Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
Anti-C Complete Blood Typing Serum, Human
NIBSC code: W1004
Instructions for use
(Version 2.0, Dated 04/04/2008)

1. INTENDED USE

This material was previously distributed by Sanquin Diagnostic Services, the Netherlands. With effect from 1st February 2007, the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK, is the custodian and distributor of this material.

For details of this International Standard, please refer to the enclosed package insert from Sanquin

The preparation is labelled International Standard for anti-C.

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

100 IU anti-C per ampoule.

4. CONTENTS

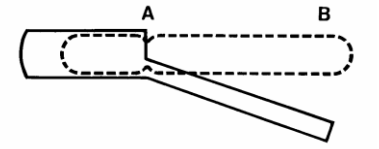
Country of origin of biological material: not known.

5. STORAGE

Store unopened ampoule at or below -20ºC.
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.



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.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

See attached package, insert from Sanquin Diganostic Services.

8. 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. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

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

N/A/

10. ACKNOWLEDGEMENTS

N/A

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

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

Physical and Chemical properties			
Physical appearance: Lyophilisate		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contains material of human origin		

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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 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 * 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: 0.07g
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_biolefstandardsrev2004.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.

PACKAGE INSERT FROM SANQUIN

DIRECTIONS FOR USE OF THE WHO INTERNATIONAL STANDARDS FOR ANTI-C AND ANTI-E SALINE AGGLUTINATING BLOOD-TYPING SERA

General

The International Standards for anti-C and anti-E saline agglutinating blood-typing sera are meant to be used exclusively in saline agglutinating techniques. The addition of additives or enzyme treatment of the cells prior to or during incubation can lead to aspecific reactions.

Anti-C. - Each ampoule of anti-C contains the freeze-dried residu (66.3 mg) of 1.0 ml of antiserum. The potency has been assigned as 100 IU per ampoule. The mean titer obtained in an international study with Ce-positive cells was 1:20, the titer with R_z cells only 1:3.
To be accepted as blood-typing serum for use in saline agglutinating techniques, the titer of the serum under investigation, obtained with R_z cells, should at least be that of the standardserum.
The anti-C serum also contains saline agglutinating anti-C^w antibodies, titer 1:10.

Anti-E. - Each ampoule of anti-E contains the freeze-dried residu (66.9 mg) of 1.0 ml of antiserum. The potency has been assigned as 100 IU per ampoule.

Reconstitution

The content of an ampoule has to be reconstituted with 1.0 ml of distilled water. After addition of the water, the ampoule should stand at room temperature for 30 min without shaking. All material should dissolve during this period. Then the serum is made homogeneous by gently shaking the ampoule. The dissolved material has to be used within 24 hours. Storage during this time has to be at 4-8^{0}C or at room temperature, the serum should not be frozen.
In case of blood-typing serum, the addition of 1.0 ml of distilled water will result in approximately 1.06 ml of serum. Nevertheless, used in twofold serial dilutions, the serum can be regarded as containing per ml the amount of IU stated on the ampoule as IU per ampoule.
In short: upon addition of 1 ml of distilled water and complete dissolving of the contents of the ampoule, IU per ampoule ~ IU per ml.

Determination

1. Wash fresh cells at least two times with an excess of a physiological phosphate buffered saline solution (PBS, pH 7.1 - 7.4).
2. Prepare a 2% suspension of these washed cells in PBS to which 2% of AB serum has been added (PBS-AB).
3. Prepare a twofold serial dilution series of the antiserum in PBS-AB (1:1 to 1:128). To avoid carry-over, a clean pipette should be used for each transfer.
4. Put 0.1 ml of each serum dilution in a separate clean, all glass testtube (10 x 75 mm), add 0.1 ml of the 2% cellsuspension, thoroughly mix the content of each tube and incubate for 15 min at 37°C (use a waterbath of 37°C for this incubation).
5. Centrifuge the tubes for 45-60 seconds at a relative centrifugal force of 120 g.
6. Examin macroscopically for agglutination by gentle dislodging the cell-buttons of each tube. The reactions should be graded according to the given notation:

   4+ : cell button remains one lump,
   3+ : cell button dislodges into several lumps,
   2+ : cell button dislodges into many small lumps of about equal size,
   1+ : cell button dislodges into finely granular, but definitely small lumps.

Calculation

The highest dilution which gives an 1+ score is the titer of the antiserum (for example: highest dilution with 1+ = 1/128 → titer = 128).

The potency of the serum under investigation is calculated with the formula:

$$\frac{\text{titer serum with cell 1}}{\text{titer standard with cell 1}} \times \text{IU/ml of the standard.}$$

If more than one cellsuspension is used, the potencies obtained with each cellsuspension are added up and then divided by the amount of cellsuspensions used:

$$\text{final potency} = \frac{\text{potency with cell 1} + \text{potency with cell 2}}{2}.$$