WHO International Standard
Sixth International Standard for Rabies vaccine
NIBSC code: 07/162
Instructions for use
(Version 2.0, Dated 15/04/2013)

1. INTENDED USE

The International Standard (IS) for rabies vaccine is used in the standardisation of rabies vaccines in NIH mouse protection tests and in in vitro assays for glycoprotein content. This material was prepared from a bulk of Vero cell derived, Pitman Moore strain, produced by the same manufacturing process as the Fifth International Standard, RAV.

The candidate standard was calibrated in IU against the Fifth International Standard in a collaborative study in which 16 participants from 10 countries assayed the International Standard (RAV) and the candidate standard (in duplicate). These samples were assayed by the participants in 37 NIH mouse protection tests, 12 enzyme immunoassays (EIA) and 13 single radial immunodiffusion (SRD) tests.

None of the invited participants offered to assay the study samples for ribonucleoprotein content. As this assay is not performed during routine quality control but manufacturers or as part of the batch release of rabies vaccines by National Regulatory Authorities, it would appear that the continuation of the International Units of rabies virus PM ribonucleoprotein is not required and has been discontinued.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

Rabies vaccine bulk containing inactivated Pitman Moore virus grown in Vero cells. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory’s safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

The unitage assigned to this material is as follows:

For use in NIH mouse protection tests: 8 IU/ampoule ie 8 IU/ml when reconstituted as directed in 1ml distilled water

For use in in vitro assays such as enzyme immunoassays and single radial immunodiffusion tests: 3.3 IU per ampoule Pitman Moore rabies virus glycoprotein antigen content or 6.6 IU/ml when the contents are reconstituted as directed in 0.5ml distilled water.

4. CONTENTS

Country of origin of biological material: France.

Each ampoule contains the freeze dried residue of 0.5 ml aliquots of a commercial rabies vaccine bulk containing inactivated Pitman Moore virus grown in Vero cells were filled in DIN ampoules and freeze dried at NIBSC following documented procedures. This fill was 0.5g fill weight with a mean dry weight of 0.0532 g. The coefficient of variation (CV) was 1.14%. Residual moisture measured on 12 samples gave a mean of 0.14 with a CV of 9.64% and oxygen headspace measured in 12 ampoules gave a mean of 0.70% with a CV of 32.81%.

Uncertainty: the proposed unitage does not carry an uncertainty associated with its calibration. The only uncertainty is therefore derived from the variability of the dry fill weight of the ampoule content.

5. STORAGE

The ampoules should be stored at -20°C or below until use. Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an ‘easy-open’ coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

For use in vivo NIH tests reconstitute the contents of each ampoule in 1ml distilled water. The resultant solution is 8 IU/ml.

For use in vitro assays (EIA, SRD) assays of glycoprotein antigen content, reconstitute the contents of each ampoule in 0.5ml distilled water. The resultant solution is 6.6 IU/ml of rabies virus glycoprotein content.

The ampoules should be shaken gently without the formation of foam to ensure that all contents are completely reconstituted. When a reconstitution volume of 0.5ml is used, the ampoule may be left at ambient temperature for 30 minutes to facilitate complete dissolution of the contents.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Stability studies on reconstituted material have not been undertaken. It is anticipated that the contents of individual ampoules will be used on the day reconstitution as the volumes required in both the in vivo potency tests and in vitro assays of glycoprotein antigen content and the contents would be used in a single assay. However, should users wish to store reconstituted material, they should determine the stability of reconstituted material according to their own method of preparation, storage, and use. Multiple freeze/thaw cycles should be avoided.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

WHO Expert Committee on Biological Standardization report WHO/BS/08.2087 available online.
http://apps.who.int/iris/bitstream/10665/70593/1/WHO_BS_08.2087_eng.pdf

10. ACKNOWLEDGEMENTS
11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK
Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION
In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze dried</td>
</tr>
<tr>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
</tr>
<tr>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
</tr>
<tr>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify): Contains human albumin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

Suggested First Aid

Inhalation: Seek medical advice
Ingestion: Seek medical advice
Contact with eyes: Wash with copious amounts of water. Seek medical advice
Contact with skin: Wash thoroughly with water.

Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS
In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC’s Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) (“Conditions”) apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient’s attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.

Net weight: 1.0 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. Attached: No

17. CERTIFICATE OF ANALYSIS
NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_esstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.