TSANZ

The Transplantation Society of Australia and New Zealand

National Histocompatibility Assessment Guideline for Solid Organ Transplantation

Produced in partnership with

Australian Government

Organ and Tissue Authority

donatelife

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1 Abbreviations

ABMR	Antibody Mediated Rejection
ASHI	American Society for Histocompatibility and Immunogenetics
AT1R	Angiotensin Type-1 receptor
CDC	Complement Dependent Cytotoxicity
DSA	Donor-specific antibody
FXM	Flow Cytometry Crossmatch
HLA	Human Leucocyte Antigen
MFI	Mean/Median Fluorescence Intensity
NATA	National Association of Testing Authorities
OLI	One Lambda
OTA	Organ and Tissue Authority
NGS	Next generation sequencing
SAB	Single Antigen Bead immunoassay
SPK	Simultaneous Pancreas Kidney
TSANZ	Transplantation Society of Australia and New Zealand
TWL	Transplant Waiting List
VXM	Virtual Crossmatch

2 Summary of recommendations

All tissue typing laboratories must be ASHI and NATA accredited and be able to provide the following testing requirements:

1. HLA typing:

- Potential transplant recipients and donors must be typed at the following HLA loci[3]:
  - HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, and -DPB1
- Deceased Donor HLA types should be confirmed by next-generation sequencing (NGS) as soon as practicable – however, NGS is not required for matching in OrganMatch at the time of organ allocation.
- For deceased donors 60ml / 8 ACD tubes for HLA typing should be collected in adult donors and 2 ACD tubes in paediatric donors

2. HLA antibody determination:

- Presence of HLA antibodies should be determined using a solid phase assay (e.g., Luminex-based immunoassay)
- HLA antibody specificity should be identified using a single HLA molecule target (e.g., Luminex-based SAB assay)
- SAB testing must be performed using EDTA-treated sera to reduce potential interference with IgM/auto antibodies or immune complexes
- If required, HLA antibodies can be confirmed using an alternative Luminex SAB assay/kit
- Where appropriate and technically feasible, a surrogate flow crossmatch (FXM) assay can be performed to confirm the binding capability or strength of an antibody to an HLA target expressed by an intact cell

3. Frequency of Serum Screening:

- A minimum of two SAB results should be obtained prior to activation on the TWL, ideally using serum samples collected > 1 month apart. It is accepted that some recipients will need to be listed urgently and only a single SAB result may be feasible
- Serum samples should be obtained regularly for patients on the transplant waiting list and sent to the local tissue typing laboratory for HLA antibody testing by SAB
- Additional screening for anti-HLA antibodies should be performed 4–6 weeks after any potential sensitising events – see section 5.2.1

4. Serum sharing for Flow Cytometry Crossmatching – refer to clinical guidance for FXM at the end of this document.

- There should not be a need to prospectively share patient sera for FXM if regular SAB testing is performed.
- Serum can be shared by arrangement with the tissue typing lab for individual patients who are clinically urgent, or where HLA antibody profile analysis is unable to resolve all antibody specificities.

5. Defining Unacceptable Antigens.

- HLA antigens to be avoided in potential donors will be defined as Unacceptable Antigens (UA) (antigens for exclusion) – see section 6.1, Table 4

6. Defining MFI Thresholds for HLA Antigens Exclusion

- To ensure consistent anti-HLA antibody assignment, a national approach to SAB interpretation and MFI threshold has been agreed for each organ group – see table 1 below:

Table 1: Usual MFI Thresholds for Defining Unacceptable Antigens

Organ group	Routine MFI threshold for UA definition
Kidney / Pancreas (including SPK)	2000 (Immucor assay) 4000 (OLI assay)
Heart / Lung	3000 (Immucor assay) 6000 (OLI assay)
Liver	UA not routinely defined but may be included by arrangement with tissue typing lab
Intestinal	2000 (Immucor assay) 4000 (OLI assay)
Multiorgan (other than SPK)	2000 (Immucor assay) 4000 (OLI assay)

7. HLA antigen / Allele Repeat Mismatches

- Repeat mismatches where a DSA has been detected previously with MFI >500 should usually be excluded
- Repeat mismatches which have never generated a DSA must have a detailed epitope analysis to explain negative HLA antibody reactivity and may be included for organ offers with approval from the clinical team
- If repeat mismatches are included as unacceptable antigens they should contribute to the mPRA irrespective of whether a DSA is present

8. Crossmatching

Virtual Crossmatching

- VXM is the first-line assessment of immunological compatibility for all solid-organ transplant offers where complete donor HLA typing is available and the recipient HLA antibody profile has been fully evaluated by SAB assay

Flow Cytometry Crossmatching

- Halifaster FXM is the preferred assay where a physical crossmatch is required
- A prospective FXM should be considered when there is clinical urgency to proceed with transplantation (e.g., heart/lung patients), but there is insufficient HLA antibody data to perform an adequate VXM / DSA assessment
- Requests for prospective FXM should be discussed with the tissue typing lab to confirm the indication for FXM and the ability of the lab to provide a result within a clinically relevant timeframe
- An immediate retrospective FXM is recommended when intending to cross multiple low MFI DSA below agreed threshold for exclusion (UA) that is present in recent sera (clinical decision)
- A retrospective FXM should be considered when:
  - There are low-level DSA present which have not been considered a contraindication to transplant
  - There is a repeat mismatch to a previous allograft

Refer to the clinical guidance for Flow Cytometric Crossmatching – see appendix

3 Introduction

The following guidelines are the consensus of the TSANZ/OTA Virtual Crossmatch Working Group and the TSANZ/OTA National Tissue Typing Committee. They are intended to establish consistent standards of practice for histocompatibility laboratories supporting clinical transplantation in Australia in identifying acceptable and unacceptable antigens for transplant recipients.

In the early era of transplantation, Donor Specific Antibodies (DSA) were a known major risk for sensitised transplant recipients. At this time, the capacity to identify DSA and to define donor human leucocyte antigens (HLA) was limited, and as a result, organs could be inadvertently transplanted in the presence of strong DSA, often failing immediately due to hyperacute rejection. In 1969, Paul Terasaki described the use of the complement-dependent cytotoxicity (CDC) assay, which revolutionised transplantation practice by allowing identification of strong DSA prior to transplantation[1].

Since the early 2000's technological advances have dramatically improved the accuracy of immunological risk assessment before transplantation. Firstly, donor HLA type is now defined for all significant HLA loci prior to transplant allocation. Secondly, serum from patients awaiting transplantation is screened for anti-HLA antibodies using Luminex-based solid phase assays. These assays provide a vastly more detailed analysis of anti-HLA sensitisation than was possible previously. Combining comprehensive donor HLA typing and well characterised recipient HLA antibody profiles allows highly accurate assessment of transplant compatibility (a virtual crossmatch (VXM)) , permitting transplantation to proceed safely in the majority of cases without a physical crossmatch[2]. In certain situations where a physical crossmatch is judged necessary at the time of transplantation, most centres internationally would now use a flow crossmatch (FXM), which provides greater sensitivity than CDC.

4 HLA typing

Effective histocompatibility assessment requires both in-depth HLA antibody analysis as well as comprehensive HLA typing for the donor and the recipient to allow accurate immunological risk assessment and epitope analysis.

4.1 Recommendations:

- All laboratories supporting solid organ transplantation in Australia must be ASHI and NATA accredited
- Potential transplant recipients and donors must be typed at the following HLA loci[3]:
  - HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, and -DPB1
- HLA typing must be performed using a molecular technique to the highest level of detail possible
  - High resolution HLA typing should not be imputed using population HLA allele frequencies (3)
- Deceased Donor HLA types should be confirmed by next-generation sequencing (NGS) as soon as practicable – however, NGS is not required for matching in OrganMatch at the time of organ allocation
- For deceased donors 60ml / 8 ACD tubes for HLA typing should be collected in adult donors and 2 ACD tubes in paediatric donors

5 Detection and Identification of antibodies to HLA

5.1 Assays for HLA antibody detection

5.1.1 Solid Phase Immunoassays

Luminex-based immunoassays have revolutionised the identification and characterisation of HLA antibodies, particularly for HLA class 2 loci including the polymorphic alpha and beta chains of HLA-DQ and -DP molecules. The antibody targets in the Luminex assay are polystyrene beads coated with single HLA molecules. The Luminex beads contain different ratios of two fluorescent dyes, allowing up to 100 beads to be discriminated in a single assay by flow cytometry. Fluorescent anti-human IgG is used to detect antibody binding to beads. There are currently two manufacturers producing Luminex assays for the detection of HLA antibodies, each producing three types of bead panel:

1. Mixed/pooled antigen beads coated with a wide selection of either HLA class I or class II molecules that can be used as an initial screening test for the presence of anti-HLA Ab
2. Phenotype beads coated with HLA class I or 2 antigens representing frequent HLA haplotypes
3. Single antigen beads (SAB) each coated with a single class I or II HLA molecule. SAB provide the greatest sensitivity and specificity, allowing precise assessment of the breadth of anti-HLA sensitisation

SAB have excellent sensitivity as well as specificity and provide a semiquantitative estimate of antibody strength (mean/median fluorescence intensity or MFI). Some laboratories have used mixed antigen beads to screen for the presence of anti-HLA antibodies, and reserved SAB for assessment of antibody specificity. However, as the range and density of HLA molecules present on the mixed beads is lower than SAB, this strategy has the potential to miss clinically significant anti-HLA antibodies[4].

There are several technical aspects of SAB testing that must be taken into account when interpreting assay results[3, 5–7]:

- The number of HLA molecules on the surface of the Luminex beads is not representative of native HLA expression on lymphocytes or tissues – Luminex beads are manufactured with a higher density of HLA molecules to provide a detectable antibody target. HLA-C, -DQ, -DP and -DR3/4/5 loci are expressed at a lower level than other HLA loci on cells and tissues, however all HLA molecule targets are present at a similar level on the Luminex beads
- HLA molecules may become denatured in the Luminex manufacturing process and/or expose cryptic epitopes which can cause false positive results in the assay
- The Luminex assay identifies both complement-fixing and non-complement-fixing antibodies
- Whilst the Luminex SAB panel has a finite number of HLA alleles in the panel, all HLA antibody verified epitopes are represented
- Antibodies to shared or public HLA epitopes may generate lower than expected MFI results in the Luminex assay, as the antibodies bind to multiple beads in the assay each displaying the same epitope – these patterns of binding can usually be identified through careful interpretation of the Luminex assay results
- Immune complexes or complement components have the capacity to cause interference with the Luminex assay, however this issue has to a large extent been resolved by the addition of EDTA to the assay

The SAB bead assay provides detailed results for HLA antibody detection for both HLA class 1 and class 2. However, the results generated from SAB testing needs careful analysis and may need additional testing to determine HLA antibody specificities for patients.

Tissue typing laboratories should be able to accurately identify the presence and specificity of antibodies to all HLA class 1 and 2 loci (as per HLA typing in section 4). Test sensitivity should be sufficient to identify low level antibodies and equivalent to any physical crossmatches performed. Tissue Typing laboratories should use a combination of techniques to meet these requirements[3, 5].

Tissue typing laboratories should have processes in place to analyse HLA antibody results in order to identify and evaluate potential false positive or false negative results (Table 2).

Table 2: Potential strategies to identify false reactivity in Luminex SAB assays

Reference to the HLA type of the patient for epitope analysis
Assessment of results using verified HLA epitopes to confirm antibodies
Confirm using serotypes / G groups / P groups in the analysis
Ensuring that self-antigens are not identified as antibodies
Further testing in cases where there is non-specific reactivity with additional serum treatment (e.g., dilution as well as EDTA) before determining whether HLA antibodies are present
Further testing with alternative kits / technologies if reactivity to denatured beads is suspected (or performance of a surrogate flow crossmatch (FXM) if cells expressing the HLA antigen in question are available)
Attention to repeated low-level reactivity against beads of the same antigen group where epitope sharing may give falsely low MFI results
Consideration that high level HLA antibodies occasionally generate a paradoxically low MFI due to prozone effect because of binding competition

5.1.2 Recommendations

- Presence of HLA antibodies should be determined using a solid phase assay (e.g., Luminex-based immunoassay)
- HLA antibody specificity should be identified using a single HLA molecule target (e.g., Luminex-based SAB assay)
- SAB testing should be performed using EDTA-treated sera to reduce potential interference with IgM/auto antibodies or immune complexes
- If required, HLA antibodies can be confirmed using an alternative Luminex SAB assay/kit
- Where appropriate and technically feasible, a surrogate flow crossmatch (FXM) assay can be performed to confirm the binding capability or strength of an antibody to an HLA target expressed by an intact cell

5.2 Frequency of monitoring for HLA antibodies

Patients who are being considered for solid organ transplantation or who are on a transplant waiting list (TWL) should be tested for HLA antibodies frequently to minimise the likelihood that the development of a clinically-relevant antibody is missed.

5.2.1 Recommendations

- A minimum of two SAB results should be obtained prior to activation on the TWL, ideally using serum samples collected ≥1 month apart. It is accepted that some recipients will need to be listed urgently and only a single SAB result may be feasible.
- Serum samples should be obtained regularly for patients on the transplant waiting list and sent to the local tissue typing laboratory for HLA antibody testing by SAB
- Serum sharing for prospective FXM if necessary – see clinical guidance for FXM for deceased donor testing serum sharing are summarised in Table 3:

Table 3: Serum collection and sharing for patients active on the TWL

Organ group	Frequency of serum collection for patients on TWL	National sharing of sera between Tissue Typing labs
Kidney / Pancreas (including SPK)	Monthly	By arrangement with tissue typing lab for individual patients who are clinically urgent, or where HLA antibody profile analysis is unable to resolve all specificities
Heart / Lung	Monthly	By arrangement with tissue typing lab for individual patients who are clinically urgent, or where HLA antibody profile analysis is unable to resolve all specificities
Liver	Pre-transplant	By arrangement with tissue typing lab
Intestinal	Monthly	By arrangement with tissue typing lab for individual patients who are clinically urgent, or where HLA antibody profile analysis is unable to resolve all specificities
Multiorgan (other than SPK)	Monthly	By arrangement with tissue typing lab for individual patients who are clinically urgent, or where HLA antibody profile analysis is unable to resolve all specificities

- Patients active on the TWL should be screened for anti-HLA antibodies every 3 months using a Luminex-based SAB assay
- Additional screening for anti-HLA antibodies should be performed 4–6 weeks after any potential sensitising events, including:
  - Pregnancy
  - Transfusion of blood products
  - Episodes of acute allograft rejection
  - Allograft nephrectomy
  - Significant changes to immunosuppressive medications in patients with an allograft in situ

- It is recommended that a day of transplant serum sample is collected and stored at the local Tissue Typing Laboratory as an immediate pre-transplant reference sample. Testing on this sample will be performed if required, for example if there is unexpected post-transplant antibody mediated rejection.

6 Assessment of HLA antibody profile and definition of unacceptable antigens

The responsibility of the tissue typing laboratory is to provide an evaluation of histocompatibility data and recipient immunologic risk factors that will allow the clinical unit to decide which approaches to transplantation are in the patient's best interest. The laboratory assessment should provide an individualised list of HLA antigens that would be unacceptable in a donor and should consider where appropriate:

1. The recipient's sensitisation history
    a. Previous transfusion of blood products
    b. Previous pregnancies and age of youngest child
    c. Donor relationship – e.g., husband to wife or child to mother
    d. Repeat mismatches from previous transplants
2. Detection and characterisation of HLA-specific antibodies
    a. The strength of the various HLA-specific antibodies
    b. Stability of antibody strength over time
3. Likelihood of repeat transplant in the future
    a. Consider avoidance of potential donor HLA mismatches with the aim of reducing risk of future sensitisation

6.1 Unacceptable antigens (UA)

HLA antigens to be avoided in potential donors will be defined Unacceptable Antigens (UA) (antigens for exclusion) and listed in the following categories – Table 4:

Table 4: Categories of Unacceptable Antigen (UA)

Category	Definition	To be included in mPRA calculation?
Antibody-sourced	Antigens to which there is evidence of historical or current HLA antibodies	Yes
Previous HLA mismatches (to HLA-A, -B, -DRB1, -DQB1)	Previous donor HLA mismatches with detectable HLA DSA	Yes
	Previous donor HLA mismatches without detectable HLA DSA (after discussion with clinical team – see section 6.3)	Yes
Other antigens for exclusion	Antigens where there is a desire to avoid with the aim of limiting future sensitisation, such as potential high eplet load HLA mismatches	No

The transplant or clinical unit must review the assignment of the UA, to ensure that this is appropriate considering the acceptable clinical/immunological risk level for the patient. This risk appetite can change over time. UA are used in the Transplant Waiting List (TWL) matching algorithms in OrganMatch to exclude potential recipients from incompatible organ offers.

If a change in HLA antibody level and/or specificity is detected, the patient's listing of antibody defined, unacceptable antigens should be reviewed in a timely manner and updated and reported in OrganMatch. All changes to UA are tracked and viewable in OrganMatch and clinical units will receive notifications of this change. An updated antibody report will be available in the OrganMatch clinical portal.

6.2 Defining MFI thresholds for HLA antigen exclusion

There is significant complexity is assigning HLA antigens for exclusion[8]. SAB assays for detection of anti-HLA antibodies are semiquantitative and there is considerable variation in MFI levels between different assay manufacturers and histocompatibility laboratories, even with strategies in place to standardise assay protocols[3,9]. Nevertheless, there is a clear correlation between increasing SAB MFI, the likelihood of a positive physical crossmatch and the immunological risk for transplantation[10].

To ensure consistent anti-HLA antibody assignment, a national approach to SAB interpretation and MFI threshold has been agreed for each organ group – Table 5. These criteria have been selected to align with typical organ acceptance practice. For individual patients these criteria may be altered by arrangement with the tissue typing lab (e.g., to increase the MFI threshold in a highly sensitised patient).

Table 5: Usual MFI Thresholds for Defining Unacceptable Antigens

Organ group	Routine MFI threshold for UA definition
Kidney / Pancreas (including SPK)	2000 (Immucor assay) 4000 (OLI assay)
Heart / Lung	3000 (Immucor assay) 6000 (OLI assay)
Liver	UA not routinely defined but may be included by arrangement with tissue typing lab
Intestinal	2000 (Immucor assay) 4000 (OLI assay)
Multiorgan (other than SPK)	2000 (Immucor assay) 4000 (OLI assay)

6.3 Repeat mismatches

Traditionally, many transplant programs have avoided transplanting recipients with organs expressing repeat HLA mismatches due to the risk of an anamnestic immune response causing accelerated rejection. However, as the precision of anti-HLA antibody detection has improved, it has become apparent that the level of risk is substantially modified by the presence or absence of DSA to the repeat mismatch. A retrospective report described clinical outcomes in 179 recipients of a second or subsequent kidney transplant, including 55 patients with a repeat mismatch[11]. In multivariate analysis, there was a significantly increased risk of ABMR (HR 8.7, 95% CI 3.4–22.1) and death-censored allograft failure (HR 3.1, 95% CI 1.2–8.1) in recipients with DSA identified to the repeat mismatch. In contrast, no increased risk of rejection, de novo DSA or graft failure was observed in recipients without DSA to the repeat mismatch. Similarly, in a large registry analysis of 13,789 recipients who received a second or subsequent kidney transplant between 1995 and 2011, repeat mismatches were present in 3,868 recipients[12]. The presence of a repeat mismatch was not a significant risk factor for all-cause or death-

censored allograft loss in a multivariable Cox proportional hazards model. The risk associated with transplantation across a repeat mismatch therefore seems acceptably low where no DSA has been identified. Clinical teams, in consultation with the tissue typing laboratory, should decide whether to exclude repeat mismatches without DSA, considering the impact that this will have on access to transplantation depending on the level of sensitisation and the frequency of the repeat mismatches in the donor population. Repeat mismatches where a DSA has been identified in multiple past sera represent an increased immunological risk and should usually be excluded even if not present in current serum.

6.3.1 Recommendations

- Repeat mismatches where a DSA (>500 MFI on SAB assay) has been detected previously should usually be excluded
- Repeat mismatches which have never generated a DSA must have a detailed epitope analysis to explain negative HLA antibody reactivity and may be included for organ offers with approval from the clinical team
- If repeat mismatches are included as unacceptable antigens they should contribute to the mPRA (whether or not a DSA has been detected previously)

6.4 Historic antibodies

Antibodies detected in historical serum may not be present at detectable levels in current serum. It should not be assumed that previously detected antibodies are not present. It is possible that are present but not at detectable levels with the assay, and if the patient was rechallenged with cognate antigen, the antibody may rebound. Alternatively, some historic antibody assignments have been based on older technology and may not be accurate, as discussed in section 5.1. Including these antibodies as antigens for exclusion may limit access to transplantation by increasing the mPRA. Therefore, histocompatibility laboratories should have a defined process to re-evaluate historic HLA antibody data where necessary. A potential algorithm could include:

1. Retesting historic sera in parallel with the current serum using more recent Luminex kits with comparison of the antibody profile
2. Retesting historic sera using an alternative SAB assay with comparison of the antibody profile
3. Performing a surrogate FXM using historic serum and cells expressing the HLA antigen in question

Individual transplant centre protocols may vary with regard to the emphasis given to historic versus current sensitisation. However, it is imperative that the listing of antibody defined UA be consistent with crossmatch practices. Therefore, if historic definition of unacceptable antigens is used for listing, appropriate historic sera must be used for final crossmatch decisions.

7 Crossmatch testing / assessment of histocompatibility

Crossmatch testing refers to techniques intended to determine whether a recipient's serum contains antibodies against a specific donor's HLA antigens. These techniques may include a physical cell-based crossmatch, or a virtual crossmatch (VXM) assessment based on knowledge of the donor and recipient HLA typing and the anti-HLA antibody profile of the recipient[13, 14].

The presence of DSA at the time of transplantation increases the risk of early antibody-mediated rejection (ABMR) and reduces the allograft survival for kidney transplants[15–18]. However, some studies suggest that the level of risk associated with low MFI DSA detected by SAB alone with negative FXM is low[19].

7.1 Virtual crossmatch (VXM)

Virtual Crossmatching (VXM) is the assessment of histocompatibility based on detailed knowledge of current HLA antibody status and the HLA type of the donor without a physical crossmatch[2, 20–22]. VXM significantly reduces the workload for donor compatibility assessment at time of donor work up and provides a faster turn-around time to generate compatible recipient lists. HLA antibody data from SAB testing has been used for many years in Australia to define UA and assess immunological risk for organ offers. However, the frequency of antibody screening and the level of detail for donor HLA typing were insufficient to permit safe VXM. However, this has changed following a series of practice changes that led to augmented antibody screening frequency (every 3 months) and comprehensive donor HLA typing for all relevant polymorphic loci.

7.1.1 Recommendations

- VXM is the first-line assessment of immunological compatibility for all solid-organ transplant offers where complete donor HLA typing is available and the recipient HLA antibody profile has been fully evaluated by SAB assay

7.2 Physical crossmatch

A physical crossmatch allows testing for HLA antibodies using cells expressing the specific donor HLA alleles. This becomes particularly important if it may not be possible to define all antibody specificities in a particular recipient's serum.

7.2.1 Complement dependent cytotoxicity (CDC) crossmatch assay

The CDC assay has been used previously as a method for detecting HLA class 1 antibodies in the serum of patients on the TWL and as a crossmatching assay testing patient serum against donor cells. This is largely a historic assay now and has been replaced by the more sensitive SAB assays on a Luminex platform for HLA antibody detection assays and flow crossmatch (FXM) when a physical crossmatch is necessary.

The CDC crossmatch assay uses donor T or B lymphocytes as targets and the presence of DSA is inferred if there is complement-mediated cell death. CDC is a whole cell assay with native expression of the HLA molecules. However, cells from deceased organ donors may have compromised viability and therefore have

suboptimal function as targets and HLA DQ and DP antigens may not be highly expressed on resting B cells. Therefore, the B cell XM may not identify HLA class 2 antibodies effectively.

Adding anti-human globulin (AHG) to the standard CDC XM improves assay sensitivity (23). Although the CDC AHG XM has been used in many international laboratories, this version of the CDC assay has never been implemented in Australia.

The CDC assay had significant limitations in identifying HLA antibodies in patients' sera:

a. IgM allo- or other autoantibodies / immune complexes (IC) may cause a positive CDC crossmatch through non-specific binding but are not thought to increase immunological risk for transplantation. Sera were treated with DTT to reduce false positive reactivity with IgM antibodies or immune complexes in the CDC assay
b. Patient sera were screened by T and B cell CDC assay.
c. HLA antibodies cannot be defined using CDC crossmatching alone.
d. The sensitivity of CDC for detection is limited, as it does not detect low level antibodies or antibodies that do not fix complement

As the CDC assay is no longer widely used, equipment and reagents are not readily available to perform this assay.

7.3 Flow cytometry Crossmatch (FXM)

Most tissue typing labs internationally now use FXM when a physical crossmatch assay is necessary. Like CDC, the FXM assay uses donor T & B lymphocytes as targets but detects the presence of DSA using fluorescent anti-human IgG rather than assessment of cell viability. The level of sensitivity is substantially improved compared to a CDC, and the FXM does not detect IgM antibodies. However, the utility of FXM is occasionally limited by the occurrence of false positive results caused by non-specific and Fc-receptor-mediated binding, particularly to B lymphocytes. A positive FXM may also occur in patients who have received medications that target lymphocytes (e.g., Rituximab) or in the presence of certain non-HLA antibodies (e.g., high level anti-AT1R antibody, ABO-incompatible transplant). The addition of pronase to the B cell FXM reduces, but does not abolish, non-specific binding[24]. Addition of pronase has been reported to modify expression of class I and II HLA and can occasionally cause false positive T cell FXM results[25].

An optimised FXM protocol has been described (Halifaster) which is more rapid and reduces non-specific binding[26]. The Halifaster flow assay protocol has been adopted by all histocompatibility labs in Australia.

The result of a FXM assay is semi-quantitative and reported as the fluorescence channel shift above the threshold for the negative control. Depending on the channel shift, the result can be classified between negative and strongly positive – Table 6:

Table 6: Reporting of FXM results

Classification	Channel shift above threshold	
	T cells	B cells
Negative	< threshold	
Weakly positive	0–50	0–100
Moderately positive	50–150	100–200
Positive	150–250	200–300
Strongly positive	>250	>300

A FXM must be clinically informative for the specific organ offer. FXM is time consuming and expensive and will need to be requested by the transplant unit. A FXM cannot be requested if there are no HLA antibodies present. The timing of a FXM (i.e., prospective or retrospective) will depend on various parameters as outlined below.

7.3.1 Recommendations

- Halifaster FXM is the preferred assay where a physical crossmatch is required
- **A prospective FXM** should be considered when: there is clinical urgency to proceed with transplantation (e.g., heart/lung patients), but there is insufficient HLA antibody data to perform an adequate VXM / DSA assessment because:
  - The recipient has not had their HLA antibodies fully defined (<2 HLA SAB assays have been performed)
  - Recipient serum has not been undergone SAB screening within the last 3 months
  - A potentially sensitising event has occurred since the most recent SAB screening
  - Assessment of the HLA antibody profile is complex, and it is unclear whether a DSA is present or not (e.g., probable non-specific reactivity in SAG assay that is inconsistent with recognised epitope-binding pattern but has not been able to be resolved by additional testing or surrogate FXM)
- Requests for prospective FXM should be discussed with the tissue typing lab to confirm the indication for FXM and the ability of the lab to provide a result within a clinically relevant timeframe
- An **immediate retrospective FXM** is recommended when intending to cross multiple low MFI DSA below agreed threshold for exclusion (UA) that is present in recent sera (clinical decision)
- **A retrospective FXM** should be considered when:
  - There are low-level DSA present which have not been considered a contraindication to transplant
  - There is a repeat mismatch to a previous allograft

For additional information please refer to the clinical guidance for Flow Cytometric Crossmatching – see appendix

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Appendix A

Guidelines for performing prospective FXM

Recipient group
Prospective FXM will be restricted to thoracic (heart and/or lung) and intestinal transplant recipients. If prospective FXM is required for an intestinal transplant to proceed, the FXM needs to be performed much earlier than for the thoracic organs. This will require upfront liaison between the VIC intestinal unit and the respective TT lab.

Prospective FXM will be performed on current sera and restricted to the following patient groups:

1. Where patient listing is urgent, and no SAB testing has been performed (or results were indeterminate) or testing was completed more than 6 months ago
2. Where there has been a known sensitising event since the previous SAB test.

Guidelines for performing urgent retrospective FXM

Recipient group
Urgent retrospective FXM, performed on the following day for local transplants (including weekends) can be performed on current sera and ONLY be requested where there is a DSA present. Urgent retrospective FXM will be restricted to thoracic (heart and/or lung) and intestinal transplant recipients. Urgent retrospective FXM may be requested on **current sera** for recipients of other organs (kidney, kidney/pancreas, islets, liver), however this will be performed on the next business day.

Urgent retrospective FXM can only be utilised in the following circumstances:

1. Where there are multiple low-level DSAs to HLA-A/B/ DR (i.e. excluding DSA to HLA- C, DQ, DP), with each individual DSA with an MFI of >1500 (using One Lambda International (OLI) beads) or MFI > 1000 Immucor beads*
2. Where there has been a known sensitising event since Where the DSA is to (HLA-C, DQ, DP) and the MFI >5000 or MFI > 1000 Immucor beads*

AND where the result of the urgent retrospective FXM will change transplant management.

Guidelines for performing routine retrospective FXM

Recipient group
Routine retrospective FXM be performed on **current sera** and can be requested for transplant recipients of any organ. Routine retrospective FXM, actioned on the following business day, can ONLY be requested where there is a DSA present.

* Pre-transplant surrogate FXM should be utilised in the work-up period wherever possible, and if results are available, this would negate the need for an urgent FXM (see section below on surrogate FXM).

Routine retrospective FXM can only be utilised in the following circumstances:

1. Where there is a DSA to HLA-A/B/ DR of MFI >1500 for OLI beads or MFI > 1000 for Immucor beads
2. Where the DSA is to (HLA-C, DQ, DP) and the MFI >5000 (OLI) or MFI >1000 (Immucor)

AND where the result of the retrospective FXM will change transplant management.

Important points to consider before requesting a prospective or urgent retrospective FXM

1. Will a positive FXM result change peri-operative management after accepting the organ?
   a. If yes: what level of positivity will change management (e.g. positive/strong positive FXM result)? NB: MFI >2000 (OLI) is a reliable predictor of FXM positivity for HLA-A, -B and -DR (see Hiho et al, 2022)
   b. If no: a retrospective FXM can be performed in routine laboratory processing times
2. Is the patient on any medication that may interfere with a FXM (e.g. rituximab or other antibody therapies targeting lymphocytes)?
3. Does the patient have any known autoantibodies that may interfere with FXM (e.g. high anti-AT1R Ab, ABOi transplant)?
4. Like other physical crossmatches (e.g. CDC), there may be circumstances where a FXM result is indeterminant or not possible due to technical reasons (e.g. point 2 and 3 above or limitations on donor cell availability/quality).

Sharing sera

Prospective FXM

Patient sera will be shared for interstate offers for urgent heart, lung and intestinal patients where the DSA profile is unknown.

Urgent retrospective FXM

Current sera will be shipped overnight to the donor home state when required.

Current sera can also be shared ahead for interstate offers

For urgent retrospective FXM for highly sensitised patients where it is intended to cross a significant DSA or for patients where there is planned desensitisation to facilitate transplant. This will be assessed on a case-by-case basis by communication between the clinical team and tissue typing lab.

Circumstances where surrogate FXM should be utilised wherever possible

1. Antibodies around FXM thresholds that may be considered for transplant (MFI 2000- 6000 (OLI), 1000- 4000 (Immucor,) HLA-A, -B, -DR) and would improve access to donors. Some of these may also be considered in the urgent retrospective FXM group, only after acceptance of the organ and where the FXM will change peri-operative management (i.e. a positive or strong positive, result)
2. HLA-C, -DP and -DQ specific antibodies MFI >5000 (OLI) >3000 (Immucor)
3. Recipients with sudden changes in antibody profiles (significant drops in MFI).