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. UNITAGE
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 pharmacopoeial maximum permissible titres of 32 from 3% IgG obtained by using the IAGT. The mean weight of the dispersed 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°C or below. 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:

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°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\textsubscript{1}, 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>Titre</th>
<th>A\textsubscript{1} cells</th>
<th>B cells</th>
<th>O cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>128</td>
<td>3.0</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>64</td>
<td>50.0</td>
<td>41.1</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>41.7</td>
<td>42.9</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>3.0</td>
<td>10.1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2.4</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>6.0</td>
<td>41.1</td>
</tr>
<tr>
<td>&lt;2</td>
<td>0</td>
<td>0</td>
<td>94.0</td>
</tr>
</tbody>
</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°C, -20°C, +4°C, +20°C, +37°C and +45°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 Matejtschuk 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/
Derivation of International Units: http://www.nibsc.org/standardisation/international_standards.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>Physical appearance:</td>
</tr>
<tr>
<td>Stable:</td>
</tr>
<tr>
<td>Hygroscopic:</td>
</tr>
<tr>
<td>Flammable:</td>
</tr>
<tr>
<td>Other (specify):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Toxicological properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effects of inhalation:</td>
</tr>
<tr>
<td>Effects of ingestion:</td>
</tr>
<tr>
<td>Effects of skin absorption:</td>
</tr>
</tbody>
</table>

### 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:** 0.05g
**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, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_esstandardsrev2004.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.