International Ref. Reagent
Anti-Human Globulin Standard
ICSH/ISBT
NIBSC code: 96/666
Instructions for use
(Version 4.0, Dated 23/01/2013)

1. INTENDED USE
This material has been prepared on behalf of the International Committee for Standardisation in Haematology (ICSH) and the International Society of Blood Transfusion (ISBT) for use in evaluating Anti-Human Globulin Reagents. The product was determined, by a working party of an ICSH/ISBT Expert Panel, to have acceptable potency of Anti-IgG and Anti-C3d. It may be used to evaluate Anti-Human Globulin reagents containing either of these components, or polyspecific reagents containing them both.

Further information can be found in the Reference (Section 9).

2. CAUTION
This preparation is not for administration to humans or animals in the human food chain.

The material consists of serum from rabbits immunized with human IgG, together with murine monoclonal C3d, blended in optimal proportions and diluted to yield optimal potency and acceptable specificity. Each ampoule contains the freeze-dried residue of 2ml of the blended liquid product. This preparation contains no preservative. The preparation does not contain material of human origin. 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. UNTAGE
N/A

4. CONTENTS
Country of origin of biological material: United Kingdom.
The material consists of serum from rabbits immunized with human IgG, together with murine monoclonal C3d, blended in optimal proportions and diluted to yield optimal potency and acceptable specificity. Each ampoule contains the freeze-dried residue of 2ml of the blended liquid product. This preparation contains no preservative.

5. STORAGE
Store unopened ampoules at -20°C or below. The reconstituted material should be stored at 2-8°C for not longer than one day.

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.

Reconstitute the entire contents of the ampoule with 2.0ml of distilled or deionised water. Allow to stand for at least five minutes at room temperature before use, with periodical gentle agitation, to allow for complete solution. No attempt should be made to weigh out any portion of the freeze dried material.

The preparation is for in-vitro use only. It is not to be used for any diagnostic purpose, but is intended solely for evaluation of Anti-Human Globulin reagents to determine their suitability for routine use.

Use of the Reconstituted Standard:
Preparation of coated red blood cells

The methods to be used in preparing coated red blood cells are at the discretion of the user, and are beyond the scope of these directions. It is the responsibility of the user to ensure that he/she has the necessary technical skills to determine the appropriateness of this product for the proposed application.

In evaluating Anti-IgG potency, it is suggested that two-fold serial dilutions of antibodies representing several different blood group systems should be prepared (e.g., anti-D, anti-K, anti-Fy and anti-Jk), covering the titration endpoint in each case. It is best if each of the antibodies is obtained from sera derived from immunized patients and having a titration endpoint not exceeding 16, rather than by diluting commercial reagents or hyperimmune sera.

Each dilution should be incubated with red blood cells of appropriate phenotypes, which are then washed to remove unattached human protein and prepared as 2% suspensions in 2% bovine albumin in physiological saline or phosphate buffered saline (PBS).

For the measurement of Anti-C3c potency, coated cells may be prepared by one of the low-ionic-strength sucrose procedures, and/or by a two stage indirect antiglobulin method using a complement-binding antibody, such as anti-Le or anti-Jk at a dilution beyond its ability to agglutinate the indicator cells directly. C3d-coated cells are prepared by treating the same C3c-coated cells with trypsin. In both cases, the coated cells are washed and resuspended for use in 2% bovine albumin in physiological saline or PBS at a concentration of 2%.

Preparing dilutions of AHG

Again, the precise method of use is at the discretion of the user, but meaningful evaluation of Anti-IgG reagents generally requires the performance of ‘chequerboard titrations’. It is accordingly suggested that master serial dilutions of the Anti-Human Globulin Standard and each AHG reagent being evaluated should be prepared, in sufficient volume that aliquots of each dilution can be tested with red blood cells incubated with dilution of the IgG antibodies chosen for the study. In the case of Anti-C3c and Anti-C3d, straight-line titrations are carried out.

Controls
To assure that any aggregation of the indicator cells in each case represents true anti-IgG or anti-C3 activity, each dilution of AHG should be tested with each indicator cell suspension in its native condition (i.e., uncoated with IgG or complement). In the case of testing for Anti-C3d activity, the control
indicator cells should be treated with trypsin to match the treatment accorded to the coated cells.

**Interpretation of Results**

It is important to remember, when reading titration results, that reactions due to anti-C3 components are improved by brief (e.g., 5 minutes) incubation at room temperature. This allows the reaction to reach stability. At the same time, delayed centrifugation may diminish anti-IgG reactivity. To assure fair comparisons between reagents, the effect of time must be kept constantly in mind when adding cells to the dilutions, so that centrifugation can be accomplished with the same delay throughout.

**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. 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. It is the policy of WHO not to assign an expiry date to their reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

**9. REFERENCES**


**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

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

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

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

**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: 0.16g

Toxicity Statement: Toxicity not assessed

Veterinary certificate or other statement if applicable.

Attached: No

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