WHO International Standard
Insulin Bovine
NIBSC code: 83/511
Instructions for use
(Version 4.0, Dated 03/03/2009)

1. INTENDED USE
Establishment of the International Standard for bovine Insulin was
authorized at the 37th Meeting of the WHO Expert Committee of
Biological Standardization. This material replaces the 4th IS of
bovine/porcine insulin as the standard for the bioassay of bovine insulin.
The 4th IS is discontinued.

2. CAUTION
This preparation is not for administration to humans or animals in
the human food chain.
The material is of bovine origin. The material is certified to be obtained
from animals taken from a closed herd in the female line since 1980, in
which no animal has been clinically suspected of having BSE & which
has not been fed rations containing ruminant derived protein during
that period. 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
ONE INTERNATIONAL UNIT of bovine insulin is the activity contained in
0.03891mg of the international standard for bovine insulin, by definition.

4. CONTENTS
Each ampoule contains approximately 50mg of hydrated crystals of
bovine insulin, together with nitrogen gas at atmospheric pressure

5. STORAGE
Unused ampoules should be stored at -20°C.
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.

7. USE OF MATERIAL
The content of an ampoule is variable. Aliquots of insulin crystals should
be accurately weighed after allowing the ampoule to equilibrate at room
temperature in a desiccator.

8. PREPARATION OF AMPOULES
8.1 Bulk Material
Approximately 400g of bovine insulin crystals were generously donated by
Eli Lilly and Co., Indianapolis, USA. The material contained approximately
92% insulin, 5% desamido insulin and 3% other insulin-related impurities,
when examined by either reverse-phase HPLC or electrophoresis.
Approximately 0.6% of insulin aggregates were detected by gel-filtration.

8.2 Distribution into ampoules
The batch of ampoules coded 83/511 was prepared by distributing
approximately 50mg aliquots of hydrated insulin crystals into glass ampoules
under conditions of controlled humidity. Ampoules were purged with
nitrogen, sealed by heat-fusion of the glass, and have since been stored at
-20°C in the dark.

9. COLLABORATIVE STUDY
The preparation in ampoules coded 83/511 was evaluated by an
international collaborative study in which twenty-three laboratories in sixteen
countries participated. The study was organized with the following aims:-
to assess the suitability of preparations of highly purified bovine, porcine and
human insulins to serve as standards for the replacement of the International
Standard for Insulin, to relate each of these preparations to the 4th
International Standard for Insulin by bioassays and to characterize these
preparations by physical, biochemical and immunological procedures used
for the analysis of insulin. Information was also sought regarding any effects
the species of origin and purity of the insulin might have on results obtained
with bioassay procedures currently in use. The study also sought, if
possible, to compare human insulin obtained by recombinant DNA
technology, by modification of porcine insulin, and from human pancreases.

9.1 Estimate of bioactivity
Bioassay data using the mouse blood-glucose, rabbit blood-glucose and
mouse convulsion methods were contributed to the study. The combined
estimate for all methods was 25.7 IU/mg (95% limits 24.9-26.4).

9.2 Assigned unifuge
The assigned potency is 0.03891mg in one International Unit of Insulin, bovine
(25.7 IU/mg). The material is intended to serve as a standard for
the assay of bovine insulin preparations, either by biological methods or
by physicochemical techniques such as HPLC. Porcine and human
insulin preparations should be assayed against the corresponding
international standards (porcine, code 83/515; human, code 83/500)

10. STABILITY
NIBSC follows the policy of WHO with respect to its reference materials.
The lower 95% limit for the predicted loss of activity of the material stored at
-20°C, based upon assays of thermally degraded samples, was less than
0.1% per year.
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.
Unopened ampoules should be stored on receipt as indicated on the
label. In addition, once reconstituted, diluted or aliquoted, users should
determine the stability of the material according to their own method
of preparation, storage and use.

11. REFERENCES
international standards for highly purified human, porcine and bovine

12. ACKNOWLEDGEMENTS
Acknowledgements are due to Novo Industri A/S, Copenhagen, Denmark,
who generously donated, through the good offices of Dr. J. Schlichtkrull, the
insulin; to the Standards Processing Division of NIBSC for ampouling; and to
the participants in the collaborative study.

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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13. 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

14. 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

15. 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.

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

### Physical and Chemical properties

<table>
<thead>
<tr>
<th>Physical appearance: solid</th>
<th>Corrosive: No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hygroscopic: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td></td>
</tr>
</tbody>
</table>

### 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.

17. 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.

18. 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: 50mg
- 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_Int_biol_refstandardsrev2004.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.