Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**WHO Reference Reagent**
**Anti-human leukocyte antigen antibodies (negative plasma)**
**NIBSC code: 10/142**
**Instructions for use**
**(Version 2.0, Dated 19/05/2023)**

This material is not for in vitro diagnostic use

## 1. INTENDED USE

10/142 is intended for use as a negative control for HLA flowcytometry cross match (FCXM) and single antigen bead Luminex (SAB- LX) assays performed for detection of anti-HLA alloantibodies. The material was evaluated in an International collaborative study involving 21 participant labroatories conducted for establishment as WHO International reference reagent, WHO-IRR (Rajagopal et al 2023).
Prior to organ transplantation, assays are performed to detect anti-HLA antibodies that may be detrimental to the performance of the organ. Findings from multicentre studies have shown not only the importance of the selection and standardization of the methods used for cross-matching, but also that the selection of the control sera is fundamental to the crossmatch, as they are the negative controls on which the definition of positivity is based (Harmer et al 1996; Shenton et al 1997).
The WHO-IRR has no assigned unitage and will serve as qualitative intra-assay variability controls, providing a means for trend monitoring for FCXM and LX assays for anti-HLA alloantibody detection. It is not intended for use as calibrator.

## 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.

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## 4. CONTENTS

Country of origin of biological material: United Kingdom.
The reference reagent was prepared from a pool of 37 donations of AB+ plasma. Prior to pooling, each donation was confirmed negative for anti-HLA antibodies before distribution into vials (1.0 ml/vial) and freeze-dried. Each vial contains the freeze-dried preparation of approximately 1.0ml of pooled normal human AB+ plasma negative for anti-HLA antibodies.

Each unit used for the production of this reference reagent was individually tested and found to be negative for the presence of HBsAg and antibody to HCV and HIV 1 and 2.

## 5. STORAGE

Reference materials should be stored on receipt as indicated on the label. Accelerated degradation studies have indicated that this material is suitably stable when stored at 2-8ºC prior to reconstitution. Once reconstituted, users should determine the stability of the material according to their own method of preparation, storage and use. It is recommended this material be used on the day of reconstitution.
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

## 6. DIRECTIONS FOR OPENING

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

## 7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution nor should aliquots be re-frozen after use.
To reconstitute this material, dissolve the entire contents of the ampoule in 1ml of sterile distilled water, keep at 2-8ºC and use on the day of reconstitution. Once reconstituted, this material should be treated as normal human AB+ plasma for use as an anti-HLA negative control. Users should be aware that by changing assay conditions or reagents e.g. incubation times or different secondary antibodies, assay results may vary. It is therefore important that each user validates this control using their own methods and reagents. Representative flowcytometry profile is shown in Figure 1.

## 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.

NIBSC follows the policy of WHO with respect to its reference materials. The stability of this preparation is monitored by NIBSC. Users with data supporting any deterioration in the characteristics of this preparation are encouraged to contact NIBSC. Prior to reconstitution, this material has an expiry date of 2025/06. It is recommended this material be used on the day of reconstitution.

## 9. REFERENCES

1. Rajagopal, D., Nowocin, A., Peraj, R., Atkinson, E., Rigsby, P., Matejtschuk, P., Diebold, S. (2023). An International collaborative study for Establishment of WHO International reference reagents for anti-HLA flow cytometry crossmatch and Luminex antibody assays. https://www.who.int/publications/m/item/WHO-BS-2023-2445.
2. Harmer, A.W., Garner, S., Bell, A.E. et al (1996). Evaluation of the flow cytometric crossmatch. Preliminary results of a multicentre study. Transplantation 61, 1108-1111.
3. Shenton, B.K., Bell, A.E., Harmer, A.W. et al (1997). Importance of methodology in the flow cytometric crossmatch: a multicentre study. Transplantation Proceedings 29, 1454-1455.

## 10. ACKNOWLEDGEMENTS

We are grateful for the valuable contributions of all participants in 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:

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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: Freeze dried powder | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: Yes | Irritant: No |
| Flammable: No | 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 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<br>* 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:** 1.0g |
| **Toxicity Statement:** Non-toxic |
| **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_biolefstandardsrev2004.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.

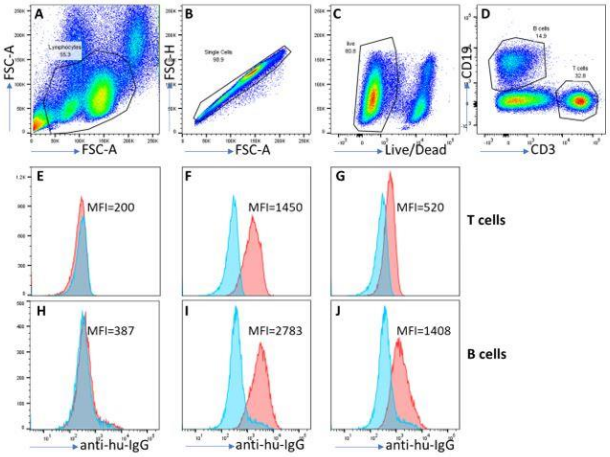


**FIGURE 1: Representative gating strategy used at NIBSC for determining HLA expression on T and B cells.** *(A) Donor PBMCs are gated for lymphocytes based on scatter profile. (B) Singlet lymphocyte subsets are identified. (C) Live lymphocytes identified using Aqua live/dead viability stain are subsequently distinguished (D) as T and B cells using anti-CD3 and anti-CD19 antibodies. Anti-HLA expression is assessed on gated T (E-G) and B cells (H-J) by histogram overlays in comparison to HLA negative RR 17/212 (E-J, blue histogram). Representative profiles for 10/142 (E, H), 17/238 (F, I) and 21/378 (G, J) are shown by the red histogram (E-J). MFI values for each RR are indicated in the plot. Plots depicted for 10/142 are from a separate assay.*