



**WHO International Standard
Calcitonin, Human
NIBSC code: 89/620
Instructions for use
(Version 5.0, Dated 28/03/2013)**

1. INTENDED USE

The International Standard (IS) consists of a batch of ampoules (coded 89/620) which was established at the 42nd Meeting of the WHO Expert Committee on Biological Standardization in 1991. This IS replaces the 1st International Reference Preparation (IRP) of Human Calcitonin, ampoule code 70/234.

2. CAUTION

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

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 of the IS contains 17.5 INTERNATIONAL UNITS and maintains continuity of the International Unit defined by the 1st IRP of Calcitonin, Human for Bioassay.

4. CONTENTS

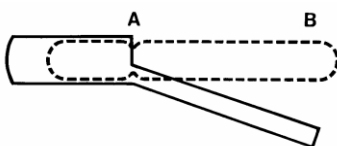
Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 0.5ml of a solution which contained:-
Synthetic human calcitonin approx 92 micrograms
Mannitol approx 10mg
and pure dry nitrogen at slightly less than atmospheric pressure.

5. STORAGE

Unopened ampoules of the IS should be stored below -20°C in the dark.
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 all practical purposes, each ampoule of the IS contains the same quantity of the substances listed above. Dissolve the total contents of the ampoule in a known volume of suitable solvent (buffer at pH 3.5) with carrier protein where extensive dilution is required.

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

For economy of use, it is recommended that the solution be sub-divided into several small containers, frozen rapidly eg. in dry ice and stored at -40°C or below. Careful evaluation will be needed to determine a feasible time of storage.

8. PREPARATION OF THE AMPOULES

Three hundred and thirty mg of human calcitonin (hCT) batch AS production standard 90 donated to WHO for ampouling was generously provided by Drs K Lingner and H P Riniker, Ciba Geigy Ltd, Basle, Switzerland. The synthetic peptide had been purified by HPLC - manufacturer's data showed that the preparation provided had a pure peptide content of 84% which was 99% monocomponent.

In order to minimize loss of peptide by surface adsorption during ampouling, the bulk peptide was dissolved in diluent to give a concentrated solution, approximately 1mg/ml for Millipore filtration (pore size 0.45 micrometre). The filter was washed several times with diluent before the concentrated peptide solution was filtered and repeated subsequent washes of the filter after filtration of the peptide were added to the filtrate to ensure maximum recovery of peptide. The final concentration of peptide was nominally 200 micrograms/ml. The diluent consisted of 0.001M acetic acid containing 20mg/ml mannitol and was purged with nitrogen before use and subsequently maintained under nitrogen in an attempt to minimize further oxidation of the peptide whilst in solution.

The mean fill weight per ampoule was 0.5038g (coefficient of variation 0.153%, n=53). The ampoule contents were freeze dried, secondarily desiccated and sealed under nitrogen according to procedures recommended by the World Health Organization Expert Committee on Biological Standardization (WHO ECBS 1990 see (1)).

High performance liquid chromatography carried out at NIBSC and at Ciba-Geigy showed minimal changes due to the ampouling and freeze drying procedures (Ciba-Geigy reported that the sulphoxide content was between 0.6 and 1.2%).

9. COLLABORATIVE STUDY

Fourteen laboratories in 10 countries took part in an international collaborative study. Raw assay data from *in vivo* bioassays in 12 laboratories and *in vitro* bioassays in one laboratory were returned to NIBSC for centralized statistical analysis. The statistical and computing methods used are based on references 3-5.

With the exception of one laboratory, all laboratories carried out the *in vivo* rat hypocalcaemia bioassay, usually according to the procedure described in the relevant pharmacopoeia although there were minor differences in the rat hypocalcaemia methodology reported by the 14 laboratories.

Laboratory mean estimates (with one exception) for hCT 89/620 relative to the first IRP hCT by *in vivo* hypocalcaemia bioassay were homogeneous within laboratories and marginally heterogeneous (0.1 > p > 0.05) between laboratories. The unweighted geometric mean (with one exclusion) was 17.55 International Units/ampoule, 95% fiducial interval 16.02 - 19.21 IU and the weighted geometric mean was 17.59 (16.92 - 18.51 IU) per ampoule.

Comparison of the hCT 89/620 with the pCT 89/540 in the same *in vivo* assays using either intravenous or subcutaneous dose administration shows clear evidence of discrimination between the two species of calcitonin - the hCT appears to be approximately equipotent with pCT when both are given by intravenous injection, but hCT appears to be



some 4-6 fold more potent than pCT when the same preparations are given by subcutaneous injection. This unexpectedly high potency of hCT when bioassayed in terms of pCT by the subcutaneous injection rat hypocalcaemia bioassay was first noted in 1970 (9) although at that time it was not realised that under those conditions of bioassay there was a clear discrimination between pCT and hCT. Differences in absorption of peptide from or degradation at the site of subcutaneous injection, half-life of peptide in circulation and metabolic clearance rate etc might provide explanations. Such species differences emphasize the need to compare "like with like".

10. STABILITY

In vivo bioassays of ampoules of 89/620 stored for 197 days at 37° in terms of 89/620 stored continuously at -20° did not show a significant difference in relative bioactivity indicating that 89/620 is sufficiently stable to serve as a biological standard. Estimates by HPLC give a predicted loss of material in 89/620 of approximately 3% per year at -20° (7,8). The apparent lack of correlation between rat hypocalcaemia bioassays and HPLC may reflect the power of HPLC to resolve particular components within a mixture of forms.

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.

11. REFERENCES

- 1.WHO Expert Committee on Biological Standardization (1990). Guidelines for the preparation, characterization and establishment of international and other standards and reference reagents for biological substances. Technical Report Series 800. Annex 4, 181-214.
- 2.Gaines Das R E & Zanelli J M (1980). The International Reference Preparation of Calcitonin, Human, for Bioassay: assessment of material and definition of the International Unit. *Acta Endocrinologica* 93, 37-42.
- 3.Finney D J (1978). Statistical method in biological assay. 3rd Edition. Charles Griffin & Co., London.
- 4.Gaines Das R E & Rice L R (1985). SCAN, an exploratory program for preliminary analysis of bioassay and immunoassay data. *Computer Methods and Programs in Biomedicine* 21, 25-33.
- 5.Gaines Das R E & Tydeman M E (1982). Iterative weighted regression analysis of logit responses. A computer program for analysis for bioassays and immunoassays. *Computer Programs and Biomedicine* 15, 13-22.
- 6.Jerne N K & Perry W L H (1956). The stability of biological standards *Bull WHO* 14, 167.
- 7.Kirkwood T B L (1977). Predicting the stability of biological standards and products. *Biometrics* 33, 736-742.
- 8.Tashjian A H Jr & Voelkel E F. (1970). Unexpectedly high biological activity of synthetic human calcitonin. *J Clin Endocr Metab* 31, 116-118.

12. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to Ciba-Geigy, Basle, Switzerland (Drs K Lingner & P H Riniker) for donating the material for ampouling; to the staff of the Standards Processing Division at NIBSC for the ampouling facilities, and to all the participants in the collaborative study

13. FURTHER INFORMATION

Further information can be obtained as follows;

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

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: freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	
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: 10mg
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_biologicalstandardsrev2004.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.