Detection and quantitation of tetanus antitoxin in blood donations

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SYNOPSIS Passive haemagglutination and IEOP have been used both to detect and to measure tetanus antitoxin in human donor sera. Forty percent of blood donors had detectable antitoxin but only 9% had levels suitable for production of human antitetanus immunoglobulin (≥ 2 IU/ml). The incidence of high titre antitoxin was significantly greater in men and was unrelated to the ABO blood group system. The prevalence of antitoxin in selected donor groups and immunized staff is shown.

Human antitetanus immunoglobulin is now recognized as the most suitable means of providing passive protection against tetanus. It does not have the disadvantages of hypersensitivity reactions and rapid elimination associated with horse antitoxin (Editorial, 1974). There is therefore an increasing demand for the blood transfusion service to provide suitable high titre plasma for preparation of this specific immunoglobulin. A minimum level of 2 IU/ml is necessary for fractionation (Eldridge and Entwistle, 1975). In the past, such plasma has been mainly obtained from either hyperimmunized volunteers or individuals with a history of recent tetanus toxoid injections. Hyperimmunized volunteers are prone to allergic reactions on repeated immunization (Annotation, 1974), and recently inoculated individuals may not necessarily produce suitable antibodies. This study was primarily undertaken to determine both the incidence and level of tetanus antitoxin in blood donors. For the last six months, we have screened donor blood for the presence of tetanus antitoxin by the method of immunoelectroosmophoresis (IEOP) described by Milne and Barr (1971). We have modified the passive haemagglutination method of Sequeira and Eldridge (1973) to sensitize human red cells with tetanus toxoid. Such cells readily allow detection of tetanus antitoxin at very low levels and can be used in a slide test, a microtitre test or an automated test.

Materials

A 25% glutaraldehyde standard solution (BDH, Laboratory reagent), and 1% tannic acid solution (Evans Medical Ltd, BP) were diluted before use. Purified tetanus toxoid preparations, satisfactory for sensitization, were obtained from Commonwealth Serum Laboratories, Melbourne, Welcome Reagents Ltd (XT 09), and Lister Institute, Elstree. Any of these preparations or tetanus vaccine in simple solution BP can be used as a source of 'free' toxoid in the final standardized cell suspension. Human antitetanus immunoglobulin, 50 IU/ml (Blood Products Laboratory, Elstree), was used for standardizing preparations of sensitized cells and for measuring antitoxin.

Methods

PREPARATION OF CELLS

Fresh human group O Rh-negative cells were washed four times in normal saline. An equal volume of 0.1% glutaraldehyde was added to these packed cells and left at room temperature for 90 minutes. The cells were then saline washed four times and a 6% suspension was prepared in 0.03 M phosphate buffered saline, pH 6.4 (PBS 6.4). An equal volume of 0.005% tannic acid was added to the 6% cell suspension, and the mixture was incubated in a water bath at 37°C for 15 minutes. Slight spontaneous aggregation occurred during incubation but was reversible on thrice washing in saline. The cells were resuspended in PBS 6.4. For sensitization, an equal volume of tetanus toxoid solution diluted in saline to 40 LF/ml was added, and the mixture was incubated at 37°C for 30 minutes, inverting twice during incubation, centrifuging, and washing once in saline. Finally, the cells were resuspended as a
2% suspension in PBS 7.2 containing 0.5% bovine serum albumin.

**Standardization of Sensitized Cells**

A slide procedure was used to determine the amount of 'free' toxoid necessary to neutralize tetanus antitoxin $\geq 5$ IU/ml. Varying amounts of tetanus toxoid 40 LF/ml were added to 1 ml volumes of the sensitized cells, and tested against various concentrations of standard human antitetanus immunoglobulin. The amount of toxoid which just prevented haemagglutination in antitoxin concentrations below 5 IU/ml determined the amount of 'free' toxoid to be added to the bulk cells for use in screening. In our experience, the final cell suspension contained 2 LF/ml of tetanus toxoid.

**Screening Tests**

**IEOP**

8 LF/ml toxoid, running for 60 minutes, detected tetanus antitoxin $\geq 3$ IU/ml; whilst 30 LF/ml toxoid, running for 30 minutes, detected levels $\geq 10$ IU/ml.

**Slide Haemagglutination**

25 $\mu$l of standardized cell suspension were added to equal volumes of test sera on a slide. After 5 minutes at room temperature the tests were examined macroscopically for agglutination, which indicated the probable presence of antitoxin $\geq 5$ IU/ml.

**Microtitre Haemagglutination**

'U'-bottomed microtitre plates were used. Sera were diluted 1:10 in PBS 7.2 containing 1% bovine serum albumin, 0.04% Tween 20 and 0.01% polyvinyl pyrrolidone, and equal volumes (25 $\mu$l) of the standardized cell suspension were added. The plate was covered and left at room temperature for 90 minutes. Haemagglutination, suggesting the presence of tetanus antitoxin, was shown by a carpet of cells on the bottom of the well.

**Autoanalyzer Haemagglutination**

The standardized cell suspension was introduced into a plasma line in a standard Technicon blood-grouping assembly; 60 ml of the cell suspension were sufficient for screening 600 plasma samples. Both positive and negative controls were included in all screening tests, and all positive results were confirmed and quantitated.

**Confirmatory Tests**

**IEOP**

Our experience has shown this test to be specific and relatively free from false positive results. Therefore, the IEOP test was suitable for use as a confirmatory test of all the haemagglutination methods.

**Microtitre Haemagglutination**

Test serum dilution, 25 $\mu$l, was added to each of eight wells. Equal volumes of tetanus toxoid solution, ranging from 0.5 LF/ml to 10 LF/ml, were added to six of the wells, and of saline to the remaining wells. After 15 minutes at room temperature, 25 $\mu$l sensitized cell suspension were added to all but one of the saline wells. To this well was added tanned but unsensitized cells to exclude anti-red cell activity. Two human antitetanus immunoglobulin solutions (5 IU/ml and 10 IU/ml) were included in the test to permit a direct comparison to be made with test serum.

**Results**

Twelve selected plasmas, previously quantitated by the described methods, were independently assayed for tetanus antitoxin by the mouse neutralization test. The results of all 12 samples in the *in vivo* method showed close agreement.

The passive haemagglutination methods, without the presence of 'free' toxoid, were capable of detecting very small amounts of tetanus antitoxin; microtitre haemagglutination 0.001 IU/ml, slide haemagglutination 0.005 IU/ml, and autoanalyzer haemagglutination 0.1 IU/ml. These limits were derived from testing 816 blood donors. Microtitre haemagglutination detected tetanus antitoxin in 40% of our donors, whereas IEOP, standardized to detect a minimum level of 3 IU/ml, indicated only 9% of these donors to be suitable for fractionation purposes.

The table shows the results using IEOP and the autoanalyzer haemagglutination method for detection of acceptable levels of tetanus antitoxin for the preparation of immunoglobulin. In this survey, 9.3% of donors had tetanus antitoxin levels of $\geq 3$ IU/ml, 5.6% levels of $\geq 5$ IU/ml, and 2.3% levels of $\geq 10$ IU/ml. Approximately 50% of donors shown as having levels $\geq 10$ IU/ml had levels very close to 10 IU/ml. These observations were very similar to those reported by Nelson (1973) and Eldridge and Entwistle (1975).

Among 738 male prisoners the incidence of tetanus antitoxin was notably higher; 26% had levels $\geq 3$ IU/ml and 9.9% $\geq 10$ IU/ml. Of the recently immunized group, 46% had levels $\geq 3$ IU/ml and 18% $\geq 10$ IU/ml.

The figure analyses the incidence and level of tetanus antitoxin in male and female donors excluding both the prison donor and the recently inoculated...
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<table>
<thead>
<tr>
<th>Population</th>
<th>Screening Method</th>
<th>Total Number Tested</th>
<th>Number with Tetanus Antitoxin Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≥ 3 IU/ml</td>
</tr>
<tr>
<td>Randomly selected donors</td>
<td>Haemagglutination</td>
<td>6461</td>
<td></td>
</tr>
<tr>
<td>Randomly selected donors</td>
<td>IEOP</td>
<td>564</td>
<td>524 (9.3%)</td>
</tr>
<tr>
<td>Prisoners (males)</td>
<td>IEOP</td>
<td>738</td>
<td>193 (25%)</td>
</tr>
<tr>
<td>Recently inoculated donors</td>
<td>IEOP</td>
<td>71</td>
<td>33 (46%)</td>
</tr>
</tbody>
</table>

Table Incidence of tetanus antitoxin suitable for fractionation

Figure Incidence of tetanus antitoxin in male and female donors (excluding the prison and recently inoculated groups).

groups. The higher incidence of antitoxin in males was not significant at low levels but became statistically highly significant at levels ≥ 3 IU/ml (χ² = 74-051; p < 0.001). It was also noted that the distribution of the ABO blood groups in randomly selected donors with antitoxin levels ≥ 3 IU/ml was normal (O 53%, A 32%, B 11%, AB 4%).

Discussion

The results of this survey show that 40% of blood donors had detectable tetanus antitoxin, but that only 9-3% possessed a level of antitoxin suitable for preparation of antitetanus immunoglobulin. Men had higher levels of antitoxin than women. This is probably due to the fact that men are immunized against tetanus because their occupations tend to expose them to the hazard of tetanus.

The higher percentage of male prisoners with high titre antibodies may be related to asocial habits which necessitate their re-immunization and boosting more frequently than that of the general population. The antitoxin levels in donors with a recent history of inoculation points to the need to screen the plasma of such donors before accepting it for immunoglobulin preparation. About 50% of these donors are likely to be acceptable. This low incidence may be due partly to an unreliable history given by donors.

The methods described are simple, inexpensive, and rapid to perform, and can be used for screening large numbers of specimens. Both the IEOP and autoanalyzer haemagglutination techniques have advantages for blood transfusion services. The screening of donors by the automated method, along with IEOP for confirmation and quantitation, offers a practical solution to the problem of finding suitable plasma for the preparation of human antitetanus immunoglobulin. The slide and microtitre haemagglutination tests could prove useful in assessing the humoral response to tetanus toxoid in individual patients.

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