Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Insulin, Human For Immunoassay. 1st International Reference Preparation
NIBSC code: 66/304
Instructions for use
(Version 6.0, Dated 16/04/2013)

1. INTENDED USE

In 1974 the Expert Committee on Biological Standardization of WHO established as the International Reference Preparation of insulin, human, for immunoassay, the batch of ampoules coded 66/304 (WHO, 1975). The Committee noted that the preparation consisted of pooled material from different sources, and had been characterized by biological assays, and by immunoassays and receptor assays, and had been found suitable to serve as standard for Immunoassays (WHO/BS/74.1084).

The potency of the preparation had been determined by bioassay with the 4th International Standard for Insulin, Bovine and Porcine, for Bioassay and, on the basis of the results obtained, the Committee defined the International Unit for Human Insulin for Immunoassay as the activity contained in 1.8233 mg of the International Reference Preparation.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA.
The preparation contains material of human origin which has been tested and found negative for HB_SAg, anti-HIV and HCV RNA by PCR. 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

Each ampoule contains 3 INTERNATIONAL UNITS, (by definition).

Uncertainty: the International Unit of 66/304 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 66/304 may be considered to be the co-efficient of variation of the fill volume, which was determined to be 1.08%.

4. CONTENTS

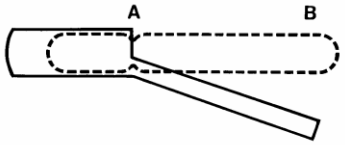
Country of origin of biological material: United Kingdom.
Human insulin approx 130 micrograms
Sucrose approx 5 mg
as the dry residue after freeze-drying from 1ml 0.00745M acetic acid, the ampoule being then sealed under nitrogen

5. STORAGE

Unopened 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.



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

The entire contents of an ampoule should be dissolved without attempting to weigh out any portion of the ampoule contents. For initial solution 0.75ml of 0.07M veronal buffer (pH 8.6) has been found satisfactory. Subsequent dilutions should be made with buffer containing carrier protein. A stock solution should be divided and frozen in small portions, one only to be thawed for each assay.

PREPARATION OF AMPOULES

(1) The bulk material consisted of contributions each of several hundred milligrams of purified human pancreatic insulin, generously contributed by:

(i) Dr I A Mirsky (University of Pittsburgh School of Medicine, United States of America);
(ii) Dr W A Bromer (The Lilly Research Laboratories, Indianapolis, United States of America);
(iii) Medical Research Council of Great Britain and extracted by Dr G H Smith (Wellcome Research Laboratories, Beckenham, England).

Contributions were subsequently pooled and recrystallized by Dr G H Smith in the Wellcome Research Laboratories, Beckenham, to yield a preparation with approximately 3% moisture content and a biological activity of 23.5 IU/mg dry weight (confidence limits, p = 0.95, 22.0 - 25.1 IU/mg dry weight), by bioassay with the 4th International Standard for Insulin, Bovine and Porcine for Bioassay.

Analysis by polyacrylamide gel electrophoresis showed the bulk insulin preparation to be essentially pure, containing a small amount of desamido insulin, and by gel filtration the preparation was found to contain a trace of proinsulin.

(2) Distribution into ampoules.

The batch of ampoules coded 66/304 was prepared according to the procedures used for international biological standards (WHO, 1978). A weighed portion of the human insulin was dissolved in a solution of acetic acid and sucrose, distributed in equal volumes into approximately 3,500 ampoules and freeze dried. After secondary desiccation to constant weight, the ampoules were filled with pure nitrogen and sealed.

ACTIVITY OF AMPOULES

(a) By bioassay against the fourth International Standard for Insulin, Bovine and Porcine for bioassay, 66/304 was estimated to contain 3 IU per ampoule.
(b) The preparation has been used as a standard in a variety of assay systems (see Cotes, 1969; Soeldner, 1974) and no adverse comment has been received despite its wide distribution.

8. STABILITY

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

Essentially no loss of *in vitro* biological activity has been detected in the accelerated degradation tests carried out, nor has there been evidence of instability from analogous studies by radioimmunoassays in 1969, or in radioreceptor assays and radioimmunoassays performed more recently.
NIBSC follows the policy of WHO with respect to its reference materials. 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. 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. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

WHO Expert Committee on Biological Standardisation, 26th Report. WHO Technical Report Series No. 565, (1975) p17.

WHO Expert Committee on Biological Standardisation, 29th Report. WHO Technical Report Series No. 626, (1978).

Unpublished working document WHO/BS/74.1084.

WHO Expert Committee on Biological Standardisation, 21st Report. WHO Technical Report Series No. 413, (1969) p16.

Campbell PJJ (1974). Biol. Standardisation, 2, 259.

Cotes PM et al (1969). J. Endocrinol., 45, 557-569.

Soeldner JS et al (1974). Metabolism, 23, 290-214.

10. ACKNOWLEDGEMENTS

Acknowledgements are made gratefully to:

Dr I.A Mirsky (University of Pittsburgh), Dr W A Bromer (Lilly Research Laboratories) and to the Medical Research Council (UK) for generous gifts of insulin; to Dr G H Smith (Wellcome Research Laboratories) for extracting and recrystallising insulin; to Dr P. J Campbell (NIBSC) for distribution into ampoules; to Mr K L Smith (Boots Pure Drug Company)

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

Physical and Chemical properties	
Physical appearance: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Contains material of human origin
Toxicological properties	
Effects of inhalation:	No adverse effects have been reported for this material
Effects of ingestion:	No adverse effects have been reported for this material
Effects of skin absorption:	No adverse effects have been reported for this material
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.

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

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: 5mg
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.