1. INTENDED USE
This consists of a batch of ampoules, coded 98/586, containing synthetic salmon calcitonin analysed by international collaborative study and established as the Third International Standard (IS) for Calcitonin, Salmon by the Expert Committee on Biological Standardisation of the World Health Organisation (1). This preparation replaces the Second International Standard of Calcitonin, (coded 87/788) (2), stocks of which are now exhausted.

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

The material is not of human or bovine origin. 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 Third IS contains 138 International Units (IU). (This value is equivalent to 23µg calcitonin per ampoule).

4. CONTENTS
Country of origin of biological material: United Kingdom.
Each ampoule contains the residue after freeze-drying of 1.0 ml of a solution which contained:

- Salmon Calcitonin: approximately 30mg/L
- Mannitol: 2g/L
- Acetic acid: 0.001M

And pure dry nitrogen at slightly less than atmospheric pressure.

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. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL
For practical purposes each ampoule contains the same quantity of calcitonin. The entire contents of each ampoule should be completely dissolved in an accurately measured amount of suitable diluent, with carrier protein where extensive dilution is required. No attempt should be made to weigh out any portion of the freeze-dried powder. The material has not been sterilized and the ampoules contain no bacteriostat. Unopened ampoules should be stored at −20ºC in the dark.

PREPARATION OF AMPOULES
A batch of highly purified synthetic peptide, lot number JO 1997007, was donated for ampouling as the replacement standard by Dr H.B. Jenny and Dr U. Merz, Novartis Pharma AG, Basle, Switzerland. Analytical data provided by Novartis stated that the peptide was >96% monocomponent by HPLC. The preparation contained 3.0% moisture and 7.9% acetic acid, had a peptide content of 90.5% by amino acid analysis and a biological activity of 5365IU/mg.

The preparation was received as a lyophilised white powder; 151mg were initially dissolved in 10ml pre-filtered (0.22µm) excipient solution (0.2% mannitol/1.74mM acetic acid), filtered (0.22µm) and added to 4.3 L excipient solution along with filter washings. The filling solution was transferred to a 4ºC cold room and stirred gently overnight to equilibrate. The solution was distributed into ampoules as 1.0ml aliquots which were then lyophilized and sealed according to procedures described by WHO for International Biological Standards (3) and stored at −20ºC in the dark.

Check weights carried out during the filling process gave a mean filling weight of 1.0083g (CV 0.98%) and the residual moisture content was 0.45%. Each ampoule coded 98/586, has a predicted content of 30µg salmon calcitonin (but see results of international collaborative study), 2mg mannitol and acetic acid (0.001M), thereby maintaining similarity with the Second IS.

COLLABORATIVE STUDY
Aims of the study
The preparation in ampoules coded 98/586 was evaluated by international collaborative study in which twelve laboratories in nine countries took part. The study was designed:

1) To determine the activity of sCT (98/586) by in vivo bioassay in terms of the second IS for sCT (87/788)
2) To assess the stability of the preparation after accelerated thermal degradation
3) To estimate the purity of the ampouled candidate preparation and to determine the sCT content in gravimetric units by HPLC.

Activity of ampoule contents
Five laboratories contributed bioassay data using the in vivo rat hypocalcaemic method with one of the laboratories also contributing in vitro data. Analysis of all bioassay data gave a homogeneous data set with a geometric mean of 140.4 (95% fiducial limits 130.6-150.8) IU per ampoule.

Twelve laboratories provided HPLC data on purity and ampoule content using methodology based on the EP method (4) or an in-house system. The mean of individual laboratory estimates for purity by all methods was 91.9% (CV4.9%). Using the salmon calcitonin CRS as a reference, the mean of individual laboratory estimates for ampoule content was 23.12µg per ampoule with a relative standard deviation of 3.8%. Since the predicted content of the ampoules was 30µg, based on dilution of a known mass of sCT and volume of formulated solution delivered to the ampoule, this indicates that material may have been lost, either prior to filling, possibly when stiring overnight, or during the filling process itself.

Assignment of unitage
On the basis of the consistency of estimates and predicted stability on storage, it was proposed that the gravimetric content of 98/586 be assigned...
in terms of HPLC measurements, and that the biological activity of the ampoule contents in IU be determined from this value and the internationally agreed figure of 6000 IU per mg for the specific activity of salmon calcitonin. Therefore using the mean laboratory estimate of ampoule content of 23µg per ampoule and a specific activity of 6000IU per mg, 98/586 was assigned an ampoule content of 138 IU per ampoule. Although from a relatively small data set, the bioassay estimate of 140 IU per ampoule was in excellent agreement with this value.

Participants
Dr K Grant, TGA, PO Box 100, Woden ACT 2606, Australia.
Dr N Dubois, Regulatory Affairs & QC Dept, UCB-Bioproducts SA, Chemin du Foreist, B-1420 Braine-l’Alleud, Belgium.
Dr E Charton & Dr F Briançon, Europena Dept for the Quality of Medicines, Council of Europe, BP 907, F-67029 Strasbourg Cedex 1, France.
Dr F Pommarat, Expansas, Route d’Avignon, BP No 6, 30390 Aramon, France.
Dr D Mannion & Dr L Husager, Danish Medicines Agency, Frederikssundsvej 378, DK-2700 Bronshoj, Denmark.
Dr K Sugimoto, Towa Pharmaceutical Co Ltd, 2-11 Shinbashicho, Kadoma, Osaka 571-8580, Japan.
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Dr H Windemann & Dr J Fluckiger, QC Division, Biochemistry, office Intercantonal de Control des Medicaments, Erlachstrasse 8, CH-3000 Bern 9 Switzerland.
Dr D Pelling & Dr D Walters, TNO BIBRA International Ltd, Woodmansterne Road, Carshalton, Surrey SM5 4DS UK.
Mr N Sutcliffe, Division of Endocrinology, NIBSC, Potters Bar, Herts, EN6 3QG UK.
Mr R Kleszynski & Mr L Antognoli, Quality Control Biology, Centeon, Kadoma, Osaka 571-8580, Japan.
Mr H Ludwig & Dr D Schwarzenbach, Novartis Pharma AG, WSJ-94.507A, CH-4002, Basle, Switzerland.

8. STABILITY
Ampoule of sCT 98/586 stored at elevated temperatures for 8 months showed little evidence of any significant loss of activity by bioassay and only a modest increase in degradation products by HPLC. The results indicate that sCT 98/586 is sufficiently stable to serve as a standard.

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.

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
4. European Pharmacopoeia, 2000 Addendum

10. ACKNOWLEDGEMENTS
We gratefully acknowledge the important contributions of all the participants; Dr H-B. Jenny and Dr U. Merz, Novartis Pharma AG, Basle, Switzerland for their generous donation of material and Dr P Dawson and Standard Division for preparation of ampouled materials.

11. FURTHER INFORMATION
Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
Derivation of International Units: http://www.nibsc.org/standardisation/international_standards.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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze dried powder</td>
<td>Corrosive: No</td>
<td></td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
<td></td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: No</td>
<td></td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
<td></td>
</tr>
<tr>
<td>Other (specify): Can react with oxidising materials. Avoid contact with acids and alkalis.</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation: Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion: Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption: Not established, avoid contact with skin</td>
</tr>
<tr>
<td>Suggested First Aid</td>
</tr>
<tr>
<td>Inhalation: Seek medical advice</td>
</tr>
<tr>
<td>Ingestion: Seek medical advice</td>
</tr>
<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
</tr>
<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
</tr>
</tbody>
</table>
**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.

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**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.

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**16. INFORMATION FOR CUSTOMS USE ONLY**

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*:</th>
<th>United Kingdom</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 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.</td>
<td></td>
</tr>
</tbody>
</table>

| Net weight: | 2 mg |

| Toxicity Statement: | Non-toxic |

Veterinary certificate or other statement if applicable.

Attached: No

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**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_biolstandardsrev2004.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.