Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO Reference Reagent
Anti-human leukocyte antigen antibodies (strong positive plasma)
NIBSC code: 17/238
Instructions for use
(Version 1.0, Dated 16/05/2023)

This material is not for in vitro diagnostic use

1. INTENDED USE

17/238 is intended for use as a strong positive 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 laboratories 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. Transplants known to have taken place after a positive FCXM result may have impaired survival (Scornik et al 2001). Additionally FCXM and Luminex based anti-HLA screen assays can be used to identify de novo alloantibody production post-transplantation. 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).
17/238 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.

3. UNITAGE

No assigned unitage. This is an anti-HLA strong positive run control that is not intended to be used for calibration purposes.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each vial contains the freeze-dried preparation of approximately 0.5ml of pooled human plasma containing anti-HLA class I and II antibodies.

5. STORAGE

This material is suitably stable when stored at -20ºC prior to reconstitution. Reference materials should be stored on receipt as indicated on the label. Once reconstituted, users should determine the stability of the material according to their own method of preparation, storage, and use. It is recommended for this material to be used on the day of reconstitution, and no later than 72h after 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 0.5ml of sterile distilled water, keep at 2-8ºC and use within 72h. Product should be centrifuged, and pellet discarded, if the presence of cryoprecipitate is noticed upon reconstitution of the freeze-dried material. Once reconstituted, this material should be treated as strong positive run control for flow cytometry cross matching (FCXM) and Luminex bead-based assays for anti-HLA alloantibody detection. Different instruments and assays may yield varying results; therefore, it is important that each user validates this control using their own platform(s). The material is not intended for use in calibration of individual laboratory standards. It is recommended that this reagent is used in combination with 21/378: Anti-human leukocyte antigen antibodies (weak positive plasma) and 17/212: Anti-human leukocyte antigen antibodies (negative serum) or 10/142: Anti-human leukocyte antigen antibodies (negative plasma). Users should be aware that by changing assay conditions or reagents e.g., incubation times or secondary antibodies, assay results may vary. FCXM results can vary depending on the donor cells used and set up of the flow cytometer. 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. Prior to reconstitution, this material has an expiry date of 01/2028. Real-time stability studies have indicated that this material is suitably stable when stored at -20ºC prior to reconstitution. Users who have data supporting any deterioration in the characteristics of this preparation are encouraged to contact NIBSC.

NIBSC follows the policy of WHO with respect to its reference materials.

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. Scornik, J.C., Clapp, W., Patton, P.R. et al (2001). Outcome of kidney transplants in patients known to be flow cytometry crossmatch positive. Transplantation 71, 1098-1102.

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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

3. 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.
4. 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:
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 * 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: 0.5g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached:** No Please add vet cert numbers separated by a space

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.

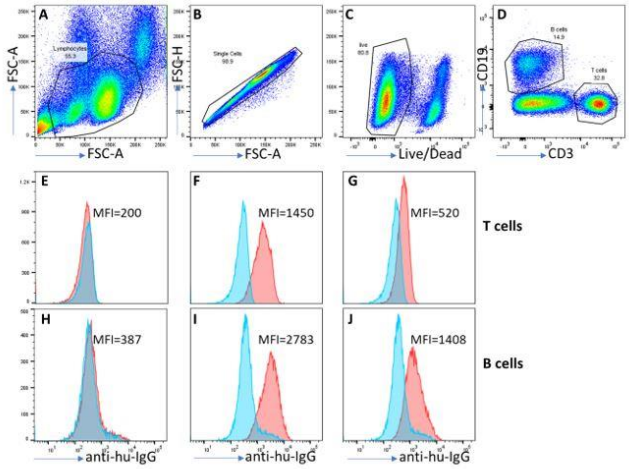

FIGURE 1: Representative gating strategy used at NIBSC for determining HLA expression on T and B cells. *(A) Donor PBMCs are gated for lymphocytes based on scatter profile. (B) Singlet lymphocyte subsets are identified. (C) Live lymphocytes identified using Aqua live/dead viability stain are subsequently 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.*