



**WHO Reference Reagent
High titre anti-A and anti-B in serum
NIBSC code: 14/300
Instructions for use
(Version 2.0, Dated 18/04/2018)**

1. INTENDED USE

Testing for high titre anti-A and anti-B in serum/plasma is important to:

- Minimise the risk of causing clinically significant haemolysis when blood components rich in plasma containing high titre anti-A/B are transfused to patients of blood groups A, B or AB e.g., platelet concentrates.
- Facilitate mismatched kidney transplants from living donors. These can be performed successfully if the recipient has sufficiently low levels of anti-A and anti-B. Patients may be considered for admission to ABO transplant programmes if anti-A/anti-B titres are within nominal cut-off values.
- Identify high titre anti-A/B plasma for exclusion from manufacture of blood products such as IVIG, where passive transfer of IgG anti-A/B to recipients can cause haemolysis.

However, anti-A and anti-B titrations are notoriously inconsistent across laboratories, even when using a common procedure and pooled red cells, often showing 32-fold variation in titres for the same sample. Defining titre 'limits' or 'cut-offs' for various applications in the absence of a reference preparation is therefore meaningless. The availability of this reference material should help overcome such inter-laboratory variability.

WHO Reference Reagent 14/300 was validated in an international collaborative study involving 24 laboratories in 13 countries, mostly using Diamed gel card technology. The mode anti-A and anti-B titres were both 128 using neutral gel cards at RT (DRT), and the mode anti-A and anti-B titres were both 256 using IAT gel cards at 37°C. Preparation 14/300, with these nominal titre assignments, is intended to be used in parallel titrations with serum and plasma samples to facilitate inter-laboratory comparisons of titre data, allow sample titres to be reported relative to the reference titres, and allow the establishment of consistent cut-off titres for various applications such as ABO-incompatible renal transplants. Sample titres may be recalculated relative to 14/300 as follows:

Note: the testing of replicate dilution series of both 14/300 and the serum/plasma samples is recommended to take into account intra-laboratory variation in repeat titrations.

Calculate geometric mean titres for 14/300 and the samples i.e., the average of the base 2 logarithmic values of each titre set, converted back to a base 2 number. For example, the base 2 log values of 6 individual titres of 128, 128, 128, 128, 128, 256 are 7 (i.e., 2^7), 7, 7, 7, 7, 8. These total 43, with an average value of 7.17. Converting this back to an antilogarithm in base 2 gives 144 (antilog calculators are available online). So 144 is the geometric mean of the 6 titres. By multiplying the ratio of the sample geometric mean to the geometric mean of 14/300 by the nominal titre assignment to 14/300, the relative titre of the sample can be determined.

For example, individual anti-A IAT titres of 512, 512, 512, 512, 512, 256 were obtained for 14/300. The geometric mean is 455. Individual anti-A IAT titres of 512, 512, 256, 256, 256, 256 were obtained for sample 1. The geometric mean is 322. The ratio of the mean titre of sample 1 to the mean titre of 14/300 is 322/455, which is 0.71. The nominal IAT anti-A titre assignment to 14/300 is 256. 256×0.71 is 182. 182 is the relative titre of sample 1 to 14/300.

14/300 may also be used to determine 'cut-off' titres for various applications. For example, a 1 in 2 dilution of 14/300 has a nominal titre of 128 (i.e., 1/2 of 256) and may be used to define high titre samples using IAT in parallel titrations regardless of the actual titres.

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 14/300 has nominal anti-A and anti-B titres of 128 for neutral gel card titrations at RT (DRT), and nominal anti-A and anti-B titres of 256 for IAT gel card titrations at 37°C, when reconstituted with 1.0 mL distilled or deionised water.

4. CONTENTS

Country of origin of biological material: United Kingdom.

Each ampoule contains the lyophilised residue of 1 mL defibrinated plasma (i.e., serum), pooled from plasma packs containing high titre anti-A and anti-B.

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 manufacturers 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 ampoule contents with 1.0 mL distilled or deionised water (containing 0.02% (w/v) sodium azide for storage at 4°C).

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.

Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. However, tests at NIBSC have shown that reconstituted 14/300 is stable for at least 1 month at 4°C in a tightly-lidded tube in the presence of 0.02% (w/v) sodium azide.

9. REFERENCES

Thorpe, S. J., Fox, B., Sharp, G., White, J. and Milkins, C. (2016), A WHO reference reagent to standardize haemagglutination testing for anti-A and anti-B in serum and plasma: international collaborative study to evaluate a candidate preparation. *Vox Sang*, 111: 161-170. doi:10.1111/vox.12399.

10. ACKNOWLEDGEMENTS

This preparation was produced in collaboration with UK NEQAS for Blood Transfusion Laboratory Practice.

We are grateful to the UK National Blood Service for providing high titre plasma donations, and the participants of the collaborative study.



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: Unknown
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains human serum:	See caution, Section 2
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 ampoule 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: 0.08g
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_efstandardsrev2004.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.