Technical method

A simple method for obtaining and storing small volumes of serum

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Many immunological tests, for example, immunoprecipitation and radioimmunoassay, can now be carried out with serum samples of less than 50 \( \mu l \). Such small volumes are difficult to manipulate, prepare, and store, and so it is normally necessary to obtain serum from larger volumes of blood, often several millilitres, by venepuncture. This can be a difficult and undesirable procedure in young children, the sick, and some animals.

Using the method described below, approximately 50 \( \mu l \) of serum can be obtained from 100 \( \mu l \) (about two drops) of blood. The blood can be collected from a human finger, the tail of a mouse, the ear of a rabbit, or any suitable site. The only materials required are a lancet, a glass capillary, a bunsen burner, a diamond or carborundum disc, paper tissue, and a centrifuge.

1. Take blood into capillary
   Allow to clot

2. Leave until the clot contracts

3. Seal one end in bunsen flame

4. Leave until serum moves along capillary

5. Break capillary and pull out clot

6. Centrifuge

\begin{figure}
\centering
\includegraphics[width=\textwidth]{method}
\caption{A method for obtaining small volumes of serum.}
\end{figure}

1. Leave about 20\% of the capillary unfilled. It is important that one end of the capillary remains clear of blood as any traces of blood will burn in the bunsen flame, causing difficulties with sealing. Several types of glass capillary have been used, for example, Hard Glass Capillary Tubes (BDH Chemicals Ltd, Poole, UK) and Plain Micro-haematocrit Tubes (Gelman-Hawksley Ltd, Lancing, Sussex, UK).

2. The clot contracts, and each end of it adheres to the inside of the capillary. It is sufficient to leave the capillary at room temperature until contraction of the clot is complete. Incubation of the capillary at 37\(^\circ\)C for 2 hours caused excessive drying of the blood at both ends of the clot which was then difficult to remove.

3. Care should be taken to avoid excessive heating of the capillary contents. This can be done by holding the capillary with the thumb and forefinger close to the end being sealed. Sufficient time (3-5 secs) should be allowed for the warm air to escape before the seal is formed.

4. As the serum moves the end of the clot usually detaches from the inside of the capillary. This makes removal of the clot (step 5) easier.

5. The capillary should be broken gently 5-10 mm from the open end after scoring with a diamond or carborundum disc. Snapping of the clot can be prevented by drawing the clot onto a paper tissue as it is gently pulled out of the capillary.

6. After centrifugation the sealed end containing the pellet can be broken off and the serum expelled and tested.
The method is suitable for obtaining and storing samples from a large number of experimental animals, from a population in a field survey, or from an individual when sequential samples are required.

The method is described in the Figure.

The capillary of serum can be stored, deep frozen, immediately after centrifugation. On removal from storage the sealed tip is broken off before the con- tents thaw, in order to prevent contamination of the serum with the products of cell lysis. This can be done either inside the deep freeze or at room temperature within a few seconds of removal.

If necessary, all the procedures described can be carried out in a sterile cabinet (Foramaflow Ltd, Windlesham, Surrey, UK).

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Letter to the Editor

An ‘insoluble’ media problem

It is a common practice in this laboratory to carry a large stock of commercially prepared culture media of the same batch so as to give uniformity. At certain unpredictable and irregular periods of time, Isosensitest agar (Oxoid Ltd) was unable to support the growth of Staphylococcus aureus, while Gram-negative bacilli seemed unaffected.

As water and dehydrated media are the sole ingredients, other batches of Isosensitest agar were tested together with water from the tap, deioniser, and glass still. The glass bottles used in the preparation of the media were also cleaned and thoroughly rinsed to obviate any likely carry-over of detergent. None of these procedures provided a solution to the problem. To overcome the problem of inhibition, and to provide the laboratory with a reliable sensitivity medium, it was found that the addition of 1% haemolysed horse blood to the medium enabled Staph. aureus to grow freely.

In September 1978, the problem seemed to be much worse than that cysteine-lactose-electrolyte-deficient agar (CLED, home-made) was also affected on an intermittent basis. As all the variables had been checked and a solution had not been found, it was postulated that the laboratory autoclaves were possibly at fault. After consultation with the hospital engineers and water treatment consultants, it was found that there had been problems with the boilers for the past three months, and

<table>
<thead>
<tr>
<th>pH</th>
<th>Number of operating boilers</th>
<th>Expected values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total dissolved solids (mg/l)</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>Phosphate (total) as PO₄ (mg/l)</td>
<td>29</td>
<td>2.5</td>
</tr>
</tbody>
</table>

at this time, out of the three hospital boilers providing steam to the hospital and thus the laboratory autoclaves, only one boiler was in full working order. This boiler was 'priming' and 'carrying-over' (see Footnote) due to overloading, contaminating the steam with boiler water. The contamination was confirmed by analysis of the steam condensate (Table). Subsequently, two boilers were put into routine working use with the consequence that a repeat steam analysis showed a reduction in suspended solids (Table). All culture media were then able to support the growth of Staph. aureus.

Since the period of malfunction of the boilers, a bottle containing Isosensitest agar has been melted daily in the autoclaves, and a plate poured and inoculated with Staph. aureus as a check for inhibition of growth. Inhibitors have not been detected except on three occasions: when one of the boilers failed to operate, causing the other boiler to 'prime'; after