Flow CrossMatching

Clinical Guidance document

Introduction

The following guidelines are a 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 requesting a flow crossmatch (FXM) to support recipient and donor crossmatching and therefore transplantation in Australia.

As the Complement Dependant Cytotoxicity (CDC) crossmatch is no longer available in Australia, there may be some rare situations where a physical crossmatch may be required. The FXM may be requested under defined circumstances to provide additional immunological data that is not provided by the Virtual Crossmatch (VXM).

Purpose

The purpose of this clinical guideline is to support the clinician in determining the requirement for a FXM following a Virtual Crossmatch Result (VXM).

User

The intended users of this guideline are both the Transplantation Clinicians and the Tissue Typing staff in Australia.

Scope

This document contains clinical operational information pertaining to the request and requirements for a FXM within Australian Laboratories.

Abbreviations

ABO	Accredited blood group
ABO-I	ABO incompatible
AT1R	Angiotensin Type-1 receptor
CDC	Complement Dependent Cytotoxicity
FXM	Flow Cytometry Crossmatch
HLA	Human Leucocyte Antigen
MFI	Mean/Median Fluorescence Intensity
OTA	Organ and Tissue Authority
SAB	Single Antigen Bead immunoassay
TSANZ	Transplantation Society of Australia and New Zealand
VXM	Virtual Crossmatch

Definitions of terms used in this document

VXM	VXM is a sensitive technique to determine compatibility between donor and recipient. Recipient HLA antibodies are defined using SAB technology which is a sensitive assay and will identify all HLA antibody targets (eplets). Donor HLA typing is compared to the recipient HLA antibodies virtually and an assessment is made on compatibility
FXM	The FXM is an established technique for assessing compatibility between a transplant recipient and a potential donor. In the FXM, donor lymphocytes are mixed with recipient serum. If donor-specific IgG antibodies (DSA) are present in sufficient amounts in the serum, these will bind to the donor lymphocytes and will result in a positive FXM. FXM is a more sensitive physical crossmatch than the outdated CDC crossmatch. However, numerous studies indicate that VXM using SAB technology is more sensitive and specific for detecting DSA than FXM.
Surrogate FXM	Surrogate FXM is a technique that can be used in the pre-transplant period to help determine the strength of antibodies identified by single SAB testing. In a surrogate FXM, the recipient's serum is mixed with lymphocytes from a third- party possessing the HLA allele(s) specific for the antibody or antibodies identified.
Prospective FXM	Prospective FXM is an urgent FXM which is performed in after-hours on call work in the TT laboratories. However, performing the FXM is a highly specialised and time-consuming technique for the TT laboratories, which is limited to patients where current antibody screening is not available (see below).
Retrospective FXM	Retrospective FXM is performed after acceptance of the organ offer where the FXM result is critical to guide patient management. This may be requested as an urgent service (urgent retrospective FXM) and can be performed on the day immediately following deceased donor processing for a local donor and recipient, or the sera will be sent to the relevant TT lab on the following day for interstate transplants. However, this is restricted to a highly targeted patient group that fulfil the criteria in the section below.

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 testing.

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)*
2. Where the DSA is to (HLA-C, DQ, DP) and the MFI >5000*

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

*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).

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 (IC), 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 (IC)
3. Recipients with sudden changes in antibody profiles (significant drops in MFI).