Technical methods

Multispot immunofluorescence: a simple semi-automatic method of processing large numbers of tests

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The value of immunofluorescence as a diagnostic procedure is now well established. However, since only one or two tests are normally carried out on each slide, the effort involved in the processing and subsequent microscopical examination of large numbers of tests may discourage adoption of the method in busy routine laboratories.

We describe here a method which uses a coating of a water-repellent material to isolate test areas from each other so that as many as 22 sera may be tested simultaneously on a standard microscope slide. These 'multispot' slides, used in conjunction with a simple device for simultaneous dilution and application of 12 samples, enable one operator to complete 100 tests in a day.

Preparation of Coated Slides

Fluoro Glide[1], as suggested by Goldman (1968), is a suitable coating material. This is an aerosol-dispensed preparation of a polymer of tetrafluoroethylene containing a bonding agent. It dries on glass in a few seconds to form a chemically inert water-repellent film, and at high magnification presents a moss-like appearance.

During spraying the test areas are masked so that they remain uncoated. This is accomplished by the initial application of drops of glycerol by means of a dispenser consisting of metal rods mounted in a Perspex block. The area masked is determined by the diameter of the rods and the depth to which they are immersed in glycerol. Test areas, 5 mm in diameter, are suitable for use with tissue sections; with bacterial suspensions, however, a diameter of 2 mm is adequate. When large numbers of tests are involved slides having 22 2-mm spots are used. A suitable dispenser for this purpose may be constructed from 19 gauge sawn-off hypodermic needles. The glycerol is easily removed after spraying by rinsing the slides under running tap water, followed by distilled water, after which they are allowed to dry and the antigen preparation applied to the clear areas. Spraying may also be carried out *after* application of antigen, eg, whole blood smears, the test areas being masked with metal discs located with a jig so that they conform to the general pattern, described below.

[1]Fluoro Glide, Chemplast Inc., N.J., obtainable from Marshall-Howlett Ltd, 293 Main Road, Sidcup, Kent.

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Equipment for Dilution and Application of Sera

A simple device for simultaneously preparing 1:5 (or 1:10) dilutions of 12 serum samples and depositing them on corresponding test areas is constructed as follows. Capillary pipettes drawn from 5 mm glass tubing are trimmed as shown in Fig. 1 to a length of about 35 mm. Pipettes matching in size and shape are selected so that the volume (V) they retain by capillarity is approximately 15 μl, and calibration marks are then made corresponding to 5V and 10V. Twelve such droppers are mounted in the base plate of an airtight chamber connected to a mouthpiece tube, their spatial arrangement conforming to the layout of the clear areas on the multispot slides.

Dilutions are made in wells (5 mm diameter) drilled in a block of Perspex according to the pattern of the general layout.

Sera to be tested are assembled in groups of 12 (usually 10 unknown sera and two controls), in tubes held in a second block drilled to the same format (Figure 2). Polystyrene disposable tubes, 13 mm external diameter, are convenient. Should the quantity of serum available in any tube be inadequate for effective sampling, the fluid level may be raised by adding glass beads.

Method of Operation

Four (or 9) volumes of diluent are first delivered into the wells as follows: The diluent is drawn by suction into all the droppers simultaneously to the 5V (or 10V) mark. By discontinuing suction 4 (or 9) volumes are released from each dropper into the wells and the 1 volume that is retained by capillarity is discarded. The droppers may be rinsed at this stage in saline and drained on blotting paper.

Before samples are taken it is necessary to rinse each dropper with its corresponding serum. The sera are drawn up to the 5V mark and allowed to run back by gravity into their tubes; this is repeated twice. One volume is

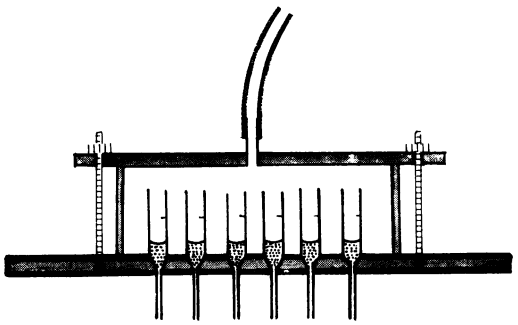

Fig. 1 *Sectional view of semi-automatic device for preparing dilutions of sera and depositing them on test areas of slides. Six capillary droppers are shown mounted in the base plate.*

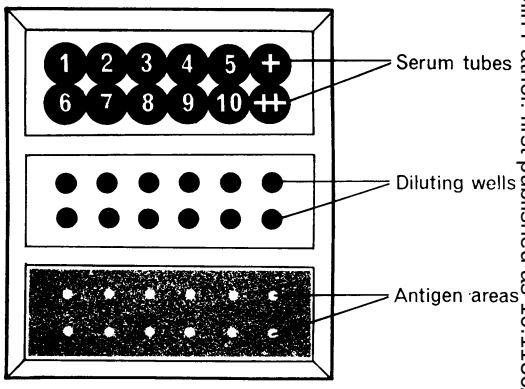

Fig. 2 *Layout of diluting block assembly showing the corresponding locations of serum tubes, dilution wells, and antigen areas.*

finally retained by capillarity, transferred to the diluent in the corresponding diluting block, and mixed by gentle suction and release. A suitable volume of diluted serum, determined by experience and dependent on the size of the test area to be covered, is drawn into the droppers. With the suction maintained, the droppers are positioned over the test areas of the corresponding multispot slide and the diluted sera released, after which the droppers are rinsed in saline and drained.

When the maximum number of tests per slide (viz 22) is required, the sampler is used twice per slide to deliver diluted sera to alternate test areas. The first (or last) pair of serum tubes contain only saline which is delivered at the end of the slide and discarded.

Titrations involving serial dilutions of sera are conveniently carried out using the procedure described. For this purpose a larger diluting block, having 10 or more rows, is required.

STAINING PROCEDURE

After incubation with the sera for the requisite time in a moist chamber, the test areas are separately washed by means of a jet of saline from a wash-bottle, and the slides then washed in a saline bath in the usual way. After removal from the bath most of the fluid may be shaken off, any remaining on the surface between the antigen spots being mopped off with paper tissue before application of the fluorescent conjugate. After the final wash each slide is mounted in buffered glycerol with a single 22 × 70 mm coverslip.

APPLICATION

The procedure described has been extensively employed in the fluorescent treponemal antibody absorbed test for syphilis, and its application to the detection of antibodies to gonococci and salmonellae is currently being investigated. We routinely use multispot slides for antinuclear factor tests on cryostat sections, which are taken direct from the microtome onto the clear areas without difficulty.

Comment

The use of multispot slides as described here affords considerable saving in the labour involved in processing, mounting, and microscopical examination when carrying out large numbers of routine tests. The microscopic structure of the intervening coating between tests greatly facilitates focusing of condenser and objective when, for example, bacterial suspensions are employed, and the consequent saving in time spent on microscopy is particularly valuable. Considerable economy in antigen and conjugated antiserum results from confining tests to a very small area of the slide. In view of the proximity of test areas, we investigated the possibility of mixing between adjacent tests. No evidence for this was found when strong positive sera were interposed between negative sera, provided that the initial washing-off procedure described above was carried out. There was also no indication of carryover of positive serum by the droppers to subsequent tests.

The method of preparing dilutions of sera depends on the volume retained by capillarity. This being a function of surface tension, it is important to consider the possible effect of the presence of surface-active agents in some systems. Multispot slides may also be used in conjunction with established methods of preparing simultaneous dilutions of multiple serum samples, for example the Takatsky system.

In addition to the applications described, the procedure is likely to prove particularly useful for the demonstration of antibodies in toxoplasmosis and malarial infection and may also be of value in the detection and serological typing of antigen by the use of specific antisera.

We wish to thank Dr E. J. Holborow for his helpful comments and encouragement, and Mr T. Plumridge who constructed the equipment.

Reference

Goldman, M. (1968). In *Fluorescent Antibody Methods*, p. 148. Academic Press, New York and London.

Single-channel AutoAnalyzer modified to provide two channels

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The existing single channel AutoAnalyzer (Technicon Instruments Co.) in this laboratory has been modified to provide two independent channels at a saving of approximately £900 over the cost of purchasing a second single-channel system.

The modifications were confined to the sampler, proportioning pump, and voltage stabilizer modules, and require the minimum of workshop facilities.

The modification of a sampler model no. 1 is described because the necessary finance to purchase the superior sampler model no. 2 (£404) was not available, and may be of interest to those laboratories in similar circumstances. The purchase price of £404 is included in the total saving.

Sampler Module (Sampler Model No. 1)

Three stainless steel rods, 6 cm long and 8 mm in diameter, were bolted onto one sample plate in place of the three studs which support a plate cover. The rods were bored at their free ends, so that they fitted snugly over the studs of a second sample plate. The block supporting the sample crook was replaced with one 9·4 cm high made from brass, to provide a fulcrum for a second crook. Both crooks were connected together with 'swinging arm' extension pieces made from steel, as shown in Figure 1. This construction permits the correct oscillation and synchronization of the upper sample crook, since both crooks are operated by the original mechanism fitted with a stronger return spring.

Proportioning Pump Module

A platen assembly (Technicon) was spring mounted in the conventional Technicon manner onto a rigid wooden box made to the same dimensions as the proportioning pump module. The box was fitted with a stainless steel top, onto which was bolted a roller head hinge block, a drive shaft guide bearing, both made from brass, and a roller head assembly locking device made from steel.

The original roller head drive shaft was replaced with one 13 cm long made from steel, with a female coupling brazed onto it at one end. This new shaft was inserted through the chain

Actual Conversion Cost	£	s	d
Colorimeter	347	0	0
Single pen recorder	438	0	0
Roller head assembly complete	62	10	0
Platen complete with springs	8	2	0
Separator plate	2	2	0
Dialyzer plates	26	8	0
Gaskets		11	0
	884	13	0
Less discount	74	18	0
Technicon account	809	15	0
Add voltage stabilizer	10	10	0
Labour costs	20	0	0
	£840	5	0

Technicon Quotation for Second Channel	£	s	d
1 Sampler II module			
1 Two-speed proportioning pump			
1 Colorimeter			
1 Transformer voltage stabilizer			
1 Single pen recorder			
1 Chart reader and general purpose comparator			
	1,733	0	0
Less chart reader and comparator	21	8	6
	1,711	11	6
Add equipment for double dialysis	29	1	0
	£1,740	12	6

Table *A single-channel AutoAnalyzer modified to provide two channels*

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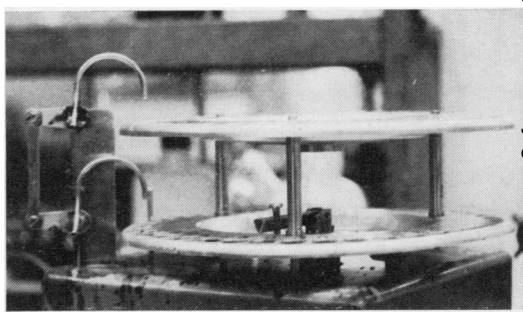

Fig. 1 *The sample plates and double sample crook arrangement. The horizontal arms from the sample crooks extend 2·5 cm. They are joined by a steel strip 6·2 cm long.*