WHO Reference Reagent  
Anti-A and anti-B in IVIG: Limit Reference Preparation  
NIBSC code: 07/310  
Instructions for use  
(Version 1.0, Dated 19/11/2008)

1. INTENDED USE
This material is a reference preparation for standardising haemagglutination tests and specifications to control the levels of anti-A and anti-B in normal intravenous immunoglobulin (IVIG) products. Preparation 07/310 defines the recommended maximum limits, where these are applicable, for those IVIG products having higher anti-A and anti-B titres than those in the 'Anti-A and Anti-B in IVIG: Positive control preparation', 07/306. Preparation 07/308 is the negative control for use in the same assay.

The preparations have been validated in an international collaborative study (coded BSP089) organised by NIBSC, EDOM and CBER/FDA. The study showed that preparation 07/310 has anti-A and anti-B titres in the 2-fold range 32-64 using direct haemagglutination of papain-treated A\textsubscript{1} and B red cells.\textsuperscript{1,2}

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. UNTAGE
Preparation 07/310 has anti-A and anti-B titres in the 2-fold range 32-64 using direct haemagglutination of A\textsubscript{1} and B red cells.\textsuperscript{1,2}

4. CONTENTS
Country of origin of biological material: United Kingdom. This preparation consists of the same batch of IVIG used to produce the Positive control preparation 07/306 except that it is 'spiked' with murine monoclonal IgG anti-A and anti-B antibodies to produce an IVIG product with nominal anti-A and anti-B titres of 64 from 5\% IgG as determined in direct haemagglutination tests at NIBSC. These titres were based on long-term studies at NIBSC and CBER/FDA of the incidence and levels of anti-A and anti-B in IVIG products using direct haemagglutination methodology, and estimated to be approximately equivalent to the present pharmacopeial maximum permissible titres of 32 from 3\% IgG obtained by using the IAGT. The mean weight of the dispensed solution was 0.5057g, the imprecision of the filling (CV) was 0.23\%, and the residual moisture was 0.21\%.

It is intended that the Limit reference preparation 07/310 should only be used for comparison with batches of IVIG which have higher titres than the Positive control preparation 07/306.

5. STORAGE
Store unopened ampoules at -20\degree C or below. Please note: because of the inherent stability of lyophilised 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. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

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

RECONSTITUTE THE CONTENTS OF THE AMPOULE WITH 0.5 ML DISTILLED OR DEIONISED WATER CONTAINING 0.02\% SODIUM AZIDE

Allow several minutes, with occasional vortexing, for reconstitution. Transfer the reconstituted contents to a capped tube and store at 4\degree C. Once reconstituted, users should determine the stability of the reconstituted material according to their own storage facilities.

The reconstituted contents are 5\% (w/v) IgG.

Preparation 07/310 defines the recommended maximum limits, where applicable, for those IVIG products having higher anti-A and anti-B titres than those in the 'Anti-A and Anti-B in IVIG: Positive control preparation', 07/306, in the direct 'spin' haemagglutination method using papain-treated red cells.\textsuperscript{1,2}

In the collaborative study, the % of tests resulting in a particular titre against A, B or O cells, using the direct method, across all laboratories was counted for preparation 07/310. The results are shown below:

<table>
<thead>
<tr>
<th>07/310</th>
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<tbody>
<tr>
<td>Titre</td>
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<tr>
<td>--------</td>
</tr>
<tr>
<td>128</td>
</tr>
<tr>
<td>64</td>
</tr>
<tr>
<td>32</td>
</tr>
<tr>
<td>16</td>
</tr>
<tr>
<td>8</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>&lt;2</td>
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</table>

% of tests | 100 | 100 | 100

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 activity and status until withdrawn or amended.

NIBSC follows the policy of WHO with respect to its reference materials. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference materials should be stored on receipt as indicated on the label.

Accelerated degradation studies on 07/310 are underway. However, an indication of the intrinsic stability of anti-A and anti-B activity in lyophilised IVIG was provided by examining ampoules of other lyophilised IVIG preparations (IRRs for Anti-D in IVIG, 02/228, and Negative (for anti-D) control IVIG, 02/226) that have been stored at -70\degree C, -20\degree C, +4\degree C, +20\degree C, +37\degree C and +45\degree C for 5 years and 9 months, and lyophilised culture supernatants containing mouse monoclonal IgM anti-A and anti-B that have been stored at elevated temperatures for nearly 16 years. Although the haemagglutination titres are not suitable for analysis using the usual Arrhenius model of accelerated degradation, there is no
haemagglutination data to suggest that the anti-A and anti-B activities in 07/310 will not be adequately stable at -20°C.

9. REFERENCES

10. ACKNOWLEDGEMENTS
We thank the manufacturer who kindly provided the IVIG batch used in the production of 07/310 (and associated Positive control 07/306). The monoclonal anti-A and anti-B antibodies used for spiking the IVIG for the production of 07/310 were gifts from Professor Marion Scott, International Blood Group Reference Laboratory, Bristol, UK. We also thank Dr Paul Matejschuk of the Technology Developments and Infrastructure Group, NIBSC for lyophilisation developmental work and staff of the Centre for Biological Reference Materials, NIBSC for lyophilising the IVIG bulks and sample despatch.

We are grateful to the study participants for contributing data.

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>
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</thead>
<tbody>
<tr>
<td>Physical appearance: Lyophilisate</td>
</tr>
<tr>
<td>Stable: Yes</td>
</tr>
<tr>
<td>Hygroscopic: Yes</td>
</tr>
<tr>
<td>Flammable: Yes</td>
</tr>
<tr>
<td>Other (specify):</td>
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</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
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</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
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<tr>
<td>Effects of ingestion:</td>
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15. LIABILITY AND LOSS
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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: 0.05g
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, characterisation and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter Biol Standardsrev2004.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.