1. INTENDED USE
The above named Standard has been developed to replace the 1st
International Standard for Alpha Thrombin (89/588) and the US
Standard Thrombin, Lot J. The potency of the new Standard is 110
IU/ampoule which is equivalent to 110 US units/ampoule.

Low stocks of two international standards for Thrombin (the WHO
International Standard for Alpha Thrombin, 89/588, and the US Standard
Thrombin, lot J) necessitated development of a replacement preparation.
Replacement of both Standards presented an opportunity to develop a
joint Standard with a common unit, thus eliminating problems caused by
the small but assay-dependent difference between the existing units and
Standards (the IU in the case of the International Standard and the US
unit in the case of the US Standard, also commonly referred to as the
"NIH Unit"). A collaborative study was organized and involved 25
laboratories from 15 countries worldwide (1). Both the International
Standard (89/588) and US Standard, lot J, were included along with two
candidate Thrombin preparations donated by manufacturers. On the
basis of results from this study, one of these preparations, 01/580, was
chosen to be the replacement Standard and is designated the WHO 2nd
International Standard for Thrombin, 01/580, and the US FDA/CBER
Thrombin Standard, Lot K. The International Standard was established by
the Expert Committee on Biological Standardisation of the WHO in
February 2003.

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
Participants in the collaborative study to calibrate the replacement
thrombin standard were requested to perform their in-house methods for
thrombin potency determinations using their usual reagents. Potency
determinations could be performed using fibrinogen or plasma substrates
or alternatively using a chromogenic method. Participants were
encouraged to perform more than one method if possible. The potency of
the candidate materials was calculated using both the International
Standard and US Standard as reference standard. Further analysis was
also performed to determine if there was any effect on results of the
different substrates used: human fibrinogen, bovine fibrinogen, plasma
and chromogenic substrates. The conclusion of the study was that
candidate material D, coded 01/580, was a suitable replacement standard
for both the International Standard and US Standards. The potency of
this material is 110 IU/ampoule. This figure was calculated using results
from all clotting assays using human or bovine fibrinogen or plasma.
Results from chromogenic assays were not used in the calculation of this
value.

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: United Kingdom.
The purified thrombin used to make the Standard was generously provided
by a manufacturer as a purified solution of human thrombin prepared from
plasma with a specific activity of 2000-3000 IU/mg. The material was
shipped to NIBSC as a frozen solution where it was then thawed and diluted
to a concentration of approximately 100 IU/m in a solution of 10 mM Hepes,
ph 7.4, containing 0.15M NaCl and 5 mg/ml human albumin solution (with
low protease activity).

This solution was dispensed into ampoules in 1 ml aliquots. The filling
operation gave a total of 9676 ampoules with a mean filling weight of
1.0061g (cv = 0.07%). After freeze drying the ampoules had a mean dry
weight of 0.0184g (cv = 1.13%) and a residual moisture of 0.17% (cv =
12.4%). The alpha thrombin content of the Thrombin Standard is not known
exactly but the international collaborative study demonstrated by the ratio
of clotting to chromogenic activity that it is very similar to the previous 1st
International Standard for Alpha Thrombin, 89/588 (1).

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. The contents of the ampoule should be
dissolved in 1 ml of distilled water and the resulting solution stored on ice
and used within 4 hours.

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.

Recent accelerated degradation studies on samples held at elevated
temperatures for 13.3 years suggest good long term stability of 01/580
and predict a loss of activity of 0.009% per year for ampoules stored at
-20°C. The product is stable for short periods at higher temperatures and is
routinely shipped under ambient conditions.

9. REFERENCES
Whitton C. Sands D. Lee T. Chong A. Longstaff C.
A reunification of the USC ("NIH") and International Unit into a single standard for Thrombin.

10. ACKNOWLEDGEMENTS
The organisers of the study gratefully acknowledge the help and materials provided by the manufacturers of the thrombin preparations included in this study, Baxter Bioscience, Vienna, Austria, and Bio Products Laboratory, Elstree, Herts UK, and all the study participants who took part in the international collaborative study.

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:
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>
<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): Contains material of human origin</td>
<td></td>
<td></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.

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: 10mg
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_refstandardsrev2004.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.