



**WHO Reference Reagent
Anti-A and anti-B in IVIG: Positive Control (for use with IVIG
Negative Control 17/240)
NIBSC code: 07/306
Instructions for use
(Version 1.0, Dated 06/01/2021)**

1. INTENDED USE

This material is a reference preparation for standardising haemagglutination tests performed to control the levels of anti-A and anti-B in normal intravenous immunoglobulin (IVIG) products, and ensuring that such tests are sufficiently sensitive and specific. This preparation has been validated in an international collaborative study (coded BSP089) organised by NIBSC, EDQM and CBER/FDA. The study showed that the anti-A and anti-B titres for the Positive control preparation 07/306 were mostly in the 2-fold ranges of 32-64 and 16-32, respectively, using direct haemagglutination of papain-treated A₁ and B red cells.^{1,2} These titres should be used as a guide for operators establishing and performing the direct method for anti-A and anti-B. Operators should investigate their assay conditions and reagents in the event of significantly different results being obtained with the Positive control. This preparation may also be used in the IAGT, in which case operators should determine their own titres for assay validity and consistency. IVIG products with anti-A and/or anti-B titres that are higher than those obtained with 07/306 should be compared with the 'Anti-A and anti-B in IVIG: Limit Reference Preparation', 07/310, using the direct method. NIBSC IVIG Negative Control reference preparation (code 17/240) is suitable for use as a negative control with 07/306.

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

The guide titres for anti-A and anti-B in the Positive control preparation 07/306 are in the 2-fold ranges of 32-64 and 16-32, respectively, using direct haemagglutination of papain-treated A₁ and B red cells.^{1,2}

4. CONTENTS

Country of origin of biological material: United Kingdom.

This Positive control IVIG preparation consists of ampoules containing the lyophilised residue of approximately 0.5 mL of a 5% IVIG donated by a European manufacturer. The levels of anti-A and anti-B in this preparation were judged close to the maximum permissible pharmacopoeial levels in haemagglutination tests, where these apply. Extensive data produced at NIBSC (~1100 batches) and CBER/FDA (~270 batches) indicate that it is uncommon for batches of IVIG to have such high anti-A and anti-B titres. The mean weight of the dispensed solution was 0.5044g, the imprecision of the filling (CV) was 0.19%, and the residual moisture was 0.23%.

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. 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°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.

The Positive control preparation 07/306 is intended to be used as a positive reference preparation for inclusion in tests alongside batches of IVIG products in the direct 'spin' haemagglutination method using papain-treated red cells.^{1,2} IVIG products with anti-A and/or anti-B titres that are higher than those obtained with 07/306 should be compared with the 'Anti-A and anti-B in IVIG: Limit Reference Preparation', 07/310, if applicable.

In the collaborative study, the % of tests resulting in a particular titre against A₁, B or O cells, using the direct method, across all laboratories was counted for each reference preparation. The results are shown below:



07/306				07/308			
Titre	A ₁ cells	B cells	O cells	Titre	A ₁ cells	B cells	O cells
128	0.4	0	0	128	0	0	0
64	29.8	7.8	0	64	0	0	0
32	45.7	48.1	0	32	0	0	0
16	21.7	38.8	0	16	0	0	0
8	2.3	5.4	0	8	0.4	0	0
4	0	0	0	4	1.2	0	0
2	0	0	8.1	2	10.1	10.5	6.6
<2	0	0	91.9	<2	88.4	89.5	93.4
% of tests	100	100	100	% of tests	100	100	100

For 07/306, the most common reported anti-A titres were 64 and 32, and the most common anti-B titres were 32 and 16. Preparation 07/308 was the IVIG negative control used in the study. Around 90% of tests with 07/308 were reported as negative with group A₁, B and O cells.

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 07/306 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 similarly 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 07/306 will not be adequately stable at -20°C.

9. REFERENCES

- Anon. Human normal immunoglobulin for intravenous administration; 2.6.26. Test for anti-D antibodies in intravenous immunoglobulin. In European Pharmacopoeia 2008; 6th edn (6.1). Strasbourg, Directorate for the Quality of Medicines of the Council of Europe.
- SJ Thorpe, BJ Fox, CD Dolman and R Thorpe. Anti-A and anti-B activity in batches of different intravenous immunoglobulin products determined using a direct haemagglutination method. *Biologicals* 2005; 33: 111-116.

10. ACKNOWLEDGEMENTS

We especially thank the manufacturer who kindly provided the Positive control IVIG batch. 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.

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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

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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: Yes	Irritant: No
Flammable: Yes	Handling: See caution, Section 2
Other (specify): Contains material of human origin	
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: 0.05g
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_biologicalstandardsrev2004.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.