Screening of blood donors for the detection of antitetanus antibodies suitable for the production of human antitetanus immunoglobulin

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SUMMARY During a 12-month period, 40 146 blood donors were tested for the presence of high titre antitetanus antibodies. An attempt has been made to define the most suitable donors, those with antitetanus antibody titres of 4 IU/ml or above, by virtue of titre, sex, location, and length of time since most recent immunisation. Particular note is made of those donors from university sessions. Towards the end of the series, donors who had an initially acceptable level of antitetanus antibody were being tested a second time.

Sensitisation of recipients to horse immunoglobulin, resulting in hypersensitivity reactions, has led to an increase in the use of human antitetanus immunoglobulin for prophylaxis (Smith et al., 1975). The average yield of antitetanus immunoglobulin is 4.5 g/litre of crude plasma (Maycock, 1976), and only plasma with an antitetanus antibody level of 3 IU/ml or above is suitable for processing. Bearing in mind the presence of anticoagulant in the blood packs, we therefore set the minimum acceptable level in donor serum at 4 IU/ml. To meet the national need for this product, large-scale blood donor screening has been instituted. Initial experience with the immunoelectrophoresis (IEOP) screening test shows that the most useful donors are recently boosted persons, although an acceptable volume of suitable plasma can be obtained by large-scale routine donor screening.

Material and methods

Antitetanus antibody is detected and quantitated by a modification of the IEOP screening technique originally used for hepatitis B surface antigen/antibody detection, developed by Milne and Barr (1971).

The buffer system and Agarose plates are used in a similar manner to that originally described, with the exception that only two sets of 30 wells plus control wells are punched into the Agarose (18 ml per plate) and the wells are 2.5 mm in diameter, designed to accept 10 μl of serum or reagent.

Tetanus toxoid is supplied by the Blood Products Laboratory of the Lister Institute at Elstree and has a concentration of 125 Lf/ml (one Lf unit of toxoid is equivalent to one unit of antitoxin when measured in the flocculation test of Ramon and Descombes (1926)). A working dilution of toxoid equivalent to 3.75 Lf/ml is prepared for use in 0.9% saline; this dilution was determined empirically and will only detect antitetanus antibody levels of 4 IU/ml or more.

Tetanus antitoxin is also supplied by the Lister Institute and although batch to batch concentration varies it is usually in the region of 100 IU/ml; from this three dilutions are prepared (the international unit is the activity contained in a stated amount of the International Standard for tetanus antitoxin) (European Pharmacopoeia, 1971):

1. 6 IU/ml for use as a control in each IEOP plate;
2. a standard 10 tube doubling dilution, tested each day to ensure the toxoid has the required detection sensitivity;
3. a 16 IU/ml dilution used in the antitetanus antibody quantitation.

The screening test plates are prepared with 30 donor samples per plate plus controls and electrophoresed for 35 minutes at 75 volts per plate, electrophoresis packs set to constant voltage.

The plates are read, the control titre being examined first. With a 110 IU/ml antitoxin the 6·5 IU/ml dilutions should be strongly positive and with the 3·25 IU/ml dilution only weakly positive or negative.

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With these results in mind the tests are read and donor samples selected for quantitation where appropriate.

QUANTITATION
The technique used closely follows that described by Entwistle and Eldridge (1973). A 16 IU/ml antitoxin control is further diluted to cover the range 16:8:4:2:1 IU/ml, and doubling dilutions of screen-test-positive donor sera are prepared to a dilution of 1 in 16. The control and test titrations are then tested together using the 3-75 LF/ml toxoid dilution and the approximate antitetanus antibody level in the donor sera obtained by comparison with the control titre. An initial series of results was confirmed by Mouse Protection assay at the Lister Institute.

Simple documentation of donations suitable for processing is kept in the laboratory, such that future donations from the same donors can be easily identified. When each day's donor blood testing is complete and the packs are sorted for various uses, as many as possible of the suitable donations are passed to the Blood Products Department. Here the plasma is separated and pooled into 5 litre plastic bags which are deep frozen (−40°C) and sent at frequent intervals to the Lister Institute for fractionation.

Results
From the inception of donor screening in this laboratory in April 1975 to March 1976, 40,146 donors have been tested for the presence of antitetanus antibodies with a titre of 4 IU/ml or greater. The total number found to have an acceptable titre was 2,285; this is an incidence of 5.69% and is in agreement with other workers (Entwistle and Eldridge, 1973).

During the period of this series those donors with an antitetanus antibody titre of 4 IU/ml or greater have had a file card prepared. Several of these donors have now attended subsequent sessions. The persistence of a useful titre in these donors is shown in Table 1.

At each blood donor session, donors are questioned regarding recent injections. A note is made on the blood drawing record of those giving a history of recent antitetanus injection, together with the time in days since injection. Results of the antitetanus antibody screening test on a series of these donors are shown in Table 2 and the Figure.

Table 1 Persistence of antitetanus antibody in previously acceptable donors

| Subsequent donations from previously acceptable donors | 535 |
| Persistence of antibody ≥ 4 IU/ml | 382 (71%) |
| Antibody level subsequently fallen below 4 IU/ml | 153 (29%) |

Table 2 Results of testing 117 donors with a history of recent antitetanus injection

| Donations tested post recent injection | 117 |
| Antitetanus antibody level ≥ 4 IU/ml | 59 (50.4%) |
| Antitetanus antibody level < 4 IU/ml | 58 (49.6%) |

In a series of 2086 screen-test-positive donations the sera were further tested to find the approximate titre of antitetanus antibody present, by comparison with known standards. These results are shown in Table 3.

The same series of 2086 donors was also studied to obtain the sex distribution of suitable donors. The results are shown in Table 4.

The final observation made was upon the number of suitable donations obtained from sessions broadly classified as urban, rural, industrial, and universities and colleges. The results obtained from analysis of 10 donor sessions in each category are given in Table 5.

Figure Antitetanus antibody levels in 59 donors with a recent history of antitetanus injections.
Screening of blood donors for the detection of antitetanus antibodies

Table 3  Quantitation of antitetanus antibody in 2086 screen-test-positive donations

<table>
<thead>
<tr>
<th>IU/ml</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>999</td>
<td>792</td>
<td>231</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td>%</td>
<td>48-0</td>
<td>38-0</td>
<td>11-0</td>
<td>2-5</td>
<td>0-5</td>
</tr>
</tbody>
</table>

Table 4  Sex distribution of 2086 screen-test-positive donors

<table>
<thead>
<tr>
<th>Male donors with antitetanus antibody level</th>
<th>1676 (80%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female donors with antitetanus antibody level</td>
<td>410 (20%)</td>
</tr>
</tbody>
</table>

Table 5  Numbers of suitable donations obtained from different categories of donor sessions

<table>
<thead>
<tr>
<th>Type of session</th>
<th>Number of donations tested</th>
<th>Number found suitable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>2093</td>
<td>136 (6-49%)</td>
</tr>
<tr>
<td>Rural</td>
<td>1912</td>
<td>104 (5-40%)</td>
</tr>
<tr>
<td>Industrial</td>
<td>1842</td>
<td>90 (4-88%)</td>
</tr>
<tr>
<td>University/college</td>
<td>1927</td>
<td>270 (14-0%)</td>
</tr>
</tbody>
</table>

During this period, therefore, it was found theoretically possible to process the plasma from 2285 units of donor blood into human antitetanus immunoglobulin. However, because of demands on donated blood for the preparation of other products and components, and also because of the particular group and genotype of some of the donations, it was possible to process only 2003 units.

Discussion

The increasing use of human immunoglobulin for tetanus prophylaxis has made it necessary to attempt to forecast and screen those donor sessions which will produce a high yield of crude plasma suitable for the preparation of this material. This knowledge will enable us to continue to produce adequate supplies of other equally important blood products, plus an increased volume of suitable plasma coupled with more efficient use of the remaining red cells.

In our example of the university sessions within the Trent Regional Health Authority area, the prior knowledge of the large numbers of potentially suitable donors, and the time interval between injection and bleeding for the optimum antibody level, could lead to further use of the 'double pack' system. Although this system is more expensive than the routinely used 'single pack' system, the two blood packs joined together with a closed transfer line enable the cells and plasma to be separated aseptically.

The concentrated red cells prepared under such a closed system can then be issued for transfusion purposes with a maximum refrigerated storage time. This is in contrast to the single pack system, in which the opening of the pack to remove plasma leaves concentrated cells suitable only for immediate transfusion use because of the possibility of contamination occurring during handling. Of equal importance is the knowledge that some sessions are likely to be less productive for antitetanus antibody plasma. These sessions need not, therefore, be screened, but instead can be used to prepare cryoprecipitate, platelet rich plasma, platelet concentrate, and other important components.

With the present system of fractionation used at the Blood Products Laboratory, a one-litre pool of crude plasma with an antitetanus antibody level of 6 IU/ml will produce 11½ doses of human antitetanus immunoglobulin with a potency of 250 IU/ml. If the antitetanus antibody level in the original pool is increased, then the number of doses produced will be increased in proportion.

From the experience gained at this centre, this technically simple procedure can conveniently be performed on 1000 donations per week. Our present detection rate, coupled with careful session selection, thus produces an acceptable volume of antitetanus antibody plasma suitable for processing into immunoglobulin with the minimum of interference with programmes for the production of whole blood and other components required in the area.

At present in this centre we do not consider it necessary to embark on the antitetanus antibody boosting and plasmapheresis programmes favoured by other workers (Cook et al., 1976).

Grateful acknowledgement is made to Dr W. d'A. Maycock and the staff of the Blood Products Laboratory at the Lister Institute of Preventive Medicine for confirmation of our original results and supplies of reagents.

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References


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