WHO International Standard
Urinary Follicle Stimulating Hormone (FSH) and Urinary Luteinizing Hormone (LH)
NIBSC code: 98/704
Instructions for use
(Version 3.0, Dated 09/11/2007)

1. INTENDED USE

This consists of a batch of ampoules (coded 98/704) containing an extract from the urine of post-menopausal women, which was established as the fourth international standard for Urinary Follicle Stimulating Hormone (FSH) and Urinary Luteinizing Hormone (LH) at the 51st meeting of the WHO Expert Committee on Biological Standardization (WHO ECBS 2002) in November 2000.

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. 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 72 INTERNATIONAL UNITS of urinary FSH (by definition) and an activity of 70 INTERNATIONAL UNITS of urinary LH (by definition).

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1ml of a solution which contained:

Extract of human menopausal urine	approx. 0.967 mg
Lactose	approx. 5mg

5. STORAGE

Unopened ampoules should be stored at –20ºC
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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

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.

The material has not been sterilized and contains no bacteriostat.

PREPARATION OF AMPOULES

BULK FSH/LH. This consisted of approximately 5.8g of menotrophin (Batch no 61662697) extracted from the urine of post-menopausal women and generously donated to WHO by the Instituto Massone, Beunos Aires, Argentina, through the good offices of Mr R Massone. It was stated to contain a total of about 500,000 IU FSH and 480,000 IU LH, by bioassay.

DISTRIBUTION INTO AMPOULES. The IS was prepared in November 1998. Some 5.8g of the bulk menotrophin was dissolved in 6000ml of 0.05% (w/v) lactose. The solution was passed through a 0.45µm membrane filter (Sartobran P, Sartorius), and distributed into ampoules as approximately 1.0ml aliquots. The solution of menotrophin was kept at +4^{o}C throughout. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen (Campbell, 1974; WHO ECBS 1978). The IS consisted of 5672 ampoules. The mean weight of filling solution in 113 weighed ampoules was found to be 1.006g with a coefficient of variation of 0.13% and a range as % of the mean of 1.09. Each ampoule of the IS contains about 0.967mg of the extract of human menopausal urine and 5mg of lactose.

COLLABORATIVE STUDY AND ASSIGNMENT OF UNITAGE

The IS was compared with the third International Standard for Urinary FSH and Urinary LH (IS 71/264; Storring, Dixon & Bangham, 1976; WHO ECBS 1994) by 10 laboratories in 9 countries using FSH and LH in-vivo bioassays.

Estimates of the FSH content of the IS by augmented ovarian weight gain assays (Steelman & Pohley, 1953) were homogeneous within each laboratory and over all laboratories. The combine weighted geometric mean estimate of FSH content of the IS (with 95% fiducial limits) in terms of IS 71/264 was 71.9 (69.0-74.9) IU/ampoule

Although estimates of seminal weight gain (SVW) assays (Van Hell, Matthijsen & Overbeek, 1964) of the relative LH activities of the IS and IS 71/264 were homogeneous within laboratories, estimates were heterogeneous between laboratories. This indicated differences between the specificities of SVW assays performed in different laboratories, which appeared to be related to the mean laboratory organ weights, and differences between the spectra of LH isoforms in the two preparations, which were obtained from different manufacturers. The combined unweighted geometric mean estimate of LH content of the IS (with 95% fiducial limits) in terms of IS 71/264 by SVW and ovarian ascorbate depletion assays (Parlow, 1961) was 70.2 (61.7-80.0) IU/ampoule.

Estimates of the FSH and LH content of ampoules of the IS kept at elevated temperatures suggested that the IS would be adequately stable under normal storage conditions.

At its 51st meeting in November 2000, the WHO Expert Committee on Biological Standardisation established the preparation in ampoules coded 98/704 as the fourth International Standard for Urinary Follicle Stimulating Hormone (FSH) and Urinary Luteinizing Hormone (LH), and, on the basis of the results of the collaborative study and with the agreement of the participants, assigned an activity of 72 International Units of urinary FSH and an activity of 70 International Units of urinary LH to the contents of each ampoule.

PARTICIPANTS IN THE COLLABORATIVE STUDY

c Wolfensen, Instituto Massone SA, Arias 4431, 1430 Buenos Aires, Argentina; K Grant, Therapeutic Goods Administration, PO Box 100, Woden ACT 2606, Australia; SL Dalmora, Departamento de Farmácia Industrial, Universidade Federal de Santa Maria, 97.105.900-Santa Maria, RS, Brazil and P Bartolini, IPEN-CNEN, Universidade de São Paulo, Travessa R, 400-Cidade Universitária, 05508.900, São Paulo, Brazil; Li-gen Xu, Deming Qian, Hong-zheng Shen, Qun-li Liu & Wei Leng, National Institute for Control of Pharmaceutical and Biological Products, 2 Tiantan Xili, Beijing 100050, PR China; A Harazonon & M Ema, National Institute of Health Sciences, Osaka Branch, 1-1-43 Hoenzaka, Chuo-ku, Osaka, 540-0006, Japan; E Pithon, Laboratoires Seron SA, CH-1170 Aubonne, Switzerland and R Bussi & G Zanolo, Instituto Ricerche Biomediche 'A Marxer', Via Ribes 1, 10010 Colleretto Giacosa, Italy; c Strijbos & V Moffat, N.V Organon, PO Box 20,

5340 BH Oss, The Netherlands; D Pelling, TNO BIBRA International Ltd, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK; P Gerson, RJ Tiplady & PL Storring, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3QG, UK; EA Raike, Qualtech Laboratories Inc., 104 Green Grove Road, Ocean, New Jersey NJ 07712, USA.

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. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

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.

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

Campbell PJ (1974). International biological standards and reference preparations. II. Procedures used for the production of biological standards and reference preparations. Journal of Biological Standardisation 2: 269-272.

Parlow AF (1961). Bioassay of pituitary luteinizing hormone by depletion of ovarian ascorbic acid. In: Human Pituitary Gonadotrophins. Ed A Albert. Springfield, Illinois: Charles C Thomas, pp 300-310.

Steelman SL & Pohley FM (1953). Assay of the follicle-stimulating hormone based on the augmentation with human chorionic gonadotrophin. Endocrinology 53: 604-616.

Storring PL, Dixon H & Bangham DR (1976). The First International Standard for Human Urinary FSH and for Human Urinary LH (ICSH), for Bioassay. Acta Endocrinologica 83:700-710.

Van Hell H, Matthijsen R & Overbeek GA(1964). Effects of human menopausal gonadotrophin preparations in different bioassay methods. Acta Endocrinologica 47: 409-418.

WHO Expert Committee on Biological Standardisation (1978). 29th Report.
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10. ACKNOWLEDGEMENTS

Grateful acknowledgments are due to the following; the participants in the menotrophin preparations; to Mr P Gerson and Mr RJ Tiplady for preliminary bioassays; and to Dr P Dawson for ampouling.

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: 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: 6 mg
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.