VIRTUAL CROSSMATCH

Frequently Asked Questions

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Transition to Virtual Crossmatch

Why are we ceasing CDC Crossmatches?

The Complement Dependent Cytotoxic (CDC) crossmatch assay is an older technology that is no longer considered an optimal test by the industry. Whilst the small plastic trays used for CDC are still available, other equipment and reagents needed for the assay are increasingly difficult to source. CDC testing has been ceased in most countries as the level of sensitivity is too low when compared to the Luminex solid phase platforms for HLA antibody testing and a CDC crossmatch does not provide an accurate reflection of DSA in the recipient.

What is a Virtual Crossmatch?

A virtual crossmatch (VXM) is an assessment of transplant compatibility using information about patient HLA antibodies and detailed donor HLA typing. Recent single antigen bead HLA antibody results are crosschecked against the donor HLA type to identify the presence of any donor specific antibodies (DSA). Since this is data-driven rather than a physical laboratory test it is termed a 'virtual' crossmatch.

Is a Virtual Crossmatch artificial intelligence (AI)?

No, a virtual crossmatch just compares the antigens for exclusion in a recipient and the donor HLA type. OrganMatch will then show whether any unacceptable donor antigens are present. The tissue typing scientists will perform DSA assessment using OrganMatch data and determine the final DSA result.

Can I still request a physical crossmatch?

Once CDC has been phased out, a flow crossmatch (FXM) can be requested however these will need to be limited as it is a very expensive and time-consuming assay.

- Prospective FXM will be limited to scenarios such as intention to cross a DSA for a particular recipient, or presence of a lot of cumulative DSA below UA cutoff which can occur in highly sensitised patients.
- A retrospective FXM will be performed the next working day when a FXM will provide immunological information that will inform transplant outcome or assist with planning post-transplant management. If no DSA has been detected, there would be no need for a FXM.

Both these assays are expensive and time consuming so this is a resource that needs to be used when it will provide information.

FXM cannot be requested for checking or screening of potential DSA.

When is VXM going to be implemented?

The transition to VXM is well underway and gradually occurring across three phases. The final phase to implement VXM nationally will occur from July 2022. Details of the changes and timeframes for each phase are available in the project communique on the OrganMatch website.

Where can I find further information?

More information is available on the OrganMatch website and a recording of the VXM webinar on 5 October is available on the TSANZ website.

Luminex Tests

What is the Luminex SAB test?

The Luminex Single Antigen Bead test is an assay which has single HLA molecules on Luminex beads that can be used to identify HLA antibodies in a patient's serum.

Luminex SAB assays do not have all HLA alleles on the bead panel but the HLA alleles in the assay cover all HLA antibody verified epitopes and will detect all HLA antibodies at a high level of sensitivity for all HLA class 1 and class 2 alleles.

Note: The tissue typing laboratories have been using the Luminex SAB test for over 15 years and there have been no cases where there was a positive CDC crossmatch due to a HLA antibody that was not detected by the Luminex SAB test.

What is MFI?

Mean/Median **F**luorescence **I**ntensity is the measurement of antibody bound to a specific Luminex bead and is how the SAB results are reported. It should be noted however that the SAB test is not quantitative – see technical issues below.

Are there technical issues with Luminex SAB tests?

There are 2 main technical issues with SAB testing:

1. **Denatured beads**

The single antigen bead assay is made up of single HLA molecules attached to a Luminex bead. The process of attaching single HLA molecule sometimes disrupts the peptide in the HLA molecule and these are called denatured beads. Denatured beads expose different antibody targets to the native cell and a positive result may not represent a true HLA antibody. Some HLA alleles are prone to damage in the manufacture of SABs as the peptide in these HLA alleles may not be bound as tightly as others. The tissue typing laboratory scientists are very experienced in interpreting SAB results and will account for denatured bead reactivity.

2. **Overexpression of HLA molecules.**

HLA molecules on Luminex beads are manufactured to work well in the assay. HLA molecules on immune cells have differential expression of HLA loci due to their function. Consequently, the Luminex SAB results measured in MFI are not an indication of quantity of antibody.

What is the difference between a HLA allele and an epitope?

The HLA alleles are the subgroups of the HLA antigen families. For example, HLA – A*11 is the antigen family and HLA-A*11:01 is one of the alleles. HLA antibody epitopes are the antibody targets (binding region) and these are common (shared) within in an antigen family but individual alleles may have specific epitopes → alleles specific antibodies. HLA alleles are a cluster of epitopes so antibodies directed against one epitope can bind to alleles within and antigen family and to any other HLA allele that shares that epitope.

Impact of epitope sharing on MFI – what is the peanut butter effect?

HLA antibodies are directed against antibody binding sites (epitopes) on the HLA molecule. Some HLA epitopes are shared across several HLA alleles causing a low MFI signal as the antibody is spread across several beads – termed the peanut butter effect. The Tissue Typing laboratory scientists will perform epitope analysis for all patient antibodies and will detect this phenomenon and advise on the best approach to manage these.

Antigens for Exclusion and Donor Specific Antibodies

What are ‘antigens for exclusion’?

Patients on a transplant waiting list are screened regularly for the presence of HLA antibodies using a single antigen bead Luminex assay. If antibodies are detected at a level that is considered too high a risk for transplantation, then the respective HLA antigens can be listed for exclusion so that donors expressing these antigens will not be offered to that recipient.

How are antibodies selected to be listed as antigens for exclusion?

Antigens will be listed for exclusion based on the presence of HLA antibodies that are above an agreed MFI threshold for a Luminex single antigen bead test. This threshold will vary for different organ groups. This value is a guide only - so antibodies below the agreed threshold may also be excluded if they are part of an antigen group or previous transplant HLA mismatch. Some antibodies may have artificially low MFI if the antibody is spread across several beads. The tissue typing laboratories are experts in Luminex SAB antibody analysis and will confirm the profile of HLA antigen exclusions for patients with the clinical units.

Why are there DSA present if we are listing HLA antibodies as antigens for exclusion?

There may be still be some low-level HLA antibodies present below the MFI cut-off that is used to determine which antibodies should be listed as antigens for exclusion.

How will I know if my patient has a DSA at time of offer?

Donor specific antibodies are clearly identified on the Organ Offer list DSA assessment and OrganMatch match event report.

What if the patient had a previous transplant but doesn't have antibodies to the previous mismatches – are these automatically excluded?

In the past repeat mismatches were automatically excluded irrespective of whether there was evidence of sensitisation to these antigens. Repeat mismatches where there are antibodies present will continue to be excluded. If there are no antibodies present to previous mismatches and an epitope analysis has been performed assessing previous donor HLA typing the clinical unit can choose to not exclude previous mismatches. In both cases these will be included in the mPRA value.

My patient has a HLA antibody to a HLA allele but the donor is only HLA typed at the antigen level, will this be a DSA?

HLA alleles are part of HLA antigen families. HLA-A*02:01 and HLA-A*02:06 are part of the HLA A2 antigen family. Antigen families share amino acid sequences across most of the antigen recognition site of the HLA molecule. Whilst there can be some differences in epitopes across the HLA alleles, most of the HLA alleles in an antigen group will share epitopes.

It should be noted that a true allele specific antibody is usually only seen when the patient has another allele of the same antigen family and has made an antibody to a restricted epitope in that antigen group. If the allele typing of the donor is not available a prospective FXM might be required however this should be discussed with the tissue typing laboratory for an epitope assessment.

If my patient has an antibody to a specific HLA allele but the donor HLA type has a different allele which is not represented on the single antigen bead test panel will this be a negative virtual crossmatch?

Not necessarily - just because the donor has a different HLA allele to the antibody specificity in the patient it does not mean that there are no shared epitopes. Across the HLA antigen groups most alleles will share epitopes and may only have a single restricted epitope which defines the different allele type.

Why can't we do HLA allele level HLA typing for donors at time of organ donation?

HLA typing requires DNA sequencing to determine the HLA alleles. HLA typing by Next Generation Sequencing is routine used in the tissue typing for HLA typing however this test can take a long time and is not able to be used for deceased organ donor HLA typing.

There are newer technologies being developed which will enable faster allele level HLA typing but these are not yet readily available for use in the deceased organ donor setting.

What if the antibody bead panel does not have a HLA allele that is present in the donor?

The Luminex Single Antigen Bead panels cover all the epitopes of clinical significance through the variety of alleles on the Luminex panel. HLA alleles are a cluster of epitopes which can be shared across the antigen group. Some specific alleles within an antigen group will have an epitope which is unique to that allele – allele specific epitope. The tissue typing laboratories have been using the Luminex SAB test for over 15 years and there have been no cases where there was a positive crossmatch due to a HLA antibody that was not detected by the Luminex SAB test.

Can I add up MFIs to determine strength of HLA antibodies?

No, the MFI result is bead specific and does not represent titre of antibody as the Luminex beads are an artificial construct and the expression of the HLA allele is not the same as it would be on a cell.

The MFI value can infer strength of antibody as it can be an expression of the amount of bound antibody on any individual Luminex beads. The Luminex beads have no consistency of the number of HLA molecules across the beads.

Adding MFI may provide a sum of bound antibody as detected in the assay but does not represent the total amount of antibody in the patient's sera.

The sum of MFI values should not be used.

What is the difference between cPRA and mPRA?

PRA is the **P**anel **R**eactivity **A**ntibody which is a way of expressing how broadly sensitised a particular recipient is. Someone with a PRA of 80% is likely to be crossmatch positive with 80% of donors and negative (compatible) with 20% of donors. The 'c' indicates that this is calculated from the Luminex HLA antibody data and not directly observed using population data.

1. cPRA is the frequency of the HLA antigens in the population of the HLA antibodies detected in a single sample.

2. mPRA is the match PRA value in OrganMatch, which is determined by the range of antigens for exclusion. Listing antigens for exclusion enables OrganMatch to exclude immunologically incompatible donors for a deceased organ donor who has these HLA antigens. This value provides an estimate of likelihood of being compatible with an organ donor. A high mPRA excludes many HLA antigens and limits the number of compatible donors.

Why do the HLA antibody tests need to be performed every 3 months on active transplant waiting list patients?

Virtual crossmatching depends on knowing the current HLA antibody profile in a patient's serum. As antibodies can change over time or there have been an unrecognised sensitising event, it is important to have regular HLA antibody testing to ensure that a virtual crossmatch is done using the patient's current antibody profile.

What is a sensitising event?

When a patient has a sudden increase in their HLA antibodies this is often due to a sensitising event such as a nephrectomy, transfusion, removal of VAD.

It is important to retest the patient for a change in their HLA antibody profile 4 weeks after a potential sensitising event.

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Offer process in VXM

What is the organ offer list (OOL)?

The Organ Offer List is the list generated by OrganMatch that will list the compatible recipients suitable for the organ offer. As not all organ groups have a specific allocation algorithm the name has been changed from Organ Allocation List to Organ Offer List.

When can I request a prospective FXM?

The number of prospective flow crossmatches will be limited as it is only possible to set up FXM for 6 samples (recipients) at a time and the assay will take just over 3 hours to complete. The donor testing lab will also need to have the patient's serum for testing and the tissue typing laboratories are working out how to send sera in small aliquots for FXM for national offers which is very different to sending CDC trays.

It is expected that prospective FXM will be limited to the accepting offer recipient where the virtual crossmatch cannot completely rule out the presence of a DSA. It maybe that highly sensitised patients where low-level antibodies are not excluded and there is a weak DSA present or where there is a DSA that needs to be crossed as the transplant is clinically urgent.

When can I request a retrospective FXM?

It is expected that retrospective FXM will be performed post-transplant when there are low level antibodies that cannot be excluded as a DSA and when the results of a FXM will inform the management of the patient post-transplant.

VXM reporting

Will all VXM determined crossmatch negative be CDC and FXM negative?

The relationship of the VXM to a physical crossmatch result will depend on the criteria chosen to define antigens for exclusion, which may vary to some degree between organ groups.

For kidney patients the threshold for defining antigens for exclusion is low so it is expected that all VXM will be CDC neg however some patients with a DSA below the threshold may have a weak positive FXM.

The VXM is only as good as the data in the system therefore it is important that patient antibody testing is performed quarterly and that the laboratory is notified of any sensitising events.

How will Flow Crossmatch results be reported?

Flow Crossmatch results will be reported as a positive weak /moderate/ strong/ very strong based on the level of channel shift or negative result for T and B cells with values for channel shift and controls.