Technical methods

A multiple sampling device for the mass screening of serum samples for hepatitis B surface antigen

C. H. CAMERON Department of Virology, Middlesex Hospital Medical School, London W1P 7LD, UK

J. A. J. BARBARA North London Blood Transfusion Centre, Deansbrook Road, Edgware, Middlesex HA8 9BD, UK

When the association between Australia antigen (HBsAg)-positive blood donations and post-transfusion hepatitis B was found, the screening of donations for antigen became desirable (Gocke and Kavey, 1969). In a blood transfusion centre handling hundreds of donations daily, streamlining of test procedures is at an obvious premium. When using the haemagglutination tests for HBsAg that are now available, the main way to facilitate testing is by dispensing the test samples more efficiently. This paper describes a machine which makes screening dilutions of serum quickly and reliably in 96-well plastic plates (eg, Cooke Microtiter).

The required volumes of serum (approximately 3 μl for a 1 in 8 ‘screen dilution’) are transferred on wire loops from donation sample tubes to 25 μl volumes of diluent already dispensed in microtitre plates. Because the width of the sample tubes is too great to allow them to be arranged in a 96-space rack corresponding in size to a 96-well microtitre plate, some other arrangement had to be devised. The sample tubes are set out in racks of 24 tubes at twice the spacing distance of the wells in a microtitre plate. Samples from four such racks of 24 are transferred in such a way as to utilise the 96 wells. After the dilutions have been completed, test cells are added to all wells.

The diluting machine (Figs. 1 and 2) is made largely of aluminium alloy and is approximately 50 cm high, 90 cm long, and 50 cm deep. A heavy base provides stability. The carriage (a) is free to move horizontally if the loop holder (b) is in the raised position.

The loop holder (b) may be lowered to a fixed level with the lever (c) only if it is directly over one of the three available microtitre plate locations on the platform (d), or the sample rack (e), or the waterbath opening (f). Once lowering has begun the carriage is no longer free to move horizontally. The platform (d) is itself movable in a horizontal plane both laterally, and backwards and forwards over a distance of one microtitre well spacing.

The rack of serum samples (e) fits snugly in its holder, which has an orientation lug matching a notch in one corner of the rack.

The 24 loops, which are made of dental wire, have stocks which are free to ride upwards relative to the loop holder when the loops touch the bottom of the microtitre wells. The diameter of the loop is chosen by experiment to hold the required volume of serum. If a wire is accidentally deformed so that the loop is no longer co-axial with the stock it may be checked and corrected on the jig shown in Figure 3.

In use, the microtitre plate is located on the platform; the four racks of 24 specimen tubes are fitted into the rack holder in turn, and sampling is carried out by lowering and raising the loops. The loops are lowered onto clean, dry blotting paper after removal from the waterbath before sampling; they are checked visually after sampling to ensure that they are all charged with serum. When the first rack has been sampled the carriage is moved laterally to the position above the microtitre plate, and the loop holder is lowered to its full extent, allowing the loops to rest on the bottom of the wells and depositing the serum in the diluent already in the wells. The loops are then withdrawn and moved along and into the waterbath (g). While the loops remain in the waterbath, the second of the four racks is put in the rack holder and the plate platform is moved into the second of its four positions, thus bringing 24 unused wells into position for the next transfer. The waterbath in which the loops are immersed after the sample has been transferred has a stirrer and a baffle plate, and a volume sufficient to prevent significant cross-contamination of samples. After use the bath can be disinfected, drained, and rinsed by continuous flow, and refilled with water.

In our screening for HBsAg we use a heat inactivated (60°C for 10 hours) positive control which is just detectable at a dilution of 1 in 8, the dilution used routinely for screening samples. Figure 4 shows the distribution of dilutions obtained using the machine; quantitative measurements were made by incorporating iodine-125 in serum samples. The distribution observed reflects the overall varia-
tion in the volumes of serum transferred by the 24 loops in four trials. One positive control sample is always included in one particular corner of each rack of 24 samples so that a pattern of four positives should appear in one corner of the microtitre plate tested (Fig. 5). These also act as an orientation check on the microtitre plate.

We have been using the Wellcome Reagents Ltd 'Hepatest' (Cayzer et al., 1974) for HBsAg testing of donations since 1974; initially, dilutions for screening were prepared using the prototype multiple sampler made at the Department of Virology, Middlesex Hospital, London. This prototype was built in 1972 in order to facilitate the testing of 50,000 donor serum samples by haemagglutination inhibition and haemagglutination (HAI and HA) (Vyas and Shulman, 1970) for HBsAg and anti-HBs. Approximately 2700 samples were tested per day. Three plate positions were provided to allow separate HA, HAI, and control cell plates to be set up rapidly. More recently, we have used the machine illustrated in Figures 1 and 2. These machines have proved very convenient and efficient for making the dilutions for mass screening with Hepatest test cells. Any screen test positives can be titrated with test and control cells, absorbed if necessary, and confirmed by specific neutralisation. The rack and the position in the rack of any screen test positive found in a plate can easily be identified from a decoding chart. A first trial of 100,000 tests (Barbara et al., 1975) has been followed up by tests on approximately a quarter of a million donations (Barbara et al., 1977).

A useful feature of the machine is the platform which can hold three microtitre plates side by side. This allows further dilutions to be made from the 1 in 8 dilution into other plates. This has been used successfully in screening large numbers of donors for high-titre complement-fixing antibody to variella-zoster virus. It also allows the serum samples to be set up rapidly at a 1 in 8 dilution in three separate plates for other serological tests. This again has been successfully used by other workers in our Transfusion Centre for the detection of antibodies to 'private' blood group antigens by mass screening (Dr M. Contreras, personal communication). This
**Fig. 2** Close-up view of the loops above the sample tubes.

**Fig. 3** Centering jig for loops: (a) closed loop; (b) dental wire; (c) stock; (d) rubber 'O' ring.
facility was also used in the evaluation of a sensitive 
HBsAg detection method based on that of Archer 
(1977) using 'Hepatest' cells diluted 1 in 10 and 
V-bottomed wells. The large numbers of 1 in 8 
serum dilutions were very conveniently made in 
parallel in the standard (U-bottomed) plates and 
in the V plates.

We are currently adapting screening methods for 
detecting several other antibodies for use with the 
multiple sampler.

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C H Cameron and J A Barbara

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