World Health Organization

WHO/BS/2023.2445
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 20 to 24 March 2023

An International collaborative study for Establishment of WHO International reference reagents for anti-HLA flow cytometry crossmatch and Luminex antibody assays

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **6 March 2023** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

Presence of donor-specific anti human leucocyte antigen (HLA) antibodies in transplant recipients can lead to hyperacute rejection and therefore prospective evaluation of potential recipients for anti-HLA antibodies is a crucial determinant for successful transplantation outcome. Recent developments in solid phase assay systems, including flowcytometric and bead-based Luminex assays have proved to be far more sensitive in detection of pre-sensitization in potential transplant recipients in comparison to conventional complement-dependent cytotoxicity (CDC) assay for antibody detection. Anti-HLA reference reagents (RR) for Flow Cytometry Crossmatch (FCXM) and Luminex (LX) antibody assays are intended for use as run controls to validate and harmonize assays for alloantibody detection. Prior to organ transplantation, FCXM is performed to examine the presence of anti-HLA antibodies that may be detrimental to performance of the transplant. FCXM and LX based anti-HLA single antigen bead (SAB) assays can further be used to identify *de novo* alloantibody generation post-transplantation. Findings from multicenter studies have not only shown the importance of selection and standardization of methods used for cross-matching, but also suggest that selection of control sera as reference materials is fundamental to the interpretation of crossmatch results since they form the basis for determination of positive/negative criteria. NIBSC has been developing and manufacturing anti-HLA controls as CE-IVD reagents for over 20 years. Provision of anti-HLA RR as WHO International RR will support global accessibility and widen the availability to user labs involved in organ transplantation programs outside of EEA.

We report here, the evaluation of four anti HLA reference materials, coded 10/142 (sample A), 17/212 (sample B), 17/238 (sample C), and 21/378 (sample D), developed for use as run controls for FCXM and LX based bead assays. Presented here are the international collaborative study results of anti HLA Reference Reagents' assessment by 21 participant laboratories from eight countries, that contributed data through concomitant measurement of coded test samples using in house procedures. A total of 86 donors were tested in FCXM assays performed by 20/21 (95%) participants. LX assays were performed by 19/21 (90%) participants using assay kits manufactured by One Lambda (Labscreen SAB) and Immucor (Lifecodes SAB) for detection of class I and II antibodies. 10/142 and 17/212 are intended for use as high and low background anti-HLA negative controls respectively; and 17/238 and 21/378, are designed as strong and weak positive anti-HLA controls respectively for use in FCXM and LX based assays for alloantibody characterization.

Relative fluorescence intensity (RFI) value calculated against 17/212-NIBSC negative anti-HLA RR, resulted in average ± SEM values reported of 0.97 ± 0.02, 14.6 ± 4.35 and 4.86 ± 2.2 (for T cells) and 1.01 ± 0.04, 22.21 ± 2.94 and 6.31 ± 0.56 (for B cells) for 10/142, 17/238 and 21/378 respectively. It is noted that these values are derived based on the in-house assay methodology used by each individual participant laboratory. Variabilities observed in flowcytometric, and solid phase single HLA-antigen bead (SAB) LX assays are further discussed in the report. One Lambda LX assays, arrived at average % PRA ± SEM values 2.63 ± 1.58%, 3.29 ± 1.91%, 92.05 ± 3.6%, 76.03 ± 7.0% for class I and 3.88 ± 2.47%, 2.99 ± 2.51%, 96.15 ± 2.06%, 74.66 ± 7.79% for class II for 10/142, 17/212, 17/238 and 21/378, respectively. Immucore LX evaluations were performed by limited number of participants. Results for class I antibodies in 10/142 were reported only by a single participant (% PRA value of 1%). 10/142 was evaluated as negative for class II and 17/212 was negative for both class I and class II. Mean % PRA ± SD values were calculated as 47 ± 4.24% and 14 .8 ± 1.13% (class I) and 22.05 ± 26.8% and 3.05 ± 0.07% (class II) for 17/238 and 21/378, respectively. Existing data based on thermally accelerated degradation study (*complete analysis for acceleration studies for 21/378 is pending*) and real time stability monitoring for all RR, indicate that the candidate reference materials are stable.

Majority of participants identified RR, 10/142 and 17/212 as negative in both FCXM and LX assays. 17/238 and 21/378 were similarly identified as positive by most collaborative study participants, however participant laboratories identified 21/378 as either strong or weak positive. Differences in assignment of strong versus weak positivity for 21/378 may be due to laboratory specific cut-off criteria and assay threshold and these attributes are further discussed in the report. Nonetheless inclusion of 17/238 in combination with 21/378 in assays will help identify different levels of alloreactivity and thereby help harmonize variations in assay cut-off criteria, threshold, and assay sensitivity.

Based on results from the collaborative study we propose the Expert Committee on Biological Standardization (ECBS) to endorse the proposal for establishment of the following four anti-HLA reference reagents for use in FCXM and SAB LX assays:

1. **10/142: WHO International reference reagent- Negative plasma for anti- HLA**
2. **17/212: WHO International reference reagent- Negative serum for anti- HLA**
3. **17/238: WHO International reference reagent- Strong positive plasma for anti-HLA**
4. **21/378: WHO International reference reagent- Weak positive plasma for anti-HLA**

The reference reagents will have no assigned unitage and will serve as qualitative intra-assay variability controls, providing a means for trend monitoring for FCXM and LX assays performed to support the evaluation of suitability of potential transplant candidates and listing criteria developed in transplant programs.

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Introduction

Reference reagents used for Flow Cytometry Cross-matching (FCXM) manufactured by NIBSC have been on the market since 2001. Conventionally used as flow cytometry and bead-based assay controls, reference materials are used as part of pre- and post-transplant alloantibody screening for donor/recipient organ/tissue matching as well as investigating adverse transfusion-related reactions, performed by clinical Histocompatibility and Immunogenicity (H&I) labs and Blood Services. Evaluation of patient's serum sample in FCXM and/or LX bead-based assay form the basis for guided decisions regarding patient treatment. Reference reagents which are used as negative, weak positive or strong positive anti-HLA controls run controls crucially influence the delivery of assays that may have impact on clinical decisions related to organ and tissue transplantations such as kidney, hematopoietic stem cell, patient specific organ transplantations and allow monitoring shifts in sensitization by anti-donor alloantibody detection in patients' sera following transplantation.

FCXM and LX bead-based assays are qualitative techniques for evaluating compatibility of potential organ transplant recipients and donors. Since presence of donor-specific antibodies leading to deleterious graft function determine the success of transplantation, several multicenter studies have emphasized the importance of selection and standardization of methods used for cross-matching. Thus, reference materials supporting recipient sample categorization are crucial to success of transplantation outcomes (Kute et al 2013[1]; Harmer et al 1996[2]; Shenton et al 1997[3]). Increase in IgG binding in comparison to a negative control indicates the presence of donor-specific antibodies which may lead to deleterious graft function. The assays can also be used to monitor levels of *de novo* alloantibody production, post transplantation (O'Rourke et al 2000[4]; Leffel et al 2005[5]). H&I labs determine individual thresholds based on negative and positive patient samples or in house controls for identification of elevated donor reactive alloantibodies in the recipient, indicative of sensitization. Interpretations of fluorescence intensity values and cut-offs based on in house negative controls, can not only be laboratory-specific but also vary based on assay sensitivity and variables intrinsic to the assay. Clinical interpretation based on the local transplant centre risk-benefit approach can therefore vary significantly in addition to the inherent assay variability. Organ transplantation into pre-sensitized patients can result in hyperacute rejection and thus inclusion of reference materials as run controls for stratification as negative, weak, or strong positive reactivity in patient samples can contribute to accurate determination of alloreactivity levels, imperative for ascertaining transplant compatibility. Clinical labs use reference reagent readouts for validation of bioassays and can aid harmonization of thresholds to evaluate the safe use of donor tissues or organs identified as compatible for transplantation. FCXM assay variability is influenced by various factors such as donor variability, sample handling, methodology adapted and flow cytometer performance. Inclusion of reference reagents as run controls with known inclusions (positive controls) and exclusions (negative controls) of anti-HLA antibodies as assessed by multicenter study can aid the Quality Assurance of the assays. Use of a stable reference preparation produced as a single batch will allow clinical labs to control for inter-assay and intra-lab variability, verify equipment setup and data analysis as

well as monitor training and qualification of new assay operators. It will also allow specific laboratories to determine stable cut-off values for determining safe alloantibody threshold in patient samples, enabling appropriate test validation.

As such, the aims of the present international collaborative study are:

1. To evaluate performance of 10/142, 17/212, 17/238 and 21/378 as negative and positive anti-HLA RR for use in FCXM and LX assays for detection of donor specific alloantibodies critical in determining post-transplantation outcome
2. To assess suitability of the indicated anti-HLA RR to in house procedures adopted by laboratories involved in clinical, pre-transplantation and post transplantation screening and monitoring
3. To assess stability of the indicated anti-HLA RR by accelerated thermal degradation studies and real time stability monitoring

Participants

21 laboratories from eight countries took part in the study and are listed in **Table 1**. Throughout the study, each participating laboratory is referred to by a randomly assigned, unique code number. The order indicated in the table does not reflect the numerical order of participant codes referenced in the report.

TABLE 1: List of participants

Country	Institution	Contact
Australia	Australian Red Cross Lifeblood Platelet & Neutrophil Reference Laboratory, 44 Musk Avenue, Kelvin Grove 4059	Mark Burton
Australia	Victorian Transplantation and Immunogenetics Service, Australian Red Cross Lifeblood, 100-154 Batman Street, West Melbourne 3003	Ian Nicholson, Mary Diviney, Cathie Hart, Megan Kummrow
Australia	PathWest Department of Clinical Immunology, 9 Robin Warren Drive, Murdoch, Perth 6150	Dianne De Santis, Jonathan Downing
Canada	Histocompatibility and Immunogenetics Laboratory, McGill University Health Centre, Room E4-5049, 1001 Decarie Boulevard, Montreal Quebec H4A 3J1	Daphnay Eliacin, Chee Loong Saw
France	Laboratoire d'histocompatibilité, Bâtiment biologie-biopathologie CHRU de Nancy, Hôpitaux de Brabois, Rue du Morvan, Vandœuvre-lès-Nancy 54500	Michaël Peres, Alice Aarnink, Sandra Clement
France	CHU De Bordeaux, Laboratoire d'Immunologie et Immunogenetique, Hopital Pellegrin, Place Amelie Raba Leon, Bordeaux 33076	Elodie Wojciechowski, Jerome Bonnet
France	Laboratoire HLA, EFS Centre-Pays de la Loire, 34, Boulevard Jean Monnet, Nantes Cedex 44011	Alexandre Walencik, Valerie Coutinho
Ireland	Histocompatibility and Immunogenicity Laboratory Beaumont Hospital, Beaumont Road, Dublin 9, DO9V2N0	Caitriona Leslie, Joseph Kelly
Portugal	Instituto Português do Sangue e da Transplantação - Porto, Rua do Bolama 133, Porto 4200-139	Paula Xavier
The Netherlands	Maastricht University Medical Center, Transplantation Immunology, Tissue typing Laboratory, P. Debyelaan 25, Maastricht, 6229HX	Lotte Wieten, Christien Voorter
UK	Anthony Nolan Round Table Laboratories, 77 Fleet Road, London NW3 2QU	Ray Fernando, Katie Butler
UK	Transplantation Laboratory, Manchester Royal Infirmary, Oxford Road, 2nd Floor, Purple Zone, Manchester M13 9WL	Steven Jervis, Patrick Flynn, Marie Hampson
UK	UK NEQAS for H&I, Welsh Blood service, Ely Valley Road, Talbot Green CF72 9WB	Deborah Pritchard, Ryan Stevens
UK	Scottish National Blood Transfusion Service, H&I Lab, Edinburgh Royal Infirmary, 51 Little France Crescent, Edinburgh EH16 4SA	Kelly Spence, Angie Abel, Sylvia McConnell
UK	Transplant Immunology Dept, Oxford transplantation Centre, Churchill hospital, Oxford OX3 7LE	Jeanette Ayers
UK	Leeds Teaching Hospitals NHS Trust, Transplant and Cellular Immunology Laboratory, Level 09 Gledhow Wing, St James's University hospital, Beckett Street, Leeds, LS9 7TF	Mark Lobb
UK	Transplant Laboratory, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW	Stephen Weston, Maureen Bloxham, David Wimbury, Paul Dunn, Kirti Odedra
USA	University of California San Diego Immunogenetics and Transplantation Laboratory, 10300 Campus Point Drive Suite 150, San Diego CA 92121-1504	Gerald P Morris, David Nguyen
USA	Histocompatibility and Molecular Genetics Laboratory American Red Cross, Penn-Jersey Region, 700 Spring Garden Street, Philadelphia 19123	Mary Carmelle Philogene, Scott Webber, Michael Gerty, Daniel Kelly, Melanie Baron
USA	Bloodworks Northwest, HLA Laboratory, 921 Terry Avenue, Seattle 98104	Danny Youngs
USA	UCSF Immunogenetics& transplantation Laboratory, Laurel Heights Campus, 3333 California Street, Suite 150, San Francisco 94403	Young Cho

Principles of FCXM and LX assays used for alloantibody detection

FCXM reference reagents produced by NIBSC over the last 20 years have been used as controls in flow cytometry and bead-based assays performed by clinical H&I labs and Blood Services for pre- and post-transplant alloantibody screening and matching of organ donors and recipients, as well as for investigating adverse transfusion-related reactions. FCXM assay consists of incubating donor peripheral blood mononuclear cells (PBMCs) with recipient patient sera/plasma samples to analyze the presence of donor reactive alloantibodies. Presence of bound IgG is detected using fluorochrome conjugated secondary anti-human antibodies. Binding of anti-HLA class I (binding to both T and B cells) versus class II antibodies (binding to B cells only) can be flow-cytometrically distinguished by staining T and B lymphocyte populations with fluorochrome labelled cell type specific antibodies. Fluorescence intensity values are subsequently determined to serve as a measure of anti IgG binding to donor cells that corresponds to the levels of HLA antibodies in the test sample. Ratio of fluorescence intensity observed in test samples to that of negative reference samples provides the relative fluorescence intensity (RFI) value as an index for presence or absence of alloreactive antibodies. H&I labs determine their individual RFI cut-offs based on in house negative and positive sera samples as indicative of elevated donor specific alloantibody levels in circulation. RFI cut-offs can therefore be laboratory-specific and clinical interpretation of risk-related level for patients can vary significantly between transplant centres.

Luminex based solid phase HLA antibody screening technology and detection methods have improved our understanding of the role of HLA antibodies in graft rejection (Tait BD 2016[6], Ravindranath et al 2021[7]). LX-based alloantibody screening is widely used in laboratories affiliated to tissue/organ transplantation programs and enables identification of positive/negative antibody status as well as alloantibody identification/monitoring with high sensitivity and specificity. Currently, two main LX-SAB kits for HLA alloantibody profiling are commercialized and marketed by One Lambda/ThermoFisher and Immucor. One Lambda Labscreen kits use microbeads, coated with purified class I or class II HLA antigens containing fluorochromes of differing intensity giving each bead group a distinct fluorescence characteristic. Immucor Lifecodes LSA uses recombinant HLA molecules for all HLA-A, HLA-B and HLA-Cw and HLA-DR, -DP and -DQ for alloantibody capture. Serum samples are incubated with beads and HLA antibodies present in the test samples bind to appropriate HLA-coated beads. Detection of serum IgG bound to bead linked antigens is subsequently performed by incubation with phycoerythrin, PE- conjugated anti-human IgG, following washes. Upon excitation by one of the lasers built into the Luminex instrument, each HLA-specific bead gives a unique signal corresponding to the specific intensity/ fluorochrome signature for the bead. Detection of PE signal from secondary anti-human IgG antibody by the second laser indicates the presence of specific HLA antibody. Based on PE fluorescence emission from each bead and reaction profile of test serum, the Luminex platform enables HLA specificity assignment based on comparison to lot-specific worksheet defining the antigen array. Data analysis is carried out using HLA Fusion software for One Lambda kits or Match

IT for Lifecodes LSA Single Antigen Antibody detection kits (Immucor). Alloantibody levels are semi-quantitatively translated into MFI readout and/or Percent Reactive Antibody (% PRA) which is the percentage of beads in the panel giving positive results with the test sample. However, it must be noted that MFI values obtained using single antigen beads may not be representative of antibody titre since degree of antigen saturation varies on individual beads and therefore HLA antibody-binding site on the bead, may not comprehensively reflect reactivity of serum antibodies on cells *in vivo*. Both FCXM and LX assays are comprehensively used as part of decisions for pre-transplantation screening and post-transplantation monitoring. Inherent to the assay methodologies are variabilities associated with each method. Since FCXM is a cell-based assay characteristics of donor PBMCs, staining antibodies, assay procedures may all contribute to variations and therefore the method is not harmonized due to use of widely different assay protocols and interpretations of results based on laboratory specific cut-off criteria. In contrast Luminex assays are performed with kits available from 2 manufacturers covering slightly different MHC molecules and having different sensitivities. Assays performed with prescribed volumes and in accordance with manufacturer's instructions might thereby reduce the variability associated with LX evaluations.

Product description and processing

10/142 consists of 1ml of freeze-dried pooled human AB plasma containing background levels of anti-HLA class I and class II antibodies and was manufactured from plasma donations screened for the absence of anti-HLA class I and II antibodies by LX assay as well as FCXM using peripheral blood mononuclear cells (PBMC) from healthy donors. The product has been on the NIBSC catalogue as a CE-marked IVD since 2010 until September 2022. The material was assessed as negative by a collaborative study conducted with 3 UK labs in 2009. Since serum and plasma negative standards give different background results, laboratories may prefer either anti-HLA negative plasma or negative serum control based on the biological test samples used for evaluation. The reference reagent 17/212 was subsequently developed as a serum-based, low background control in 2017. The material consists of 0.5ml of freeze-dried pooled human AB sera obtained from Welsh branch of National Blood Service (NBS). Both 10/142 and 17/212, with varying background levels are designed to be used as negative controls in FCXM and in anti HLA- bead-based LX assays.

Anti-HLA Positive Control for FCXM and LX assays, 17/238 was manufactured at NIBSC in 2017. The pool was generated from individual donations of anti-HLA antibody-positive plasma that have been obtained historically from the National Blood Service (NBS) as H&I material from highly sensitized patients or were purchased from LabSera from multiple-pregnancy donors. The product was validated in house as reference material to provide robust positive readouts in FCXM assay and LX bead-based assays. An international collaborative study CS617, was earlier conducted with 81 laboratories, to establish fitness for purpose and performance evaluation in the hands of end users for both 17/238 and 17/212 and both products have been on the NIBSC catalogue as CE-marked IVD since 2018 until September 2022.

21/378 was developed in 2021 as a replacement for an earlier HLA weak positive reference 07/214, that was on the catalogue since 2008 and currently the stocks are depleted. Many H&I laboratories use weak positive controls for detection of alloantibodies just above the cut off point for anti-HLA antibody presence. Intermediate and weak positive controls serve as run controls that allow assay validation, trend monitoring and verification of acceptance criteria for sensitivity. Less sensitive assays, or assays that use negative controls which give high background are unable to detect weak positive samples, highlighting the significance of complementary use of strong and weak positive reference reagents to identify varying thresholds of alloreactivity. 21/378 was initially evaluated in a study conducted with UK NEQAS and found fit for purpose as having a lower level of positivity in comparison to the strong positive, 17/238. Both 21/378 and 17/238 are designed as positive controls with different levels of anti-HLA antibodies.

Production and fill details for anti-HLA RR

As described earlier, 10/142, 17/212 and 17/238 were available on NIBSC catalogue as CE-marked IVDs since 2010 and 2018 respectively. 7712 vials of 1ml freeze-dried human plasma were produced in October 2010 as 10/142. Since then, they were stored at 4°C. 9652 vials of 0.5ml freeze-dried human AB serum negative for anti-HLA antibodies was produced in November 2017 as 17/212 and 10,273 vials containing 0.5ml freeze-dried human plasma positive for anti-HLA antibodies, were produced in January 2018. Both 17/212 and 17/238 are stored at -20°C. An identical cycle was used for freeze-drying of all four reference materials 10/142, 17/212, 17/238 and 21/378. Since 21/378 is a new product, the report will describe the production in further detail. All reference materials held at NIBSC are stored within assured, temperature-controlled storage facilities.

Bulk material was sterile filtered prior to fill. Filling of bulk material for 21/378 was undertaken at the Centre for Biological Reference Materials, NIBSC, in March 2022. A 5-day lyophilization cycle was carried out in the CS-150 freeze drier (Serail, France). The bulk was constantly stirred during the filling process and was dispensed in 0.5g aliquots into 5ml type I glass screw cap vials (Adelphi Healthcare Products, Haywards Heath, UK) using a Bausch and Strobel vial filling line (AFV 5060). Vials were weighed before and after filling ensuring weights stayed within filling limits and enabling the calculation of the coefficient of variation (CV). Filled vials were then partially stoppered and placed in the freeze dryer with a shelf temperature of 4ºC. The shelf temperature was then dropped to -50ºC and then a vacuum pulled to 100µb before the temperature was raised to -35ºC for primary drying for 2884 minutes. The shelf temperature was then ramped to 25ºC over 420 minutes and vacuum increased to 30 µb and held for 2760 minutes for secondary drying. Once lyophilization was complete, the freeze dryer chamber was backfilled with nitrogen obtained from the boil off from liquid nitrogen, and stoppers fully inserted into the vial before removing them from the freeze dryer. The vials were then capped on the same line. Capped vials were placed into continuously temperature monitored storage at -20 °C for the lifetime of the product. A total of 8966 21/378 vials were

put onto stock. Coefficient of variation (CV) was calculated at 0.40% using 302 check weights (3% of vials filled). Oxygen concentration in the headspace of the ampoule was measured at 0.63% using non-invasive frequency modulated NIR spectroscopy at 760nm using FMS instrument equipment (Lighthouse Instruments, Charlottesville, Virginia, USA). 12 samples were measured from across the batch. Residual moisture in the lyophilized cake was measured at 0.41% by manual Karl Fisher analysis using a CA-200 moisture meter (Mitsubishi, Tokyo, Japan). During the manufacturing process for 17/212, the oxygen concentration in freeze-dried vials was slightly over the normal acceptance limits. Re-testing performed by production, ensured that the stability of the product should not be affected because of this anomaly.

There is no claim for sterility of the reference materials, however individual donation of candidate materials and final pool prior to filling are sterile filtered. In addition, all vesicles, tubing, pumps, and needles used for the filling of the product are autoclaved to reduce the risk of contamination. Bioburden (total viable count) was tested in the prefilled bulk, liquid ampoules and lyophilized cake and showed no counts in the bulk, liquid vials, and lyophilized cake. Fill details of all four RR are summarized below (**Table 2**)

TABLE 2: Anti HLA RR fill summary

Code number	10/142	17/212	17/238	21/378
Presentation	5ml screw cap vials	5ml screw cap vials	5ml screw cap vials	5ml screw cap vials
Number of ampoules	7712	9590	10273	8966
Date filled	August 2010	December 2017	December 2018	March 2022
Mean fill mass (g)	1.0060 (n=78)	0.5010 (n=324)	0.5138 (n=344)	0.5126 (n=302)
CV of fill mass (%)	0.1932	0.4261	0.3064	0.3954
Mean dry weight	0.0802	0.04270	0.04113	0.04065
CV of dry weight (%; n=6)	0.28	0.4127	0.55	0.61
Mean Residual moisture (%; n=12)	0.5432	0.27951	0.0749	0.4
CV of residual moisture (%; n=12)	20.64	22.05	12.99	13.03
Mean oxygen head space (%; n=12)	0.06 (n=9)	1.36	0.75	0.63
CV of oxygen space (%; n=12)	77.06	6.31	9.4	18.21
Microbiological results	Negative	Negative	Negative	Negative
Storage conditions	4°C	-20°C	-20°C	-20°C
Address of processing facility	NIBSC, Potters Bar, EN6 3QG, UK			
Address of custodian	NIBSC, Potters Bar, EN6 3QG, UK			

Current RR status and stocks

Three NIBSC manufactured anti-HLA controls (10/142, 17/212 and 17/238) have been marketed as CE-IVD reagents for over 5-12 years. However, since September 2022, 10/142, 17/212 and 17/238 are no longer CE-marked IVDs and currently unavailable on the NIBSC catalogue. There is demand for the reference materials and customers for the RR are from national and international labs including those outside the EU. Details of current stocks of the reference materials (**Table 3**).

TABLE 3: Current NIBSC stocks of anti HLA RR

NIBSC code	Vials in stock
10/142	3087
17/212	6193
17/238	8560
21/378	8786

Post fill characterization and stability monitoring

Anti-HLA RR are monitored annually for stability by FCXM, and the products are found to be stable at the designated storage temperatures (-20°C for all except 10/142, which is stored at 4°C). Post fill characterization of samples from beginning, middle and end of fill lines were performed for all anti-HLA RR for homogeneity assessments at production and results obtained with 21/378 that is recently manufactured are shown as a representative for the current report. Characterization of 21/378 material was performed by FCXM assays using 4 PBMC donors and LX (One Lambda) assays from samples collected at the beginning, middle and end of the fill line (**Figure 1**). Statistical significance for T cells and B cells in FCXM assay was determined using one way ANOVA, Tukey's multiple comparison test with GraphPad Prism (version 8.1.1) software; and there were no significant differences (p values > 0.5). These results indicate the homogeneity of fill samples at production.

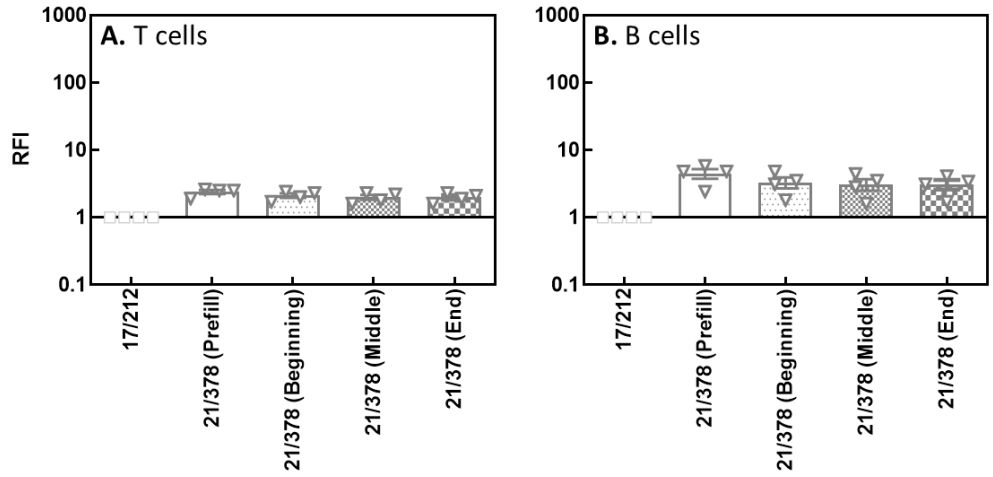

FIGURE 1: Post fill characterization of 21/378 by FCXM assay. *Samples of RR from beginning middle and end of the definitive fill line were tested in FCXM assay. Each symbol on the plots indicates an individual donor and a total of 4 donor PBMCs were testes. RFI values were calculated by determining ratio of the fluorescence values obtained for the test samples against negative RR, 17/212. RFI values for T cells (A) and B cells (B) are depicted.*

One Lambda LX assay showed comparable %PRA values for the pre-fill, and post fill samples for 21/238 collected from the beginning, middle and end of the fill line (47%, 46%, 48% and 52% for MHC class I and 29%, 37%, 31% and 45% for MHC class II). Representative antibody specificities as identified by OneLambda against the following class I and class II molecules were detected in the test samples.

MHC class I: A2, A24, A34, A68, A69, B7, B8, B18, B27, B37, B38, B39, B41, B42, B44, B45, B46, B54, B55, B59, B60, B61, B64, B67, B73, B76, B81, Cw1, Cw5, Cw6 Cw7, Cw8, Cw9, Cw10, Cw12, Cw14Cw15, Cw16, Cw17, Cw18

MHC class II: DR1, DR4, DR8, DR9, DR10, DR11, DR12, DR13, DR14, DR15, DR16, DR17, DR18, DR52, DR53, DP1, DQ5, DQ6

Accelerated degradation testing on anti-HLA reference materials produced at NIBSC are routinely carried out from sample vials stored at 37°C, 20°C, 4°C and -20°C for 3-, 6- and 9-months post-production. Samples are evaluated for stability using FCXM assay with at least 3 individual donor PBMC. Extrapolation of activity data obtained in accelerated stability studies is extrapolated to shelf life using the Arrhenius equation which relates chemical reaction rate to the absolute temperature. The equation has been widely applied to determining chemical reaction rates and model temperature variation effects on thermal degradation of test samples. Historic monitoring data from studies conducted for earlier manufactured reference materials similar to 10/142 indicate that the products are very stable over an extended number of years and storage at 4°C would result in less than 0.3% loss in potency. Based on these results, an initial expiry date of 10 years from the date of freeze-drying was assigned to 10/142 and further extension to this initial expiry date was applied following real time stability monitoring and a

current expiry date of June 2025 was designated. Accelerated degradation studies conducted earlier for 17/212 and 17/238, however did not fit the Arrhenius equation model due to inherent variability of the FCXM as cell-based assay and no annual loss of potency could therefore be established. Based on the performance of all anti-HLA standards manufactured at NIBSC, an initial expiry date of 10 years from the date of freeze-drying was similarly assigned to 17/212 and 17/238. Expiry dates were assigned to the reference materials as a requirement of CE-marking. We will now continue to monitor the reference materials for stability in real time and the reference materials will not have an assigned expiry date. 21/378 is a newly manufactured RR and results from accelerated degradation studies are discussed in detail here as an example. Samples stored at indicated temperatures (37°C, 20°C, 4°C and -20°C) were tested in 2-3 independent FCXM assays using 4 individual PBMC donors for each time point (**Figure 2**). At the time of writing the report, data for the 9-month time point (to be tested after 15th January 2023), are not available and preliminary results from the 3 and 6-month monitoring are depicted. Data will be re-analyzed to evaluate fit to the Arrhenius equation once samples from 9-month time point are assessed, since this may change following inclusion of the 9-month evaluation results.

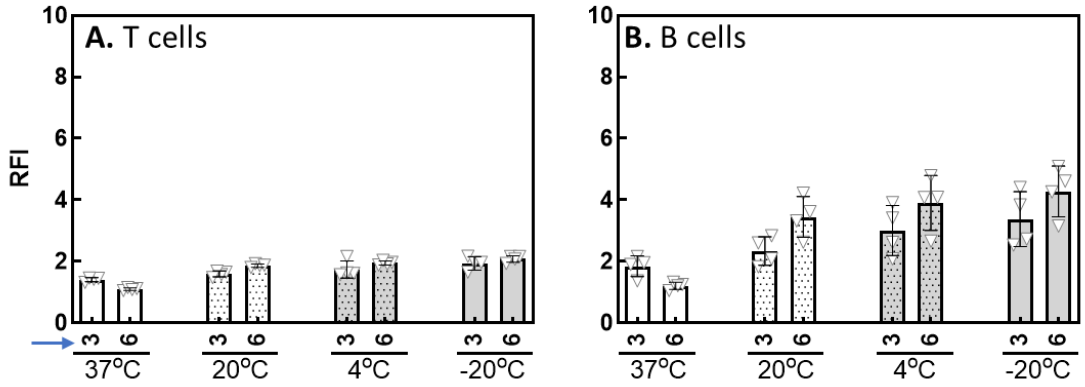

FIGURE 2: Accelerated degradation study for 21/378 by FCXM assay. *21/378 samples were tested at 3- or 6-months post-production by FCXM assay with donor PBMC. Samples were stored at indicated temperatures for 3 or 6 months prior to analysis. Each symbol represents average RFI values from at least 2 independent FCXM assays performed with the same donor PBMC. RFI values were determined as ratio of the fluorescence values obtained for the test samples against negative RR, 17/212. Four individual donors were tested in each assay. RFI values were calculated against anti-HLA negative RR, 17/212. RFI values for T cells (A) and B cells (B) are depicted. Blue arrow indicated the time in months after production.*

Based on the Arrhenius equation the following annual cumulative loss percent were calculated for T cells as follows:

- A cumulative 2.41% loss in potency per year when stored at -20°C
- A cumulative 14.45% loss in potency per year when stored at 4°C
- A cumulative 36.504% loss in potency per year when stored at 20°C
- A cumulative 71.415% loss in potency per year when stored at 37°C

RFI values generated for B cells did not fit the Arrhenius equation model and therefore activity values relative to storage at -20°C were calculated as 0.57%, 0.71%, 0.87% at 37°C, 20°C and 4°C respectively at 3 months and 0.29%, 0.8%, 0.9% at 37°C, 20°C and 4°C respectively at 6 months.

Due to the qualitative nature of the assays, readouts can vary based on the methodology and instrumentation performance on the day of assay in addition to donor variability making it more appropriate to monitor real-time performance of the standard in relation to other anti-HLA controls such as 10/142 or 17/212 for FCXM. Real-time performance monitoring data for 10/142 over 139 months and for 17/212 and 17/238 covering 49 and 56 months respectively are represented (**Figure 3**). Data for 10/142 illustrate that these RR are stable for more than 10 years which also has been observed for previous FXCM RR (**Figure 3**). In-house cut-off acceptance criteria for stability monitoring established based on real time data for each RR are represented by dotted lines. 21/378 is a new RR and since the available data are limited to a few time points, acceptance criteria represented are based on an earlier weak positive RR. Stability of the RR can be proven based on the data aligning with the indicated acceptance criteria and the following results demonstrate that the RR are stable.

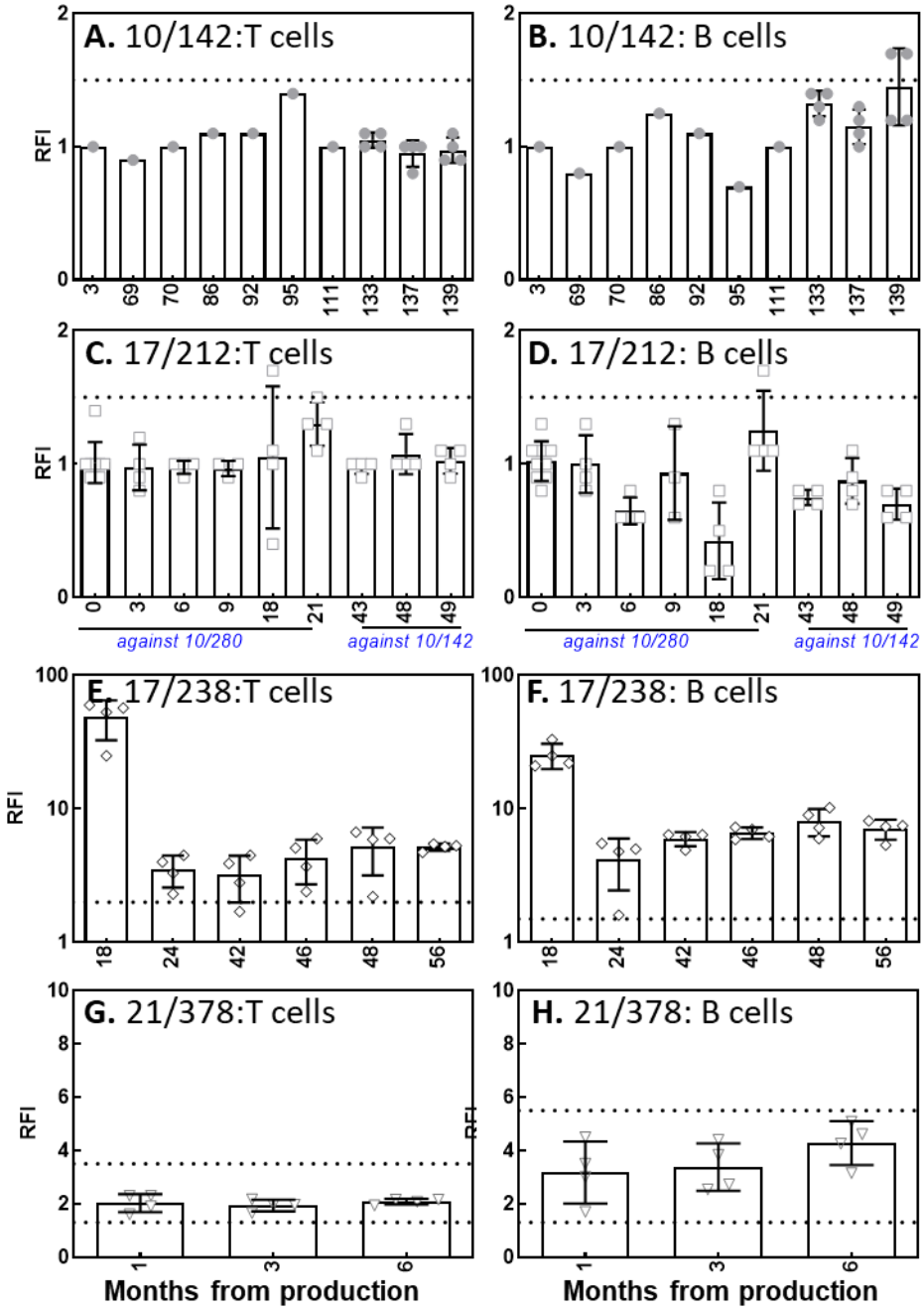

FIGURE 3: Real time stability monitoring of anti-HLA RR post-production by FCXM assay. *Samples of RR were tested at indicated time points by FCXM assay with donor PBMC. Each symbol represents results corresponding to 1 donor. RFI values (calculated by determining ratio of the fluorescence values obtained for the test samples against the negative RR 17/212) for 10/142 (A, B); 17/238 (E, F) and 21/378 (G, H) are represented. RFI values for 17/212 (C, D) were calculated against an earlier anti-HLA negative control, 10/280, manufactured at NIBSC and for time points after 49 months and later, values are calculated against anti-HLA negative control 10/142. RFI values for T cells (A, C, E, G) and B cells (B, D, F, H) are depicted. Dotted*

lines indicate in house acceptance criteria for each RR. The upper cut-off for negative RR (A-D), lower cut-off for 17/238 (E, F) and acceptance range for 21/378 (G, H) are represented.

As discussed above, it may not always be possible to identify a fit to the Arrhenius equation to assess product's degradation rates and it is proposed that real-time stability of samples stored at 4°C (for 10/142) and -20°C (for 17/212, 17/238 and 21/378) will continue to be assessed annually at NIBSC. In case there is evidence of substantial product degradation at any point, as evidenced in real-time stability monitoring studies or reported by the customers, the product will be removed from the catalogue.

WHO collaborative study design for evaluation of anti HLA RR

The present international collaborative study, **CS708**, was organized by NIBSC. The study was conducted to support the proposal for conversion of the indicated anti-HLA RR to WHO International RR. The proposed conversion will allow use of these reagents as run controls to support pre- and post-transplantation screening programmes conducted in labs outside of EEA, thereby aiding global public health and standardisation.

Participants were provided 6x vials of each RR coded as sample A, B, C and D for evaluation. Samples were shipped by the sales and dispatch team at NIBSC during July 2022. Instructions for evaluation and use (**Appendix 1 and 2**) were provided with the samples. Sample vials provided for the study, which were identified only by a single letter code during the evaluation, are listed in **Table 4.**

TABLE 4: Collaborative study samples provided to participants

Code	Reference material code	Description
A	**10/142**	Negative plasma for anti HLA
B	**17/212**	Negative serum for anti HLA
C	**17/238**	Strong positive plasma for anti HLA
D	**21/378**	Weak positive plasma for anti HLA

Participants were requested to perform FCXM and LX assays in accordance with their in-house SOPs to evaluate the RR and report methodology details and evaluation results for FCXM and LX bead-based assays in the questionnaires shown in **Appendix 3-6**. Additionally, participants were asked to comment on suitability of the candidate materials as anti-HLA RR for their individual needs. Participant returned results during Oct-November 2022 and all results were analyzed centrally at NIBSC. Participants were requested to send raw data alongside their in-house negative and positive controls readouts, run in the same assay. Additionally, labs were

asked to assign positive/negative/weak positive or equivocal sample with details on the cut-off criteria for each of the assays. Participants were requested to evaluate samples with at least 3 different donor PBMCs in FCXM assays and Immucore and One Lambda LX assays for determination of anti-HLA reactivity. Participant labs were also asked to provide information on the H&I guidelines followed to ensure the international standards of assay performance. Methodology details were received from 90.5% of the participants. Of the participants who returned methodology details, 37% and 21% followed either American Society for Histocompatibility and Immunogenetics (ASHI) or European Federation for Immunogenetics (EFI) guidelines respectively, 26% followed both EFI and British Society for Histocompatibility & Immunogenetics/ British transplantation society (BSHI/BTS) guidelines. In addition, 5% of participants each followed BSHI/BTS exclusively or in combination with ASHI. Similarly, a further 5% participants followed a combination of ASHI and/or other guidelines. This suggest that all the participants taking part in the study have been identified as following one of the internationally renowned H&I standards of high quality.

From a total of 21 participants, 20 evaluated the material in FCXM assays and 18 laboratories performed both FCXM and LX bead-based evaluation (Figure **4**)

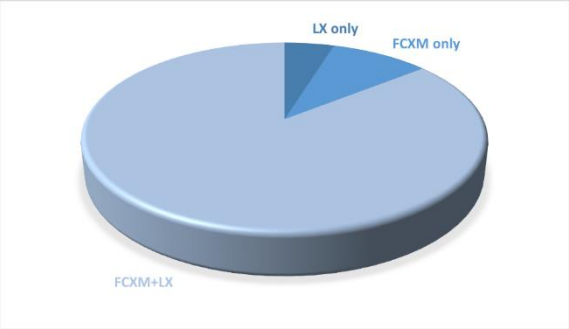

FIGURE 4: Relative proportion of participants performing FCXM or LX assays. *Participants conducted either FCXM or LX assays and 85.7% participants performed both evaluations, 9.5% only FCXM and 4.8% only LX assay.*

Participants' interpretation of the sample's identity as positive, weak positive or negative was also requested**.**

Assessment by FCXM assay

FCXM is a sensitive cell-based, flowcytometric method for detection of anti-HLA antibodies. A positive readout is contraindicated for transplantation. Participant laboratories were requested information on the scope of FCXM assay in their respective laboratories. All participants reported the use of FCXM for pre-transplant cross matching and 44% used the assay for post-transplantation cross matching. 61% labs had a clinical/diagnostic usage, 17% performed FCXM as part of research and development and 6% reported other usage including cell lineage purity analysis. FCXM gating strategy employed at NIBSC and representative profiles for 10/142, 17/212, 17/238 and 21/378 obtained in house are shown below (**Figure 5**). As seen with the

histogram overlays, HLA expression on 10/142 and 17/212 are overlapping and negative. 17/238 shows high expression on both T and B cells as a strong positive RR and while 21/378 is also positive, levels of HLA expression are lower in comparison to the strong positive RR,17/238.

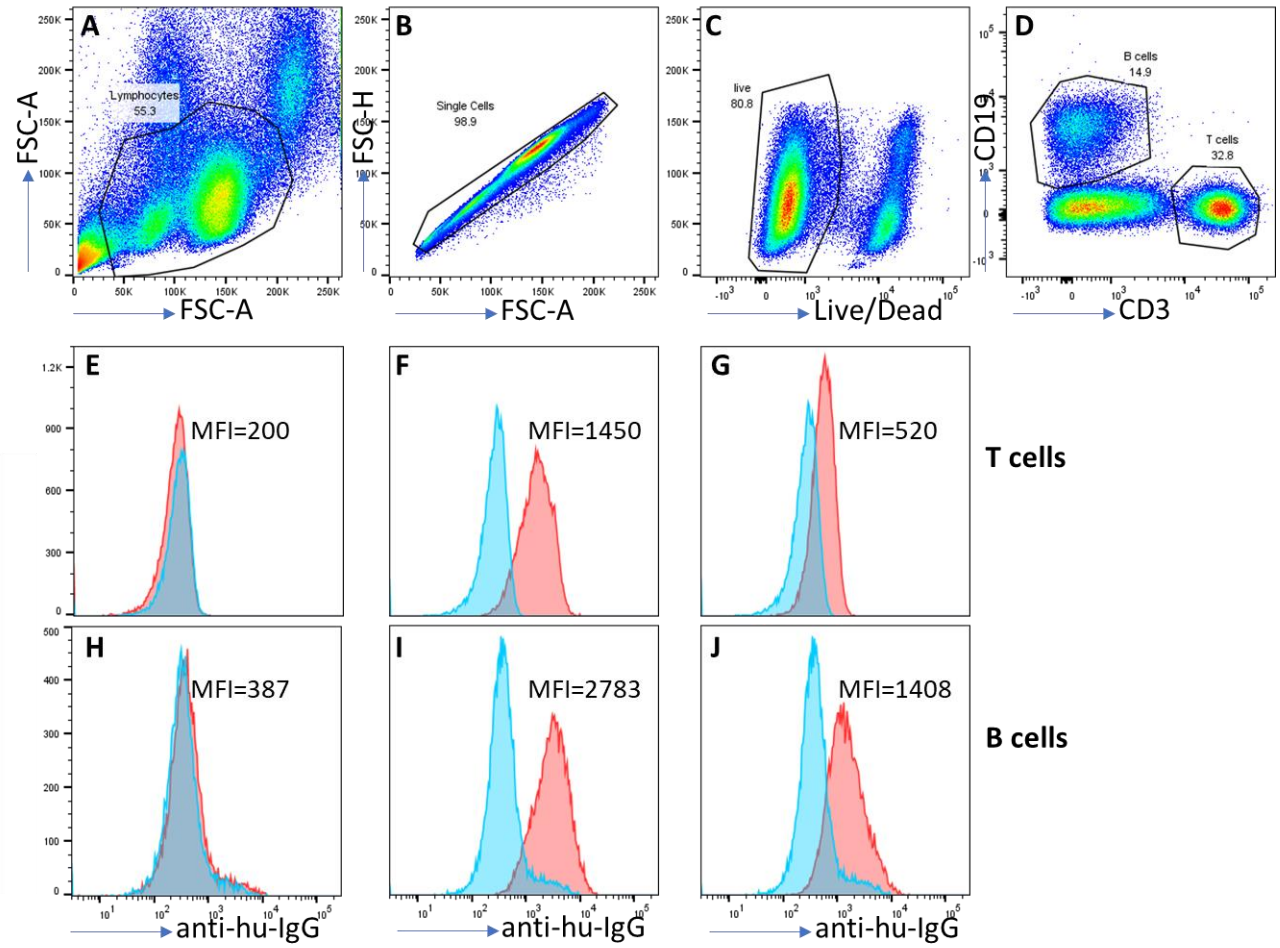

FIGURE 5: Representative gating strategy used at NIBSC for determining HLA expression on T and B cells. *(A) Donor PBMCs are gated for lymphocytes based on scatter profile. (B) Singlet lymphocyte subsets are identified. (C) Live lymphocytes identified using Aqua live/dead viability stain are subsequently 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.*

FCXM assays are sensitive and time-consuming assays, critically influenced by the cell isolation method, cell numbers used for the assay, incubation times and flowcytometry platform used for analysis. In order to gain an insight into the methodology used for the evaluation, participants were requested to test the samples in accordance with their in-house protocols, alongside in-house controls. Samples were to be ideally and as possible tested with 3 different donor cells and in 2-3 replicates, however participants could test on more than the recommended 3 different sets of donor

cells. Participants isolated PBMCs according to their in-house procedures. Donor lymphocyte enrichment procedure and haplotype characteristics of donor PBMCs contribute to variations inherent to the FCXM assay. A wide range of cell isolation media were utilized in the study. Of the participants, who returned methodology questionnaires, majority (67%) used density gradient methods for PBMC isolation such as Ficoll, Lymphoprep and other preparations. 33% participants used EasySep direct lymphocyte enrichment technology or automated RoboSep instruments, without the requirement for density gradient centrifugation. Implementation of Halifax (traditional density gradient based) or Halifaster (non- density gradient based) protocols (Liwski et al 2018[8]) may have a significant impact on isolation times and cell purity in pre-transplantation clinical facilities and thereby influence the outcome of FCXM assay.

Nonspecific binding of immunoglobulins to Fc receptors on B cells can result in background reactivity in FCXM assays. While most participant laboratories used isolated lymphocytes without pre-treatment, 28% included pronase/DNAse pre-treatment following lymphocyte enrichment, that could enhance specificity and sensitivity in FCXM assays by reducing background Fc receptor binding. However, since functional targets of such treatments are not completely understood and pre-treatment may also affect HLA molecules, in turn impacting FCXM reactivity, there are varying viewpoints in the field regarding inclusion of a pre-treatment step for PBMCs.

Similarly, pre-treatment of serum/plasma samples prior to inclusion in FCXM assay is another approach used to reduce the background in FCXM assay. Most participants used the serum sample for testing without any prior treatment, however 33% used de-complementation, DTT treatment, flash-freeze procedure and/or airfuging methods. Various parameters were recorded in the methodology followed by participants including the staining volumes, temperatures, cell numbers and incubation times. It must be noted that assays conducted in the current study are multivariant and therefore it is not possible to conclusively assess the impact of a particular individual parameter as part of the study however it is of importance to note the methodology variabilities that can affect the intra-laboratory comparison. An important characteristic of the FCXM assay is determined by the antibody combination used for staining. All participants who returned FCXM methodology questionnaires used anti-CD3 for identifying T cells. 94% of participants used CD19 as a marker for B cells and 1 participant (6%) reported the use of CD20 to identify B cells. HLA antibody detection was performed by using Fab_2 (89%) or whole (12%) anti-human IgG as secondary antibody. Several fluorophore combinations, antibody clones, host species and suppliers for various antibodies used during the study are summarized below (**Table 5**). Overall perCP/percy5.5 (50%), PE (56%) and FITC (100%), were the predominantly used by participants as fluorophores to identify T, B cells and IgG respectively.

TABLE 5: Summary of staining antibodies used for FCXM in the collaborative study

Antibody detail	Fluorochrome	Host species	Supplier	Participant (%)
Anti-human IgG-Fab$_2$	FITC	Goat	Beckman Coulter, Southern biotech, Sigma, Jackson ImmunoResearch Labs	72
	FITC	Rabbit	Dako	17
Anti-human IgG-whole	FITC	Goat	Southern biotech	6
	FITC	Mouse	BD	6
Anti-human CD3	perCP/perCPcy5.5	Mouse	BD	50
	PE/RPE	Mouse	BD, Beckman Coulter, Dako	28
	APC	Mouse	BD, Beckman Coulter	17
	PC5	Mouse	Beckman Coulter	6
Anti-human CD20	PE	Mouse	BD	6
Anti-human CD19	PE	Mouse	BD	50
	perCPcy5.5	Mouse	BD	6
	RD1-PE	Mouse	BD	6
	APC	Mouse	Dako	6
	BV421	Mouse	BD	6
	PC7	Mouse	Beckman Coulter	6
	PE-cy7	Mouse	BD	6
	R-PEcy5	Mouse	Biorad	6
	PC5 (R-Phycoerythrincyanin 5.1)	Mouse	Beckman Coulter	6

Flowcytometry platforms used in the collaborative study included instruments from Beckman Coulter such as Navios (11%) and Dx Flex (6%) in addition to FACS Lyric (56%) and FACS

Canto platforms (28%) from BD. Majority of participants (83%) acquired samples directly following staining however 17% participants acquired samples following fixation with formaldehyde or paraformaldehyde-based fixation. Sample acquisition was carried out in tubes by most (61%) participants, while 39% acquired samples in plates.

A total of 86 donor PBMCs were evaluated during the collaborative study and while majority (65%) of participants performed assays with 3 donor PBMCs, 35% participants tested up to 12 donor PBMCs for the FCXM assays.

Relative fluorescence index was calculated centrally at NIBSC, from average raw fluorescence readouts provided for each donor PMBC, separately based on fluorescence values for NIBSC negative control, 17/212, sample B (**Figure 6A, C**) and the in-house negative control (**Figure 6B, D**), tested in the same assay. No significant difference was observed for samples A, B, C and D, when RFI values were calculated using NIBSC negative RR (17/212) as opposed to in-house negative controls. RFI values from participant 5 could not be calculated against local negative control, since the fluorescence values reported were set as zero and similarly RFI values for only 2 of 6 donors could be calculated against 17/212 for samples B, C, D and local positive due to negative fluorescence values for 4 of 6 donors. Since donor 2 was not tested for sample B, RFI values could not be determined for all the test samples for this donor against 17/212 and for samples A & B against local negative control from participant 21. The total number of RFI values recorded for each test sample are indicated in **Table 6**. Statistical significance for T cells and B cells was determined using One way ANOVA, Tukey's multiple comparison test with GraphPad Prism (version 8.1.1) software; and these statistical analysis of the data indicate that the reference material samples perform comparably when RFI values are calculated against NIBSC anti-HLA negative 17/212, or negative control used by each participant laboratory (p values > 0.5).

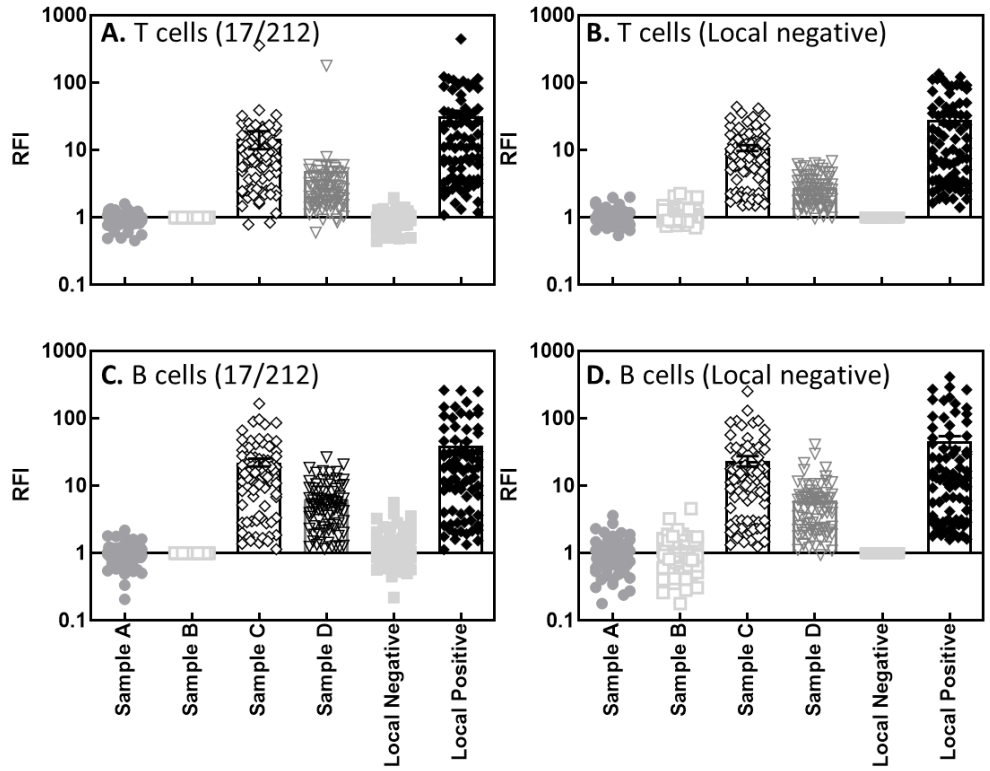

FIGURE 6: FCXM assay results for 10/142, 17/212, 17/238 and 21/378. *RFI values were calculated by determining ratio of the fluorescence values obtained for the test samples against NIBSC negative control, 17/212 (A, C) and local negative control (B, D) used by study participants in their assays. Values are separately calculated for T cells (A, B) and B cells (C, D) for each donor PBMC tested (represented by a symbol on the plot). Sample A: 10/142; sample B: 17/21; sample C: 17/238 and sample D: 21/378.*

Results from calculated RFI values as indicated above were combined to generate minimum and maximum value ranges, mean values and corrected n-values for each RR are summarized in table 5. Observed variability between laboratories has been expressed using coefficients of variation (%CV) and was found to be high for 17/238 and 21/378 on both T and B cells (**Table 6**). Mean RFI values calculated for 10/142 against 17/212 were 0.97$\pm$ 0.02 (T cells) and 1.01$\pm$ 0.04 (B cells), showing that both 10/142 and 17/212 behave comparably as negative RR. Mean RFI value calculated for negative RR (10/142 and 17/212) against the local negative controls were comparable for both T and B cells (**Table 6**). Similarly average RFI value of 14.6 $\pm$ 4.35 (T cells) and 22.21 $\pm$ 2.94 (for B cells) was calculated for 17/238. Mean RFI values for 21/378 calculated similarly as 4.86 $\pm$ 2.2 (T cells) and 6.31 $\pm$ 0.56 (B cells) fall within an intermediate range in comparison to strongly positive 17/238. Comparison of RFI values derived for each RR against local/in house, follow a similar pattern and 21/378 shows intermediate values for both T and B cells when compared to the strong positive control 17/238.

TABLE 6: Descriptive statistics for FCXM results

A.	T cells: 17/212 negative			
	10/142	17/212	17/238	21/378
MIN	0.45	1.00	0.79	0.59
MAX	1.59	1.00	354.90	178.00
MEAN	0.97	1.00	14.60	4.86
STDEV	0.19	0.00	39.18	19.66
SEM	0.02	0.00	4.35	2.20
N	79.00	81.00	81.00	80.00
RSD or %CV	19.29	0.00	268.41	404.48

B.	B cells: 17/212 negative			
	10/142	17/212	17/238	21/378
MIN	0.21	1.00	1.14	1.15
MAX	2.18	1.00	164.69	26.40
MEAN	1.01	1.00	22.21	6.31
STDEV	0.31	0.00	26.50	4.97
SEM	0.04	0.00	2.94	0.56
N	79.00	81.00	81.00	80.00
RSD or %CV	30.73	0.00	119.32	78.74

C.	T cells: Local negative			
	10/142	17/212	17/238	21/378
MIN	0.54	0.70	1.49	0.96
MAX	2.01	2.24	43.97	6.86
MEAN	1.02	1.08	10.76	2.71
STDEV	0.25	0.29	9.53	1.45
SEM	0.03	0.03	1.07	0.16
N	79.00	79.00	80.00	79.00
RSD or %CV	24.85	27.18	88.60	53.45

D.	B cells: Local negative			
	10/142	17/212	17/238	21/378
MIN	0.18	0.18	1.25	0.93
MAX	3.62	4.54	252.33	40.44
MEAN	1.02	1.07	23.40	6.05
STDEV	0.55	0.63	36.54	6.29
SEM	0.06	0.07	4.09	0.71
N	79.00	79.00	80.00	79.00
RSD or %CV	53.77	58.92	156.19	103.87

Based on assignments/interpretations reported by participant laboratories, test samples were classified as negative, weak, or strong positive and equivocal as detailed in the FCXM results questionnaire (**Appendix 3**). Majority of participants, 96.4% and 95.2% (for T cells) and 96.4% and 92.9% (for B cells), identified 10/142 and 17/212 as negative in FCXM assays. 17/238 was identified as strong positive for T cells by 94.1% participants and 100% for B cells. 21/378 was identified as negative, weak positive or strong positive by 8.2%, 37.6% and 48.2% respectively for T cells, 4.7%, 27.1% and 67.1% respectively for B cells (**Figure 7**). Based on results from the current collaborative study both 10/142 and 17/212 are identified as negative RR and 17/238 as a strong positive RR for both T and B cells. In order to differentiate the observed lower level of alloreactivity for 21/378 in comparison to strong positive 17/238, it is proposed to indicate 21/378 as a weak positive reference material, that maybe used in conjunction with strong positive control to identify different assay thresholds and increase sensitivity of alloantibody detection. It is noted that the proposed nomenclature is adopted as a differentiating nomenclature and positivity assignments may consequently vary depending on the in-house cut-off criteria used for evaluation at each individual laboratory. To sum up, majority of the laboratories assign samples as negative/positive with high accuracy and differences in assignment of strong versus weak positivity maybe attributed to laboratory and/or assay specific variations in threshold and assay sensitivity.

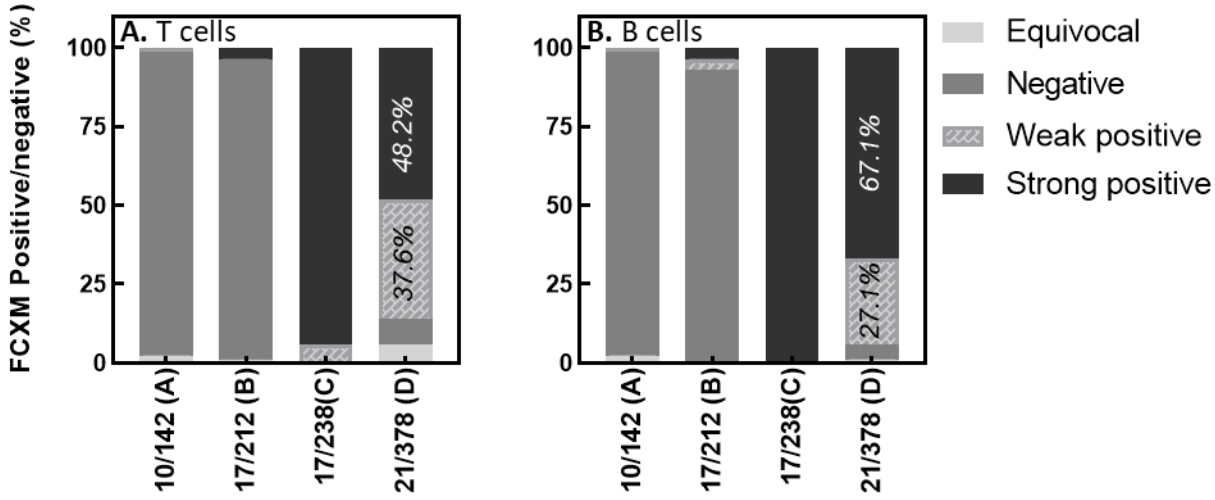

FIGURE 7: FCXM assay based positive/negative assignment for 10/142, 17/212, 17/238 and 21/378. *Participants reported interpretation of FCXM results based on the in-house criteria as negative, strong or weak positive for T (A) and B (B) cells. A total of 84 interpretations were received for negative controls (10/142 and 17/212) and 84 for positive controls (17/238 and 21/378). Percentage values were calculated based on the total assignment for donors reported/tested.*

Interestingly, raw fluorescence values reported by participants for positive samples 17/238 and 21/378 were variable and this could be based on the instrument setup performed at each laboratory. Representative plot of raw fluorescence values for 17/238 are shown in **Figure 8**.

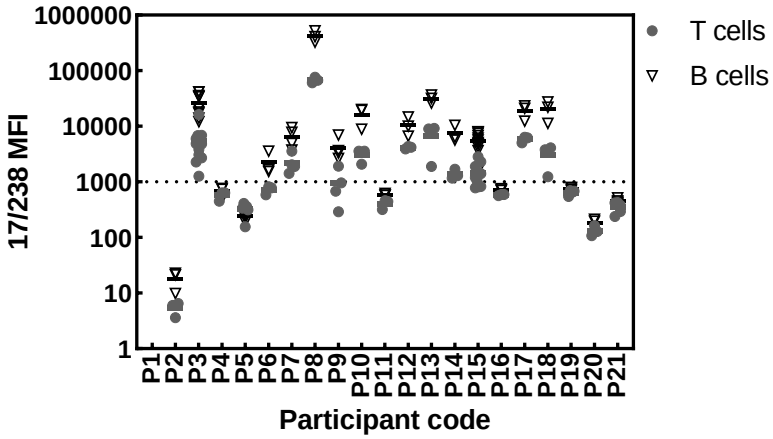

FIGURE 8: Variability in fluorescence values reported by participants for 17/238 values. *Participants reported raw fluorescence values from FCXM assay. The readouts for T and B cells are represented.*

As an example, in laboratories reporting 17/238 fluorescence values ranging from (0-999), fluorescence values for T and B cells were of a similar order, in contrast to participant

laboratories reporting ≥ 1000 fluorescence values, where mean values observed for T versus B cells was more divergent and fluorescence values for B cells were almost a log higher. Interestingly, 54.1% vs. 40.5% of participants classify 21/378 as weak positive vs. strong positive on T cells and 36.7% vs. 53.3% participants classify 21/378 as weak positive vs. strong positive on B cells in the group where reported fluorescence values are in the range from 0-999. Contrastingly, relatively higher proportion of participants 72.2% and 72.5% identify 21/378 as strong positive in comparison to relatively lower proportions 19.4% and 23.5% assign 21/378 as weak positive in the second cohort reporting fluorescence values of 1000 or higher. These results suggest that the instrument setup including voltage settings and cut off criteria may vary greatly between laboratories and add to variability of the results obtained in multicenter collaborative studies. This is also evident from the high %CV values observed for both 17/238 and 21/378 in FCXM assays. Inclusion of reference materials with intermediate positivity such as 21/378 may help refine measurements and harmonize thresholds for cut-off criteria, thereby supporting a closer conformity in values obtained with patient samples.

Luminex (LX) based assay for HLA alloantibody detection

Luminex bead-based assays comprise an additional technique employed for detecting clinically relevant HLA antibodies. Presence of HLA antibodies were evaluated by collaborative study participants using Luminex SAB analysis by either one or both, One Lambda and Lifecodes (Immucor) kits. Of 19 participants that evaluated the test samples using Luminex platform, 89.5% used only One Lambda kits and 10.5% used both available kits. Participants were requested to test the samples in accordance with their in-house protocols, alongside in-house controls and record results and methodology in the questionnaires (**Appendix 5, 6**).

All participants performing One Lambda Luminex assay, reported use of kit reference numbers: LS1A04, LABScreen SA Class I and LS2A01, LABScreen SA Class II. Few participants also indicated the use of LS12PRA, LABScreen PRA Class I&II (5%) and LSM12, LABScreen Mixed Class I&II kits (16%). Immucore evaluation, performed by 2 participants used LSAI, Lifecodes Lifescreen SA Class I and LSAII, Lifecodes Lifescreen SA Class II kits. Several studies in literature report the use of chelating agents such as EDTA or dithiothreitol (DTT) reduction, preheating, and/or dilution of serum as a step to eliminate prozone effect caused by covalent binding and accumulation of C4 and C3 degradation products on the immune complex leading to a reduction in detection of IgG by fluorescent labelled anti-IgG (Schwaiger et al 2014[9]). Since Luminex SAB assay use MFI values as a semi-quantitative readout for antibody measurement, serum pre-treatment could reduce effects of complement interference and enhance positive signal. In the current study, 83% and 50% participants included pre-treatment step with EDTA for One Lambda and Immucore Luminex evaluations respectively, however different pre-treatment conditions including concentrations, incubation times and methods were used across different laboratories. 11% and 50% participants reported the use of serum samples without pre-treatment for One Lambda and Immucor assays and 6% reported the use of DTT for One Lambda assays. Interestingly, only 22% participants reported following manufacturer's SOP and 78% indicated variations in the serum/ bead volume used,

higher centrifugation speed for washing step or use of rapid Halifax method in the assay. Of the 2 participants that evaluated samples using Immucor kits, 1 deviation from the manufacturer's SOP with regard to bead and serum sample volumes was reported. All participants used various versions of HLA fusion software for data analysis for One Lambda LabScreen assays, while analysis for Immucor assays was performed using MatchIT software.

Antigen coverage for different kits vary, and participants were requested to report all specificities that a particular assay kit allows testing for. Analysis for various specificities were performed independently for class I and II IgG in accordance with the external quality assessment (EQA) study conducted in 2018 for development of 17/238 and 17/212 as CE-IVD controls. Similarly, FCXM assay, cut off points for Luminex assays differ from lab to lab and cut-off criteria reported by collaborative study participants and the proportion of laboratories reporting a particular cut-off values are summarized in **Table 7**.

TABLE 7: Luminex cut-off criteria reported by collaborative study participants

MFI (cut-off criteria)	Participant %
>500	11
>1000	39
>1500	17
>2000	17
>3000	6
>4000 or higher	11

Majority participant laboratories reported use of MFI readout of 2,000 as a negative sample cut-off in the earlier EQA study performed in 2018 and also in-house assays at NIBSC conform to this cut-off value. Both negative RR 10/142 and 17/212 were developed not to exceed 2,000 MFI for any of the single antigen specificities for class I and II antibodies with Luminex kits produced by both vendors, Immucor and One Lambda.

To harmonize current results for HLA assignments reported by participants, we applied a > 2000 cut-off criteria, that is applied during in house evaluations leading to development of the RR, and also applied for the EQA study, CS617. In addition, and in concordance with the EQA study, consensus presence of a specificity was determined when at least 75% of laboratories report the specificity and absence of a specificity was determined when less than 5% report a specificity or a particular specificity is not reported by $\geq$ 95% participants. Consensus MHC

class I and class II specificities identified by participants using One lambda kit, analyzed based on the above criteria, are depicted below (**Figure 9 and Table 8)**.

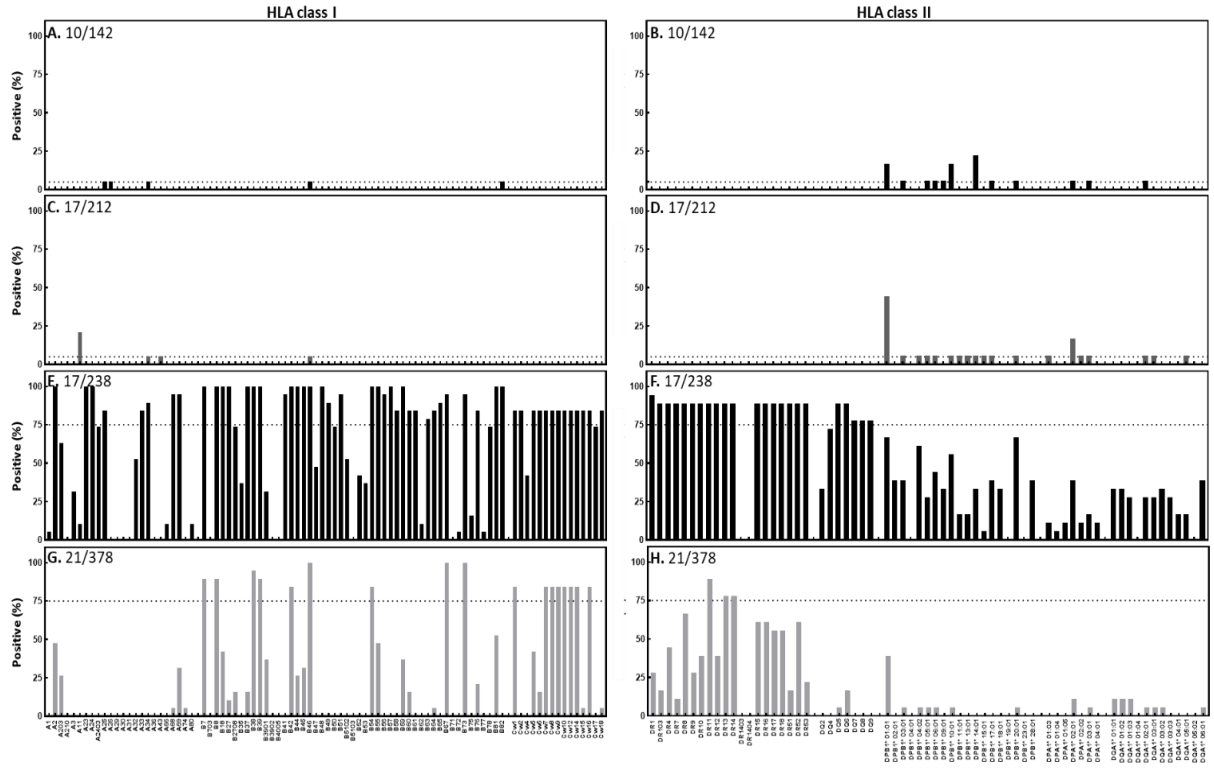

FIGURE 9: HLA class I and class II specificities reported based on One Lambda Luminex assay. *Luminex assay values reported by participants corresponding to indicated specificities were analyzed with a cut-off criteria of $\geq$ 2000 individually for class I and class II. Percentage of participants reporting a positive value for a particular specificity on HLA class I (A, C, E, G) or HLA class II (B, D, F, H) are represented. HLA specificities for 10/142 (A, B), 17/212 (C, D), 17/238 (E, F) and 21/378 (G, H) are represented. Dotted line indicates 75% value for a positive consensus and 5% for negative consensus.*

TABLE 8: Summary of specificity assignments for HLA class I and class II based on One Lambda Luminex evaluation

NIBSC code	Consensus positive (HLA class I)	Consensus negative (HLA class I)	Additional specificity (≥ 60%)
10/142	None	83/88 specificities	None
17/212	None	84/88 specificities	None
17/238	A2, A23, A24, A25, A33, A34, A68, A69, B7, B8, B18, B27, B37, B38, B39, B41, B42, B44, B45, B46, B48, B49, B51, B54, B55, B56, B57, B58, B59, B60, B61, B63, B64, B65, B67, B73, B76, B81, B82, Cw1, Cw2, Cw5, Cw6, Cw7, Cw8, Cw9, Cw10, Cw12, Cw14, Cw15, Cw16, Cw18 (52/88 specificities)	A210, B5103, B71, Cw17, A26, A29, A30, A31, A36, A43, A74, B703, B3902, B4005 (13/88 specificities)	B13, A*33:03 (63%)
21/378	B7, B8, B38, B39, B42, B46, B54, B67, B73, Cw1, Cw7, Cw8 Cw9, Cw10, Cw12, Cw14, Cw16 (17/88 specificities)	A1, A210, A3, A11, A23, A24, A2403, A25, A26, A29, A30, A31, A32, A33, A34, A36, A43, A66, A80, B703, B35, B3902, B4005, B41, B47, B48, B49, B50, B51, B5102, B5103, B52, B53, B56, B57, B58, B61, B62, B63, B65, B71, B72, B75, B77, B78, B82, Cw2, Cw4, Cw17 (49/88 specificities)	None

NIBSC code	Consensus positive (HLA class II)	Consensus negative (HLA class II)	Additional specificity (≥ 60%)
10/142	None	53/65 specificities	None
17/212	None	45/65 specificities	None
17/238	DR1, DR103, DR4, DR7, DR8, DR9, DR10, DR11, DR12, DR13, DR14, DR15, DR16, DR17, DR18, DR51, DR52, DR53, DQ5, DQ6, DQ7, DQ8, DQ9 (23/65 specificities)	DR1403, DR1404, DPB1* 04:01, DPB1* 19:01, DPB1* 23:01, DQA1* 01:04, DQA1* 05:02 (7/65 specificities)	DQ4 (72%), DPB1* 01:01 (66.7%), DPB1* 04:02 (61.1%), DPB1* 20:01 (66.7%)
21/378	DR11, DR13, DR14 (3/65 specificities)	DR1403, DR1404, DQ2, DQ4, DPB1* 02:01, DPB1* 09:01, DPB1* 11:01, DPB1* 13:01, DPB1* 14:01, DPB1* 15:01, DPB1* 17:01, DPB1* 18:01, DPB1* 19:01, DPB1* 23:01, DPB1* 28:01, DPA1* 01:03, DPA1* 01:04, DPA1* 01:05, DPA1* 02:02, DPA1* 04:01, DQA1* 01:04, DQA1* 03:03, DQA1* 04:01, DQA1* 05:01, DQA1* 05:02 (30/65 specificities)	DR8 (66.7%), DR15 (61.1%), DR16(61.1%), DR52 (61.1%)

Participants arrived at a consensus negative identification by One Lambda evaluations of 10/142 and 17/212 with 94.3% and 95.5% for HLA class I specificities and 81.5% and 69.2% for HLA class II specificities respectively (**Figure 9 and Table 8**). One of the participants was unable to report specificities for 10/142 due to high assay background when using Immucor kits and A*26:01 was detected as an additional specificity by one participant, however consensus could not be determined due to the limited number of evaluations. 17/212 was identified as negative using Immucor kits for both class I and II antibodies. Anti-HLA specificities for 17/238 using One Lambda LX assays, reached consensus on positivity for

59.1% class I and 35.4% class II antigens which are greater than the values arrived during the earlier EQA evaluation, where consensus positivity was reached for 53% of class I antigens and 29% class II antigens. As indicated above, a limited number of participants performed Immucor LX evaluations and consensus was reached for 26.1% class I, however no consensus was observed for class II antigens. These results suggest that 10/142, 17/212 and 17/238 can be used as negative and positive run control respectively for anti HLA detection by Luminex assay platform. Antigen assignments derived for 21/378 were intermediate in comparison to the negative and positive RR and consensus positivity values for 21/378 were 19.3% for HLA class I and 4.6% for HLA class II respectively as determined by One Lambda LX assays. Though some antigen assignments were identified with Immucor LX assay, a clear consensus could not be identified due to the limited number of evaluation using Immucor kits.

In addition to identification of antibody specificities, percentage panel reactive antibodies (PRA%) as an index of anti-HLA reactivity was recorded. Participants reported class I and class II %PRA values for LSA kits from OneLambda and Immucor (**Figure 10**). A relatively small cohort of participants performed evaluations using Immucor kits and this may contribute to the observed differences in interpretations reported by participants using this particular assay kit. Antigen coverage for different kits vary and in general the %PRA values were lower for Immucor assays in comparison to One Lambda evaluations.

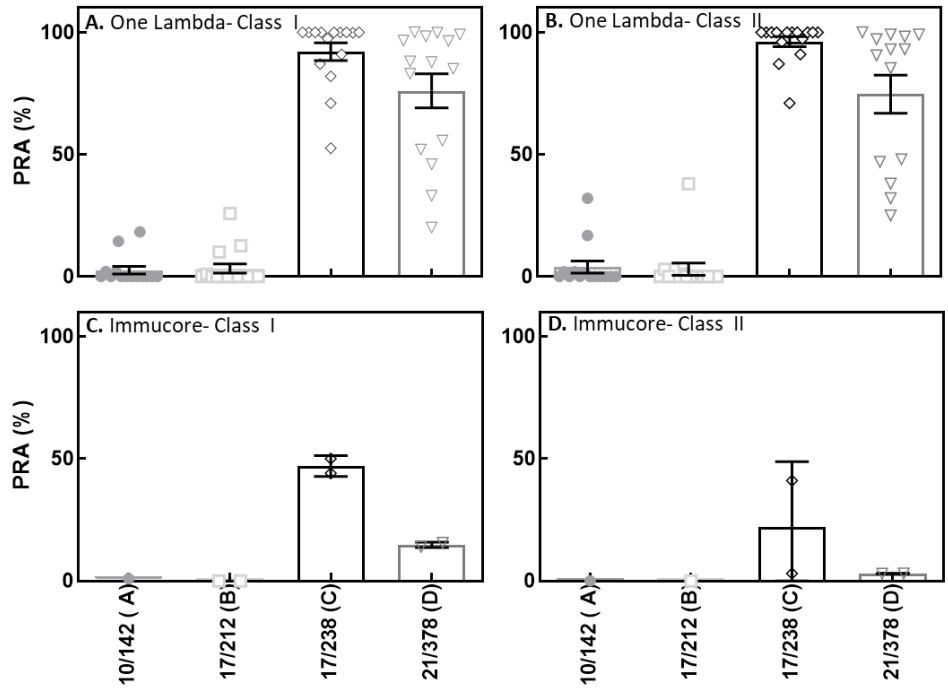

FIGURE 10: PRA % values reported from Luminex SAB assays. *Participants reported PRA (%) values for test samples evaluated by One Lambda (A, B) or Immucor assay kits (C, D). Values generated for class I (A, C) and class II (B, D).*

Mean and modal %PRA values were calculated from participant data as representative of average and most frequently reported values from the reported data set. Interestingly, 71.4%

and 73.3% of participants reported 0% PRA value calculated from One Lambda class I assay, indicating the RR are identified as negative for anti-HLA class I by majority of participants. Similarly, for One Lambda class II assays, 64% and 71% of participants respectively reported 0% PRA values, classifying both 10/142 and 17/212 as negative for anti-HLA class II antibodies. Higher variability was recorded with One Lambda class I kit for negative RR (10/142 and 17/212) in comparison to that for positive RR (17/238 and 21/378) (**Table 9**). 53.3% and 66.7% participants reported 100% PRA value for 17/238 on HLA class I and class II respectively, when assayed by One Lambda LX kits. Similarly, what was observed with FCXM assay readouts, values for 21/378 were intermediate between negative and strong positive RR concurrently evaluated in the current study. Descriptive statistics for One Lambda evaluations are summarized in **Table 9**. The minimum and maximum range and mean values $\pm$ SEM are represented in addition to the most frequently observed reported value and percentage of participants reporting the value calculated as mode and modal frequency (%) respectively. Immucor evaluation was performed by 2 participants and mean $\pm$ SD values for class I were calculated as 47 $\pm$ 4.24%, 14.8 $\pm$ 1.13% for 17/238 and 21/378 respectively; for class II mean values were calculated as 22.05 $\pm$ 26.8% and 3.05 $\pm$ 0.07% for 17/238 and 21/378 respectively. Due to a single reported value for 10/142, statistical analysis could not be carried out, however 10/142 was classified as negative for both class I and class II by the participant. Similarly, 17/212 was evaluated as negative on both HLA class I and class II. These results conform to the design criteria for the negative RR, 10/142 and 17/212 where the %PRA caused by non-specific binding to denatured antigens on the surface of the Luminex beads was designed not to exceed 5-10%, depending on the kit used.

TABLE 9: Descriptive statistics for % PRA values reported for One Lambda LX assay

One Lambda- class I (% PRA)							
NIBSC code	**Range (Min)**	**Range (Max)**	**Mode**	**Modal frequency (%)**	**Total values reported**	**Mean +/- SEM**	**CV%/ RSD**
10/142	0	18.26	0	71.4	14	2.63 +/- 1.58	224.80
17/212	0	25.8	0	73.3	15	3.29 +/- 1.91	224.14
17/238	52.6	100	100	53.3	15	92.05 +/- 3.6	15.15
21/378	20	100	N/A	N/A	15	76.03 +/- 7.0	35.66

One Lambda- class II (% PRA)							
NIBSC code	**Range (Min)**	**Range (Max)**	**Mode**	**Modal frequency (%)**	**Total values reported**	**Mean +/- SEM**	**CV%/ RSD**
10/142	0	32.07	0	64.3	14	3.88 +/- 2.47	237.97
17/212	0	37.9	0	73.3	15	2.99 +/- 2.51	324.51
17/238	71	100	100	66.7	15	96.15 +/- 2.06	8.30
21/378	25	100	99	14.3	14	74.66 +/- 7.79	39.02

Collaborative study participants were requested to interpret IgG anti-HLA specificities of 17/238 in LSA according to their in-house cut off criteria. As indicated earlier, 9.5% total participants did not use the Luminex platform for evaluations and of those, 2 participants (11%) did not report assignments/interpretations for test samples. A total of 17 participants (89.5%) returned interpretations for classification of test samples as negative, weak, or strong positive and equivocal results as requested in the results questionnaire. Percentage values are calculated based on the number of total participants who returned the results (17 for One Lambda and 2 for Immucor assays). 10/142 and 17/212 are reported as negative by 76% and 65% respectively for class I and 71% (both RR) for class II One Lambda LX kits. 17/238 was identified as strong positive by all participants for both class I and class II. 21/378 was identified as class I strong positive and class II weak positive by 65% participants (**Figure 11**).

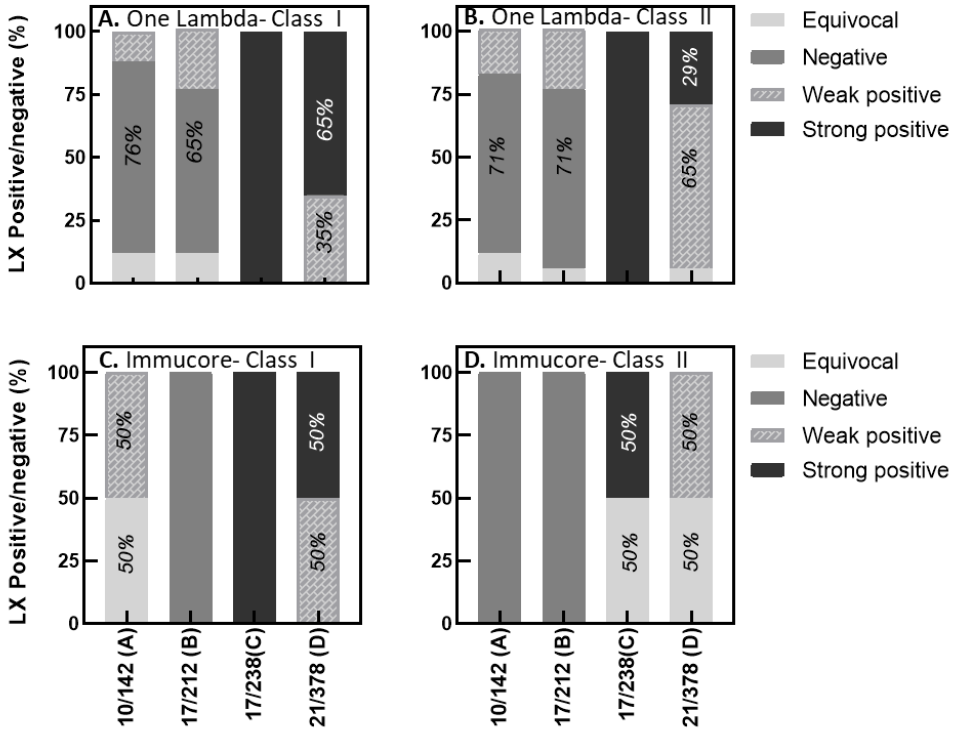

FIGURE 11: Positive/negative assignments for 10/142, 17/212, 17/238 and 21/378 using LX One Lambda and Immucor kits. *Participants reported LX results interpretation based on their in-house criteria as negative, strong or weak positive for HLA class I (A, C) and HLA class II (B, D). Percentage values for One Lambda (A, B) and Immucor (C, D) evaluations were calculated based on the total number of reported assignments.*

Report dissemination to participants

The completed report was disseminated to all participants on 23rd December 2022. Participants were requested to confirm by email on whether they agree with and are happy to endorse the proposal for establishment of the 10/142, 17/212, 17/238 and 21/378 as WHO International anti-HLA reference reagents for use in FCXM and SAB LX assays. Response/ comments were invited by 10th January 2023. Responses were received from all participants and 20 participants confirmed agreement for endorsement of the proposal. One participant supported the standardisation of reagents for FCXM and LX assays, however added that they are not in agreement with description of 17/238 and 21/378 as strong and weak positive RR respectively. The participant suggested that 17/238 anti-HLA be referred to as Broad Spectrum Reactivity, with HLA calculated reaction frequency (cRF): 100% and 21/378 anti-HLA be referred to as Lower Spectrum Reactivity, with HLA calculated reaction frequency (cRF): 92%. However, as discussed in the report, the reference materials are proposed as qualitative reference reagents and will not have an assigned unitage. Description as strong versus weak positive RR is proposed to differentiate the observed lower level of alloreactivity for 21/378 in comparison to strong positive 17/238 as observed in both FCXM and LX readouts.

Conclusions

The international collaborative study was conducted to ascertain fitness for purpose of anti-HLA RR for use in FCXM and LX bead-based assays. Detection of donor specific antibodies in patient serum and plasma samples is a cornerstone of ascertaining HLA compatibility prior to transplantation and for monitoring engraftment success, post transplantation. The RR were tested using in house procedures adopted at each participant laboratory. As may be expected, reported data show variations in several aspects of assay set up and cut-off criteria. Inclusion of negative, strong, and intermediate/weak positive RR will therefore help harmonizing flowcytometry and Luminex based readouts for alloantibody characterization. There is an increasing requirement for such RR in transplantation compatibility testing and availability of these references as WHO International RR will expand availability and increment utility for tissue typing laboratories outside EU. Consensus on sample positivity or negativity can be affected by many variables, even when factors such as donor-to-donor variability and non-specific anti-IgG binding are controlled for. Both 10/142 and 17/212 were evaluated as negative RR by majority of participants in FCXM and LX assays suggesting that the products work reliably in the hands of the end users across assay platforms and methodologies. Similarly, 17/238 was evaluated as strong positive in FCXM and LX assays by vast majority of participants and methodologies. 21/378 performs as an HLA positive RR, however at a lower

threshold and mean value in comparison to 17/238. Inclusion of weak positive RR in conjunction with strong positive control can potentially improve sensitivity of these complex assays and increment sensitivity.

In conclusion, the indicated RR perform consistently for HLA antibody detection assays on both FCXM and LX platforms.

Proposal for endorsement

Based on results from the collaborative study, the endorsement for establishment of the following four anti-HLA reference reagents for use in FCXM and SAB LX assays is recommended.

1. **10/142: WHO International reference reagent- Negative plasma for anti- HLA**
2. **17/212: WHO International reference reagent- Negative serum for anti- HLA**
3. **17/238: WHO International reference reagent- Strong positive plasma for anti-HLA**
4. **21/378: WHO International reference reagent- Weak positive plasma for anti-HLA**

The reference materials will have no assigned unitage and will serve as qualitative intra-assay variability controls, providing a means for trend monitoring for FCXM and LX assays for alloantibody detection.

Acknowledgements

We are grateful for the important contributions of all participants in the collaborative study. Support from standardization Science Group at NIBSC for trial fills, Standards Processing Division at NIBSC for the preparation and dispatch of materials for collaborative study is gratefully acknowledged. We are thankful to Chris Burns for helpful comments and review of the report.

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APPENDIX-1: Instructions for CS708 participant

Instructions for CS708 collaborative study participants

1. Receiving samples

Four freeze-dried preparations (2-6 vials of each depending on which assays are performed) will be sent to study participants. They will be labelled:

a. CS708sampleA
b. CS708sampleB
c. CS708sampleC
d. CS708sampleD

Upon receiving, the freeze-dried material should be stored Sample A at 4°C and the rest at -20°C. The instructions for use (IFU) will be sent to participants with the samples as a hard copy and electronically beforehand. They include safety specifications and the instructions for re-constituting the freeze-dried material. The IFU states that the samples should be used on the day of the reconstitution but short-term storage (up to a week) of the samples after re-constitution has been shown not to affect the sample stability. Before using the material that has been re-constituted and then stored it is recommended to spin down any cryoprecipitates that can form in the samples.

2. Performing the assays

The collaborative study's aim is to evaluate the performance of the four freeze-dried preparations as intra-assay controls and reference material. A number of H&I laboratories will be performing FCXM and anti-HLA Luminex assays. Participants are free to test the samples at any additional assays they routinely perform.

Assays and samples to be tested:

a. Flow Cytometry Crossmatch-FCXM
b. Luminex solid-phase alloantibody identification in the sample

FCXM considerations

Please test the samples as you would be testing patient sera, alongside in-house controls. Ideally, and as possible, all samples should be tested with 3 different donor cells (in 2-3 replicates). Participants can test the samples on more than the recommended 3 different sets of donor cells.

Luminex alloantibodies assay considerations

Please test the samples as you would be testing patient sera, alongside in-house controls. Ideally, and as possible, all four samples should be tested for class I and II antibodies using one or both Immucore and OneLambda kits (in accordance with the

local SOP). If a participant cannot test both samples for single antigen specificity it is asked that only samples C and D are tested (with at least one vendor for class I and II). If single antigen kits are not routinely used by the participants, the positive/negative screening kits can be used instead.

Filling in the collaborative study questionnaires

Four questionnaire forms (2 methodology forms and 2 results forms) will be sent to participants. They will include information that will allow appropriate data stratification when performing biostatistical analysis. Only the forms for the assays performed are to be return to the Study Director (not all the participants will be performing all the assays). The participants are asked to return filled in questionnaires as soon as they have finished evaluating the material but not later than 3 moths from the date of receiving the samples.

3. **What happens at the end of the study**

The results of the study (with coded participant details) will be communicated to the participants. The participants will be unblinded to the sample codes at the end of the study. If the fitness for purpose of tested in this study freeze-dried preparation is confirmed, the study report will be prepared. As part of this report participants will be asked to give their expert opinion on the tested material as possible reference reagents. The study report will be submitted to WHO's Expert Committee in Biological Standardisation (ECBS). The report will also be published on WHO's website for public consultation. Then it will be presented by NIBSC at ECBS meeting in April 2023 at earliest. The ECBS will endorse or reject the proposal within the report. If it is endorsed then NIBSC will become custodian of the WHO Reference Reagents (WHO RR) and will place the material in their catalogue of WHO RR where they can be accessed by external requestors.

The participation in this study is voluntary and gives the testing laboratories ability to test the material that is developed in order to aid global biological standardisation of FCXM and anti-HLA Luminex assays.
If data collected as a part of this collaborative study is of publishable quality, it may be submitted into a per-review journal and presented at scientific and clinical meetings. All the data used for publications and scientific presentations will be coded and no identity of any participants will be revealed.

If at any point study participants are unclear about any of the above please contact the study director.

Study Director:

Dr Anna Nowocin

Biotherapeutics

National Institute for Biological Standards and Control (NIBSC)

APPENDIX-2: Collaborative study IFU and material safety sheet

National Institute for Biological Standards and Control

CS708sample-A, -B, -C and -D

VERSION 1.0 22.03.2022

"This material is not for *in vitro* diagnostic use"

1. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS OR ANIMALS IN THE HUMAN FOOD CHAIN.

The preparation contains material of human origin, which has been tested and found negative for HBsAg, HIV antibody, HCV antibody and HCV RNA by PCR.

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 probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

2. DIRECTIONS FOR OPENING THE SCREW CAP VIALS.

Vials have a screw cap and stopper. The cap should be removed by turning anti-clockwise, please note on removal of the cap, the stopper may remain in the vial or be removed with the cap. Care should be taken on removal of cap to prevent the contents escaping.

3. USE OF MATERIAL

To reconstitute this material, dissolve the entire content of the ampoule in 0.5ml of sterile distilled water, keep at 2-8°C and use on the day of reconstitution. No attempts should be made to weight out a portion of the freeze-dried material, nor should aliquots be frozen after re-constitution for future use. Upon re-constitution material should be transferred to a centrifuge tube and spun down to pellet any visible precipitates. The material is intended to be used in accordance with internal laboratory procedures and upon re-constitution should be treated in the same way as the serum samples obtained from patients. The material can be tested in FCXM and/or Luminex anti-HLA head based assays.

4. LIABILITY AND LOSS

4.1 Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (http://www.nibsc.org/terms_and_conditions.aspx) apply to the exclusion of all other terms and are hereby incorporated into this document by reference.

4.2 Unless the context otherwise requires, the definitions in the Conditions shall apply.

4.3 Nothing in this document or the Conditions shall limit or exclude NIBSC's liability for fraud or fraudulent misrepresentation, death or personal injury caused by its negligence, or the negligence of its employees.

4.4 Subject to clause 4.3:

4.4.1 NIBSC shall under no circumstances whatsoever be liable to the Recipient, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of data, loss of profit, loss of business or goodwill, or any indirect or consequential loss or damage suffered or incurred by the Recipient arising in relation to the supply of the Materials or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or by any other person; and

4.4.2 NIBSC's total liability to the Recipient in respect of all other losses arising under or in connection with the Contract, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed 100% of the fees paid to NIBSC for the Materials.

4.5 The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

5. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	Freeze-dried powder		
Fire hazard	None		
Chemical properties			
Stable	Yes	Stable	Yes
Hygroscopic	No	Hygroscopic	No
Flammable	No	Flammable	No
Other *Contains material of human origin*			
Handling:	See caution, section 1		
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 a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			

APPENDIX-3: CS708-FCXM results questionnaire

NIBSC

CROSSMATCHING BY FLOW CYTOMETRY RESULTS QUESTIONAIRE

Laboratory Name/Address: Click or tap here to enter text.

Date samples received: Click or tap here to enter text.

Samples tested:

☐CS708sample A

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

FCXM date: Click or tap here to enter text.

☐CS708sample B

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

FCXM date: Click or tap here to enter text.

☐CS708sample C

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

FCXM date: Click or tap here to enter text.

☐CS708sample D

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

FCXM date: Click or tap here to enter text.

Units of cytometer readings: Click or tap here to enter text.

Bw4/Bw6 positive control cells used: YES/NO

If YES please specify: Click or tap here to enter text.

Bw4 positive control cells used: YES/NO

If YES please specify: Click or tap here to enter text.

Bw6 positive control cells used: YES/NO

If YES please specify: Click or tap here to enter text.

T cell Results: please fill in the cytometer readings

Cells	Replicates	T cells						
		CS708 sample A	CS708 sample B	CS708 sample C	CS708 sample D	Local negative control	Local positive control	Any other controls used in assay (please specify)
Donor 1	1							
	2							
	3							
Donor 2	1							
	2							
	3							
Donor 3	1							
	2							
	3							
Bw4/Bw6 positive cells	1							
	2							
	3							
Bw4 positive cells	1							
	2							
	3							
Bw4 positive cells	1							
	2							
	3							

Donor 1 HLA class I type if known: Click or tap here to enter text.

Donor 2 HLA class I type if known: Click or tap here to enter text.

Donor 3 HLA class I type if known: Click or tap here to enter text.

B cell Results: please fill in the cytometer readings

Cells	Replicates	B cells						
		CS708 sample A	CS708 sample B	CS708 sample C	CS708 sample D	Local negative control	Local positive control	Any other controls used in assay (please specify)
Donor 1	1							
	2							
	3							
Donor 2	1							
	2							
	3							
Donor 3	1							
	2							
	3							

Donor 1 HLA class II type if known: Click or tap here to enter text.

Donor 2 HLA class II type if known: Click or tap here to enter text.

Donor 3 HLA class II type if known: Click or tap here to enter text.

Results Interpretation: please check the appropriate box

Sample	Donor	T cells				
		Strong Positive	Weak Positive	Negative	Equivocal	Not tested
CS708 sample A	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample B	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample C	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample D	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐

Sample	Donor	B cells				
		Strong Positive	Weak Positive	Negative	Equivocal	Not tested
CS708 sample A	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample B	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample C	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐
CS708 sample D	1	☐	☐	☐	☐	☐
	2	☐	☐	☐	☐	☐
	3	☐	☐	☐	☐	☐

Comments (with explanation if the results were equivocal):

Click or tap here to enter text.

APPENDIX-4: CS708-FCXM methodology questionnaire

Collaborative study questionnaire for the new anti-HLA reference material CS708

CROSSMATCHING BY FLOW CYTOMETRY:

METHODS QUESTIONAIRE

Laboratory Name/Address: Click or tap here to enter text.

Date: Click or tap here to enter text.

Main scope of the work performed in the lab: Click or tap here to enter text.

Guidelines followed:

☐BSHI/BTS ☐EFI ☐ASHI ☐Other

If Other please specify: Click or tap here to enter text.

FCXM assay use:

☐Clinical/Diagnostic

☐Research/Development

☐Antibody Screen

☐Pre-Transplant crossmatching

☐Post-Transplant crossmatching

☐Other

If Other please specify: Click or tap here to enter text.

1. **Cell Preparation**
    a. Method of cell preparation: Click or tap here to enter text.
    b. Cells volume/test [µl]: Click or tap here to enter text.
    c. Cells concentration/test: Click or tap here to enter text.
    d. Cells diluent/wash buffer: Click or tap here to enter text.
    e. Other specifications: Click or tap here to enter text.

2. **Serum and controls**
    a. Volume/test [µl]: Click or tap here to enter text.
    b. Serum treatment (additives, dilutions, time): Click or tap here to enter text.
    c. Source of negative control (if NIBSC please provide product code): Click or tap here to enter text.
    d. Source of positive control (if NIBSC please provide product code): Click or tap here to enter text.
    e. Incubation time [min]: Click or tap here to enter text.
    f. Incubation temperature [°C]: Click or tap here to enter text.
    g. Wash buffer: Click or tap here to enter text.
    h. Number of washes: Click or tap here to enter text.
    i. Other specifications: Click or tap here to enter text.

3. **Detection reagents**

    a. **Anti-Human IgG**

    ☐ Whole IgG ☐ Fab_2 fragment

    Raised in: Click or tap here to enter text.

    Labelled with: Click or tap here to enter text.

    Manufacturer: Click or tap here to enter text.

    Product code: Click or tap here to enter text.

    Dilution: Click or tap here to enter text.

    Diluent: Click or tap here to enter text.

    Volume/test [µl]: Click or tap here to enter text.

Incubation time [min]: Click or tap here to enter text.

Incubation temperature [°C]: Click or tap here to enter text.

Other specifications: Click or tap here to enter text.

b. Anti-T cell reagent

☐ CD3 ☐Other

If Other please specify: Click or tap here to enter text.

Raised in: Click or tap here to enter text.

Labelled with: Click or tap here to enter text.

Manufacturer: Click or tap here to enter text.

Product code: Click or tap here to enter text.

Dilution: Click or tap here to enter text.

Diluent: Click or tap here to enter text.

Volume/test [µl]: Click or tap here to enter text.

Incubation time [min]: Click or tap here to enter text.

Incubation temperature [°C]: Click or tap here to enter text.

Other specifications: Click or tap here to enter text.

c. Anti-B cell reagent

☐ CD19 ☐CD20 ☐Other

If Other please specify: Click or tap here to enter text.

Raised in: Click or tap here to enter text.

Labelled with: Click or tap here to enter text.

Manufacturer: Click or tap here to enter text.

Product code: Click or tap here to enter text.

Dilution: Click or tap here to enter text.

Diluent: Click or tap here to enter text.

Volume/test [µl]: Click or tap here to enter text.

Incubation time [min]: Click or tap here to enter text.

Incubation temperature [°C]: Click or tap here to enter text.

Other specifications: Click or tap here to enter text.

d. **Other reagents:** Click or tap here to enter text.

4. **Detection procedure:**

☐ Tubes ☐Plates ☐Other

If Other please specify: Click or tap here to enter text.

a. Number of crossmatch/control replicates: Click or tap here to enter text.
b. Is the anti-human IgG reagent added with anti-T cell reagent: YES/NO
c. Is the anti-human IgG reagent added with anti-B cell reagent: YES/NO
d. Is the anti-human anti-T cell reagent added with anti-B cell reagent: YES/NO
e. If NO: are there any wash steps in between anti-human IgG, T cells and B cells reagent incubations: Click or tap here to enter text.
f. Final wash buffer: Click or tap here to enter text.
g. Number of final washes: Click or tap here to enter text.
h. Final volume/test for acquisition: Click or tap here to enter text.
i. Cells resuspension buffer: Click or tap here to enter text.
j. If the cells were fixed, please provide the fixative used: Click or tap here to enter text.
k. Other specifications: Click or tap here to enter text.

5. **Data Acquisition/Analysis:**

a. Make and model of flow cytometer: Click or tap here to enter text.
b. Calibration beads used: YES/NO

c. If yes please specify what type of beads are used and in what capacity (e.g. alignment, laser, fluorescence): Click or tap here to enter text.
d. Gating strategy (please briefly describe the sequence of gates applied when analysing the data): Click or tap here to enter text.
e. Minimum events recorded (gated on which population): Click or tap here to enter text.
f. Fluorescence readout (Median, mode, mean, other): Click or tap here to enter text.
g. Scale used (linear, logarithmic, other): Click or tap here to enter text.
h. Human-IgG fluorescence shift parameter (Relative Fluorescence Intensity against negative control, peak shift, MFI difference, %, other): Click or tap here to enter text.
i. T cell clinical cut-off value for a positive readout (e.g. RFI=2.0): Click or tap here to enter text.
j. B cell clinical cut-off value for a positive readout (e.g. RFI=2.0): Click or tap here to enter text.
k. Form of reporting the result (e.g. positive/negative): Click or tap here to enter text.
l. Other specifications: Click or tap here to enter text.

6. **Comments/Other Methodology Specifications:**

Click or tap here to enter text.

APPENDIX-5: CS708-Luminex results questionnaire

Collaborative study questionnaire for the new anti-HLA reference material CS708

NIBSC

LUMINEX BEAD-BASED ALLOANTIBODY DETECTION AND IDENTIFICATION:

RESULTS QUESTIONAIRE

Laboratory Name/Address: Click or tap here to enter text.

Date samples received: Click or tap here to enter text.

Samples tested:

☐CS708sample A

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

Assays dates: Click or tap here to enter text.

☐CS708sample B

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

Assays dates: Click or tap here to enter text.

☐CS708sample C

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

Assays dates: Click or tap here to enter text.

☐CS708sample D

Reconstitution date: Click or tap here to enter text.

Reconstituted with water: YES/NO

If NO please specify the diluent: Click or tap here to enter text.

Sample reconstituted easily: YES/NO

If NO please specify why: Click or tap here to enter text.

Appearance upon reconstitution: Click or tap here to enter text.

If samples reconstituted not on the day of crossmatch then please provide the storage conditions of reconstituted material: Click or tap here to enter text.

Spun down before use: YES/NO

Assays dates: Click or tap here to enter text.

CLASS I

Class I IgG Results for anti-HLA ID with Immucore Kits: CS708SampleA

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			

B7 ☐			
B703 ☐			
B8 ☐			
B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			

B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			
Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with Immucore Kits: CS708SampleB

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			

B8 ☐			
B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			

B82 ☐			
Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with Immucore Kits: CS708SampleC

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			
B8 ☐			

B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			

Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with Immucore Kits: CS708SampleD

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			
B8 ☐			

B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			

Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with OneLambda Kits: CS708SampleA

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			
B8 ☐			

B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			

Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with OneLambda Kits: CS708SampleB

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			
B8 ☐			

B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			

Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

CLASS II

Class I IgG Results for anti-HLA ID with OneLambda Kits: CS708SampleC

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			

B703 ☐			
B8 ☐			
B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			

B81 ☐			
B82 ☐			
Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class I IgG Results for anti-HLA ID with OneLambda Kits: CS708 SampleD

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
A1 ☐			
A2 ☐			
A203 ☐			
A210 ☐			
A3 ☐			
A11 ☐			
A23(9) ☐			
A24(9) ☐			
A2403 ☐			
A25(10) ☐			
A26(10) ☐			
A29(19) ☐			
A30(19) ☐			
A31(19) ☐			
A32(19) ☐			
A33(19) ☐			
A34(10) ☐			
A36 ☐			
A43 ☐			
A66(10) ☐			
A68(28) ☐			
A69(28) ☐			
A74(19) ☐			
A80 ☐			
B7 ☐			
B703 ☐			
B8 ☐			

B18 ☐			
B27 ☐			
B2708 ☐			
B35 ☐			
B37 ☐			
B38(16) ☐			
B39(16) ☐			
B3901 ☐			
B3902 ☐			
B4005 ☐			
B41 ☐			
B42 ☐			
B44(12) ☐			
B45(12) ☐			
B46 ☐			
B47 ☐			
B48 ☐			
B49(21) ☐			
B50(21) ☐			
B51(5) ☐			
B5102 ☐			
B5103 ☐			
B52(5) ☐			
B53 ☐			
B54(22) ☐			
B55(22) ☐			
B56(22) ☐			
B57(17) ☐			
B58(17) ☐			
B59 ☐			
B60(40) ☐			
B61(40) ☐			
B62(15) ☐			
B63(15) ☐			
B64(14) ☐			
B65(14) ☐			
B67 ☐			
B71(70) ☐			
B72(70) ☐			
B73 ☐			
B75(15) ☐			
B76(15) ☐			
B77(15) ☐			
B78 ☐			
B81 ☐			
B82 ☐			

Cw1 ☐			
Cw2 ☐			
Cw4 ☐			
Cw5 ☐			
Cw6 ☐			
Cw7 ☐			
Cw8 ☐			
Cw9 ☐			
Cw10 ☐			
Cw12 ☐			
Cw14 ☐			
Cw15 ☐			
Cw16 ☐			
Cw17 ☐			
Cw18 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with Immucore Kits: CS708SampleA

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with Immucore Kits: CS708SampleB

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with Immucore Kits: CS708SampleC

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with Immucore Kits: CS708 SampleD

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with OneLambda Kits: CS708SampleA

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
  Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with OneLambda Kits: CS708 SampleB

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with OneLambda Kits: CS708SampleC

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Class II IgG Results for anti-HLA ID with OneLambda Kits: CS708 SampleD

Bead lot specifics: Click or tap here to enter text.

PRA%: Click or tap here to enter text.

Results:

- Please **check appropriate box** (in sero-specificity column) if the HLA- specificities were identified as **positive** according to your local cut-off value.
- If antibodies were found **positive for one but negantive for other allel(s)** please specify in the second column which allel-specificity was found positive.
- Please provide **raw MFI** value only for HLA-specificities identified in the sample as positive according to your local cut-off values.
- Please specify the Minimum Bead Count for any readout (positive or negative) only if the **Bead Count is below 50**

Sero-specificity	If differentaial allel-specificity identified please specify here	Raw MFI [only for positive readouts]	Minimum Bead Count [only for counts below 50]
DR1 ☐			
DR103 ☐			
DR4 ☐			
DR7 ☐			
DR8 ☐			
DR9 ☐			
DR10 ☐			
DR11(5) ☐			
DR12(5) ☐			
DR13(6) ☐			
DR14(6) ☐			
DR1403 ☐			
DR1404 ☐			
DR15(2) ☐			
DR16(2) ☐			
DR17(3) ☐			
DR18(3) ☐			
DR51 ☐			
DR52 ☐			
DR53 ☐			
DQ2 ☐			
DQ4 ☐			
DQ5(1) ☐			
DQ6(1) ☐			
DQ7(3) ☐			
DQ8(3) ☐			
DQ9(3) ☐			

DPB1* 01:01 ☐			
DPB1* 02:01 ☐			
DPB1* 03:01 ☐			
DPB1* 04:01 ☐			
DPB1* 04:02 ☐			
DPB1* 05:01 ☐			
DPB1* 06:01 ☐			
DPB1* 09:01 ☐			
DPB1* 10:01 ☐			
DPB1* 11:01 ☐			
DPB1* 13:01 ☐			
DPB1* 14:01 ☐			
DPB1* 15:01 ☐			
DPB1* 17:01 ☐			
DPB1* 18:01 ☐			
DPB1* 19:01 ☐			
DPB1* 20:01 ☐			
DPB1* 23:01 ☐			
DPB1* 28:01 ☐			
DPA1* 01:03 ☐			
DPA1* 01:04 ☐			
DPA1* 01:05 ☐			
DPA1* 02:01 ☐			
DPA1* 02:02 ☐			
DPA1* 03:01 ☐			
DPA1* 04:01 ☐			
DQA1* 01:01 ☐			
DQA1* 01:02 ☐			
DQA1* 01:03 ☐			
DQA1* 01:04 ☐			
DQA1* 02:01 ☐			
DQA1* 03:01☐			
DQA1* 03:02 ☐			
DQA1* 03:03 ☐			
DQA1* 04:01☐			
DQA1* 05:01☐			
DQA1* 05:02 ☐			
DQA1* 06:01 ☐			

Other specificieties/comments: Click or tap here to enter text.

Results Interpretation: please check appropriate boxes

Sample classification:

Sample	Kit		Class I			
		Weak Positive	Strong Positive	Negative	Equivocal	Not tested
CS708 sampleA	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleB	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleC	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleD	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐

Sample	Kit		Class II			
		Weak Positive	Strong Positive	Negative	Equivocal	Not tested
CS708 sampleA	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleB	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleC	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐
CS708 sampleD	Immucore	☐	☐	☐	☐	☐
	One Lambda	☐	☐	☐	☐	☐

Comments (with explanation if the results were equivocal):

Click or tap here to enter text.

If other analysis were performed on the samples (e.g. IgM, MICA, solid phase assay-complement fixing antibody detection) please specify the kits used and the results here:

Click or tap here to enter text.

APPENDIX-6: CS708-Luminex methodology questionnaire

NIBSC

LUMINEX BEAD-BASED ALLOANTIBODY DETECTION AND IDENTIFICATION:

METHODS QUESTIONAIRE

Laboratory Name/Address: Click or tap here to enter text.

Date: Click or tap here to enter text.

Main scope of the work performed in the lab: Click or tap here to enter text.

Guidelines followed:

☒BSHI/BTS ☐EFI ☐ASHI ☐Other

If Other please specify: Click or tap here to enter text.

Luminex assay use: please check the box next to the assays used **routinely in your lab** and specify the criteria for a selection of the specific assay in free-form editing field.

IMMUCORE

☐LM1, Class I ID Kit: Click or tap here to enter text.

☐LM2, Class II ID Kit: Click or tap here to enter text.

☐LMX, Lifecodes Lifescreen Deluxe Kit: Click or tap here to enter text.

☐LSAI, Lifecodes Lifescreen SA Class I Kit: Click or tap here to enter text.

☐LSAII, Lifecodes Lifescreen SA Class II Kit: Click or tap here to enter text.

☐LSAI&II, Lifecodes Lifescreen SA Class I&II Kit: Click or tap here to enter text.

☐LSAMIC, Lifecodes Lifescreen SA MICA: Click or tap here to enter text.

☐Other

If Other please specify: Click or tap here to enter text.

ONELAMBDA

☐LS1PRA, LABScreen PRA Class I: Click or tap here to enter text.

☐LS2PRA, LABScreen PRA Class II: Click or tap here to enter text.

☐LS12PRA, LABScreen PRA Class I&II: Click or tap here to enter text.

☐LS1A04, LABScreen SA Class I: Click or tap here to enter text.

☐LS2A01, LABScreen SA Class II: Click or tap here to enter text.

☐LSM12, LABScreen Mixed Class I&II: Click or tap here to enter text.

☐LSMUTR, LABScreen Multi: Click or tap here to enter text.

☐Other

If Other please specify: Click or tap here to enter text.

1. **Immucore Kits used for testing the collaborative study sample(s):**

☐LM1, Class I ID Kit

☐LM2, Class II ID Kit

☐LMX, Lifecodes Lifescreen Deluxe Kit

☐LSAI, Lifecodes Lifescreen SA Class I Kit

☐LSAII, Lifecodes Lifescreen SA Class II Kit

☐LSAI&II, Lifecodes Lifescreen SA Class I&II Kit

☐Other

If Other please specify: Click or tap here to enter text.

Class I antibody detection with Immucore kits:

a. Bead Volume [µl]: Click or tap here to enter text.
b. Serum Volume [µl]: Click or tap here to enter text.
c. Negative control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
d. Positive control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
e. Details of cut-off criteria (e.g. 2,000 MFI): Click or tap here to enter text.
f. Serum treatment (additives/dilutions/heat, volume and concentration of additives, time of treatment, added to sera/wash buffer, at which step in protocol are sera treated, other specifications): Click or tap here to enter text.
g. Were both (A and B) samples treated: YES/NO
h. If NO please specify why: Click or tap here to enter text.
i. Are the manufacturers instructions for the detection followed: YES/NO
j. If NO please specify the alterations to manufacturer recommended protocol: Click or tap here to enter text.
k. Which software is used to analyse the data: Click or tap here to enter text.

l. What is the minimum bead count accepted by your laboratory: Click or tap here to enter text.
m. What is the minimum 'Positive Control' bead MFI value accepted by your laboratory: Click or tap here to enter text.
n. What is the criteria (if any) for classifying a sample as having 'High Background': Click or tap here to enter text.

Class II antibody detection with Immucore kits:

a. Bead Volume [µl]: Click or tap here to enter text.
b. Serum Volume [µl]: Click or tap here to enter text.
c. Negative control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
d. Positive control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
e. Details of cut-off criteria (e.g. 2,000 MFI): Click or tap here to enter text.
f. Serum treatment (additives/dilutions/heat, volume and concentration of additives, time of treatment, added to sera/wash buffer, at which step in protocol are sera treated, other specifications): Click or tap here to enter text.
g. Were all samples treated: YES/NO
h. If NO please specify why: Click or tap here to enter text.
i. Are the manufacturers instructions for the detection followed: YES/NO
j. If NO please specify the alterations to manufacturer recommended protocol: Click or tap here to enter text.
k. Which software is used to analyse the data: Click or tap here to enter text.
l. What is the minimum bead count accepted by your laboratory: Click or tap here to enter text.
m. What is the minimum 'Positive Control' bead MFI value accepted by your laboratory: Click or tap here to enter text.
n. What is the criteria (if any) for classifying a sample as having 'High Background': Click or tap here to enter text.

2. OneLambda Kits used for testing the collaborative study sample(s):

☐LS1PRA, LABScreen PRA Class I

☐LS2PRA, LABScreen PRA Class II

☐LS12PRA, LABScreen PRA Class I&II

☐LS1A04, LABScreen SA Class I

☐LS2A01, LABScreen SA Class II

☐LSM12, LABScreen Mixed Class I&II

☐Other

If Other please specify: Click or tap here to enter text.

Class I antibody detection with OneLambda kits:

a. Bead Volume [µl]: Click or tap here to enter text.
b. Serum Volume [µl]: Click or tap here to enter text.
c. Negative control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
d. Positive control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
e. Details of cut-off criteria (e.g. 2,000 MFI): Click or tap here to enter text.
f. Serum treatment (additives/dilutions/heat, volume and concentration of additives, time of treatment, added to sera/wash buffer, at which step in protocol are sera treated, other specifications): Click or tap here to enter text.
g. Were all samples treated: YES/NO
h. If NO please specify why: Click or tap here to enter text.
i. Are the manufacturers instructions for the detection followed: YES/NO
j. If NO please specify the alterations to manufacturer recommended protocol: Click or tap here to enter text.
k. Which software is used to analyse the data: Click or tap here to enter text.

l. What is the minimum bead count accepted by your laboratory: Click or tap here to enter text.
m. What is the minimum 'Positive Control' bead MFI value accepted by your laboratory: Click or tap here to enter text.
n. What is the criteria (if any) for classifying a sample as having 'High Background': Click or tap here to enter text.

Class II antibody detection with OneLambda kits:

a. Bead Volume [µl]: Click or tap here to enter text.
b. Serum Volume [µl]: Click or tap here to enter text.
c. Negative control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
d. Positive control used in assay (if NIBSC please provide the product code): Click or tap here to enter text.
e. Details of cut-off criteria (e.g. 2,000 MFI): Click or tap here to enter text.
f. Serum treatment (additives/dilutions/heat, volume and concentration of additives, time of treatment, added to sera/wash buffer, at which step in protocol are sera treated, other specifications): Click or tap here to enter text.
g. Were all samples treated: YES/NO
h. If NO please specify why: Click or tap here to enter text.
i. Are the manufacturers instructions for the detection followed: YES/NO
j. If NO please specify the alterations to manufacturer recommended protocol: Click or tap here to enter text.
k. Which software is used to analyse the data: Click or tap here to enter text.
l. What is the minimum bead count accepted by your laboratory: Click or tap here to enter text.
m. What is the minimum 'Positive Control' bead MFI value accepted by your laboratory: Click or tap here to enter text.
n. What is the criteria (if any) for classifying a sample as having 'High Background': Click or tap here to enter text.

Other methodology specifications: Click or tap here to enter text.