Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Swine Erysipelas Serum (Anti-N)
NIBSC code: SES
Instructions for use
(Version 8.0, Dated 23/01/2014)

1. INTENDED USE

This material has been prepared by immunising a horse with type A and B strain of Erysipelothrix rhusiopathiae at the Veterinary Laboratories Agency, Weybridge (UK). The standard should be used to immunise a minimum of three groups of 20 mice with app. 0.2 to 2 IU of the standard. After one hour immunised mice and control mice (n=10) are challenged i.p. with 10.000.000 organisms of E. rhusiopathiae type N, derived from an active culture. Mice are observed for 8 days and deaths are recorded. Immunised mice should have survival rate of 10-90% depending on the unitage administered. Control mice should die within 2-5 days. For calibration of an in-house standard two groups of mice should be immunised with dilutions of either the in-house standard or the reference standard. It is suggested that the in-house standard should contain at least 100 IU/ml.

2. CAUTION

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

Equine source material. 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

628 International Units per ampoule. Assigned content of vial valid at time of manufacture – no information on long term stability.

4. CONTENTS

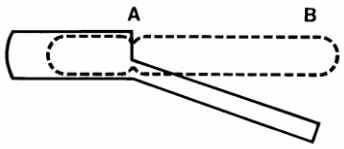
Country of origin of biological material: United Kingdom.
See attached insert from the Veterinary Laboratories Agency for further details.

5. STORAGE

This material should be used immediately after reconstitution with 1 ml water.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

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

8. STABILITY

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.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

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

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

Ewald FW. Mh Tierheilk. 1955; 7:109.

10. ACKNOWLEDGEMENTS

N/A.

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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

Physical and Chemical properties	
Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not Physical appearance: Freeze dried powder.	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains horse serum	
Toxicological properties	
Effects of inhalation: Not established, avoid inhalation	
Effects of ingestion: Not established, avoid ingestion	
Effects of skin absorption: Not established, avoid contact with skin	
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: 0.09 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_biolefstandardsrev2004.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.

Central Veterinary Laboratory
New Haw, Addlestone, Surrey KT15 3NB United Kingdom
Telephone: 01932 357641 Facsimile: 01932 357701 Telex: 262318 VetWey G

Veterinary Laboratories Agency

INTERNATIONAL STANDARD FOR SWINE ERYSIPELAS SERUM (ANTI-N) (SES)

Description

The International Standard for Swine Erysipelas Serum (Anti-N) was established in 1954. The Standard consists of serum from a horse immunized against a type A and a type B strain* of *Erysipelothrix rhusiopathiae*. The serum was distributed in 1ml amounts into glass ampoules and freeze-dried. The ampoules were sealed under vacuum.

The average weight of dry material per ampoule has been determined as 87.9mg with a standard deviation of ± 2.4%.

The Standard is suitable for assaying the potency of all swine erysipelas sera, irrespective of serotype, provided only that a type N strain is used for challenge.

*For an account of the serotypes of *E. rhusiopathiae* see Ewald (1955).

International Unit

The International Unit is defined as the activity contained in 0.14mg of the International Standard.

Distribution

The Standard is distributed by the International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Addlestone, Surrey, England on behalf of the World Health Organisation. It is available free of charge in limited amounts. If a laboratory needs more than one sample every six month, it is expected to prepare its own standard and to calibrate it against the International Standard. A quantity of this latter sufficient for the purpose will be supplied on request.

Reconstitution of the International Standard

The Standard should be reconstituted immediately before it is to be used.

Ampoules may be opened by scoring with a small saw specifically designed for the purpose, or a hard mineral edge, for approximately one third of the circumference. Application of a piece of red hot glass rod to this scratch will give a clean line of fracture.

If the scoring is made firmly and the glass rod is hot enough, it is possible to produce a fine crack without disturbing the ampoule top until needed. Then slight pressure will complete the separation.

The freeze-dried material in each ampoule may be reconstituted in any convenient volume of a suitable diluent, which will not alter the final pH.

Care should be taken to ensure that the entire contents of the ampoule are completely resuspended, this can be achieved by suspending the bulk of the contents of the ampoule in some of the fluid, and using the remainder of the diluent to rinse out the ampoule three times. There will be a need to weigh the contents to accurately gauge the quantity of diluent required.

National and Laboratory Standards

National and laboratory standards should be prepared in a stable form. This may be achieved by freeze-drying aliquots of the reference preparation in neutral-glass ampoules and sealing them in an oxygen-free atmosphere by fusion of the glass. The ampoules should be stored in the dark at a low temperature, e.g. -20°C.

Calibration of National and Laboratory Standards

The following method is suggested.

Healthy white mice falling within a limited range of weight (e.g. 18-22g) are distributed into groups of a least 20 mice each. A minimum of three groups is injected subcutaneously with a series of doses of the International Standard equally spaced on a logarithmic scale. The same number of groups is injected with a similar series of doses of the preparation being calibrated. The doses are chosen to cover the range from 10% to 90% protection. This can usually be achieved somewhere within the range of 0.2 to 2 Units per mouse. (If the appropriate range for the mouse stain and technique being used is not known, a small-scale, preliminary trial should be carried out to determine this). A group of at least 10 mice is injected with saline and kept as a control. One hour later all the mice, including the control group, are injected intraperitoneally with sufficient quantity of an actively growing culture of *E. rhusiopathiae* type N, or a dilution thereof, to give a dose of about 10^6 organisms. The mice are observed for at least eight days and deaths are recorded. The control mice which should all die during the test, usually die within two and five days after injection.

The potency of the new standard relative to that of the International Standard is calculated by the method of probit analysis, and the results expressed in International Units.

It is suggested that the potency of a National or Laboratory Standard should be checked against that of a fresh sample of the International Standard about once a year.

The International Laboratory for Biological Standards at Weybridge is willing to advise and assist laboratories in providing national and laboratory standards.

Assaying Routine Production Batches of Serum

Once a National or Laboratory Standard has been prepared, it can be used to assay routine batches of serum, thus defining their potencies in terms of International Units. The method used can be similar to the one described in the preceding section, but the number of mice used can often be reduced. When it has been demonstrated that the assay method gives consistent results with any particular serum, a four point assay may be used, i.e. an assay with two groups of mice for the standard preparation and two for the serum being tested.

Although no definite recommendations concerning potency requirements can be made at present, it is suggested that sera should contain at least 100 International Units per ml (Anon, 1950).

References

Anon. (1950). Staats-Anzeiger fur das Land hessen. No. 6, p. 52.
Ewald, F.W. (1955). Mh. Tierheilk. 7, 109.