WHO International Standard
1st International Standard For Alpha-1-Antitrypsin, Plasma-Derived
NIBSC code: 05/162
Instructions for use
(Version 2.1, Dated 27/10/2009)

1. INTENDED USE
Alpha-1-Antitrypsin (AAT) belongs to the serpin (serine proteinase inhibitor) family of inhibitors and elastase is the main physiological target. Plasma-derived therapeutic AAT products are used to treat AAT deficiency (Alpha-1), a genetic disorder identified in virtually all populations that can cause liver and lung disease in adults and children. The 1st International Standard for AAT is made from AAT purified from plasma, was provided by a manufacturer of therapeutic concentrates and is intended primarily for use to standardise the determination of potencies of AAT used for replacement therapy including plasma-derived, recombinant and transgenic products. The standard is also suitable for assigning total protein and antigen concentrations to AAT preparations. For antigen determinations individual laboratories should investigate the suitability of the antibody used for the material to be tested. This is particularly important when recombinant products are being tested where differences in glycosylation patterns may affect the results. Reports of the studies are available as BS working documents WHO/BS/06.2044, and WHO/BS/08.2092.

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
The potency of the 1st International Standard for AAT was determined as part of a collaborative study where the inhibitory activity of AAT was determined by titration against trypsin. The molar concentration of active trypsin was determined by active site titration using 4-Nitrophenyl 4-guanidinobenzoate hydrochloride (NPGB). AAT and trypsin form a tight 1:1 stoichiometric complex so titration of AAT against a known concentration of trypsin allows expression of active AAT concentration in molar units. The concentration of active AAT in this International Standard is 243 nmoles per ampoule. The total protein and antigen concentration of the standard was also determined, using a number of methods including amino acid analysis, and found to be consistent with the active concentration. This is equivalent to 12.4 mg AAT per ampoule applying a molecular weight of 51 000 g/mole, determined by mass spectrometry for this preparation. Recombinant preparations with different molecular weights will require a different conversion factor to calculate the mg amount from moles of active inhibitor of the recombinant preparation determined in titration assays which include this Standard. International Standards are traditionally assigned units based on consensus values and represent the primary standard for a particular measurand and as such are not assigned an uncertainty. The uncertainty of the ampoule content of 05/162 may be considered as the coefficient of variation of the fill which was 0.15%.

4. CONTENTS
Country of origin of biological material: United Kingdom.

After reconstitution each ampoule will contain, in addition to active AAT, phosphate buffer and saline (17 mM phosphate; 38 mM chloride, 81 mM sodium at pH 7.0) and 144 mM mannitol added as a bulking agent. No other proteins were added. A total of 9792 ampoules of 05/162 were prepared with a mean filling weight of 1.0057 g (cv = 0.15 %), a mean dry weight of 0.0468 g (cv = 3.74 %) and residual moisture of 0.6615 % (cv = 11.31 %).

5. STORAGE
Unopened ampoules 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 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 (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

This International Standard is intended to be a primary reference material for local calibrators and should not be used as a reagent. Ampoules should be stored at -20 °C or below (but are shipped at ambient temperatures and are stable for short periods). Before use, the ampoules should be warmed to room temperature and the contents of the ampoule should be reconstituted in 1 ml of distilled water and stored on ice for use within the same day.

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.

9. REFERENCES

10. ACKNOWLEDGEMENTS

11. FURTHER INFORMATION
Detailed 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

<table>
<thead>
<tr>
<th>Physical and Chemical properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance: White powder</td>
<td>Corrosive: No</td>
</tr>
<tr>
<td>Stable: Yes</td>
<td>Oxidising: No</td>
</tr>
<tr>
<td>Hygroscopic: No</td>
<td>Irritant: No</td>
</tr>
<tr>
<td>Flammable: No</td>
<td>Handling: See caution, Section 2</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>Contains material of human origin</td>
</tr>
</tbody>
</table>

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 |
| Toxicity Statement: Toxicity not assessed |
| 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.