INTERNATIONAL STANDARD FOR CHOLERA ANTITOXIN

A note by the Secretariat

The WHO Diarrhoeal Diseases Control Programme has an urgent need for an international standard for cholera antitoxin which would be useful for the comparison of cholera antitoxin levels in man and for the calibration of enterotoxins and toxoids.

Since 1965 two batches of cholera antitoxin have been prepared, one in rabbits by Craig (1) and one in horses by the Swiss Serum and Vaccine Institute in Berne (2). The antigens used in the preparation of these antitoxins were crude toxins which could not be well defined.

For several reasons the US Cholera Panel of the NIAID did not consider these to be suitable as a US Standard or as an antitoxin proposed as an international standard. The US Cholera Panel, therefore, sponsored work to produce and standardize a more suitable preparation.

Accordingly, cholera toxin prepared by Finklestein and Peterson (3) was detoxified at a concentration of 550 μg/ml with 0.1% formalin at pH 7.8 for 72 hours at 30°C and then dialysed against phosphate-buffered saline for 72 hours.

This preparation was adjusted to a concentration of 360 mg/ml with aluminium chloride and the toxoid was adsorbed onto the aluminium phosphate thus formed in situ. The adsorbed antigen was then used to immunize four goats injecting the antigen by the subcutaneous route at four sites (180 μg toxin protein) with booster injections (215 μg) at 8, 16 and 24 weeks. Plasma was obtained from the goats by plasmapheresis after 25, 26 and 27 weeks, the plasma from two goats were converted to serum, pooled and filtered under sterile conditions. The serum was dispensed in 0.5 ml quantities in 2.0 ml ampoules and freeze-dried. The residual moisture was 1.12%. This material was designated NIH Lot 1 US Standard Cholera Antitoxin and sent to investigators for a national collaborative study of the antitoxic activity when measured by several systems.

The properties of the antitoxin in comparison with the two other antitoxins mentioned above are shown in Table 2.

The antitoxin was compared by several methods with the antitoxin prepared in horses (SSVI) and to which a neutralizing activity of 4470 units had been assigned when measured by a rabbit intracutaneous method.

2 The list of participants is shown in Table 1
The tests used and results obtained are summarized in Table 2.

As can be seen the gel diffusion study against purified and crude cholera toxin indicate that NIH Lot 1 contains no detectable precipitating antibody against non-toxin vibrio antigens. It is not uncommon to have poor flocculation and separate reference sera for flocculation are often used.

1. A test measuring the vascular permeability activity in rabbits by the limit of blueing (Lb) method showed that NIH Lot 1 had an activity of about 4400 units/ml.

2. Toxin neutralization as determined by the intravenous injection of toxin/antitoxin mixtures in mice indicated an activity of about 4400 units/ml.

3. Titration of the antitoxin in ligated segments of the small intestines of rabbits gave a geometric mean titre of 5594 antitoxin units/ml; the individual results, however, varied from 4080 units/ml to 9260 units/ml.

4. The results obtained using a haemagglutination test showed an activity of 4470 units/ml.

5. A microtitre method using Y-1 adrenal cells treated with cholera toxin and dilutions of antitoxin gave a mean titre of 5016 units/ml.

As it is considered that the neutralization to toxin is the most important property the NIH Lot 1 antitoxin was assigned an activity of 4400 units/ml based on the neutralization of the vascular permeability activity in rabbit skin as measured by the limit of blueing (Lb) method.

The committee may wish to accept the generous offer to WHO of 400 ampoules of this US standard Lot 1 and, in order to maintain the unitage, to assign to it an activity of 2200 international units to the contents of each ampoule.

REFERENCES


Table 1

List of Participants in the Collaborative Assay of Cholera Antitoxin

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<table>
<thead>
<tr>
<th>Criterion</th>
<th>Antitoxin</th>
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<tbody>
<tr>
<td></td>
<td>Anti-Choleragenoid</td>
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<tr>
<td>Origin</td>
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<td>Immunizing Antigen(s)</td>
<td>Choleragenoid Purified</td>
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<tr>
<td>Vibriocidal Antibody</td>
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</tr>
<tr>
<td>Unitage (Lb)</td>
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<tr>
<td>Avidity</td>
<td>Low</td>
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1 Taken from Craig, J.B., Develop biol. Standard., Vol. 41, pp. 415-422, Karger, Basel 1978