

## Review Article

# Understanding crossmatch testing in organ transplantation: A case-based guide for the general nephrologist

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antibody specificity, crossmatching, flow cytometry, graft rejection, kidney transplantation, tissue.

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**ABSTRACT:**

**Crossmatching of potential renal donors against potential renal transplant recipients has been performed for over 40 years and is a mandatory component of the transplant work-up process. However, gone are the days when all that was available was the T-cell complement-dependent cytotoxicity crossmatch. There are now many more options available for determining the likelihood of donor-specific antibody-mediated responses including flow crossmatching and the ‘virtual’ crossmatch. In addition, assays to determine the extent of sensitization of cell-mediated responses are being examined. This article builds an understanding of modern day crossmatch interpretation using a case-based approach in order to provide a framework for the general nephrologist to determine the likely immune consequences of a particular donor–recipient pairing.**

**SUMMARY AT A GLANCE**

Crossmatching and tissue typing are crucial components of renal transplantation. There have however been a number of new developments in the last decade. This paper discusses current crossmatching techniques and is a very useful aid for nephrologists to interpret the crossmatch results of their patients.

**INTRODUCTION**

Crossmatching was developed in an attempt to identify recipients who are likely to develop acute vascular rejection of a graft from a given donor. This phenomenon, hyperacute rejection (HAR), is a result of preformed antibodies to one or more human leucocyte antigens (HLA) of the donor; referred to as donor-specific antibodies (DSAbs). Such antibodies are formed as the result of previous exposure to HLA, generally through pregnancy, blood transfusion or previous transplantation.<sup>1</sup> There are other debated forms of developing anti-HLA Abs such as via microbial exposure but the three exposures mentioned above are thought to be the most relevant. Particularly relevant is the exposure of women during pregnancy, to their partner’s HLA. This commonly results in direct sensitization against the partner, potentially making him an unsuitable donor. HAR may also occur in blood group incompatible transplantation without desensitization.

Preformed antibodies cause rejection by binding to HLA antigens expressed on the endothelium of vessels in the transplanted kidney, resulting in activation of the complement cascade with resultant thrombosis and infarction of the graft (reviewed in<sup>2</sup>). HAR can occur immediately upon reperfusion of the donor kidney. This catastrophic outcome necessitates the immediate removal of the graft. Clearly avoiding HAR is desirable and crossmatching helps predict and hence prevent this.<sup>3</sup>

**UNDERSTANDING THE CDC CROSSMATCH**

In brief, a crossmatch involves placing recipient serum (potentially containing donor-specific anti-HLA antibodies) onto donor lymphocytes (containing HLA antigens). A cytotoxic reaction (deemed ‘positive’) suggests the presence of preformed DSAbs. A more detailed description is provided later in this manuscript.

**Table 1** Case 1 typing and crossmatch

HLA Typing	
Recipient	A 1,3 B 7,44 DR 17,4
Donor	A 2,23 B 52,57 DR 1,14
CDC crossmatch results	
CDC T-cell XM	+ve
CDC B-cell XM	+ve

+ve, positive; CDC, complement-dependent cytotoxicity; HLA, human leucocyte antigen; XM, crossmatch.

## Case 1

A 44-year-old woman with end-stage renal failure secondary to reflux nephropathy is interested in a renal transplant and her husband has offered to be a donor. They are of the same blood group but are unmatched on tissue typing (0/6 HLA matches at the HLA-A, -B and -DR loci). They have a complement-dependent cytotoxicity (CDC) crossmatch performed as part of their initial assessment, which shows a positive result for both the T- and B-cell crossmatch (Table 1). Is it safe to proceed?

## Interpretation

It is not safe to proceed in light of these crossmatch results but clarification steps are needed to better understand the reason for the positive results. This could be a falsely positive result (technical issue) or there may be autoantibodies (against lymphocyte antigens) present in the recipient serum.

Autoantibodies are generally IgM rather than IgG antibodies. To establish if autoantibodies are responsible for the result an auto-crossmatch should be performed. In this assay, recipient serum is crossmatched against recipient (rather than donor) lymphocytes. Second, the original crossmatch should be repeated with the addition of the agent Dithiothreitol (DTT). DTT reduces the disulfide bonds in IgM thereby preventing IgM antibodies from generating a positive result. IgM antibodies are generally regarded as having no pathological significance in transplantation.<sup>4-7</sup>

If a repeat crossmatch with DTT is negative then it may be safe to proceed with the transplant. An auto-crossmatch adds weight to this analysis by determining if the recipients are reacting against their own T or B cells in a similar way (Table 2). These results suggest that the reaction of the recipient to the donor is on the basis of autoantibodies. This means that the transplant could proceed using this pairing; however, before most live donor transplants and indeed cadaveric transplants more information is routinely available that aids in forming a more complete assessment of immunologic risk.

When interpreting crossmatches with the addition of DTT it is important to check the reaction in the wells to which a

**Table 2** Case 1. Further crossmatch testing

Crossmatch with DTT added (to cleave IgM)	
DTT – CDC T-cell XM	–ve
DTT – CDC B-cell XM	–ve
Crossmatch with PBS added (to control for effect of dilution)	
PBS – CDC T-cell XM	+ve
PBS – CDC B-cell XM	+ve
Auto-crossmatch (recipient lymphocytes used rather than donor lymphocytes)	
Auto – CDC T-cell XM	+ve
Auto – CDC B-cell XM	+ve

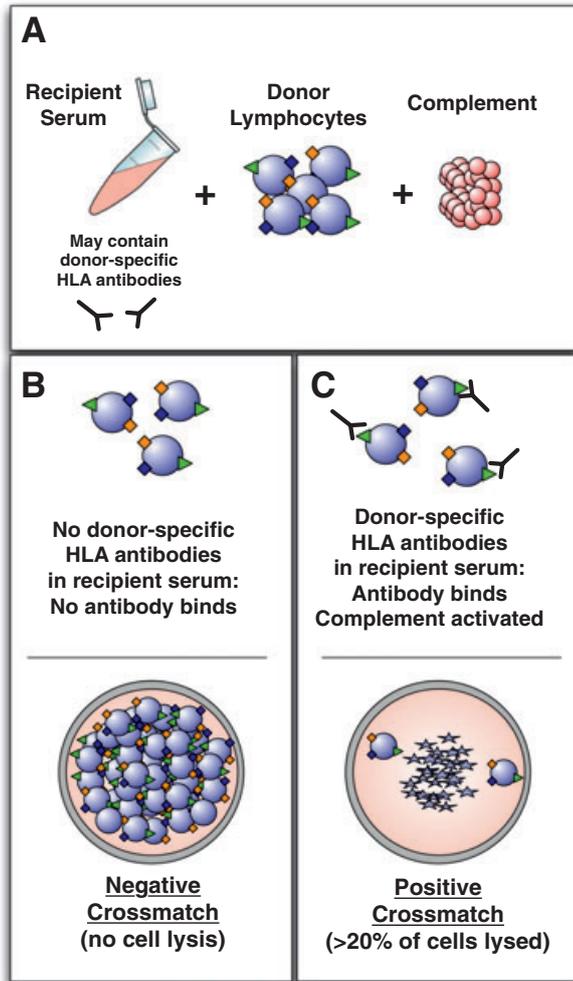
+ve, positive; –ve, negative; CDC, complement-dependent cytotoxicity; DTT, Dithiothreitol; PBS, phosphate-buffered saline, XM: crossmatch.

control agent has been added, usually phosphate-buffered saline. This controls for the effect of diluting the level of antibodies when adding DTT to the reaction. Hence, if the crossmatch becomes negative with the addition of phosphate-buffered saline, the results with DTT cannot be fully interpreted as the result may have become negative by diluting the antibody level.

## The CDC crossmatching technique

Complement-dependent cytotoxicity crossmatching was pioneered by Terasaki and colleagues in the 1960s.<sup>3,8</sup> It seeks to identify clinically significant donor specific HLA antibody mediated responses for a given recipient. Lymphocytes from the donor are isolated and separated into T and B cells. Serum from the recipient is mixed with the lymphocytes in a multi-well plate. Complement is then added (usually derived from rabbit serum). If donor-specific antibody is present and binds to donor cells, the complement cascade will be activated via the classical pathway resulting in lysis of the lymphocytes (see Fig. 1).

The read-out of the test is the percentage of dead cells relative to live cells as determined by microscopy. The result can thus be scored on the percentage of dead cells, with 0 correlating to no dead cells; scores of 2, 4 and 6 represent increasing levels of lysis. On this basis, a score of 2 is positive at a low level, consistent with approximately 20% lysis (generally taken as the cut-off for a positive result). A score of 8 represents all cells having lysed and indicates the strongest possible reaction. The use of a scoring system allows a semi-quantitative analysis of the strength of reaction. Another way to determine the strength of the reaction is to repeat the crossmatch using serial doubling dilutions of the recipient serum (often known as a 'tired crossmatch'). In this way, dilutions are usually performed to 1 in 2, 4, 8, 16, 32, 64 and so on. In the situation of a high titre of high avidity DSAb it may be that many dilutions are required for the test to become negative (e.g. 1 in 128). With antibody at a low level or one with a low affinity, a single dilution may be enough to render the crossmatch result negative. This



**Fig. 1** The CDC crossmatch. Recipient serum potentially containing donor-specific anti-HLA antibodies is added to donor T or B lymphocytes, along with complement (A). If donor-specific antibodies are not present, no lysis occurs and the result is deemed negative (B). If donor-specific anti-HLA antibodies bind to the lymphocytes and then activate complement, cell lysis will occur and the crossmatch result will be deemed positive (C). The proportion of lysed cells is assessed and the crossmatch is graded as being weakly, moderately or strongly positive.

may also give an indication as to the likelihood that a negative crossmatch could be achieved with a desensitization protocol.

### Additional CDC crossmatch considerations

The basic CDC crossmatch can be enhanced by the addition of antihuman globulin (AHG). This technique increases the sensitivity of the CDC crossmatch as a result of multiple AHG molecules binding to each DSAb attached to the donor cells thereby amplifying the total number of Fc receptors available for interaction with complement component 1, which increases the likelihood of complement

activation and cell lysis. In Australia this assay is not routinely used.

It is also possible to have a negative crossmatch in the presence of a DSAb. Situations where this might occur include: (i) when the antibody titre is too low to cause complement activation; (ii) when the antibody is of a type that does not activate complement; (iii) and when the antigen for which the antibody is specific is expressed only at very low levels on the donor's lymphocytes.

A further consideration relates to variations in antibody levels in a given individual's serum samples, collected at different times. The most reactive serum is generally called the 'peak serum'. This may have been collected several years earlier, with the 'current serum' showing quite different reactivity. As an example, the peak serum may show a clear positive CDC crossmatch result, but as the antibody levels have fallen in subsequent sera, so too may the degree of cell lysis in the assay. This may render the CDC crossmatch negative. Nevertheless, the antibodies found in the peak sera may still be of relevance, increasing the risk of early rejection as a result of this prior sensitization and the resulting immunological memory. For this reason, patients on transplant waiting lists have sera collected at frequent intervals; variations can be monitored and newly appearing HLA antibodies can be detected.

### Basic immunology of the crossmatch

In interpreting crossmatches a basic understanding of HLA expression is required. The genes encoding HLA are found on chromosome 6 and are inherited en bloc; such that half of each individual's HLA (an allele) will be from each parent.<sup>9</sup> HLA is divided into class I and class II. Class I molecules are HLA A, B and C while class II molecules are HLA DR, DP and DQ.

Class I molecules are expressed on all nucleated cells while class II molecule expression is restricted to cells such as antigen presenting cells, for example, dendritic cells, macrophages and B cells. Importantly for transplant rejection pathophysiology, both class I and II HLA can be expressed by vascular endothelial cells.<sup>9</sup> Most rejection responses are thought to be due to differences in HLA between donor and recipient, with the HLA mismatched antigens serving as the targets in antibody-mediated rejection. Non-HLA antigens may generate rejection responses but in general this is thought to be less common.<sup>1</sup>

There are important differences in HLA expression between T and B cells, which influence the interpretation of the crossmatch. T cells do not constitutively express HLA class II so the result of a T-cell crossmatch generally reflects antibodies to HLA class I only. B cells on the other hand express both HLA class I and II so a positive B-cell crossmatch may be due to antibodies directed against HLA class I or II or both. Hence, if the T- and B-cell crossmatches are positive the interpretation is that there may be either single or multiple

**Table 3** Interpretation of Crossmatch result

T-Cell XM	B-Cell XM	Interpretation
-ve	-ve	No DSAb to HLA class I or II OR DSAb titre too low to cause positive reaction OR (DSAb that is not complement-fixing – relevance unclear)
+ve	+ve	DSAb/s to HLA class I OR Multiple DSAb/s to HLA class I +/- II
-ve	+ve	DSAb/s to HLA class II OR Low level DSAb/s to HLA class I
+ve	-ve	Technical error (possibly related to B-cell viability). The test should be repeated

+ve, positive; -ve, negative, DSAb: donor-specific anti-HLA antibody; HLA, human leucocyte antigen, XM: crossmatch.

HLA class I DSAb/s or a mixture of HLA class I and II DSAb/s. While a negative T-cell crossmatch in the setting of a positive B-cell crossmatch suggests either that: (i) there is/are one or more class II DSAb/s but no class I antibodies; or (ii) that there is a low-level DSAb to a class I antigen with greater lysis of B cells relative to T cells. This is often due to the fact that B cells express higher levels of HLA class I than do T cells.<sup>10</sup> When class I complement fixing HLA DSAb/s are present at a significant level one would expect both the T- and B-cell crossmatches to be positive. A negative B-cell crossmatch in the presence of a positive T-cell crossmatch therefore suggests a technical error. This is not unusual as B cells tend to be less resilient than T cells and their viability can often be a concern in the assays. These points are summarized in Table 3.

### The relevance of a positive T-cell CDC crossmatch

Proceeding with a transplant in the setting of a positive T-cell crossmatch, which is not due to an autoantibody, is likely to generate a very poor outcome. In their seminal work in this area Patel and Terasaki described the outcomes of 30 such transplants.<sup>3</sup> Twenty four (24) patients lost their grafts immediately to HAR while another three lost their grafts within 3 months. It is not clear why the other three patients had less severe reactions but it may relate to false positive crossmatches generated by autoantibodies given that DTT was not used in their assays. Other possibilities include false positive tests or lower immunogenicity of the antibodies or antigens in those cases.

More recently, a study investigated whether IVIg or plasma exchange was more effective at desensitizing crossmatch-positive recipients so that they might be crossmatch-negative at the time of transplant.<sup>11</sup> While most patients were successfully desensitized there was a group of 10 patients who did not achieve a negative crossmatch but were still transplanted. Of this group 70% developed AMR with 50% losing their grafts. Given this data, even after reducing the antibody

titre with a desensitization protocol before transplant, a persistent positive T-cell crossmatch remains an absolute contraindication to transplantation.

### The relevance of a positive B-cell CDC crossmatch

B-cell CDC crossmatching is not as predictive of HAR as the T-cell CDC crossmatch and there has been much controversy about its role.<sup>12</sup> Many centres do not perform B-cell crossmatching for cadaveric renal transplantation because of uncertainty about the significance of a positive result. The major limitation is a rate of false positive results of up to 50%.<sup>13</sup> While a negative result is reassuring a positive result may mean a transplant is cancelled when it was safe to proceed. Another argument against the routine use of B-cell crossmatching is that antibodies to class II antigens are of less significance in generating antibody-mediated rejection. More recently it has been found that they are not so benign.<sup>14</sup>

B-cell crossmatches are often performed as part of the immunologic assessment before live donor transplantation when there is more time to determine the significance of the result. Paired with information about the presence of DSAb/s, determined by more specific means such as antigen-coated beads (Luminex, discussed below) the B-cell CDC crossmatch results may be more meaningful.<sup>15</sup> If a B-cell crossmatch is positive and there are no detectable antibodies to class I or II antigens, the result may be falsely positive while a positive result in the presence of detectable DSAb/s signifies that the identified DSAb may be functionally relevant in that it can activate complement. This has led to the suggestion that the B-cell CDC crossmatch should not be used alone to determine transplant suitability and that it be interpreted only in the light of accompanying Luminex results.<sup>15</sup> One could argue it now has no role at all; however, its strength lies in having a functional read-out that is not the case with Luminex or flow crossmatching.

## UNDERSTANDING THE FLOW CROSSMATCH

In brief, a flow crossmatch involves adding recipient serum to donor lymphocytes and then incubating them with fluorescein-labelled antibodies against human IgG (anti-human IgG F(ab)/FITC). This fluorescein-labelled antibody will bind to all the IgG antibodies in the recipient serum. If a DSAb in this serum then binds to the donor lymphocytes, it will be detectable by flow cytometry.

### Case 2

A 30-year-old mother of four has end-stage renal failure as a result of reflux nephropathy. Her husband offers to donate a kidney to her. They are of matching blood groups and their tissue typing and crossmatch results are shown below. Is it safe to proceed? (Table 4)

**Table 4** Case 2 typing and crossmatch

HLA Typing		
Recipient		A 1,3 B 7,44 DR 4,17
Donor		A 1,23 B 7,57 DR 14,17
CDC Crossmatch results		
CDC T-cell XM		-ve
CDC B-cell XM		-ve
Flow Crossmatch results		
Flow – CDC T-cell XM		+ve
Flow – CDC B-cell XM		+ve

+ve, positive; -ve, negative; CDC, complement-dependent cytotoxicity, HLA: human leucocyte antigen; XM, crossmatch.

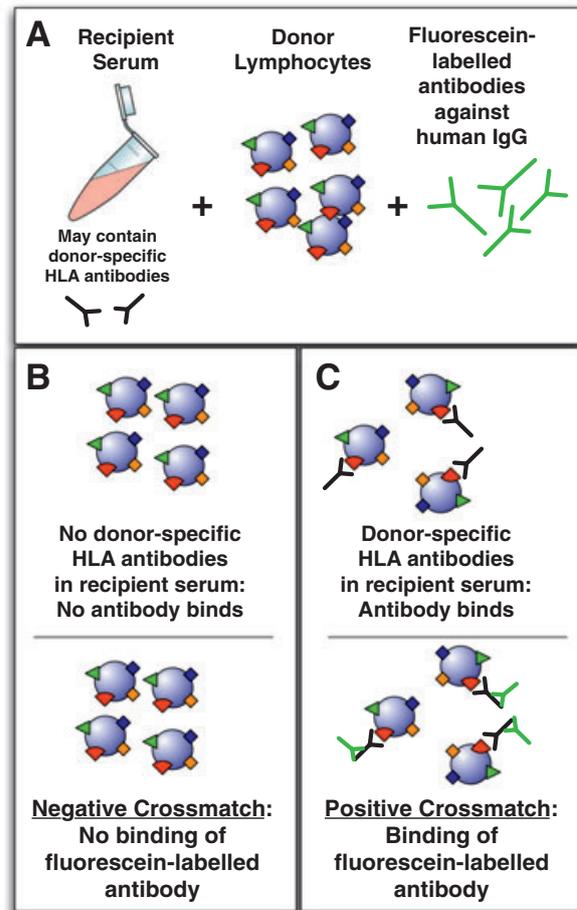
## Interpretation

Simple interpretations of these results include: (i) there is a low-level DSAb (or several antibodies); and (ii) there is/are one or more DSAb that are not complement fixing. There are, however, other considerations. If the donor in this instance was a cadaveric donor the flow crossmatch result would generally not be available at the time of organ allocation. Without further information most transplant clinicians would accept this offer, on the basis of the negative CDC crossmatch. Viewed in that light we could conclude that it may be reasonable to proceed; however, in the live donor setting there is more time to reflect on the immunological aspects of the pairing and potentially desensitize the recipient before transplantation.

Flow crossmatching detects antibodies binding to donor lymphocytes and suggests an increased likelihood of antibody-mediated rejection.<sup>16,17</sup> Flow crossmatches are more sensitive for detecting DSABs compared with CDC crossmatching.<sup>18,19</sup> Hence, the negative CDC crossmatches suggest that the DSAb titre is low or of a type that does not activate complement. The positive T-cell flow crossmatch suggests that there is a DSAb to a class I antigen while the positive B-cell crossmatch may be due to the same class I Ab or due to that and other antibodies directed against either class I or II.

Based on the above results proceeding with the transplant is not entirely clear-cut. Alternative options may need to be considered as they may result in a better short- or long-term outcome (alternative donors, paired kidney donation, blood group incompatible options). The degree of risk will need to be further assessed and information from antibody detection assays will certainly need to be closely examined. If a low-level DSAb is responsible for the positive flow crossmatch, then it may be reasonable to proceed; however, many clinicians would use a desensitization protocol to decrease the risk of early rejection.

In order to confirm the presence of anti-HLA antibodies as the cause of the positive flow crossmatch (as opposed to antibodies to non-HLA antigens) antibody specificity should be determined by Luminex testing. This will also provide some information regarding the antibody levels.



**Fig. 2** The flow crossmatch. Recipient serum potentially containing donor-specific anti-HLA antibodies is added to donor T or B lymphocytes, along with fluorescein-labelled antibodies against human IgG (A). If donor-specific antibodies are not present, no binding occurs and the result is deemed negative (B). If donor-specific anti-HLA antibodies bind to the lymphocytes these can then bind the fluorescein-labelled antihuman IgG antibody, and this will be detectable by flow cytometry (C). The strength of the fluorescence can be measured and expressed as 'channel shifts' above the control sample.

## The flow crossmatching technique

Flow crossmatching is performed using the same initial base ingredients as CDC crossmatching (i.e. donor lymphocytes and recipient serum) and was first described in 1983.<sup>18</sup> The two are mixed to allow antibody binding and after washing, fluoresceinated AHG is added to bind attached DSABs and hence allow detection by flow cytometry (see Fig. 2). The read-out may be reported simply as positive or negative or can be further quantitated. Intensity of fluorescence above control, referred to as channel shifts, may be reported while another means of quantitation is to determine the number of dilutions of recipient serum required to generate a negative result.

The subtype of antibody can also be determined by the isotype specificity of the fluorescently labelled detection

antibody. Hence if only IgG antibodies are of interest the detection antibody chosen will be of the type that binds only to IgG and not IgM or IgA.<sup>20</sup> Furthermore the subtype of IgG can be elucidated by choosing a detection antibody that binds only to IgG1, 2, 3 or 4. Refining the analysis in this way provides information about the likelihood of complement activation *in vivo* as IgG4 does not activate complement.

### Interpreting Flow Crossmatch Results

The role of flow crossmatching in the pre-transplant assessment is controversial. The significance of a positive result is mainly of interest when the CDC crossmatch is negative. In this setting the positive flow crossmatch is likely to be caused by a non-complement fixing antibody, a non-HLA antibody or a low-level antibody. In patients who are not known to be sensitized several studies have suggested that a positive T- or B-cell flow crossmatch was not predictive of increased rejection rates or worse graft survival while in sensitized patients other studies have suggested inferior graft survival.<sup>5,14,16,17,20–22</sup> A possible reason for this difference is that there would be a higher false positive rate in non-sensitized patients than in sensitized patients given that they are not expected to have a positive result. Another factor determining the significance of the result is the cut-off values used to determine a positive test.<sup>20</sup> These are not applied uniformly between centres and those that apply a very low cut-off value will increase sensitivity at the expense of specificity.

Some transplant clinicians do not use flow crossmatching as part of their pre-transplant assessment and rely on CDC crossmatching along with defining DSABs by Luminex, otherwise known as 'virtual crossmatching'. Others contend that flow crossmatching adds important information on the strength of donor-specific antibody reactivity and should be considered in the context of donor-specific antibody results and CDC crossmatching to help develop an overall opinion on the likelihood of immune complications. The area remains controversial and no clear recommendation can be made at this time.

## UNDERSTANDING THE VIRTUAL CROSSMATCH

### Case 3

A 65-year-old man who has end-stage renal failure as a result of ANCA vasculitis has been on dialysis for 4 months. He has had three blood transfusions in the past. His wife has been assessed as a possible renal donor for him. Their immune compatibility is defined below. Is it safe to proceed with transplantation? (Table 5)

**Table 5** Case 3 Typing, Crossmatch and HLA antibody detection

HLA Typing	
Recipient	A 1,3 B 7,44 DR 17,4
Donor	A 1,23 B 7,17 DR 17,14
CDC Crossmatch results	
CDC T-cell XM	–ve
CDC B-cell XM	–ve
Flow crossmatch results	
Flow – CDC T-cell XM	–ve
Flow – CDC B-cell XM	–ve
Antibodies detected by Luminex assay	
Class I	anti HLA – A2, A23, B13
Class II	nil

+ve, positive; –ve, negative; CDC, complement-dependent cytotoxicity; HLA, human leucocyte antigen; XM, crossmatch.

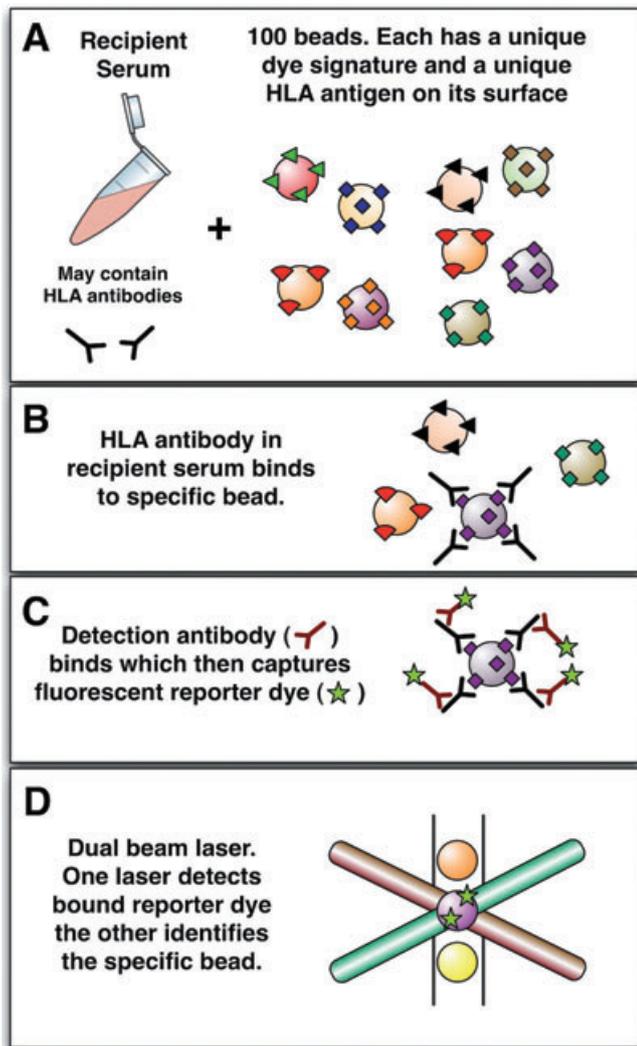
### Interpretation

Proceeding with transplantation in the setting of a negative CDC and flow crossmatch is generally considered as low risk and is reasonable without a desensitization protocol. The issue here is the HLA A23 DSAB detected by Luminex antigen-coated beads (Luminex). Despite the lack of reaction on crossmatching the presence of a DSAB may have prognostic significance for the transplanted kidney and should be further considered before proceeding.<sup>23,24</sup> Many transplant units screen all patients on their cadaveric waiting list for anti-HLA antibodies using Luminex and if positive the specificity of the anti-HLA Abs are defined. This means that the transplant clinician can perform a 'virtual crossmatch' at the time of a cadaveric renal transplant offer as well as in the live donor transplant setting. While outcomes for DSAB positive transplants are inferior to DSAB negative transplants a decision to proceed with a DSAB-positive, CDC crossmatch-negative transplant, in a highly sensitized recipient, may in some cases be in the patient's best interests.

### The virtual crossmatch 'technique'

Virtual crossmatching refers to the comparison of the anti-HLA antibodies of the recipient, as defined by Luminex, with the HLA of the donor.<sup>25</sup> If there is a DSAB present this would represent a positive virtual crossmatch. Antibodies are defined against HLA class I and II antigens.

Synthetic microspheres (beads) coated with HLA antigens are commercially available for this testing. Beads may be coated with multiple HLA antigens for screening purposes or a single HLA antigen for defining specificity of antibodies more precisely (see Fig. 3). For the virtual crossmatch, multiple beads each coated with a single HLA antigen are mixed with recipient serum. Anti-HLA antibodies present bind to the beads and are detected by an isotype-specific (e.g. IgG) detection antibody via flow cytometry. Unique fluorochromes within the beads mark the HLA antigen specificity



**Fig. 3** The virtual crossmatch. Recipient serum potentially containing anti-HLA antibodies is added to a mixture of synthetic beads. Each bead is coated with a set of antigens (screening beads) or for more precise detail, with a single antigen (single antigen beads). A unique dye signature (up to 100) specifies the identity of each bead (A). If anti-HLA antibodies are present these will bind to the appropriate bead (B) and a detection antibody can subsequently bind and capture a reporter dye (C). Each unique bead can then be interrogated for the presence of the reporter dye on its surface using a dual beam laser (D). A profile of antibodies can thus be identified in the recipient and compared with the known HLA identity of any potential donor, allowing a prediction of the crossmatch result.

of each bead (reviewed in<sup>26</sup>). This technique is as sensitive as flow crossmatching and provides the specificity of the antibody.<sup>27</sup>

### Interpreting results of antibody detection assays

It has long been established that the presence of antibodies that react with human leucocytes portend worse long-term graft survival.<sup>28</sup> This information has been further refined

by more sensitive antibody detection systems, particularly Luminex. It has been shown that recipients with third party anti-HLA Abs (antibodies against HLA antigens that are not donor-specific) have reduced graft survival compared with recipients without any anti-HLA antibodies and furthermore those with DSABs have worse graft survival than those with third-party anti-HLA Abs.<sup>24</sup> Therefore, the presence of a DSAB suggests inferior graft survival compared with no DSAB even in the presence of a negative CDC crossmatch.<sup>23</sup>

One advantage Luminex testing has over other forms of crossmatching is the removal of false positives because of antibody binding to non-HLA antigens. In addition, because the antigens present on Luminex can be controlled, confusion regarding the class of HLA they are binding to is eliminated; remembering that in B-cell crossmatching class I and II antigens are present.

The presence of a DSAB detected by Luminex in the setting of a negative CDC crossmatch appears to have prognostic importance in terms of graft survival and acute rejection risk; however, there is insufficient data to determine the meaning of a DSAB with a negative flow crossmatch.<sup>19,23,25,29</sup>

In each assay negative control beads provide a minimum threshold for a positive result. Positive results can then be graded as weak, moderate or strong on the basis of the degree of fluorescence of the positive bead. This result can be scored as a mean fluorescence index or molecules of equivalent soluble fluorescence. The molecules of equivalent soluble fluorescence of a DSAB has been shown to correlate with antibody titre and predict graft failure.<sup>30</sup>

Recently, it has become evident that while adding significantly to the area of crossmatching, Luminex testing has some limitations including possible interference of the assay by IgM, incomplete antigen representation on bead sets and variation in HLA density on beads.<sup>29,31,32</sup> Those interested in more detail regarding Luminex testing should read the recent review paper in this journal covering the topic.<sup>26</sup>

### Cellular crossmatching

All the above-mentioned crossmatching techniques attempt to detect a donor-reactive antibody likely to result in acute or chronic antibody-mediated rejection. The presence of sensitization of the cellular arm of the immune system, particularly T cells, can be assessed by cytokine assays such as ELISPOTs. These assays detect the number of recipient T cells producing cytokines such as interferon gamma when encountering donor antigen presenting cells. The assays are conducted in plates coated with a capture antibody for the cytokine of interest. The mixed donor and recipient leucocytes are added to the plate and incubated. After washing to remove the cells the reaction is developed by adding a second antibody for the cytokine of interest and then stained for that antibody.<sup>33</sup>

African American transplant recipients with higher levels of donor-reactive T cells producing interferon gamma were shown to have an increased frequency of acute rejection episodes and worse renal function at 12 months than those with lower levels.<sup>33</sup> The overall utility of this type of assessment requires more investigation and remains experimental at this stage.

## CONCLUSION

Crossmatching is a vital tool in assessing the immune compatibility of a particular donor/recipient pairing. A positive T-cell CDC crossmatch would usually mean that a particular pairing should not proceed. In some cases, a desensitization protocol may allow such a transplant to occur, avoiding hyperacute or early acute rejection albeit with inferior long-term graft outcomes compared with patients who are not sensitized to their donor. The advent of flow crossmatching and Luminex assays has allowed detection of lower titre but potentially clinically relevant anti-HLA antibodies by approximately 10-fold. Further studies are required to better define the significance of very low-level DSABs, non-complement fixing antibodies, IgM antibodies and non-HLA antibodies as well as the importance of assessing T cellular sensitization.

The authors' view is that the tried and trusted technique of CDC crossmatching remains essential and should be coupled with a determination of the specificity of anti-HLA antibodies by Luminex. With these two assays the role of flow crossmatching is less clear and is rarely helpful in decision making. The ideal future crossmatch will be highly sensitive in identifying DSABs and provide accurate prediction of the functional significance of the antibody. This will allow transplant physicians to confidently proceed with a transplant in the face of a clinically irrelevant DSAB while providing clear prognostic information in the setting of more serious antibodies.

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