MANUAL OF DETAILS OF TESTS REQUIRED ON FINAL VACCINES
USED IN THE WHO EXPANDED PROGRAMME OF IMMUNIZATION

In view of the recent updating of this manual, the following should be amended:

1. All pages marked "Corr." should replace corresponding former pages;

2. All pages marked "Add." should be inserted as new pages.

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INTRODUCTION

This manual has been prepared, in the first instance, to give details of the tests applied to vaccines in the final form. Such tests may be applied by control authorities to vaccines as they are given to children in order to check that they have adequate potency and do not have undue toxicity.

The tests are identified by the vaccine to which they are applicable or as a general test and are classified according to the complexity of the equipment and facilities required for their execution.

**Class A:** is a class of tests requiring only the laboratory equipment expected to be present in any microbiological laboratory.

**Class B:** is a class of tests requiring special items of equipment but does not require the use of animals.

**Class C:** is a class of tests requiring the use of animals and, thereby, demanding the facilities of an animal holding unit, an animal breeding station or a reliable source of good quality animals.

Each of the following sections contains recommendations. The parts of each section that cover half the width of the page are comments and recommendations for guidance.

The essential tests applicable to diphtheria, tetanus, pertussis and combined vaccines are summarized on Table 1.
TABLE OF CONTENTS
# INTRODUCTION

## TESTS APPLIED TO VACCINES

### General Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Sterility</td>
<td>GEN-1 6</td>
</tr>
<tr>
<td>2. Innocuity</td>
<td>GEN-2 19</td>
</tr>
<tr>
<td>3. Aluminium content</td>
<td>GEN-3 20</td>
</tr>
<tr>
<td>4. Thiomersal</td>
<td>GEN-4 22</td>
</tr>
<tr>
<td>5. Hydrogen ion concentration (pH)</td>
<td>GEN-5 24</td>
</tr>
<tr>
<td>6. Free formaldehyde</td>
<td>GEN-6 25</td>
</tr>
<tr>
<td>7. Opacity</td>
<td>GEN-7 27</td>
</tr>
<tr>
<td>8. Moisture content</td>
<td>GEN-8 29</td>
</tr>
</tbody>
</table>

### Special tests

#### B.C.G. Vaccine

<table>
<thead>
<tr>
<th>Test</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Identity</td>
<td>BCG-1 33</td>
</tr>
<tr>
<td>2. Sterility</td>
<td>see GEN-1</td>
</tr>
<tr>
<td>3. Absence of contaminating microorganisms</td>
<td>BCG-2 37</td>
</tr>
<tr>
<td>4. Absence of virulent mycobacteria</td>
<td>BCG-3 38</td>
</tr>
<tr>
<td>5. Total bacterial content</td>
<td>BCG-4 41</td>
</tr>
<tr>
<td>6. Number of culturable particles</td>
<td>BCG-5 42</td>
</tr>
<tr>
<td>7. Stability</td>
<td>BCG-6 50</td>
</tr>
</tbody>
</table>
Poliomyelitis Vaccine (Oral)

1. Virus titration (potency)  
2. Test for bacteria and fungi  
3. Innocuity  
4. Test for pH  
5. Identity

Measles Vaccine (Live)

1. Virus concentration (potency)  
2. Sterility  
3. Innocuity  
4. Identity  
5. Stability

Table 1

List of tests applicable to diphtheria (D), tetanus (T), pertussis (P) vaccines and to combined vaccines DT, DPT.

Appendices

Appendix 1: Media for the detection of aerobic and anaerobic bacteria and fungi  
Appendix 2: Tests for mycoplasmas  
Appendix 3: Individual identification of guinea-pigs in an animal room  
Appendix 4: Probit analysis of potency tests  
Appendix 5: Media for the growth of B. pertussis  
Appendix 6: Media for the assay of live measles vaccine by plaque assay on Vero cells  
Appendix 7: Suggested sources of cell culture materials
### Summary protocols for the reporting of results of tests

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria, pertussis, tetanus</td>
<td>124-131</td>
</tr>
<tr>
<td>Diphtheria toxoid</td>
<td>132-136</td>
</tr>
<tr>
<td>Pertussis Vaccine</td>
<td>137-140</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>141-144</td>
</tr>
<tr>
<td>BCG Vaccine</td>
<td>145-149</td>
</tr>
<tr>
<td>Poliomyelitis Vaccine (Oral) Sabin strains</td>
<td>150-161</td>
</tr>
<tr>
<td>Measles Vaccine (Live)</td>
<td>162-167</td>
</tr>
</tbody>
</table>
TESTS APPLIED TO VACCINES
CLASS OF TEST: A
GEN-1

VACCINE: All vaccines

TEST: Test for Sterility

THE TEST

1. Sampling from final lot

Samples of final containers from each final lot to be tested shall be taken in such a manner as to be representative of the lot to be tested. Appropriate periodic samples shall be taken including samples at the beginning and the end of the filling operation.

If a product lot is filled through several outlets from a single bulk, samples should be taken from each outlet (filling lot) so as to be representative of the filling assembly.

The number of samples taken shall be at least that approved by the national control authority, provided that for final lots containing 500 or more containers, at least 20 samples shall be taken, including samples at the beginning and the end of the filling operation.

2(a) Press: 1
   2nd
   Write

   Pass blank card side 1 (number 1 appears in display)

(b) Press: 2
   2nd
   Write

   Pass blank card reversed (side 2)

(c) Press: 3
   2nd
   Write

   Pass second blank card (3)

The new cards contain the programme now
Check the new cards.
B. WITH A HEWLETT PACKARD 41 CV CALCULATOR

If the "RELPOT" Programme is already stored in the machine, the user does not need to repeat steps 1.1 to 1.4 and can analyse directly the results as indicated under 2. Otherwise, proceed as follows:

1. Loading the machine with the "RELPOT" programme

In order to be able to use the preprogrammed magnetic cards\(^1\), the machine should be equipped with the ad hoc card reader.

1.1 Make sure that the machine does not contain any information. The machine can be considered as "empty" if, for example, it has contained no battery for several minutes.

1.2 Setting up the required partition of registers.

<table>
<thead>
<tr>
<th>Press</th>
<th>On display</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON</td>
<td>0.0000</td>
</tr>
<tr>
<td>XEQ</td>
<td>XEQ--</td>
</tr>
<tr>
<td>ALPHA</td>
<td>XEQ-(ALPHA)</td>
</tr>
<tr>
<td>SIZE</td>
<td>XEQ SIZE-(ALPHA)</td>
</tr>
<tr>
<td>ALPHA</td>
<td>SIZE---</td>
</tr>
<tr>
<td>054</td>
<td>SIZE 054</td>
</tr>
</tbody>
</table>

1.3 Reading the cards

\(^1\) Available from Chief, Biologicals, WHO Geneva.
Then insert card No. 1 placed in normal position into slot at upper right hand side of machine. "RDY 02 OF 10" comes on display. Insert the same card placed in the upside-down position. "RDY 03 OF 10" comes on display.

Proceed in a similar way with each of the four other cards. "WORKING" comes under display at the end, followed by "0.0000"(USER).

The reading system can then be removed and the machine will store the programme as long as batteries are effective, even if the machine is switched off.

1.4 Assigning the programme to key R

Press

On display

(Yellow key) 0.0000(USER SHIFT)
ASN-USER
ASN-(USER ALPHA)
ASN RELPOT-(USER ALPHA)
ASN RELPOT-(USER)
0.0000(USER)
2. Probit analysis

The following example is based upon a test in which after immunization of each of three groups of animals with five-fold concentrations of a standard vaccine and three other groups with five-fold concentrations of a vaccine under test, all the animals were challenged and survivors recorded at the end of the observation period.

The data can be summarized as follows:

<table>
<thead>
<tr>
<th>Point</th>
<th>Dose</th>
<th>Survivors</th>
<th>Total number challenged</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>7</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>18</td>
<td>20</td>
</tr>
</tbody>
</table>

2.1 Entering data

<table>
<thead>
<tr>
<th>Press</th>
<th>Comment</th>
<th>Output*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R↓</td>
<td></td>
<td>5.000000</td>
</tr>
<tr>
<td>A</td>
<td>POINT?</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>first point</td>
<td>1-</td>
</tr>
</tbody>
</table>

* Under the output, the calculator will also systematically display the status indicators "USER 1".
Proceed similarly for the rest of the data until the last figure (20) has been entered. In case a wrong number has been entered, if \( \text{R/S} \) has not yet been pressed, press \( \leftarrow \) in order to delete and then enter the correct figure; if \( \text{R/S} \) has already been pressed, start anew by pressing \( \text{A} \), then introducing point, dose, survivors and total number challenged.

2.2 Checking that data have been correctly entered

<table>
<thead>
<tr>
<th>Press</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>POINT?</td>
</tr>
<tr>
<td>1</td>
<td>1-</td>
</tr>
<tr>
<td>R/S</td>
<td>4.0000</td>
</tr>
<tr>
<td>R/S</td>
<td>2.0000</td>
</tr>
<tr>
<td>R/S</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td>POINT?</td>
</tr>
</tbody>
</table>

etc...
Proceed similarly until all data have been checked; in this particular case, "20" comes under display at the end of the process. In case of data wrongly entered, press A and introduce again the right sequence.

### 2.3 Calculations

<table>
<thead>
<tr>
<th>Press</th>
<th>Output*</th>
<th>Nature of information</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>NO.IT = 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Number of iterations</td>
</tr>
<tr>
<td>D</td>
<td>x²2.H = 0.498</td>
<td>Chisquare for linearity</td>
</tr>
<tr>
<td>R/S</td>
<td>HET. = 1.000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Heterogeneity factor</td>
</tr>
<tr>
<td>R/S</td>
<td>x²2.P = 0.014&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Chisquare for parallelism</td>
</tr>
<tr>
<td>R/S</td>
<td>B = 1.502</td>
<td>Common slope</td>
</tr>
<tr>
<td>R/S</td>
<td>SE.B = 0.251</td>
<td>Standard error of common slope</td>
</tr>
<tr>
<td>R/S</td>
<td>ED50.S = 30.087</td>
<td>50% immunizing dose for the standard</td>
</tr>
<tr>
<td>R/S</td>
<td>ED50.T = 40.674</td>
<td>50% immunizing dose for the vaccine</td>
</tr>
<tr>
<td>E</td>
<td>R.POT = 0.740&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Relative potency</td>
</tr>
<tr>
<td>R/S</td>
<td>G = 0.107&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Coefficient G</td>
</tr>
<tr>
<td>R/S</td>
<td>LL = 0.330</td>
<td>Lower limit of relative potency (for the 95% confidence interval)</td>
</tr>
<tr>
<td>R/S</td>
<td>UL = 1.796</td>
<td>Upper limit of relative potency (for the 95% confidence interval)</td>
</tr>
<tr>
<td>R/S</td>
<td>0.000</td>
<td>End of calculation</td>
</tr>
</tbody>
</table>

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* For footnotes, refer on next page.
Analysis of further data can be achieved as indicated under 2. Any further analysis can be performed again by starting directly as indicated under 2. If the machine is no longer needed, switch it off.

This process can take several minutes; its completion is indicated by a beep. The figure which is on display indicates the number of iterations which have been executed in order for the difference between two consecutive estimates of the common slope to be within 5% of the standard deviation of the last estimate. The programme allows for eight consecutive iterations; in case figure "9" is on display, the required level of precision (in this case 5%) has not been reached; however, the rest of the programme can be executed.

If the heterogeneity factor of linearity (which is equal to $X^2$/number of degrees of freedom) displayed is greater than one, it is significant at 5% probability level. This is indicated by a beep. As a consequence, the confidence interval for the relative potency will be wider.

Figure on display indicates the Chi Square value for parallelism; in case of non-parallelism (at the 5% probability level), "NON PARAL" comes under display and the rest of the programme cannot be executed.

The potency of the vaccine under test is equal to that of the standard multiplied by 0.74.

If coefficient $G 1$, the confidence limits cannot be calculated. This is indicated by a beep and the programme will not proceed further.
VACCINE: Diphtheria

TEST: Test for identity

THE TEST

The identity tests on a batch of toxoid can be carried out by both in vitro and in vivo methods. Although it is a simple procedure to establish the identity of fluid toxoid by a flocculation test, it is necessary, in the case of adsorbed toxoids, to dissolve the adsorbent (adjuvant) by the addition of sodium citrate.

For fluid toxoid, a diphtheria antitoxin is diluted, so as to contain 20 IU per ml. Increasing volumes of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 ml are pipetted into a series of tubes; the volumes are made up with normal saline to 1 ml, and 1 ml of fluid toxoid, diluted to approximately 10 Lf/ml, is added to each of the tubes. The tubes are placed in a water bath at 50°C and observed continuously. The tube containing 0.5 ml of antitoxin or the tube on either side of it may show flocculation and such flocculation establishes the identity of the product.

For an adsorbed toxoid about 0.5 g of sodium citrate is added to 10 ml of the adsorbed toxoid. The mixture is incubated at 37°C for one or two days to dissolve. After

1 Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid and Combined Vaccine (Requirements for Biological Substances, Nos 8 and 10, Revised 1978), Part A, Section 5.1, p. 51.
the aluminium salt has dissolved the flocculation test similar to the one stated above is used. The flocculation occurs in the same way though it may take a longer time.

In the in vivo test a suitable dose of toxoid is injected into guinea pigs. The two guinea pigs used in the test for innocuity (GEN-2) can be used also for this purpose, provided the response is not assessed until 14 days after immunization; the presence of antitoxin in the blood is demonstrated either by intradermal challenge of the test animals or by a passive protection test in guinea pig skin.

The identity of diphtheria toxoids can also be demonstrated by double immunodiffusion in gels, also called "Ouchterlony reaction", described below.

Identity test by the immunodiffusion method

1. **Aim**

   The object of the test is to demonstrate the presence of diphtheria toxoid in the vaccine.

2. **Principle**

   This test is carried out by precipitation in a gel medium through diffusion of the product undergoing test against a specific antiserum.

3. **Material and equipment - reagents - methods**

   3.1 **Material and equipment**

   - microscope slides 75 x 25 mm
   - slide holder
   - staining solutions in appropriate containers
   - punches for 3 mm diameter trocarts
   - horizontal stage
3.2 Reagents

Antisera: diphtheria toxoid antiserum

This antiserum must have been thoroughly tested to verify its specificity.

References: diphtheria toxoid reference solution.

Gel, buffers, staining solutions, desorption reagent: as described below.

3.3 Preparation of reagents

3.3.1 Buffer pH 8.6 ionic strength 0.1

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veronal</td>
<td>0.01 M</td>
<td>1.482 g</td>
</tr>
<tr>
<td>Veronal sodium</td>
<td>0.05 M</td>
<td>10.309 g</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>0.05 M</td>
<td>6.800 g</td>
</tr>
</tbody>
</table>

Dissolve and make up to 1 litre with distilled water.
Check pH.

3.3.2 Gel:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose</td>
<td>12.50 g</td>
</tr>
<tr>
<td>Buffer pH 8.6</td>
<td>250 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>750 ml</td>
</tr>
</tbody>
</table>
Weigh 12.50g of agarose in an Erlenmeyer flask and slowly add the buffer and the distilled water. Place this Erlenmeyer flask stoppered with a non-impermeable stopper in a boiling water bath until the solution becomes clear (about half-an-hour). Divide up into sterile bottles and store at +4°C.

3.3.3 NaCl solution:

\[
\text{NaCl} \quad 9 \text{ g} \\
\text{Distilled water q. suff.} \quad 1000 \text{ ml} \\
\text{Dissolve.}
\]

3.3.4 Rinsing solution:

\[
\text{Methanol} \quad 450 \text{ ml} \\
\text{Acetic acid} \quad 100 \text{ ml} \\
\text{Distilled water} \quad 450 \text{ ml} \\
\text{Mix.}
\]

3.3.5 Stain:

\[
\text{Amido Black} \quad 9 \text{ g} \\
\text{Rinsing solution} \quad 1000 \text{ ml} \\
\text{Dissolve.}
\]

3.3.6 Desorption reagents: Prepare one of the following solutions

\[
\text{Disodium phosphate (2H}_2\text{O)} \quad 5 \text{ g} \\
\text{Distilled water q. suff.} \quad 100 \text{ ml}
\]

or

\[
\text{Sodium citrate (5H}_2\text{O)} \quad 5 \text{ g} \\
\text{Distilled water q. suff.} \quad 100 \text{ ml}
\]
3.3.7 Preparation of plates:

- liquefy the agarose in a warm water bath at 85-90°C;
- place the glass slides, which have first been degreased with alcohol at 95°C, on the slide holder;
- place the holder on a horizontal stage;
- using a paint brush, coat the slides and their junctions with the holders with a fine layer of agarose;
- allow to dry for 15-30 minutes at +37°C;
- let the agar run thoroughly so as to produce a uniform layer;
- allow to cool for 5 minutes at the ambient temperature and under cover.

4. Carrying out the test

Pierce the gel with the punch. Gently suck out the contents of the hole with a rubber tube fitted to a 3 mm diameter trocart. Introduce the products to be tested by Pasteur pipette as indicated on the scheme below. Place the reference substance in holes 1 and 4 so that the content is 15 Lf/ml. Place the antiserum corresponding to the reference substance in hole 7. Holes 2-3 and 5-6 will take the test samples.

Should the vaccine for testing be adsorbed, mix equal amounts of the vaccine and one or other of the desorption reagents; leave in contact for 30 minutes at laboratory temperature. Centrifuge in order to clear the supernatant. Take an aliquot and test as indicated above.

Distance between two holes: 5 mm.
Leave to diffuse for 24 hours in a moist chamber. Wash in 9% NaCl for 72 hours. Wash in distilled water for one hour. Dry the plates by placing strips of filter-paper on the gel for 12 hours in the drying cabinet at 37°C. Remove the strips and allow to dry at the ambient temperature for as long as necessary.

5. **Staining**

Staining is carried out by immersing the slides in the stain for five minutes. After that, dip the slides successively in six baths of rinsing solution for 10-15 minutes in each bath and allow to dry.

6. **Interpretation of results**

Each antiserum should react with the reference solution specific to it.

The products being tested should exhibit a precipitin line fusing with that obtained with the reference solution.
CLASS OF TEST B

TET-2

VACCINE: Tetanus toxoid

TEST: Test for identity

THE TEST$^{1,2}$

The identity tests on a batch of toxoid can be carried out by both in vitro and in vivo methods. Although it is a simple procedure to establish the identity of fluid toxoid by a flocculation test, in the case of adsorbed toxoids it is necessary to dissolve the adsorbed material by the addition of sodium citrate.

For non adsorbed toxoids a tetanus antitoxin is diluted so as to contain 20 IU per ml. Increasing volumes of 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 ml are pipetted into a series of tubes, the volumes are made up with normal saline to 1 ml, and 1 ml of fluid toxoid, diluted to approximately 10 Lf/ml, is added to each of the tubes. The tubes are placed in a water bath at 50°C and observed continuously. The tube containing 0.5 ml of antitoxin or the tube on either side of it may show flocculation and such flocculation establishes the identity of the product.

For an adsorbed toxoid about 0.5 g of 2H$_2$O sodium citrate is added to 10 ml of the adsorbed toxoid. The mixture is incubated at 37°C for one or two days to

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1 Requirement for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid, and Combined Vaccines (Requirements for Biological Substances Nos. 8 and 10) (Revised 1978), Part A, Section 5.1, p. 92.
2 WHO Manual for the Production and Control of Vaccines: Tetanus Toxoid, BLG/UNDP/77.2, Rev.1, Appendix T27.
dissolve. After the aluminium salt has dissolved the flocculation test similar to the one stated above is used. The flocculation occurs in the same way though it may take a longer time.

The identity of tetanus toxoids can also be demonstrated by double immunodiffusion in gel, also called "Ouchterlony reaction", as described in test DIP-2 for diphtheria toxoid.

In the **in vivo** test a suitable dose of toxoid is injected into guinea pigs and the presence of antitoxin in the guinea pigs' blood can be demonstrated by the protection test carried out in mice.
CLASS OF TEST C

DT-1

VACCINE: Diphtheria and tetanus toxoid combined

TEST: Test for potency

THE TEST

There are no international reference preparations specifically for the combined vaccines, and the potency of each component is expressed in international units by comparison with reference materials calibrated against the reference materials for the single components. This is not an ideal situation, because the dose-response relationships in animals differ when the components are in the combined form. Nevertheless, meaningful potency data can be obtained.

Final bulk

The test for potency of the diphtheria component is that specified for diphtheria.

The test for potency of the tetanus component is that specified for tetanus.

Final lot

If a test for potency has not been performed on the final bulk, the tests specified above for bulk combined vaccines shall apply.

1 Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid and Combined Vaccines (Requirements for Biological Substances Nos. 8 and 10) (Revised 1978) Part A, Section 17, p. 105.
CLASS OF TEST A

DT-2

VACCINE: Diphtheria and tetanus combined

TEST: Test for identity

THE TEST

This test is applied to the final vaccine.

The identity of diphtheria and tetanus toxoid shall be tested as specified for diphtheria and for tetanus.

The tests for DT adsorbed vaccine shall be made after dissolving the mineral carrier with sodium citrate.

The identity test can be performed by double immunodiffusion in gel, also called the "Ouchterlony reaction", as described for diphtheria in test DIP-2.

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1 Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid and Combined Vaccines (Requirements for Biological Substances Nos 8 and 10) (Revised 1978) Part A, Section 17, p. 105.
CLASS OF TEST C

DPT-1

VACCINES: Diphtheria, pertussis and tetanus combined

TEST: Potency

THE TEST

There are no international reference preparations specifically for the combined vaccines, and the potency of each component is expressed in international units by comparison with reference materials calibrated against the reference materials for the single components. This is not an ideal situation, because the dose-response relationships in animals differ when the components are in the combined form. Nevertheless, meaningful potency data can be obtained.

Final bulk

For the test for the potency of diphtheria component, the requirements for diphtheria shall apply.

For the test for the potency of pertussis component, the requirements for pertussis shall apply.

For the test for the potency of tetanus component, the requirements for tetanus shall apply, with the following addition:

1  Requirements for Diphtheria Toxoid, Pertussis Vaccine, Tetanus Toxoid and Combined Vaccines (Requirements for Biological Substances Nos 8 and 10) (Revised 1978) Part A, Section 12, p. 104.
The potency of the tetanus component shall not be less than 40 IU per single human dose if the test is performed in guinea pigs. It has been found that the presence of pertussis vaccine has a strong adjuvant effect on the tetanus potency in mice; usually, DPT vaccines show a higher tetanus potency of about 50% when tested in mice than when tested in guinea pigs; this is why 60 IU of tetanus toxoid is required per human dose when DPT vaccines are tested in mice. The 95% confidence interval of the tests shall be smaller than 50–200%.

**Final lot**

If a test for potency has not been performed on the final bulk, the tests specified above for bulk combined vaccines shall apply.
2. **Single harvests included in final bulk**

- **Medium**
- **Period of incubation**
- **Date of earliest harvest included**
- **Conditions of storage**

3. **Bulk purified toxoid**

- **Results of test for antigenic purity**
  - (Lf/mg protein N)

  **Test of irreversibility (on toxoid diluted to reach the concentration of the final bulk)**

  - **Concentration (Lf/ml) of the toxoid solution**
  - **Incubation temperature**
  - **Length of time of incubation**
  - **Volume injected to each guinea-pig**
  - **Route of injection**
  - **No. of guinea-pigs injected**
  - **Time of observation of guinea-pigs**
  - **Result of the test**

---

**PERTUSSIS VACCINE**

**Information on Manufacture**

1. **Strains**

- **Identity of B. pertusis strain used in vaccine**
- **Serological type of strain**
- **Date(s) of reconstitution of ampoule(s) for manufacture**

---

* A list of the identification numbers of the single harvest and bulk purified toxoids should be included.
2. Single harvests included in final bulk

   Medium
   Date of inoculation
   Date of harvest
   Method of killing
   Opacity
   Conditions of storage

3. Bulk material

   Results of tests for living organisms

   TETANUS TOXOID
   Information on Manufacture

1. Strain

   Identity of \textit{C. tetani} strain used in vaccine

2. Single harvest included in final bulk*

   Medium
   Period of incubation
   Date of earliest harvest included
   Condition of storage

3. Bulk toxoid

   Nature of bulk toxoid
   Purified/Unpurified
   Results of test for antigenic purity, if applicable (Lf/mg protein N)

* A list of the identification numbers of the single harvest and bulk purified toxoids should be included.
Test of irreversibility (on toxoid diluted to reach the concentration of the final bulk)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (Lf/ml) of the toxoid solution</td>
<td></td>
</tr>
<tr>
<td>Incubation temperature</td>
<td></td>
</tr>
<tr>
<td>Length of time of incubation</td>
<td></td>
</tr>
<tr>
<td>Volume injected to each guinea-pig</td>
<td></td>
</tr>
<tr>
<td>No. of guinea-pigs injected</td>
<td></td>
</tr>
<tr>
<td>Time of observation of guinea-pigs</td>
<td></td>
</tr>
<tr>
<td>Result of the test</td>
<td></td>
</tr>
</tbody>
</table>

INFORMATION ON BLENDING
Composition of the Final Bulk

1. **Diphtheria toxoid component**

   Reference                          |                              |
   Lf/ml                              |                              |
   Volume added                       |                              |

2. **Tetanus toxoid component**

   Reference                          |                              |
   Lf/ml                              |                              |
   Volume added                       |                              |

3. **Pertussis vaccine component**

   Reference                          |                              |
   Opacity units                      |                              |
   Volume added                       |                              |
4. **Innocuity**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
</tr>
<tr>
<td>Route of injection</td>
<td></td>
</tr>
<tr>
<td>Volume of injection</td>
<td></td>
</tr>
<tr>
<td>Date of start of test</td>
<td></td>
</tr>
<tr>
<td>Date of end of test</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

5. **Preservative**

Nature and amount of preservative per dose

6. **pH**

Results of pH test

7. **Adjuvant**

Nature and amount of adjuvant per dose (in mg Ca or Al)

Signature of head of laboratory

Certification by personne taking overall responsibility for production of the vaccine:

I certify that lot No. ...... of the vaccine satisfies Part A of the WHO Requirements for Diphtheria Toxoid, Pertussis Vaccine, and Tetanus Toxoid.

Signature

Name typed

The protocol must be accompanied by a sample of the label and a copy of the leaflet.
If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National as well as the WHO requirements.

1 If the product does not meet the National requirement, the reason should be given.
SUMMARY PROTOCOL FOR DIPHTHERIA TOXOID PRODUCTION AND TESTING

Identification of Final Lot

Name and address of manufacturer

Lot No.
Date of manufacture of final lot
Nature of final product (plain or adsorbed)
Volume of recommended single human dose
No. of containers in final lot for each filling volume

Information on Manufacture

1. Strain

Identity of \textit{C. diphtheriae} strain used in vaccine

2. Single harvests included in final bulk*

Medium
Period of incubation
Date of earliest harvest included
Conditions of storage

3. Bulk purified toxoid

Results of test for antigenic purity
(Lf/mg protein N)

* A list of the identification numbers of the single harvests and bulk purified toxoids should be included.
Test of irreversibility (on toxoid diluted to reach the concentration of the final bulk)

Concentration (Lf/ml) of the toxoid solution
Incubation temperature
Length of time of incubation
Volume injected to each guinea-pig.
Route of injection
No. of guinea-pigs injected
Time of observation of guinea-pigs.
Result of the test

4. **Final bulk**

Date of preparation
Lf per ml
Results of test for residual free formaldehyde
pH
TEST ON FINAL LOT

1. Identity

Test for diphtheria toxoid and results
Test for pertussis vaccine and results
Test for tetanus toxoid and results

2. Sterility

No. of containers examined
Method of test
Date of start of test
Date of end of test
Results

3. Potency

If this test has not been performed on the final bulk, report these date in the space provided under "Tests on final bulk".

4. Innocuity

<table>
<thead>
<tr>
<th>Mice</th>
<th>Guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
</tr>
<tr>
<td>Route of injection</td>
<td></td>
</tr>
<tr>
<td>Volume of injection</td>
<td></td>
</tr>
<tr>
<td>Date of start of test</td>
<td></td>
</tr>
<tr>
<td>Date of end of test</td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
</tr>
</tbody>
</table>

5. Preservative

Nature and amount of preservative per dose

6. pH

Results of pH test
7. **Adjuvant**

Nature and amount of adjuvant per dose (in mg Ca or Al) ............................

Signature of head of laboratory ............................

Certification by person taking overall responsibility for production of the vaccine:

I certify that lot No. ....... of the vaccine satisfies Part A of the WHO Requirements for Diphtheria Toxoid.

Signature ............................

Name typed ............................

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National and as well as the WHO requirements.

---

1 If the product does not meet the National requirement, the reason should be given.
3. Potency

Weight and sex of mice
No. of mice per dose of vaccine
Date of immunization
Challenge dose
Date of challenge
Date of end of test

Results of potency tests

<table>
<thead>
<tr>
<th>Dilution</th>
<th>No. of survivors</th>
<th>ED₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference vaccine</td>
<td>... ...</td>
<td>... .ml</td>
</tr>
<tr>
<td>...IU/ml)</td>
<td>... ...</td>
<td>... .ml</td>
</tr>
<tr>
<td>Test vaccine</td>
<td>... ...</td>
<td>... .ml</td>
</tr>
</tbody>
</table>

Potency of test vaccine .... IU per single human dose
95% confidence limits of potency ........

Tests on Final lot

1. Identity

Test for diphtheria toxoid and results ....
Test for pertussis vaccine and results ....
Test for tetanus toxoid and results ....

2. Sterility

No. of containers examined ....
Method of test .... 
Date of start of test ...........................................
Date of end of test ...........................................
Results .........................................................

3. Potency

If this test has not been performed on the final bulk, report these date in the space provided under "Tests on final bulk".

4. Innocuity

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>Guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
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<tr>
<td>Volume of injection</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Date of end of test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Preservative

Nature and amount of preservative per dose ...........................................

6. pH

Results of pH test .............................................

7. Adjuvant

Nature and amount of adjuvant per dose (in mg Ca or Al) ...........................................

Signature of head of laboratory ....................................................

Certification by person taking overall responsibility for production of the vaccine:
I certify that lot No. ...... of the vaccine satisfies Part A of the WHO Requirements for Pertussis Vaccine.

Signature ........................................

Name typed ........................................

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National\(^1\) as well as the WHO requirements.

---

\(^1\) If the product does not meet the National requirement, the reason should be given.
SUMMARY PROTOCOL FOR TETANUS TOXOID
PRODUCTION AND TESTING

Identification of Final Lot

Name and address of manufacturer

Lot No.

Date of manufacture of final lot

Nature of final product (plain or adsorbed)

Volume of recommended single human dose

No. of containers in final lot for each filling volume

Information on Manufacture

1. Strain

Identity of C. tetani strain used in vaccine

2. Single harvests included in final bulk*

Medium

Period of incubation

Date of earliest harvest included

Conditions of storage

3. Bulk toxoid

Nature of bulk toxoid

Purified/Unpurified

Results of test for antigenic purity, if applicable (Lf/mg protein N)

* A list of the identification numbers of the single harvest and bulk purified toxoids should be included.
Test of irreversibility (on toxoid diluted to reach the concentration of the final bulk)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (Lf/ml) of the toxoid solution</td>
<td></td>
</tr>
<tr>
<td>Incubation temperature</td>
<td></td>
</tr>
<tr>
<td>Length of time of incubation</td>
<td></td>
</tr>
<tr>
<td>Volume injected to each guinea-pig</td>
<td></td>
</tr>
<tr>
<td>No. of guinea-pigs injected</td>
<td></td>
</tr>
<tr>
<td>Time of observation of guinea-pigs</td>
<td></td>
</tr>
<tr>
<td>Result of the test</td>
<td></td>
</tr>
</tbody>
</table>

4. **Final bulk**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of preparation</td>
<td></td>
</tr>
<tr>
<td>Lf per ml</td>
<td></td>
</tr>
<tr>
<td>Results of test for residual free formaldehyde</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
</tr>
</tbody>
</table>

5. **Adjuvant**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td></td>
</tr>
<tr>
<td>mg/Al or Ca/dose</td>
<td></td>
</tr>
</tbody>
</table>

6. **Preservative**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td></td>
</tr>
<tr>
<td>Concentration in final product (by assay or calculation)</td>
<td></td>
</tr>
</tbody>
</table>

7. **Buffer**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td></td>
</tr>
</tbody>
</table>
# TEST ON FINAL BULK

## 1. Sterility

- **Date of test and results**
- **Was a repeat test necessary?**

## 2. Specific toxicity

<table>
<thead>
<tr>
<th></th>
<th>Bulk purified toxoid</th>
<th>Final bulk or final lot</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose of toxoid injected (Lf per animal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period of observation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results of test</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## 3. Potency

- **Species of animals**
- **Weight and sex of animals**
- **No. of animals per dose of toxoid**
- **Date of immunization and volume of dilutions administered**
- **Date of challenge or bleed**
- **Challenge dose**
- **Date of end of test**
5. **Adjuvant**

   Nature
   mg/Al or Ca

6. **Preservative**

   Nature
   Concentration in final product
   (by assay or calculation)

7. **Buffer**

   Concentration

**TESTS ON FINAL BULK**

1. **Sterility**

   Date of test and results
   Was a repeat test necessary?

2. **Specific toxicity**

   No. of animals
   Date of injection
   Dose of toxoid injected
   (Lf per animal)
   Route of injection
   Period of observation
   Results of test

<table>
<thead>
<tr>
<th>Bulk purified toxoid</th>
<th>Final bulk or final lot</th>
</tr>
</thead>
</table>

3. **Potency**

   Species of animals
   Weight and sex of animals
   No. of animals per dose of toxoid
   Date of immunization and volume
   of dilutions administered
   Date of challenge or bleed
   Challenge dose
   Date of end of test
Results of challenge test

<table>
<thead>
<tr>
<th>Dilution</th>
<th>No. of survivors</th>
<th>ED50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference toxoid (IU/ml)</td>
<td>...</td>
<td>... ml</td>
</tr>
<tr>
<td>Test toxoid</td>
<td>...</td>
<td>... ml</td>
</tr>
</tbody>
</table>

Potency of test toxoid ... IU per single dose
95% confidence limits of potency ...............

TESTS ON FINAL LOT

1. Identity

Test for diphtheria toxoid and results ...........
Test for pertussis vaccine and results ...........
Test for tetanus toxoid and results ............

2. Sterility

No. of containers examined .................
Method of test ................................
Date of start of test ..................
Date of end of test ...................
Results ..................................

3. Potency

If this test has not been performed on the final bulk, report these data in the space provided under "Tests on final bulk".
4. **Innocuity**

<table>
<thead>
<tr>
<th></th>
<th>Mice</th>
<th>Guinea-pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume of injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of start of test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date of end of test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. **Preservative**

Nature and amount of preservative per dose

6. **pH**

Results of pH test

7. **Adjuvant**

Nature and amount of adjuvant per dose (in mg Ca or Al)

Signature of head of laboratory

Certification by person taking overall responsibility for production of the vaccine:

I certify that lot No. ...... of the vaccine satisfies Part A of the WHO Requirements for Tetanus Toxoid.

Signature

Name typed
The protocol must be accompanied by a sample of the label and a copy of the leaflet.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National\textsuperscript{1} as well as the WHO requirements.

\begin{enumerate}
\item If the product does not meet the National requirement, the reason should be given.
\end{enumerate}
9. Stability test

Date of start of test
Temperature of incubation
Time of incubation
No. of containers tested
Mean percentage of survival

INFORMATION ON RELEASE

Is the vaccine satisfactory? yes/no
Has the lot been released by the national control authority? yes/no
If yes, date
Can a certificate be supplied by the national control laboratory? yes/no
Which laboratory would supply such a certificate?

When was the vaccine tested in children in your country? (summary of results)

Signature of head of laboratory

Certification by personne taking overall responsibility for production of the vaccine:

I certify that lot No. ..... of BCG vaccine satisfies Part A of the WHO Requirements for BCG Vaccine.

Signature
Name typed
The protocol must be accompanied by a sample of the label, a copy of the leaflet, and a copy of the national control release certificate, if issued.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National as well as the WHO requirements.

1 If the product does not meet the National requirement, the reason should be given.
SUMMARY PROTOCOL FOR POLIOMYELITIS VACCINE (ORAL)
SABIN STRAINS BASED ON REQUIREMENTS FOR
POLIOMYELITIS VACCINE (ORAL)

(REQUIREMENTS FOR BIOLOGICAL SUBSTANCES NO. 7)

REVISED 1971 AND ADDENDUM 1980

| NAME AND ADDRESS OF MANUFACTURER | __________________________ |
| PROPRIETARY NAME | __________________________ |
| LOT NO. OF VACCINE TRIVALENT BLEND | __________________________ |
| FILLING LOT NO. | NO OF FILLED CONTAINERS |
| DATE OF INITAITION OF LAST TEST FOR VIRUS CONCENTRATION | __________________________ |
| EXPIRY DATE | __________________________ |
| NATURE AND CONCENTRATION OF STABILIZER | __________________________ |
| VOLUME OF VACCINE CONTAINER | __________________________ |
| VOLUME OF HUMAN DOSE (IN DROP AND/OR ML) | __________________________ |
| PRESCRIBED VIRUS CONCENTRATION PER HUMAN DOSE - TYPE 1 | __________________________ |
| TYPE 2 | __________________________ |
| TYPE 3 | __________________________ |
| CONCENTRATION OF ANTIBIOTICS/HUMAN DOSE | __________________________ |
| MAXIMUM RESIDUAL CONCENTRATION OF SERUM PER HUMAN DOSE | __________________________ |
| PRODUCTION CELL TISSUE: MONOVALENT BULK SUSPENSIONS BLENDED IN TRIVALENT VACCINE | TYPE 1 | TYPE 2 | TYPE 3 |
| BULK NO. | ______ | ______ | ______ |
| DATE OF APPROVAL OF PROTOCOL OF BULK INDICATING COMPLIANCE WITH WHO REQUIREMENTS | ______ | ______ | ______ |
FINAL FILLING

LOT NO. ____________________________

BLENDING DETAILS

VOLUME OF TRIVALENT BLEND ____________________________

NATURE AND VOLUME OF STABILIZER ____________________________

NATURE AND VOLUME OF DILUENT ____________________________

TOTAL VOLUME OF FINAL FILLING ____________________________

DATE OF FINAL FILLING ____________________________

NO. OF VIALS FILLED ____________________________

TESTS ON FINAL FILLING

1. STERILITY TESTS:
   DATE ____________________________
   NO. OF CONTAINERS TESTED ____________________________
   MEDIA USED ____________________________
   RESULT ____________________________

2. TESTS FOR VIRUS CONTENT:

2.1 TOTAL VIRUS CONTENT
   DATE OF TEST ____________________________
   Not heated ____________________________
   heated¹ ____________________________
   NO. OF CONTAINERS EXAMINED ____________________________
   TOTAL VIRUS CONTENT PER DOSE IN EACH CONTAINER ____________________________
   AVERAGE REDUCTION OF TITRE AFTER HEATING ____________________________

2.2 INDIVIDUAL VIRUSES CONTENT
   DATE OF TEST ____________________________

¹ Specify conditions of heating
### 2.2.1 REFERENCE VACCINES

<table>
<thead>
<tr>
<th>IDENTIFICATION</th>
<th>DECLARED TITRE PER DOSE</th>
<th>NO. OF CONTAINERS TESTED</th>
<th>GEOMETRIC MEAN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 2.2.2 TEST VACCINE

<table>
<thead>
<tr>
<th>NO. CONTAINERS TESTED</th>
<th>RESULTS FOR EACH CONTAINER&lt;sup&gt;1&lt;/sup&gt;</th>
<th>GEOMETRIC MEAN&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### 3. INNOCUITY:

- **DATE**
- **NO. OF MICE OR RABBITS (SPECIFY) GIVEN INJECTIONS**
- **VOLUME AND ROUTE**
- **OBSERVATION PERIOD**
- **RESULTS (GIVE DETAILS IN CASE OF DEATHS)**

### 4. PH:

<sup>1</sup> Per human dose
Signature of head of laboratory .................

Certification by person taking overall responsibility for production of the vaccine:
I certify that lot No. ...... of the vaccine satisfies Part A of the WHO Requirements for Oral Polio Vaccine.

Signature ...........................................
Name typed ...........................................

The protocol must be accompanied by a sample of the label and a copy of the leaflet.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National as well as the WHO requirements.

1 If the product does not meet the National requirement, the reason should be given.
<table>
<thead>
<tr>
<th><strong>NAME AND ADDRESS OF MANUFACTURER</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>LOT NO. OF VACCINE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>NO. OF FREEZE-DRYING LOT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>DATE OF INITIATION OF LAST TEST FOR VIRUS CONCENTRATION</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>EXPIRY DATE</strong></th>
</tr>
</thead>
<tbody>
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<td></td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>PROPRIETARY NAME OF VACCINE</strong></th>
</tr>
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<table>
<thead>
<tr>
<th><strong>NO. AND VOLUME OF AMPOULES OR VIALS IN THE LOT</strong></th>
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<table>
<thead>
<tr>
<th><strong>SEED VIRUS</strong></th>
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<table>
<thead>
<tr>
<th><strong>SEED VIRUS STRAIN</strong></th>
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<table>
<thead>
<tr>
<th><strong>TISSUE USED FOR PREPARING SEED LOT</strong></th>
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<thead>
<tr>
<th><strong>DATE(S) OF SATISFACTORY TEST(S) FOR FREEDOM FROM EXTRANEOUS AGENTS</strong></th>
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<thead>
<tr>
<th><strong>NUMBER OF PASSAGES BETWEEN VACCINE AND SEED</strong></th>
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<tr>
<th><strong>NUMBER OF MONKEYS INOCULATED</strong></th>
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<table>
<thead>
<tr>
<th><strong>QUANTITY INOCULATED</strong></th>
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<thead>
<tr>
<th><strong>NUMBER OF MONKEYS SURVIVING WITHOUT SPECIFIC SYMPTOMS</strong></th>
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<tr>
<th><strong>RESULT OF BLOOD SERUM TEST</strong></th>
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<tr>
<th><strong>RESULT OF HISTOPATHOLOGICAL EXAMINATION (SPECIFY ANY ABNORMAL FINDINGS)</strong></th>
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INNOCUITY TESTS

DATE

NO. OF MICE GIVEN INJECTIONS

VOLUME AND ROUTE

OBSERVATION PERIOD

RESULTS (SPECIFY DETAILS IN CASE OF DEATHS)

NO. OF GUINEA PIGS

VOLUME AND ROUTE

OBSERVATION PERIOD

RESULTS (SPECIFY DETAILS IN CASE OF DEATHS)

RESIDUAL MOISTURE

SIZE OF SAMPLE

MOISTURE CONTENT (%)

Signature of head of laboratory

Certification by personne taking overall responsibility for production of the vaccine:

I certify that lot No. ...... of the vaccine satisfies Part A of the WHO Requirements for Measles Vaccine (live).

Signature

Name typed
The protocol must be accompanied by a sample of the label and a copy of the leaflet.

If the vaccine is imported, the protocol must be accompanied by a Certificate of the National Control Authority from the exporting country stating that the product meets the National as well as the WHO requirements.

1 If the product does not meet the National requirement, the reason should be given.
APPENDIX 7

SUGGESTED SOURCES OF CELL CULTURE MATERIALS

The list shown below is representative of manufacturers of cell culture materials from whom it is possible to obtain certain equipment, chemicals, etc. It should be stressed that in most cases there are other manufacturers from whom similar materials of equal quality may be obtained. This list, therefore, does not express any preference for the companies mentioned.

Becton Dickinson AG (Flacon)
Alte Reinacherstrasse 1-3
CH-4142 Münchenstein
Telex: 64784

Bio-Mérieux
Marcy-l'Etoile
69260 Charbonnières les Bains
France
Telex: 3900972
(Sales Office)

Difco Laboratories Inc.
P.O. Box 1058A
Detroit, Michigan 48232
United States of America
Telex: 23-5683

Dynatech Produkte AG
Oberfeldstrasse 20
CH-8302 Kloten
Telex: 57 575

Flow Laboratories Ltd.
P.O. Box 17
Second Av. Industrial Estate
Irvine, Ayrshire, KA12 8NB
Scotland
Telex: 77231