others have used these samples for population studies on antiviral antibodies (Brody, McAlister, Haseley, and Lee, 1964; Draper and Kelly, 1969). It is likely that similar methods could be applied in other immunological surveys, research programmes, and screening procedures.

I acknowledge the excellent technical work of Mrs. Sandra Campbell. I am grateful to Dr J. S. Stevenson, Dr G. Lindsay, and Mr R. Kennedy who gave much useful advice about screening procedures and sample processing, and to the doctors and nurses who have collected specimens for this preliminary analysis. I thank Professor R. G. White for his encouragement and for the gift of antiserum. This work (departmental publication no. 7226) was supported by a grant from the higher medicine funds of the Western Regional Hospital Board.

References

- Brody, J. A., McAlister, R., Haseley, R., and Lee, P. (1964). Use of dried whole blood collected on filter paper disks in adenovirus complement fixation and measles haemagglutination inhibition tests. *J. Immunol.*, 92, 854-857.
- Draper, C. C., and Kelly, A. (1969). Haemagglutination-inhibition tests for rubella. *Brit. med. J.*, 1, 177-178.

A simple, inexpensive gel filtration technique for use in diagnostic serology

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Previously described methods (Best, Banatvala, and Watson, 1969; Gupta, Peterson, Stout, and Murphy, 1971; Haire and Hadden, 1972) for the separation of IgM and IgG as applied in diagnostic serology all involve at least moderate capital expenditure and/or a skilled, highly standardized technique. In investigating gel filtration techniques in order to define a simple, inexpensive, robust technique for the fractionation of human sera the following system has been devised. The basic technique has been applied in this laboratory for the past five years in the serological diagnosis of recent rubella and no difficulties in interpretation of the results have been encountered.

Methods

APPARATUS

The cheapest automatic fraction collector available is the Boulting fraction collector (James A. Jobling, Laboratory Division, Stone, Staffs). The remainder of the system has been designed around this fraction collector so as to achieve the desired separation of IgM and IgG when collecting 4-5 ml fractions. The most convenient column for this has been found to be the 500 × 20 mm Quickfit chromatographic column (Baird and Tatlock, Romford, Essex) packed to a length of 50 to 52 cm with Sephadex G-200 (Pharmacia, Uppsala, Sweden) suspended in

¹Requests for reprints should be addressed to J. R. Pattison from whom more detailed instructions are available for those not familiar with gel filtration techniques.

Received for publication 10 January 1973.

A screening test for IgG deficiency—continued.

Guthrie, R., and Susi, A. (1963). A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics*, 32, 338-343.

Medical Research Council (1971). Hypogammaglobulinaemia in the United Kingdom. *Spec. Rep. Ser. med. Res. Coun. (Lond.)*, 310.

Newman, R. L., and Starr, D. J. T. (1971). Technology of a regional Guthrie test service. *J. clin. Path.*, 24, 564-575.

Nairn PBS (Nairn, 1964) plus 0.02% sodium azide (PBS-NA). The remainder of the apparatus consists of a constant level solvent reservoir with an airtight feed to the top of the column. As illustrated this consists of a transfusion bottle, an intravenous infusion set with the filter removed, and a serum needle passing through a rubber bung (fig 1).

Fig. 1 The apparatus.

FRACTIONATION OF SERA

The technique is illustrated by its application to the serological diagnosis of recent rubella. Taking care not to disturb the gel itself, layer onto the top surface of the gel a sample (determined as one tenth the fraction volume, ie, 0.4-0.5 ml) of serum pretreated with $MnCl_2$ /heparin/50% pigeon RBCs (Dold and Northrop, 1968). Elute the serum sample at a rate of not more than 20 ml/hr. After 40 fractions have been collected the column is cleared for the next run, and with such a system a single column will perform 30-50 fractionations. Rubella haemagglutination inhibition (HAI) tests are performed on the appropriate fractions (for methods of selec-