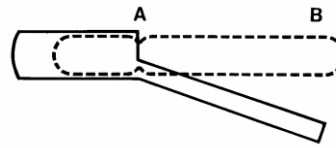




**WHO Reference Reagent**  
**Erythropoietin, Human, Urinary. 2nd International Reference**  
**Preparation**  
**NIBSC code: 67/343**  
**Instructions for use**  
**(Version 5.0, Dated 28/03/2013)**



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.

### 1. INTENDED USE

This consists of a batch of ampoules (coded 67/343) which was established by the WHO Expert Committee on Biological Standardization (WHO ECBS 1970). For further details of this Standard and of its collaborative study see Annable et al (1972).

### 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

Each ampoule contains 10 INTERNATIONAL UNITS (by definition)

Uncertainty: the International Unit of 67/343 is assigned without uncertainty. Where required, the uncertainty of the ampoule content of 67/343 may be considered to be the coefficient of variation of the fill volume, which was determined to be 0.5%.

### 4. CONTENTS

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

Human urinary extract approx 2 mg  
Sodium chloride approx 3 mg  
Nitrogen gas at slightly less than atmospheric pressure.

### 5. STORAGE

Unopened ampoules should be stored at -20°C.

**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.

### 7. PREPARATION OF AMPOULES

**Bulk material** The bulk material comprised some 16g of human urinary erythropoietin pool D-1, generously given by the US National Heart Institute Committee on Erythropoietin, obtained by the Department of Physiology, University of the Northeast, Corrientes, Argentina (from patients with anaemia attributable to hookworm infection and iron deficiency), and processed by Dr Hammond in the Haematology Research Laboratories, Children's Hospital of Los Angeles, California USA. Dr Hammond reported that pool D-1 was made from 35 individual batches of concentrates of erythropoietin prepared by the collodion adsorption method from the urine of anaemic patients, that it contained 72% protein (Lowry method), and that it was estimated to contain 2.4 IU/mg by bioassay in the polycythaemic mouse.

**Distribution into ampoules** In June 1968 an aqueous saline extract was prepared at the National Institute for Medical Research from 16.05g of erythropoietin pool D-1. The erythropoietin powder was triturated with water (93.6 ml/g); the resulting suspension was heated to 80-82°C, immediately cooled on ice, and then centrifuged at 10,000g for 15 min at 4-6°C. The supernatant was separated and stored at 4°C and the sediment was re-extracted twice by the same method using water for the first re-extraction and 0.154M sodium chloride for the second. The combined supernatants (4,370 ml) were filtered serially through membrane filters (Millipore grades RA, AA, and HA with average pore diameters of 1.2, 0.8 and 0.45 micrometres respectively). The solution was distributed into ampoules (1.019g ± 0.5% per ampoule) and the contents were freeze-dried, secondarily desiccated and sealed under nitrogen.

### 8. USE OF MATERIAL

For practical purposes each ampoule contains the same amount of the same materials. Dissolve all the contents in a known amount of buffer solution. No attempt should be made to weigh portions of the freeze-dried powder.

For economy of use the solution can be kept for several months if an anti-bacterial preservative is added and the solution is subdivided into several small containers, which are frozen rapidly to below -70°C and then stored below -30°C in the dark; repeated freezing and thawing should be avoided. If extensive dilutions are prepared, a carrier protein (0.1% w/v) should be added, which is free of peptidase. The material has not been sterilized and contains no bacteriostat.

### 9. STABILITY

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. Unopened ampoules 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.



**10. REFERENCES**

Annable L, Cotes PM & Mussett MV (1972). Bull Wld Hlth Org, 47, 99-112

WHO Expert Committee on Biological Standardization (1970). 23rd Report. World Health Organization Technical Report Series No. 463, 14.

**11. ACKNOWLEDGEMENTS**

Grateful acknowledgements are due to the National Heart Institute Committee on Erythropoietin for providing the material for the Standard and to the participants in the international collaborative study.

**12. FURTHER INFORMATION**

Further information can be obtained as follows;

This material: [enquiries@nibsc.org](mailto: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](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](http://www.nibsc.org/terms_and_conditions.aspx)

**13. 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](mailto:enquiries@nibsc.org)

**14. 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.

**15. 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):	Contains material of human origin
Toxicological properties	
Effects of inhalation:	<i>No adverse effects reported for this material</i>

Effects of ingestion: No adverse effects reported for this material
Effects of skin absorption: No adverse effects reported for this material
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.

**16. 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](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.

**17. INFORMATION FOR CUSTOMS USE ONLY**

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 5mg
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> 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](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.