WHO International Standard

5th International Standard For Unfractionated Heparin, (established 1998)
NIBSC code: 97/578
Instructions for use
(Version 4.0, Dated 01/04/2008)

1. INTENDED USE
The 5th International Standard for Unfractionated heparin, consists of ampolules, coded 97/578, containing aliquots of a freeze-dried material prepared from porcine mucosa. This preparation was established as the 5th International Standard for Unfractionated Heparin, by the Expert Committee on Biological Standardisation of the World Health Organisation in 1998, with labelled potency of 2031 IU/ampoule.

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.

3. UNITAGE
The standard was calibrated by 24 laboratories in 14 countries against the 4th International Standard for Unfractionated Heparin (82/502). Seven different methods were employed in the study: anti-Xa chromogenic assay, anti-IIa chromogenic assay, activated partial thromboplastin time (APTT), Hep test, the European Pharmacopoeia (EP) assay, the United State Pharmacopoeia (USP) assay and the Japanese Pharmacopoeia (JP) assay. A total of 184 assays were carried out. The potency of 2031 IU/ampoule was assigned by taking the geometric mean of all the valid assay results.

Uncertainty: the assigned unitage 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 determined to be ±0.07%

4. CONTENTS
Country of origin of biological material: Denmark.

The bulk starting material consisted of a single batch or porcine mucosal sodium heparin. 50.05 g of dried powder were dissolved with 5000 ml of sterile distilled water. The solution was distributed at 4°C into 5000 ampolules, coded 97/578. The contents of the ampolules were them freeze-dried under conditions normally used for international biological standards.

The mean weight of the liquid content of 99 checkweight samples was 1.0063g, with the limits 1.0034 to 1.0082 (coefficient of variation 0.07%). The mean weights of the freeze dried plug was 8.85 ± 0.16mg (mean of 25 estimates)

5. STORAGE
Unopened ampolules should be stored in the dark at or below –20°C. Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING
DIN ampolules have an ‘easy-open’ coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

The tap ampoule gently to collect the material at the bottom (labeled) 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
No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

Allow ampolules to warm to room temperature. Open ampoule, taking care to ensure that all material is in the lower part, and reconstitute with 1.0 ml distilled water. Heparin is very stable and aliquots of the reconstituted solution, at a suitable concentration (eg 10 IU/ml) could be stored frozen at –40°C or below for subsequent use.

8. STABILITY
Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

Accelerated degradation studies have shown that the 5th International Standard is very stable in unopened ampolules stored at –20°C. No loss of activity was observed even when the material has been stored at +45°C for 6 months.

9. REFERENCES

10. ACKNOWLEDGEMENTS
All participants in the international collaborative study. We are also grateful to the following manufacturers for their kind donation of heparin samples, two of which were used as candidates for the collaborative study:

Diosynth bv, 5340 BH Oss, The Netherlands
Cirisos Industria Farmacobiologica SpA, 22079 Villa Guardia, Como, Italy
Laboratori Derivati Organici, SS 31 bis Trino, Italy
Leo Pharmaceutical Products Ltd, 55 Industriparken, DK-2750 Ballerup, Denmark
Pharmacia & Upjohn, 160 Industrial Drive, Franklin, Ohio 45005, USA
Scientific Protein Laboratories, 700 E Main Street, Waunakee, USA
New Zealand Pharmaceuticals Ltd, Palmerston North 5330, Linton, New Zealand

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

National Institute for Biological Standards and Control, PO Box 28, Hertfordshire. EN6 3QG. Tel: (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory

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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>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: Freeze dried solid</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
<td>Irritant: Yes</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material of porcine origin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
<td>Not established, avoid inhalation</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
<td>Not established, avoid ingestion</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
<td>Not established, avoid contact with skin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Suggested First Aid</th>
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<tbody>
<tr>
<td>Inhalation: Seek medical advice</td>
<td></td>
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<tr>
<td>Ingestion: Seek medical advice</td>
<td></td>
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<tr>
<td>Contact with eyes: Wash with copious amounts of water. Seek medical advice</td>
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<tr>
<td>Contact with skin: Wash thoroughly with water.</td>
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<thead>
<tr>
<th>Action on Spillage and Method of Disposal</th>
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<tbody>
<tr>
<td>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.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Country of origin for customs purposes*: United Kingdom</th>
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<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>
</tr>
<tr>
<td>Net weight: 8.85mg</td>
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<tr>
<td>Toxicity Statement: Toxicity not assessed</td>
</tr>
<tr>
<td>Veterinary certificate or other statement if applicable. Attached: No</td>
</tr>
</tbody>
</table>

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_e stdardsrev2004.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.