Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Luteinizing Hormone, Human, Pituitary
NIBSC code: 80/552
Instructions for use
(Version 5.0, Dated 13/01/2014)

1. INTENDED USE

This consists of a batch of ampoules (coded 80/552) containing highly purified human pituitary LH. It was established as the Second International Standard for Pituitary LH (IS) at the 39th Meeting of WHO Expert Committee on Biological Standardization (WHO ECBS) in 1988 (WHO ECBS, 1989), to replace the International Reference Preparation of Pituitary LH for Immunoassay (IRP 68/40; WHO ECBS, 1975; Storring et al, 1978) of which stocks had become exhausted.
For further details of this Standard and its collaborative study see Storring & Gaines Das (1993).

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.
However, as with all materials of human origin, the preparation cannot be assumed to be free from infectious agents. The container and its contents should be used and discarded according to your own laboratory procedures. Such procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening the container to avoid cuts.

3. UNITAGE

35 INTERNATIONAL UNITS per ampoule (by definition).
Uncertainty: the assigned unitage is arbitrary and does not carry an uncertainty associated with its calibration. The uncertainty may therefore be considered to be the variance of the ampoule content, and was experimentally measured as ± 0.25% (cv)

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue, after freeze-drying, of 0.5ml of a solution which contained:

LH extract	approx	5.8 µg
Lactose	"	5 mg
Human plasma albumin	"	1 mg
Sodium chloride	"	90 µg

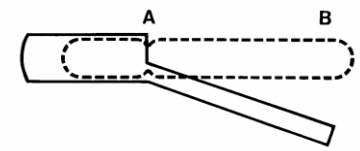
Nitrogen gas at slightly less than atmospheric pressure.

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

For practical purposes each ampoule contains the same amount of the same materials. Dissolve all the contents in a known amount of buffer solution. No attempt should be made to weigh portions of the freeze-dried powder.
For economy of use the solution can be kept for several months if an anti-bacterial preservative is added and the solution is subdivided into several small containers, which are frozen rapidly to below -70°C and then stored below -30°C in the dark; repeated freezing and thawing should be avoided. If extensive dilutions are prepared, a carrier protein (0.1% w/v) should be added, which is free of peptidase.
The material has not been sterilized and contains no bacteriostat.

8. PREPARATION OF AMPOULES

Some 50mg of highly purified pituitary LH, batch no. NM15, were generously donated to WHO by Drs R.M. Lequin and J.G. Loeber (Nijmegen, the Netherlands) and Dr G. Hennen (Liége, Belgium). This material was isolated by Drs Loeber and Lequin from acetone-dried human pituitary glands (Loeber, 1977) as described by Closset et al (1975).
Some 24.08mg of LH batch No. NM15 was dissolved in 30ml of a solution containing 0.2% (w/v) purified human plasma albumin which was free of peptidase activity (Batch AK3; Lister Institute, Elstree), 1% w/v lactose and 3mM-sodium chloride.
The solution was centrifuged at 10,000 g for 15 min at 4°C and the supernatant (29.445g) was diluted with the diluent above to a final concentration of about 11.6µg of LH/ml. The solution was distributed into ampoules as approximately 0.5ml aliquots. The mean weight of filling solution in 79 weighed ampoules was 0.501g with a range as % of the mean of 0.5%. The ampoule contents were then freeze-dried, secondarily desiccated and sealed under nitrogen (Campbell, 1974; WHO ECBS, 1978). The batch consisted of 3698 ampoules.

9. ACTIVITY OF AMPOULE CONTENTS

The LH potencies of the IS were estimated in terms of IRP 68/40 by in-vivo and in-vitro bioassays, a receptor binding assay and immunoassays, in a collaborative study carried out by 19 laboratories in 11 countries (Storring & Gaines Das, 1993).

10. STABILITY

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. 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

Campbell PJ (1974). Journal of Biological Standardization 2, 259-267.
Closset K, Vandalem J-L, Hennen G & Lequin RM (1975). European Journal of Biochemistry 57, 325-333.
Loeber JG (1977). Acta Endocrinologica 85 (Suppl 210), 1-130.
Storring PL, Bangham DR, Cotes PM, Gaines-Das RE & Jeffcoate SL (1978). Acta Endocrinologica 88, 250-259.
Storring PL & Gaines Das, RE (1993). Journal of Endocrinology 138, 345-359.

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

World Health Organization

WHO Expert Committee on Biological Standardization (1975). 26th Report. World Health Organization Technical Report Series No. 565.
WHO Expert Committee on Biological Standardization (1978). 29th Report. World Health Organization Technical Report Series No. 626.
WHO Expert Committee on Biological Standardization (1989). 39th Report. World Health Organization Technical Report Series No. 786.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the following: Dr R.M. Lequin and Dr J.G. Loeber for isolating and characterizing the bulk LH from which the IS was prepared, and with Dr G. Hennen for providing the material; the participants in the collaborative study; Dr A.F. Bristow, Dr E. Diczfalusy, Mr K.M. Ferguson, Mr Y.G. Mistry and Dr W.R. Robertson for preliminary characterization of the Standard; and Dr P.J. Campbell for ampouling.

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	
Physical appearance: Freezed dried powder	Corrosive: No
Stable: No	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: 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: 6mg
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

efstandardsrev2004.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.