Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation
A collaborative publication by:

**British Society for Histocompatibility & Immunogenetics**

*12 Coldbath Square, London, EC1 5HL*

[www.bshi.org.uk](http://www.bshi.org.uk)

and

**British Transplantation Society**

*South Park Road, Macclesfield, Cheshire, SK11 6SH*

[www.bts.org.uk](http://www.bts.org.uk)

Publication at [www.bts.org.uk](http://www.bts.org.uk) & [www.bshi.org.uk](http://www.bshi.org.uk), May 2010

Revision due May 2012

Based on search of publications at March 2010

*The British Society for Histocompatibility & Immunogenetics is the professional body for healthcare and basic scientists supporting clinical transplantation and transplantation research.*

*The British Transplantation Society is the professional body for Surgeons, Physicians, Specialist Nurses for Organ Donations, Nurses, Healthcare Scientists, Allied Health Professionals and Basic Scientists actively working in clinical transplantation and transplantation research.*
Disclaimer:
These Guidelines are guides to best practice which inevitably change with the passage of time. All practitioners need to undertake clinical care on an individual basis and keep themselves up to date with changes in practice of clinical medicine. The British Transplantation Society and The British Society for Histocompatibility & Immunogenetics Guidelines ("the Guidelines") were compiled by a joint working party of the Societies. The Guidelines represent the collective opinions of a number of experts in the field and do not have the force of law. The Guidelines contain information and guidance for use by practitioners as a best practice tool; it follows that the Guidelines should be interpreted as such rather than the letter of their contents. The opinions presented in the Guidelines are subject to change and should not be considered to be a treatment recommendation for any individual patient.

The British Transplantation Society and The British Society for Histocompatibility & Immunogenetics cannot attest to the accuracy, completeness or currency of the opinions contained herein and does not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on the Guidelines or as a result of any inaccurate or misleading opinion contained in the Guidelines.
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

Contents:

1 Process of Guidelines development 6
2 Evidence base 7
3 Writing committee 7
4 Overview, recommendations and best practice 10
   4.1 Immunological risk 10
   4.2 Recommendations 11
      4.2.1 Kidney and pancreas 11
      4.2.2 Islets 13
      4.2.3 Thoracic organs 14
      4.2.4 Liver 15
      4.2.5 Intestinal and multi-visceral 15
      4.2.6 HLA-specific antibody incompatible transplantation 16
5 Introduction 17
   5.1 The immune system 18
      5.1.1 T cell recognition of foreign antigens 18
      5.1.2 Effector cell activation and functions 18
      5.1.3 Control of allorecognition 20
   5.2 Priming sources 21
   5.3 Acute antibody mediated rejection mechanisms 22
      5.3.1 Hyperacute rejection 22
      5.3.2 Acute rejection 22
6 Defining risk 23
7 Identification of HLA specific antibodies 24
   7.1 Cell based assays 24
      7.1.1 Complement dependent cytotoxicity 24
      7.1.2 Flow cytometry 26
   7.2 Solid phase assays 27
      7.2.1 Enzyme-linked immunosorbent assays 27
      7.2.2 Flow cytometry 28
      7.2.3 X-map (Luminex) 28
   7.3 Interpretation of HLA specific antibody data in the clinical setting 31
   7.4 Screening strategies 33
   7.5 Collection & storage of samples for antibody screening & donor crossmatching
      7.5.1 pre-transplant 35
      7.5.2 post-transplant 35
      7.5.3 sample storage 36
   7.6 Recommendations 36
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8  Crossmatching</td>
<td>37</td>
</tr>
<tr>
<td>8.1 Crossmatch techniques</td>
<td>38</td>
</tr>
<tr>
<td>8.2 Pre-transplant virtual crossmatching</td>
<td>39</td>
</tr>
<tr>
<td>8.3 Sample selection</td>
<td>40</td>
</tr>
<tr>
<td>8.4 Results and interpretation</td>
<td>41</td>
</tr>
<tr>
<td>8.5 Recommendations</td>
<td>43</td>
</tr>
<tr>
<td>9  Kidney and pancreas transplantation</td>
<td>44</td>
</tr>
<tr>
<td>9.1 Pre-transplant antibody screening</td>
<td>45</td>
</tr>
<tr>
<td>9.2 Definition of unacceptable mismatches</td>
<td>47</td>
</tr>
<tr>
<td>9.3 Clinical relevance of crossmatching</td>
<td>48</td>
</tr>
<tr>
<td>9.3.1 The cytotoxic crossmatch</td>
<td>48</td>
</tr>
<tr>
<td>9.3.2 Flow cytometric crossmatching</td>
<td>50</td>
</tr>
<tr>
<td>9.3.3 Virtual crossmatching</td>
<td>52</td>
</tr>
<tr>
<td>9.3.4 Reporting crossmatch results</td>
<td>53</td>
</tr>
<tr>
<td>9.4 Development of HLA specific antibodies after kidney transplantation</td>
<td>53</td>
</tr>
<tr>
<td>9.5 Recommendations</td>
<td>56</td>
</tr>
<tr>
<td>10 Islet transplantation</td>
<td>57</td>
</tr>
<tr>
<td>10.1 Pre-transplant antibody screening</td>
<td>57</td>
</tr>
<tr>
<td>10.2 Post first transplant and pre-second transplant antibodies</td>
<td>58</td>
</tr>
<tr>
<td>10.3 Crossmatching</td>
<td>59</td>
</tr>
<tr>
<td>10.4 Recommendations</td>
<td>59</td>
</tr>
<tr>
<td>11 Thoracic organ transplantation</td>
<td>60</td>
</tr>
<tr>
<td>11.1 Pre-transplant HLA specific antibodies</td>
<td>61</td>
</tr>
<tr>
<td>11.2 Post-transplant production of HLA specific antibodies</td>
<td>63</td>
</tr>
<tr>
<td>11.3 Non-HLA specific antibodies</td>
<td>64</td>
</tr>
<tr>
<td>11.4 Recommendations</td>
<td>65</td>
</tr>
<tr>
<td>12 Liver transplantation</td>
<td>66</td>
</tr>
<tr>
<td>12.1 Recommendations</td>
<td>69</td>
</tr>
<tr>
<td>13 Intestinal and multi-visceral transplantation</td>
<td>69</td>
</tr>
<tr>
<td>13.1 Recommendations</td>
<td>72</td>
</tr>
<tr>
<td>14 HLA-specific antibody incompatible transplantation (AiT)</td>
<td>72</td>
</tr>
<tr>
<td>14.1 Recommendations</td>
<td>76</td>
</tr>
<tr>
<td>15 Appendices</td>
<td>79</td>
</tr>
<tr>
<td>15.1 Laboratory resources and relationship</td>
<td>80</td>
</tr>
<tr>
<td>15.2 Glossary</td>
<td>90</td>
</tr>
<tr>
<td>15.3 References</td>
<td>101</td>
</tr>
<tr>
<td>15.4 Statements of potential conflicts of interests</td>
<td></td>
</tr>
</tbody>
</table>
1: Process of Guidelines development

The past decade witnessed a revolution in technology allowing the histocompatibility laboratory to define the presence of HLA specific antibodies with a high degree of sensitivity, resulting in ongoing re-definition of the crossmatch boundary to allow successful transplantation of allo-sensitised patients. These developments started early in the past decade leading to the publication of the 2004 Guidelines. Subsequently technological developments continued allowing relative quantitation of HLA specific antibody levels which, combined with crossmatching results allowed a graded assessment of the immunological risk should a transplant proceed, rather than a simple ‘positive’ or ‘negative’ crossmatch assessment. This facilitated the establishment and growth of HLA antibody incompatible living donor kidney transplantation. Together, these and other developments have driven a thorough revision of these Guidelines, so that optimum approaches can be applied to maximise safe and effective use of the donor organ pool and to help expand this pool. These revised Guidelines accept ABO blood group compatibility as a pre-requisite and focus on HLA specific antibodies in allotransplantation, although selective references to non-HLA specific antibodies are included. ABO blood group incompatible kidney transplantation is addressed as a separate issue. The role of HLA specific antibodies in some forms of HLA mismatched haematopoietic progenitor cell transplantation is also of interest, but for clarity is excluded from these Guidelines. The Histocompatibility laboratory will also play a critical role in the development of cell therapies and although the Writers discussed these developments, it was decided to omit this exciting area.
2: THE EVIDENCE BASE:

The Writers drew the evidence to support their recommendations from peer-review publications up to March 2010. The specialist nature of histocompatibility testing in the context of clinical allotransplantation means that there are few large or multicentre studies in this field and meta analyses are not possible. Furthermore, recent changes in technology to detect and define HLA specific sensitisation have made many historic publications less relevant or obsolete.

3: WRITING COMMITTEE:

Executive bodies from the BSHI and the BTS invited the persons below to compile the original Guidelines which were published in 2004. A revision process, involving the original authors and Martin Howell as Chair of the writing committee, resulted in production of these Guidelines. One meeting, chaired by Martin Howell, was held to complete this process and a final draft was sent to selected clinical colleagues for review and comment. Finally, by email to all BSHI and BTS Members, comments were invited by placing the draft Guidelines on both Society websites for a four week period. Comments were reviewed and the Guidelines amended by Phil Dyer, who also formatted this publication. There was no specific request for non-professional comment.
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

Writing Committee:

Chair:

Martin Howell PhD FRCPath, Consultant Clinical Scientist
Histocompatibility & Immunogenetics Laboratory, NHS Blood & Transplant
Newcastle upon Tyne NE2 4NQ

Chair of Writing Committee for 2004 Guidelines:

Andrea Harmer PhD FRCPath, Consultant Clinical Scientist
Histocompatibility & Immunogenetics Laboratory, NHS Blood & Transplant
Sheffield S5 7JN

Members:

David Briggs PhD, Consultant Clinical Scientist
Histocompatibility & Immunogenetics Laboratory, NHS Blood & Transplant
Birmingham B15 2TT

Philip Dyer PhD FRCPath, Consultant Clinical Scientist
Histocompatibility & Immunogenetics, Scottish National Blood Transfusion Service
Edinburgh EH16 4SA

Susan Fuggle DPhil FRCPath, Consultant Clinical Scientist
Transplant Immunology and Immunogenetics Laboratory, Churchill Hospital
Oxford, OX3 7LJ

Susan Martin PhD FRCPath, Consultant Clinical Scientist
Transplantation Laboratory, Manchester Royal Infirmary
Manchester, M13 9WL

Paul Sinnott PhD FRCPath, Consultant Clinical Scientist
The Royal London Hospital,
London E1 1BB

John Smith PhD, Consultant Head of Tissue Typing Service
Tissue Typing Laboratory, Harefield Hospital
Harefield UB9 6JH

Craig Taylor PhD, FRCPath, Consultant Clinical Scientist,
Tissue Typing Laboratory, Addenbrooke's Hospital
Cambridge CB2 0QQ

Robert Vaughan PhD FRCPath, Consultant Clinical Scientist
Tissue Typing Laboratory, Guy’s Hospital
London SE1 9RT
Clinical reviewers:

Miss Lorna Marson, Consultant Transplant Surgeon and Senior Lecturer
Royal Infirmary Edinburgh, Edinburgh EH16 4SA

Dr Chas Newstead, Consultant Nephrologist
St James’ University Hospital, Leeds LS9 7TF

Dr Jayan Parameshwar, Consultant Cardiologist
Papworth Hospital, Cambridge CB23 3RE

Mr Keith Rigg, President – British Transplantation Society and Consultant Surgeon
Nottingham University Hospitals NHS Trust, Nottingham NG5 1PB

Dr Michael Sheaff, Consultant Histopathologist
St Bartholomew’s Hospital, London EC1A 7BE

Dr Raj Thuraisingham, Honorary Clinical Senior Lecturer and Consultant Nephrologist
Royal London Hospital, London E1 1BB

Dr Jane Tizard, Consultant Paediatric Nephrologist
Bristol Royal Hospital for Children, Bristol BS32 8BJ

Dr Nicholas Torpey, Consultant Transplant Nephrologist
Addenbrooke’s Hospital, Cambridge CB2 0QQ

Professor Anthony Warrens, Professor of Renal and Transplant Medicine and Honorary Consultant Physician
Hammersmith Hospital, London W12 0NN

Mr Christopher Watson, Reader in Surgery and Honorary Consultant Surgeon
University of Cambridge Department of Surgery, Addenbrooke’s Hospital Cambridge CB2 0QQ

Comments received from BSHI and BTS Members:

Dr Peter ANDREWS, Consultant Nephrologist, Chair BTS Standards Committee
SW Thames Renal & Transplantation Unit, St Helier Hospital
Carshalton SM5 1AA

Vaughan CARTER PhD FRCPath, Clinical Scientist
Histocompatibility & Immunogenetics Laboratory, NHS Blood & Transplant
Newcastle upon Tyne NE2 4NQ

Brendan CLARKE PhD, Consultant Clinical Scientist
Histocompatibility & Immunogenetics Laboratory, St James’ University Hospital, Leeds LS9 7TF

Ms Sussie SHRESTHA, Clinical Research Fellow
Royal Infirmary Edinburgh, Edinburgh EH16 4SA
4: OVERVIEW, RECOMMENDATIONS AND BEST PRACTICE

4.1 Immunological risk

Gebel et al [17] have suggested three broad risk categories for a given donor-recipient combination (adapted below):

1) High immunological risk is indicated when there are high titre circulating antibodies specific for mismatched donor HLA antigens present at the time of transplantation. In most cases the high risk of hyperacute rejection would constitute a veto to transplantation. Some centres may advocate carefully planned pre-transplant desensitisation regimes together with close post-transplant immunological monitoring.

2) Intermediate immunological risk is indicated by the presence of risk factors such as prior donor reactive sensitisation (that is absent at the time of transplantation) and in selected donor-recipient combinations with only weak sensitisation to certain mismatched HLA specificities. In such cases, it may be justified to consider augmented immunosuppression and post-transplant immunological monitoring.

3) Low (or standard) immunological risk is indicated when non-sensitised or sensitised patients receive a minimally HLA mismatched organ in the absence of current or historical donor reactive antibodies.
4.2 Recommendations

4.2.1 Kidney and pancreas transplantation:

- Laboratories must have a strategy in place for the detection and characterisation of clinically relevant antibodies.
- The techniques adopted must have the capability of defining antibody class and specificity.
- A combination of tests should be considered in order to fully resolve complex antibody profiles.
- Laboratories should have a programme for investigating and evaluating newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments.
- Patient serum samples must be sent to the histocompatibility laboratory no less than three monthly for routine antibody monitoring and also following transfusion of any blood products.
- Serum samples must be stored for potential use in future antibody screening and crossmatch tests.
- Laboratory crossmatch tests should distinguish T cell and B cell populations and between IgG and IgM antibodies.
- Serum samples used for crossmatching must include a current sample and, where HLA specific antibodies have been detected, samples that are representative of the patient’s antibody profile, over time.
• The timing, duration, priming source, antibody titre and donor specificity should be considered when interpreting the crossmatch result.

• The reporting of results to clinical teams should include appropriate advice on the clinical relevance of the result.

• Laboratories providing services for kidney transplant programmes must have the capability of precisely defining HLA-A, -B, -Cw, -DR, -DQ and -DP antibody specificities in their patients so that donors who should be crossmatch negative can be identified.

• A patient’s HLA alloantibody profile must be assessed to delineate the antigens regarded as unacceptable for transplant.

• A pre-transplant crossmatch should be performed for all patients unless a programme exists for identifying those individuals who can confidently be defined as unsensitised. Patients with no detectable HLA specific antibodies can be transplanted on the basis of a negative virtual crossmatch (vXM) without waiting for a crossmatch test to be performed.

• Sensitised patients should be crossmatched using flow cytometric techniques.

• Post-transplant antibody monitoring should be performed at agreed regular intervals, at the time of biopsy and in cases of suspected rejection. Samples should also be tested at times of declining graft function when there is no other clinical cause.
• The clinical team must inform the laboratory of potential sensitisation events, such as previous transplantation, skin grafting, transfusion of blood products, pregnancy (including known miscarriages) and recent infection or vaccination.

4.2.2 Islets
• Prior to listing a patient for transplant, it is recommended that antibody screening and specificity analysis are performed on two separate samples.
• Once the patient is listed, samples for antibody analysis should be obtained no less than three monthly.
• Potential priming events should be notified promptly to the laboratory and samples sent approximately 2-4 weeks after the event.
• Antibody testing should be performed by two different assays, including a highly sensitive technique to determine the specificity of the antibodies.
• Both cytotoxic and flow cytometry crossmatching is recommended.
• Proceeding to transplant on the basis of a virtual crossmatch may be acceptable if the laboratory and transplant centre have a validated policy.
• It is recommended that samples are taken regularly following the first and any subsequent transplants.
4.2.3 Thoracic organs:

- Laboratories must be able to precisely define antibodies specific for HLA-A, -B, -Cw, -DR, -DQ and -DP using sensitive solid phase assays.
- HLA antigens to which a patient has produced antibodies should be listed as “unacceptable mismatches”
- Before transplantation, patients must be screened for HLA specific antibodies on at least two occasions prior to listing, preferably taken at least 24 hours apart, although this may not be possible for urgent patients.
- Samples must also be collected following any sensitising events such as transfusion of blood products or pregnancy, and every three months whilst on the waiting list so that a complete antibody profile is available prior to transplantation.
- Patients with no detectable HLA-specific antibodies can be transplanted following virtual crossmatching in the histocompatibility laboratory.
- Sensitised patients with fully defined HLA-specific antibodies and with no residual reactivity can also be transplanted without a prospective crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. Prospective crossmatching must be performed for all other sensitised patients.
• For patients where a prospective crossmatch test is not performed a retrospective crossmatch using serum collected within 48 hours prior to transplantation must be performed.

• Crossmatch techniques should distinguish between IgG and IgM as well as T and B cells as targets.

• Following transplantation patients should be screened for HLA specific antibodies at regular intervals and must be tested when clinically necessary.

4.2.4 Liver

• Prospective crossmatching is not indicated prior to liver transplantation.

• Identification of donor specific antibody can be used to identify patients at high risk of acute rejection and can aid post-transplant management, such as changes in immunosuppression regimen or antibody removal.

4.2.5 Intestinal and Multi-visceral

• Pre-transplant HLA-specific antibody screening and pre-transplant donor lymphocyte crossmatching can identify those patients at high risk of acute cellular or vascular rejection.
• A positive donor cytotoxic crossmatch caused by IgG HLA class I specific antibodies is a relative contra-indication to isolated intestinal transplantation (in the absence of a liver transplant from the same donor) and the final decision to proceed with transplantation will depend on a careful evaluation of the relative risk of proceeding versus the risk of delayed transplantation.

4.2.6 HLA-specific antibody incompatible transplantation (AiT)

• The HLA specificity and level of donor specific antibodies must be fully determined prior to antibody reduction.

• Antibody levels must be monitored regularly throughout the duration of treatment to determine its effectiveness using a method that can unequivocally distinguish between antibodies directed against donor HLA mismatches, other HLA-specific antibodies and non-HLA antibodies.

• The frequency of post-transplant donor specific antibody monitoring should match the risk of adverse immunological events and this is advised for all HLA AiT cases whether or not pretransplant removal has been used.

• Antibody reduction should only be undertaken following establishment of a clinical and laboratory protocol.
5  INTRODUCTION

The adaptive immune response to infection elicits antigen specific cells and antibodies that bind with high affinity to foreign antigens, resulting in recovery from infection and also protection against re-infection. An unwanted ‘side effect’ of this adaptive immune response is the response to non-infectious agents (e.g. allotrafts, pollen, drugs) and even to an individual’s own body constituents (autoimmunity).

Exposure of an individual’s immune system to tissue or cells from another individual can result in immunological priming (sensitisation) to alloantigens. Subsequent re-exposure to the same or structurally related, cross-reactive antigens causes a vigorous humoral and/or cellular immune response. In the context of organ transplantation, previous immunological priming to alloantigens can cause hyperacute rejection due to circulating pre-formed donor reactive antibodies, or accelerated acute rejection, which is difficult to control using conventional immunosuppressive agents. Patient exposure to alloantigens of another individual is a common occurrence and takes place through pregnancies, blood transfusions or previous transplants. An audit of the UK’s national kidney transplant waiting list in March 2009 showed that 41% of adult patients and 58% of paediatric patients were sensitised (calculated donor specific antibody reaction frequency >10%). A critical function of the histocompatibility laboratory is to identify sensitisation in patients to reduce the immunological risks of allotransplantation.
5.1 The Immune System

5.1.1 T cell recognition of foreign antigens.

The essential feature guiding the evolution of the immune system of all vertebrate species is the need to distinguish between ‘self’ and ‘non-self’. In humans this is achieved through T cell recognition of self-human leukocyte antigens (HLA) which bind and present antigens in the form of processed peptides (Figure 1).

Antigen specific T cell clones with T cell receptors (TCR) that recognise foreign peptide bound to self-HLA engage the antigen presenting cells (APC). In the presence of co-stimulatory molecules present on mature APCs (e.g. CD28/CD80 interaction), T cells receive the second signal that triggers their activation. Activated T cells undergo clonal expansion and secrete cytokines that initiate and control the inflammatory response and are involved in recruitment of other effector cells such as B cells, cytotoxic T cells, macrophages and natural killer cells. In addition, a sub-population of activated T cells express the CD45RO molecule and become long-lived memory T cells that offer a rapid and vigorous response on re-encounter with the same priming antigen.

5.1.2 Effector cell activation and functions.

The cytokines secreted by T helper cells direct the immune response by regulating effector cell pathways towards a humoral and/or cellular response. T cell secretion of the cytokines IL-2, IL-4, IL-5, IL-6 and IL-13 induces activation and differentiation of antigen specific B cells. In the presence of these cytokines, naïve B cells that express cell surface IgM undergo immunoglobulin class
switching so that high affinity IgG antibodies can be produced. Under T cell help, B cells differentiate into antibody producing plasma cells with the initial production of IgM antibodies and subsequently IgG antibodies due to class switching and into memory B cells that respond rapidly upon repeat exposure to the same antigenic stimulus (Figure 1). Antibody binding to its target antigen facilitates opsonisation by phagocytes, and chemotaxis and lysis via the classical complement pathway.

B cells can also function as antigen-presenting cells to T cells, providing the second signal for T cell activation. Unlike T cells that recognise processed antigen in the context of self-HLA, B cells express cell surface immunoglobulin that can recognise and bind native antigen enabling the selection of antigen specific B cell clones. B cells bind exogenous antigen through their cell surface immunoglobulin which is internalised and broken down into peptide fragments. These peptides are loaded into the antigen binding cleft of HLA class II molecules for presentation at the cell surface to T cells. The interaction of the TCR with HLA/peptide complex together with co-stimulatory molecules (CD40/CD154) stimulates antigen-specific T cell activation for the provision of B cell help.

T cell cooperation is needed for B cells to develop into alloantibody producing plasma cells and the characterisation of the epitope(s) to which an alloantibody is directed can be used as a measure of the extent of T cell sensitisation. Although the original donor cell epitopes recognised by T and B cells are not the same, this use of antibody to characterise the memory T cell response is an important tool in the prevention of accelerated acute rejection,
the “second set” response. The dynamics of an antibody response may include "epitope spreading", whereby as the immune response develops, the specificity of HLA directed antibodies may broaden from the original ‘dominant’ HLA epitope. One way of investigating this is to test a serum in dilution; when titrated, the main specificities should become apparent. It is possible that the main specificity approximates more closely to the T cell epitope. It may therefore be conjectured that the removal of the broad or secondary antibody specificities may not engender an accelerated memory T cell response in a graft expressing these HLA mismatches.

Helper T cells can initiate cellular immune responses by the activation of antigen specific cytotoxic T cells and are also involved in the non-specific activation of macrophages and natural killer cells causing cell-mediated cytotoxicity. Currently there is no routine assay of T cell sensitisation.

5.1.3 Control of allore cognition.
In kidney transplantation, attempts are made to reduce the immunogenicity and alloantigen load through avoiding HLA mismatching of donors to recipients. This is effective at reducing the number and severity of acute rejection episodes and is also translated into improved long-term graft survival [1-4]. In addition, conventional calcineurin inhibitor based immunosuppressive regimens are potent inhibitors of naïve T cells and effectively control the primary immune response to HLA alloantigens expressed on transplanted tissue. In individuals who are
already primed to donor HLA antigens, both humoral and cellular secondary responses are poorly controlled by current immunosuppressive agents.

5.2 Priming sources

Exposure of an individual’s immune system to alloantigens of another individual by pregnancy, blood transfusion or transplantation can result in immunological priming. In addition, HLA-specific antibodies have been observed in patients in the absence of obvious priming events. Idiopathic HLA-specific antibodies are usually IgM and may arise through cross-reactivity with infectious microorganisms. More recently, antibody screening by highly sensitive solid phase binding assays using purified HLA molecules has identified IgG HLA-specific antibodies in the sera of normal healthy individuals with no history of allosensitisation events, although it is not yet known whether these antibodies are clinically relevant to graft rejection [5-6].

It has been suggested that the use of leukodepleted blood negates the risk of post transfusion allosensitisation but a randomised trial found that buffy coat removal and additional white blood cell reduction by filtration resulted in similar post-transfusion alloimmunisation frequencies after a single transfusion event [7]. The risk of sensitisation after blood transfusion is highly variable and is influenced by recipient factors such as genetic control of the immune response and previous exposure to alloantigens. In addition the storage time of transfused blood is relevant.
5.3 Acute Antibody Mediated Rejection Mechanisms

5.3.1 Hyperacute rejection.
Circulating antibodies which bind to donor ABO blood group or HLA antigens expressed on endothelial cells of the transplanted organ cause activation of the complement system which can lead to direct damage of the endothelial cells and to cell lysis. There is an accumulation of granulocytes and platelets, endothelial cell activation and loss of anti-thrombotic state with coagulation leading to formation of microthrombi. The vessels become obstructed by thrombi leading to ischaemia and infarction of the graft. Direct involvement of antibodies in this process has been shown with deposition of IgG in the capillaries of hyperacutely rejected kidneys due to antibodies to ABO blood group or HLA antigens [8]. Perfusion of kidneys with plasma containing antibodies directed against HLA antigens present in the kidney has been shown to cause hyperacute rejection [9].

5.3.2 Acute rejection.
Whilst acute rejection is regarded as primarily a cell mediated process, acute antibody-mediated rejection is well defined. The onset of acute rejection may be preceded by, or accompanied by the appearance of antibodies specific for donor HLA antigens [10,11]. Recovery of both lymphocytes and donor HLA specific antibodies from rejected grafts together with the identification of immunoglobulin deposition in the vessel walls of some grafts [12] and the demonstration of plasma cells amongst infiltrating cells recovered from failed kidney grafts [13] indicate that both cellular and humoral responses may be
present in acute rejection. Further evidence comes from the identification of de novo donor-specific antibody in patient sera and C4d in biopsies obtained from kidney and heart transplants undergoing acute or chronic rejection [14,15]. C4d is a product of complement indicating antibody dependent activation.

Antibodies may also initiate graft damage by the mechanism of antibody dependent cell-mediated cytotoxicity (ADCC). Graft infiltrating cells have been shown to mediate cellular lysis of antibody coated cells [16] and antibodies eluted from rejected grafts have been found to mediate ADCC activity to donor cells [12].

6: DEFINING RISK

Recent advances in immunosuppressive therapy have enabled successful transplantation in some sensitised recipients and it is important for clinicians and histocompatibility scientists to identify the immunological risk and understand its clinical significance. Gebel et al [17] have suggested three broad risk categories for a given donor-recipient combination (adapted below):

1) High immunological risk is attributed when there are high titre circulating antibodies specific for mismatched donor antigens present at the time of transplantation. In most cases the high risk of hyperacute rejection would constitute a veto to transplantation. Nevertheless, some centres may advocate carefully planned pre-transplant desensitisation regimes together with close post-transplant immunological monitoring in certain selected patients with a compelling reason to proceed in such circumstances.
2) Intermediate immunological risk may be an appropriate categorisation in the presence of risk factors such as prior donor reactive sensitisation (that is absent at the time of transplantation) and in selected donor-recipient combinations with only weak sensitisation to certain mismatched HLA specificities. In such cases, it may be justified to consider augmented immunosuppression and post-transplant immunological monitoring.

3) Low (or standard) immunological risk may be attributed to non-sensitised patients or to sensitised patients receiving a minimally HLA mismatched organ in absence of known current or historical donor reactive antibodies.

A summary of immunological pre-transplant risk assessment based on donor crossmatch and antibody screening results [18] is given in Table 1.

7: IDENTIFICATION OF HLA SPECIFIC ANTIBODIES

There have been significant advances in the ability of the histocompatibility laboratory to precisely detect and characterise HLA antibodies in recent years.

Methods in current use are outlined as follows:-

7.1 Cell based assays

7.1.1 Complement dependent cytotoxicity

The first established method for the detection and definition of HLA-specific antibodies was the complement dependent cytotoxicity (CDC) test that employs lymphocyte targets to detect complement-fixing IgG and IgM antibodies. The CDC test is used widely in both antibody screening and crossmatching protocols. Despite its widespread use, the CDC assay has several inherent problems [19];
an adequate cell panel can be difficult to obtain and test specificity and sensitivity are influenced by cell viability and the rabbit complement used.

Furthermore, only complement-fixing antibodies are detected but these may not be HLA-specific. Results from the CDC test are presented as the percentage of the cell panel with which a serum has reacted (%PRA). The “% PRA” is absolutely dependent on the composition of the cell panel and is therefore of limited value. For example, a serum from a patient with a monospecific antibody to HLA-A2, that is present at high frequency in the population, may react with 50% of a random cell panel but with a much lower percentage of a selected panel. PRA results cannot therefore be compared between cell panels or between laboratories and do not necessarily reflect the proportion of the donor pool to which a patient is sensitised. This problem is compounded by false positive results due to the presence of autoreactive lymphocytotoxic antibodies which could give “100% PRA” but are probably irrelevant to transplant outcome [20]. Hence patients should not be defined as sensitised simply on the basis of “%PRA”. Importantly, the HLA-specificities of the antibodies must be defined. To facilitate this, autoantibodies can be removed by absorption with autologous lymphocytes and/or treatment of the serum with dithiothreitol (DTT) to remove IgM antibodies. The detection of IgM antibodies in the CDC test is seen as a disadvantage and DTT is used to remove ‘false’ positive autoreactivity due to IgM. However, it will also remove reactivity due to IgM alloantibodies as well as autoantibodies.

A number of developments have improved the detection and characterisation of alloantibodies in sensitised patients. A method has been
described that employs the non-HLA expressing cell line K562, transfected with cDNA encoding single HLA class I alleles [21,22]. These cells can be used in cytotoxicity and flow cytometry assays and because they express only a single HLA antigen their use facilitates the characterisation of HLA antibody specificity, particularly in highly sensitised patients.

7.1.2 Flow Cytometry
As flow cytometry crossmatching (FCXM) became widely used, particularly for sensitised and/or repeat transplant patients, the increased sensitivity of the FCXM meant that antibody results derived from screening by CDC were not necessarily predictive of the FCXM result. There was therefore a requirement for increased sensitivity in the screening test.

Flow cytometry screening tests were originally developed using cell pools designed to cover all major HLA specificities or serological cross reactive groups. Cells from chronic lymphocytic leukaemia (CLL) patients [23], Epstein Barr Virus (EBV) transformed lymphoblastoid cell line cells [24,25] and peripheral blood lymphocytes (PBL) have all been used [26,27].

Screening sera using flow cytometry with individual cell panels is cumbersome for large-scale use, so the option of pooled cells allows for a large number of sera to be tested over a short period and is more sensitive than CDC. Antibody positive sera can then be investigated to define specificity using flow cytometry against individual cell panels, ELISA or CDC. More recently, the use of a single HLA antigen expressing cell line in a flow cytometry assay has enabled
the identification of HLA antigens to which sensitised patients do and do not have antibodies [22]. However, although cell based flow cytometry assays do not detect IgM autoreactive antibodies, non HLA-specific IgG antibodies will be detected and must be taken into account in the interpretation of results.

7.2 Solid phase assays

The development of solid phase ELISA and flow cytometry based assays using purified or recombinant HLA class I and class II molecules has significantly improved the detection and characterisation of alloantibodies in sensitised patients. Solid phase assays offer a number of advantages over CDC:

- no requirement for viable lymphocytes and complement
- designed to detect only HLA-specific antibodies
- detect non-complement fixing antibodies
- objective and can be partially automated
- commercially available

7.2.1 Enzyme-linked immunosorbent assays

ELISA-based tests were the first solid phase assays to be introduced in the 1990s [28,29]. There are two types of ELISA based assays which are commercially available. The first detects the presence or absence of HLA-specific antibody and the second defines antibody specificity. These tests are advantageous since positive reactions can be ranked according to optical density in the test readout and antibody specificities can be clustered to aid
interpretation facilitating analysis of sera from sensitised patients with a high reaction frequency [30,31]. It is important to note that discrepancies are occasionally observed in the results obtained with kits from different manufacturers due to differences in the HLA composition of the antigen pools used in each kit. Sensitivity of ELISA testing is higher than CDC but lower than flow cytometry [32].

7.2.2 Flow Cytometry

A flow cytometry solid phase assay using microparticles coated with soluble HLA antigens to detect alloantibodies was developed in the mid 1990s [33]. The presence of HLA class I specific and/or class II specific antibodies in serum can be determined in a single test using this method [34]. Following the same principles, microparticles are available that enable the separate definition of antibodies to HLA-A, -B, -Cw and HLA-DR, -DQ, -DP. Studies have shown these microparticles to be more sensitive and more specific than CDC for the detection of HLA-specific antibodies [30]. A further advance was the development of microparticles coated with a single antigen. These facilitate antibody specificity definition and in particular enable the identification of HLA specificities to which a highly sensitised patient is not sensitised, termed “acceptable mismatches” [35,36].

7.2.3 X-Map (Luminex)

An assay using multiplexed beads (or microparticles) and flow cytometry [37] has been applied to the detection and characterisation of HLA-specific antibodies. X-Map (Luminex) technology uses pools of HLA class I or class II
antigen-coated microbeads. These are coloured with a combination of two dyes and for each set of beads the dyes are in different proportions so that the bead sets can be distinguished. Typically, 100 bead populations can be combined in a single test and so this method allows simultaneous analysis of a far greater number of HLA antigen coated microbeads than other current methods. HLA-specific antibody binding to the microbeads is detected using R-phycoerythrin-conjugated anti-human immunoglobulin and a flow analyzer. As for flow cytometry solid phase assays, the construction of HLA class I and II recombinant single antigens from transfected cell lines has allowed the production of microparticles coated with single HLA antigens which enables identification of antibody specificities in highly reactive sera [38]. This method can be applied to monitoring patient antibody profiles for particular specificities, such as in monitoring antibody removal pre-transplantation [39] and identification of donor-specific antibodies post-transplantation [40].

Another advantage of this solid phase ‘single antigen bead’ technology is that the data can be used to define epitopes accurately and hence understand patterns of antibody reactivity [41]. HLA Matchmaker is a computer algorithm that compares HLA compatibility at a structural level by considering triplet amino acid sequences or amino acids clustered together structurally but not in sequence (eplets) [42]. It has been demonstrated that triplet mismatches correlate with antibody production as detected using Luminex single antigen beads [43,44]. The combined use of these tools enables a patient sensitisation profile to be established identifying not only those HLA epitopes to which the
patient has produced antibodies but also to which mismatches they are likely to react.

The development and use of single antigen beads has enabled the identification of antibodies to HLA-DQA1 and HLA-DPB1 epitopes [45,46] and also allele-specific antibodies [47] that it had previously been impossible to characterise. It is now crucial to establish the clinical relevance of these antibodies in order to inform the selection of patients for transplantation and their management post-transplant.

Since their introduction, there have been a number of modifications to the commercially available bead array assays that have sought to facilitate analysis of the results and their interpretation in the clinical setting. Recent reports have shown that IgM HLA-specific antibodies present in some patient sera can block binding of IgG HLA-specific antibodies to single antigen beads and give a misleadingly low or negative assessment of alloantibody levels that may be clinically important [48,49]. Pre-treatment of patient sera with DTT to destroy inhibitory IgM HLA-specific antibodies has revealed previously undetected IgG HLA-specific antibody binding that is masked during routine single antigen bead screening [50]. These reports suggest that when using single antigen beads for monitoring IgG HLA-specific antibodies in sensitised patients, it is important to consider pre-treatment of sera with DTT to reveal HLA class I and class II antibody specificities of potential clinical relevance that may otherwise be masked by IgM and other blocking factors.

Although it has been widely believed that IgM HLA-specific antibodies are not generally harmful to the graft, data from CDC testing have not always been
easy to interpret. In an attempt to address this, a modification to the flow
cytometry bead based assay was developed to detect IgM HLA-specific
antibodies [51] and a similar modification to a Luminex based bead array assay
has been reported [52].

Another reported modification to the bead array for the detection of HLA-
specific antibodies is designed to detect only those antibodies that fix
complement by detecting the complement fragment C4d fixed on to the Luminex
beads [53]. Again, the aim of this modification is to detect those antibodies that
help stratify the risk to determine clinical relevance.

7.3 Interpretation of HLA-specific antibody data in the clinical setting

One key question that is asked on behalf of sensitised patients is how their HLA-
specific antibody profile will influence their chance of being offered a transplant
from a deceased donor. Previously, the %PRA determined from screening
against cell panels has been used in this context; the higher the %PRA, the
lower the chance of a transplant. However, as discussed above, the %PRA will
reflect the composition of the cell panel rather than the potential donor pool. In
addition, %PRA is not applicable when single antigen bead assays have been
used.

For patients listed for a deceased donor transplant in the UK, a calculated
HLA antibody reaction frequency (cRF) is determined at NHS Blood & Transplant
- Organ Donation and Transplantation Directorate (NHSBT – ODT) from the
unacceptable HLA specificities reported for each patient. The unacceptable
specificities are compared with the HLA types of blood group identical donors
from a pool of 10,000 UK donors and the resulting HLA antibody reaction frequency (cRF) is expressed as a % of HLA incompatible donors. The advantage of this approach is that the figure can be calculated objectively and, as long as the HLA antibodies in the patient’s antibody profile have been specified, it represents an accurate reflection of the chance of a patient receiving an HLA compatible deceased donor transplant in the UK.

The solid phase assays described above provide specific and sensitive tools for the detection and characterisation of HLA-specific antibodies that have, by and large, replaced cell based assays and become the gold standard. However, there is still considerable debate as to the clinical significance of antibodies detected by solid phase assays, particularly if they are not detected by cell-based CDC or flow cytometry assays. Interpretation of the results is further complicated by recent reports of naturally occurring HLA-specific antibodies in sera from healthy males with no history of alloimmunisation [6]. Many of these antibodies reacted with dissociated antigens and have been reported to be irrelevant to transplant outcome [54]. Nevertheless, the possibility of their existence in patient sera must be considered when the results of bead based assays are being interpreted.

One of the main purposes of characterising a patient’s HLA-specific antibody profile is to define those HLA antigens that would be unacceptable in a donor for that patient so that positive crossmatches and unnecessary shipping of organs are avoided, whilst at the other end of the spectrum, transplantation is allowed when it is safe to proceed prior to performing the crossmatching test (a process recently termed a ‘virtual crossmatch (vXM)’) [55]. Critical to the
success of this approach is an understanding of the relationship between antibodies detected in a solid phase assay and a crossmatch test result [55,38]. It is not possible to directly correlate the mean fluorescence intensity for the bead to which antibody has bound and the crossmatch result for that antibody because the amount of HLA protein bound to each bead varies between beads within a batch and between batches [52]. Attempts have been made to correct for this variation by testing each lot of beads with monoclonal antibodies to determine the antigen density.

When using data from single antigen bead testing to define HLA antigens that would be unacceptable in a donor for that patient, it is also important to note that these assays now allow the identification of allele-specific antibodies [47]. For example, a patient with HLA-A*68:01 genotype could have antibodies to the antigen encoded by HLA-A*68:02. It is therefore now possible to identify potentially graft damaging antibodies that might previously have been dismissed as self (HLA-A68) reactive and therefore not graft damaging.

Consequently, it is crucial to have a full understanding of the results generated from solid phase assays in order to assess the clinical significance of the antibodies identified.

### 7.4 Screening strategies

A comprehensive programme for antibody detection and characterisation is an essential component of histocompatibility laboratory support for solid organ transplantation.
As only those transplant candidates who have previously been exposed to allosensitisation are likely to be positive when screened for HLA-specific antibodies, a rapid screening test is required to determine whether a serum sample is antibody positive or negative. Effort can then be focused on antibody definition in the positive samples.

A number of laboratory techniques are available for the definition of recipient sensitisation. These tests have often been considered as alternatives, but they each yield different information and have their individual advantages and limitations. It is possible to devise a strategy that employs a combination of assays to maximise the information obtained from minimal effort. Approaches may differ between laboratories and each centre should evaluate which combination of currently available technologies will most efficiently and accurately define antibody specificities in their sensitised patients.

The aim of a laboratory’s screening strategy should be to support their clinical transplantation service by:

- Identifying HLA-specific antibody positive sera that can be used in the pre-transplant crossmatch test.
- Identifying HLA-specific antibodies in order to assess the risk associated with transplantation from a given donor.
- Allowing a pre-transplant virtual crossmatch assessment (where appropriate).
- Providing data to support a clinical antibody reduction protocol.
7.5 Sample collection and storage for antibody screening and donor crossmatching

7.5.1 Pre-transplant.

The sensitisation status of a patient can vary over time, and therefore regular monitoring of antibody levels is necessary until a time that transplantation is permanently excluded as a treatment option. In order to define an individual’s sensitisation status and interpret antibody screening results it is essential to have accurate information about the timing and nature of potential priming events including transfusions, pregnancies, transplantation and infections. It is the responsibility of the clinical team to inform the histocompatibility laboratory of potential allo-sensitisation events and ensure that samples for antibody screening are sent to the laboratory at the agreed frequency. Patient serum samples should be obtained following transfusion of any blood products in order to detect any consequent sensitisation; this will optimally be between two and four weeks after the transfusion. Ideally, samples will be obtained approximately three-monthly for routine antibody monitoring.

7.5.2 Post-transplant.

De-novo synthesis of donor-specific antibodies after transplantation is associated with acute rejection and chronic graft attrition. Following transplantation, it is recommended that recipients are monitored for the presence of donor reactive HLA-specific antibodies and that the histocompatibility laboratory continues to receive serum samples for antibody screening. Failure to provide these samples may jeopardise a patient's future chances of transplantation. Local policy should stipulate the frequency of testing as well as testing following reduction or
cessation of immunosuppression and or transplant nephrectomy. It is recommended that, post-transplant, antibody monitoring should be related to the patient’s clinical course and the immunological risk associated with the transplant. Serum samples should be obtained and tested at times of biopsy for graft dysfunction to support the diagnosis of antibody-mediated rejection [56].

7.5.3 Sample storage
The consideration of historical patient serum samples for donor crossmatching is essential to provide information on the clinical risk associated with a transplant from a given donor because the use of only contemporary patient sera in the donor crossmatch test has been associated with sub-optimal graft survival [31]. Serum samples must be stored indefinitely for potential use in future antibody screening and crossmatch tests. For patients transferring to a different transplant centre, samples of all archived serum specimens, records and test results must be made available to the centre currently responsible for that patient.

7.6 Recommendations

- Laboratories must have a strategy in place for the detection and characterisation of clinically relevant antibodies.
- The techniques adopted must have the capability of defining antibody class and specificity.
- A combination of tests should be considered in order to fully resolve complex antibody profiles.
• Laboratories should have a programme for investigating and evaluating newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments.
• Regular patient serum samples must be sent to the histocompatibility laboratory as well as samples following transfusion of any blood products.
• Post-transplant serum samples should be obtained at times of graft dysfunction.
• Serum samples must be stored for potential use in future antibody screening and crossmatch tests.

8: CROSSMATCHING

The purpose of the crossmatch test is to determine whether a patient has antibodies which react with antigens expressed by a given donor and to inform the immunological risk assessment for that patient/donor combination. Pre-formed antibodies present in recipient serum at the time of kidney transplantation and directed against donor ABO blood group and or HLA antigens have been shown to cause hyperacute rejection [57]. A crossmatch between donor and recipient is the definitive pre-transplantation test to avoid hyperacute rejection although naturally occurring ABO blood group-specific antibodies must be avoided in the same manner as for blood transfusion. The pre-transplant crossmatch together with data on HLA-specific antibodies can also indicate patients with an increased risk for graft loss.
8.1 Crossmatch Techniques

The first technique to be developed to detect donor-specific antibodies was based on the complement dependent cytotoxicity test (CDC) [58]. A positive donor-specific cytotoxic crossmatch test was shown to be predictive of hyperacute rejection in 1969 [59]. The standard CDC technique detects both HLA and non HLA-specific complement fixing antibodies. The flow cytometric crossmatch was later developed [60] and is also recognised as a reliable and highly sensitive method for the detection of donor HLA-specific antibodies. Like the CDC test, this technique detects HLA and non HLA-specific antibodies. The test can be adapted to detect different immunoglobulin classes although the majority of methods in routine use detect both complement fixing and non-complement fixing IgG subclasses. The flow cytometric crossmatch is therefore able to detect antibody classes which are not identified by the standard CDC test.

The target cells used for the crossmatch test are donor lymphocytes. These are routinely isolated from peripheral blood, spleen or lymph node. T cells are used for the detection of donor HLA class I specific antibodies and B cells for donor HLA class I and II specific antibodies. Target cells isolated from peripheral blood contain relatively low numbers of B cells and this can affect the reliability of the test for the detection of HLA class II specific antibodies.

More recently solid phase crossmatch tests have been developed which mirror the antibody screening techniques in routine use. However, the take up of solid phase crossmatch tests has been low and there are few data to support their use at present. This low take up may be due to the documented utility of
the highly specific and sensitive X-Map Luminex antibody screening to reliably predict crossmatch outcome [38], leading laboratories to regard an additional crossmatch test as unnecessary.

8.2 Pre-transplant Virtual Crossmatching

The purpose of the pre-transplant crossmatch is the detection of pre-formed donor HLA-specific antibodies. It should follow that if the recipient has never experienced any potential sensitising events and/or has never produced HLA-specific antibodies the crossmatch is superfluous to requirements. The difficulty in translating this theoretical standpoint into practice has been the uncertainty that sufficient information exists regarding potential sensitising events and the ability to prove definitively that a patient has never, at any time, produced HLA-specific antibodies. Initially some transplant units speculated on the possibility of being able to define a sub-set of patients where the pre-transplant crossmatch could be omitted [61,62] with the aim of reducing cold storage time. It was first demonstrated that this works in practice with a study omitting the pre-transplant crossmatch for a well defined group of patients [63]. Crossmatches which were performed retrospectively in this group were all negative indicating that prediction of a negative crossmatch was reliable in this carefully selected sub-set of patients. A more recent study by the same group looked at the 10 year experience of omitting the pre-transplant crossmatch and in all cases the retrospective crossmatch confirmed the prediction of a negative crossmatch [64]. This large study also demonstrated that the omission of the
crossmatch did result in reduced cold storage time and additionally that there may be a reduction in delayed graft function for these patients.

The term virtual crossmatch (vXM) is applied to the use of antibody data to predict crossmatch outcome based on a comprehensive knowledge of the specificity of any detected antibody and the potential reactivity with a donor of given HLA type. As described above the virtual crossmatch can be used to facilitate omission of the pre-transplant test. It has also been very effectively used for many years in the UK by the listing of unacceptable antigens for all patients on the waiting list which allows a virtual crossmatch to be performed within the matching run so that offers are not made to potential recipients with a predicted positive crossmatch [4].

8.3 Sample Selection

The selection of patient serum samples is of great importance and will affect the interpretation of the crossmatch. A sample taken immediately prior to the crossmatch test being performed is the most reliable means of determining the current status of donor-specific sensitisation. In some cases a sample which has been collected within the last 3 months may be accepted as a current sample where it is known that the patient has had no potential sensitising events in the intervening period. In addition it is advisable to crossmatch a selection of historic serum samples which are representative of the patient’s sensitisation status over time. This should include samples in which all the antibody specificities which have been detected are represented. The laboratory must inform the clinical team whenever more than three months has lapsed since a patient’s serum
sample has been received. The onus is on the referring centre to provide appropriate screening samples so as to avoid an increasing risk of unknown sensitisation and governance issues associated with failure to screen recipients correctly.

8.4 Results and Interpretation

For each serum sample tested the result of a crossmatch is positive or negative. A negative crossmatch indicates donor-specific antibodies are absent from the recipient serum or are below the level detectable by the test. A positive result is usually due to donor-specific antibodies but may also occur due to the presence of non HLA-specific antibodies. The specificity and strength of the antibodies causing the positive result is the most important factor in the interpretation of the crossmatch and where it can be demonstrated that the antibodies are not HLA-specific the positive result is not generally regarded as a veto to transplantation. The demonstration of antibody binding to both T and B cells suggests the antibody detected is likely to be directed at the HLA class I antigens. B cell positive FCXMs may occur when the T cell FCXM is negative and could be due to antibody directed at HLA class I or class II antigens or to autoantibodies. The latter are distinguished by also giving positive results in auto-flow crossmatches. A T cell positive FCXM where there is no antibody binding with B cells suggests that the antibody may not be HLA-specific.

In addition the antibody class and the timing of samples giving a positive result (i.e. historic versus current) are important in determining the clinical relevance of the result.
The issue of clinical relevance is of paramount importance in interpreting crossmatch results and in assigning the potential risk associated with any transplant. As outlined in the following sections of these guidelines, whilst the clinical relevance of a positive cytotoxic crossmatch due to donor-specific HLA antibody is not generally questioned, in almost every other aspect of crossmatching there is a lack of absolute correlation between results and clinical outcome, although significant associations and trends are identifiable. This has increasingly become the case as antibody detection techniques have become more sensitive and at the same time immunosuppression and other interventions have led to a greater ability to transplant in the face of donor-specific HLA antibodies. The interpretation of crossmatch results by experienced histocompatibility scientists in possession of all the details of the patient’s allosensitisation history is essential if an appropriate risk assessment is to be made (see Risk assessment Table 1). In certain urgent cases, a patient-specific assessment may be necessary, involving dialogue between the histocompatibility laboratory and the clinicians directly responsible for patient care.

A recent finding has been the increasing incidence of negative crossmatches in patients where bead-based antibody testing has identified donor-specific antibody. This phenomenon causes ‘false positive’ virtual crossmatches i.e. cases where the crossmatch test does not confirm the predicted result. Detailed analysis of the strength, specificity and, where known, the patient’s exposure to potential sensitising events are important in the risk analysis in such cases. Studies have shown associations between such antibodies detected by bead-based assays only and acute rejection episodes [65] and with
long term but not short term graft outcome [66]. However it has also been shown that antibodies reacting in bead-based assays are found in some non-transfused, non-transplanted males indicating that these assays detect antibodies in unsensitised individuals [5]. Such antibodies have been described as “natural” antibodies. The authors suggested that the antibodies may be produced to pathogens or allergens and the reactivity with HLA coated beads is due to cross-reactive epitopes. However, they also acknowledged the possibility that the process of attaching the HLA molecules to the beads causes denaturation of the molecules and that epitopes are exposed which would not be present in naturally expressed HLA molecules on the cell surface. These factors have given rise to the suggestion that whilst a negative virtual crossmatch is a very reliable indicator for graft survival, the interpretation of a positive virtual crossmatch is less straightforward [67].

It is essential that the interpretation of crossmatch results is undertaken by experienced personnel who are able to determine and provide appropriate advice on the clinical relevance of the result obtained.

### 8.5 Recommendations

- A prospective crossmatch must be performed (except for liver transplants) by carrying out a pre-transplant crossmatch test or by performing a virtual crossmatch in selected cases.
- Laboratory crossmatch tests should distinguish donor T cell and B cell populations and between IgG and IgM antibodies.
• Serum samples used for crossmatching must include a current sample and, where HLA-specific antibodies have been detected, samples that are representative of the patient’s antibody profile over time.
• The timing, duration, priming source, antibody titre and donor specificity should be considered when interpreting the clinical relevance of the crossmatch result.
• The reporting of results to clinical teams should include appropriate advice on the clinical relevance of the result.

9: KIDNEY AND PANCREAS TRANSPLANTATION.
Analysis of (as yet unpublished) UK pancreas transplant outcome by NHSBT-ODT indicates that the two factors with greatest impact on pancreas graft function are increasing donor age and cold ischaemia time. In this section, since there is little other specific pancreas data, the kidney and pancreas are considered together in terms of pre-transplant workup, crossmatching and follow up.

Particular care is required when a donor kidney or pancreas is transplanted into a sensitised patient. Special consideration has to be given to the donor HLA mismatch grade and to avoid HLA mismatched specificities to which the patient is sensitised. The more stringent kidney allocation criteria with respect to HLA match and negative pre-transplant crossmatch means that sensitised patients can expect longer than average waiting times. In highly sensitised patients who have an HLA antibody profile which excludes >85% of potential donors, the increased immunological risk of transplant rejection may have to be balanced against clinical risks of remaining on dialysis. In some
highly sensitised patients the likelihood of locating a suitable deceased donor is remote and specific strategies such as antibody removal to facilitate living donation may be considered.

Registry data from a large number of transplant centres has shown that kidney transplant outcome in sensitised patients and regrafts is inferior to that in non-sensitised patients [68]. Data from the UK are consistent with these findings. Recent analysis shows that for first transplants and retransplants performed between 2000-2008, five year graft survival was poorer in all groups of sensitised patients compared with non-sensitised patients (first transplants 74-81% vs 84%; p=0.008; retransplants 75-78% vs 82%; p=0.002).

9.1 Pre-transplant Antibody Screening

Antibody characterisation aids the interpretation of crossmatch results and also contributes to the success of organ sharing schemes set up to facilitate successful transplantation of sensitised patients with well matched kidneys and less well matched but compatible pancreata. Although the importance of HLA matching in kidney transplantation is well accepted, it is not the only factor to influence transplant outcome. Investigators have reported the detrimental effect of prolonged cold storage times and delayed graft function on transplant outcome [69,70]. It is important that transplant centres participating in organ sharing programmes ensure that laboratory processes are in place to minimise the chance of kidneys and/or pancreases being shipped and then being crossmatch positive.

If sera are carefully screened during patient work-up for transplantation, then HLA-specific antibodies can be defined and hence a patient’s crossmatch
reactivity against a particular donor of known HLA type predicted [71-73]. The ability to accurately define the specificity allows the UK national allocation scheme to perform a virtual crossmatch prior to allocation. This has been successfully instituted for many years. In the case of zero HLA-A, -B, -DR mismatched transplants, antibodies specific for these loci would not be expected to have a role. However, the immunological loss of some HLA-A, -B, -DR matched transplants suggests that antibodies specific for HLA-Cw or -DQ or -DP antigens may have a role in transplant failure [74,75]. Definition of antibodies to HLA-Cw and -DQ and –DP antigens in addition to -A, -B and -DR is therefore necessary in order to predict whether a crossmatch with a particular donor will be positive. This may also involve careful characterisation of any allele-specific antibodies present. After initial sample testing, sera must be screened at three monthly intervals and following each sensitising event such as blood transfusion so that at the time of crossmatch against a potential organ donor each patient has a comprehensive antibody profile available. This avoids unnecessary crossmatching and facilitates interpretation of positive results. A significant influence of matching for HLA-DP in repeat transplant patients also suggests a possible role for HLA-DP specific antibodies in transplant failure [76]. Similarly, the presence of IgM autoantibodies can be identified during patient work-up through antibody screening and performing an autologous crossmatch. For patients known to have IgM autoantibodies, the crossmatch can be carried out in the presence of DTT and false positive crossmatch results can therefore be avoided.
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

HLA-specific antibodies that are not apparently generated by exposure to alloantigens have been detected with the latest sensitive screening techniques. Their origin and clinical relevance is unclear [5].

9.2 Definition of Unacceptable Mismatches

The results of antibody screening should be used to define unacceptable mismatches. These will include HLA antigens for which the patient has been shown to develop specific antibodies. Further unacceptable mismatches may be identified, which can include mismatched antigens on previous failed transplants to which specific antibody has not been demonstrated. This is because there may be immunological memory of exposure even if there is no antibody currently detectable. Mismatches which do not elicit an antibody response are repeated with no apparent detriment but it is important that there are sufficient screening data to determine that there has been no antibody response. This can only be the case where regular post transplant serum samples have been collected and analysed, in particular samples taken at the time of and subsequent to graft loss. Where it is judged that the screening history is incomplete, such as when mismatches from a past pregnancy are unknown, all mismatched antigens should be regarded as representing a potentially increased immunological risk.

For patients with a functioning transplant requiring transplantation of an additional organ (e.g. thoracic organ or liver transplant patients requiring a kidney transplant, or kidney transplant patients requiring a subsequent pancreas transplant), previous mismatched antigens should not be listed as unacceptable
unless antibody specific for the mismatched antigens has been demonstrated. This recommendation is based mostly on case reports. However, a limited UK analysis of recipients of cardiothoracic organs who subsequently received a sequential kidney transplant, did not show an adverse effect of a repeated mismatch on kidney transplant outcome [77].

Other HLA antigens may be listed as unacceptable where it is desirable to avoid sensitisation to these antigens. This could be the case for those patients who may be considered for living donor transplantation at a future date, especially if a particular donor was being considered.

9.3 The Clinical Relevance of Crossmatching

The crucial factors determining the clinical significance of any crossmatch are the specificity and immunoglobulin class of the antibodies causing a positive result. In addition the timing of the patient samples and the strength of the reaction are of relevance.

9.3.1 The cytotoxic crossmatch.

It is generally accepted for kidney transplantation that IgG antibodies directed against donor HLA-A or -B specificities and present at the time of transplant will cause hyperacute rejection in the majority of cases [59,78]. Although less data are available, donor HLA-DR specific antibodies present in the recipient may also result in rejection [48]. The outcome will differ between individuals depending on antibody titre and level of expression of HLA-DR on the donor organ. Hyperacute rejection has been described in cases of positive B cell crossmatches due to HLA class II specific antibody and the elution of class II
specific antibody from the rejected kidney provides strong evidence of a role for this antibody in the rejection process [79,80]. There is little information on the role of antibodies to HLA-Cw, -DQ or –DP specificities in transplant failure. Kidney transplant failure in a patient who had antibodies to HLA-Cw5 present in the donor has been reported [74] and acute humoral rejection has been associated with high titre IgG HLA-DQ specific antibodies [81]. If patients have antibodies to HLA-Cw or HLA-DQ, many centres now consider it advisable to avoid organ donors with those specificities just as they would when a patient has antibodies to HLA-A, -B and -DR.

IgM autoreactive antibodies react with autologous as well as allogeneic lymphocytes in the CDC crossmatch test and have been shown to be irrelevant to transplant outcome [82]. They therefore give rise to false positive results. The clinical relevance of IgM HLA-specific antibodies is not clear and whilst in many cases they appear not to be detrimental, in some circumstances they may be an association with rejection [83].

The clinical relevance of antibodies in non-current sera is also a point of debate. There have been reports of successful kidney transplantation with a “peak positive, current negative” crossmatch [84] but again it is the specificity of the antibodies that may be the crucial factor. IgG HLA-A or -B specific antibodies present in historic sera are associated with accelerated rejection and decreased graft survival [78,85]. It has been suggested that the earlier antibody response might be associated with T cell activation which then later mediates acute graft rejection [86]. This hypothesis is supported by evidence of rejection associated
with the presence of ciclosporin-resistant cytotoxic T lymphocytes specific for antigens to which antibody had previously been detected [87,88]. Rejection could also be antibody mediated as there has been a report of a kidney transplant following a “peak positive, current negative” crossmatch due to an IgM alloantibody, which resulted in a secondary antibody response within 5 days post-transplant, vascular rejection and transplant failure [89]. Decisions regarding the transplantation of patients with antibodies in non-current sera should take into account the requirement for effective post-transplant management.

9.3.2 Flow cytometric crossmatching.

Early studies of FCXM showed the method to be more sensitive than conventional CDC crossmatches for the detection of antibody [60]. The greater sensitivity of flow cytometry and an association of a positive flow crossmatch with graft rejection have been confirmed [90]. The technique has also been shown to be more sensitive than the anti-human globulin (AHG) augmented CDC crossmatch [91].

The application of FCXM to specific groups of potential recipients is a matter on which evidence varies. The first clear association between a positive flow crossmatch and graft failure in CDC crossmatch negative kidney allograft recipients was shown in 1987 [92]. This association was significant only in sensitised recipients (those with previous failed grafts or with panel reactive antibodies). Other studies have demonstrated associations between positive FCXMs and complications in both first and re-grafts [93,94]. In one of the largest studies of FCXM and primary kidney transplantation, there was a
significant association between a positive T cell FCXM and reduced graft survival at one year [95]. In contrast other studies have not found a significant association between a positive FCXM and graft function [96,97], although the latter study did show a non-significant trend towards poor graft survival in the small number of regrafts studied.

The above studies focused on the T cell FCXM. B cell FCXMs are also performed by increasing numbers of laboratories. A significant association between positive T and B cell FCXMs and graft failure has been described [98]. The demonstration of antibody binding to both T and B cells suggests the antibody detected is likely to be directed at the HLA class I antigens. A T cell positive FCXM where there is no antibody binding with B cells suggests that the antibody may not be HLA-specific. B cell positive FCXMs may occur when the T cell FCXM is negative. A strongly positive B cell FCXM was significantly associated with poorer graft survival at one year compared with those where negative, or weakly positive, B cell FCXMs occurred [99]. This association was found only in those patients receiving allografts mismatched for at least one HLA-DR antigen, suggesting that the antibody detected may be specific for HLA class II.

Stratification of outcome according to the FCXM results has been shown with the highest survival in patients with T and B cell negative FCXM, intermediate survival with a B cell positive FCXM and poorest survival with T and B cell positive FCXM [100,101]. This stratification has also been shown in relation to the development of chronic rejection, with the incidence highest in T and B positive, intermediate in B positive and lowest in T and B negative FCXM.
groups [102]. As with the CDC crossmatch the specificity of the antibody causing the positive crossmatch is a critical factor.

Although some published studies have found no significant association between a positive FCXM and graft outcome, the majority indicate that a positive FCXM is predictive of early graft rejection and failure. In particular large multi-centre studies do indicate a significant association between FCXM and graft outcome [95,101].

9.3.3 Virtual crossmatching.

The purpose of the pre-transplant crossmatch is the detection of pre-formed donor HLA-specific antibodies. It should follow that if the recipient has never experienced any potential sensitising events and/or has never produced HLA-specific antibodies the crossmatch is superfluous to requirements. The difficulty in translating this theoretical standpoint into practice is the uncertainty that sufficient information exists regarding potential sensitising events and the ability to prove definitively that a patient has never, at any time, produced HLA-specific antibodies. Some transplant units have speculated on the possibility of being able to define a sub-set of patients where the pre-transplant crossmatch could be omitted [61,62] with the aim of reducing cold storage time. It has been demonstrated that this works in practice with a study omitting the pre-transplant crossmatch for a well-defined group of patients [63]. Crossmatches which were performed retrospectively in this group were all negative indicating that prediction of a negative crossmatch was reliable in this carefully selected sub-set of patients. More recently this approach has been shown to significantly lower the cold ischaemic time. [64]. If a vXM strategy is to be implemented for
selected patients, close liaison between the transplant team and the histocompatibility laboratory is essential.

9.3.4 Reporting crossmatch results.

A positive crossmatch result is obtained when the patient’s serum sample contains one or more antibodies which are assessed to bind to the potential donor’s cells sufficiently. The evidence indicates that when this antibody is IgG with specificity for HLA, there is a high risk of rejection and/or complications. Usually the risk constitutes a veto to transplantation. Where the antibody is not HLA-specific, the positive crossmatch is not a veto to transplantation. The reporting of crossmatch results must clearly distinguish between positive reactions thought to be clinically relevant and those thought not to be.

9.4 Development of HLA-specific antibodies after kidney transplantation

Following transplantation, de novo HLA-specific antibodies have been identified in kidney allograft recipients [89,102-116]. Antibodies have been identified either by specifically crossmatching against donor cells or by demonstrating HLA-specific antibody reactivity in conventional antibody screening assays. The proportion of recipients reported to develop antibodies varies between 12 and 60% [117]. Clearly a number of factors could influence these figures, including the type and sensitivity of assay used and clinical factors such as the degree of mismatching between donor and recipient and immunosuppressive protocols. Modification of immunosuppressive treatment can affect antibody production.
The development of HLA-specific antibodies following kidney transplantation has been shown to be associated with a poorer transplant outcome. Recipients developing HLA-specific antibodies have a higher incidence of acute rejection and of chronic graft dysfunction than those patients without antibodies. Many of the early reports demonstrated the presence of HLA-specific antibodies using complement dependent cytotoxicity (CDC) assays, but with the availability of new technology, donor-reactive antibodies have been more accurately defined using sensitive and specific solid phase assays. In order to identify de novo production of post transplant antibodies, it is important to specify reactivity against mismatched donor antigens. In a recent report where donor HLA-specificity was assigned to the antibody produced de novo following kidney transplantation, antibodies directed against donor HLA-A, -B, -Cw, -DR, -DQ and -DP mismatches were shown to be strongly predictive of transplant failure [89]. Accordingly, samples should be taken from transplant recipients at regular intervals, on an agreed basis (this may be determined on an individual patient basis on the basis of perceived immunological risk), and at the time of biopsy, suspected rejection and in cases of declining graft function where there is no other clinical cause.

Whilst mismatched classical HLA antigens present targets for antibody responses, other polymorphic antigens may also be important in this context. Antibodies to the mismatched MICA antigens have recently been described in the sera of transplant recipients [88, 89]. These antibodies may be of particular interest because MICA expression has been described on kidney tubular epithelia in rejecting allografts [119] and on endothelium in vitro, but not on lymphocytes
Therefore pre-existing MICA antibodies would not be detected by current crossmatching tests.

While circulating donor-specific antibodies can easily be detected following transplantation, the histological detection of immunoglobulins bound to the endothelium in a transplant has proved difficult, because antibody is rapidly removed from the endothelial surface. However, after antibody-mediated activation of the classical complement pathway, the complement protein C4d is covalently bound to the endothelial surface leaving a marker of antibody activity that persists after complement activation. Following the initial report from Feucht and colleagues [121], the presence of C4d on peritubular capillaries of kidney transplant biopsies has been shown to be a reliable marker for humoral rejection and as such an immunohistochemical marker of post-transplant donor reactive antibody responses. However, the absence of detectable C4d positivity does not preclude antibody-mediated rejection. Studies of biopsies obtained during kidney allograft dysfunction have revealed that C4d deposition in the peritubular capillaries is present in approximately 30% of acute rejection biopsies [122-126] and analysis of early protocol biopsies has shown C4d to be a specific marker of humoral rejection. [127]. There is now very strong evidence that circulating donor reactive antibodies detected by post-transplant crossmatching and screening are significantly associated with C4d deposition [14,122,124-126,128,129].

The Banff 2007 classification of kidney allograft rejection recognises negative, minimal, focal and diffuse C4d staining. However C4d deposition without morphological evidence of active rejection has been added to the Banff
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

diagnoses under the antibody mediated category. This is largely to acknowledge the diffuse C4d staining that is common in ABO incompatible transplantation but is not associated with graft dysfunction [55]. Since the production of donor HLA-specific antibodies following transplantation is associated with poor outcome, there is a potential benefit to monitoring patients for production of post transplant antibody. There is some evidence to suggest that the introduction of agents such as mycophenolate mofetil into immunosuppressive regimens decreases antibody production [130-132].

### 9.5 Recommendations

- Laboratories providing services for kidney transplant programmes must have the capability of precisely defining HLA-A, -B, -Cw, -DR, -DQ and -DP antibody specificities in their patients so that donors who will be crossmatch negative can be identified.
- A patient's HLA alloantibody profile must be assessed to delineate the antigens regarded as unacceptable.
- Pre-transplant, samples should be obtained and tested at three monthly intervals and after known sensitising events.
- A pre-transplant crossmatch test should be performed for all patients unless a programme exists for identifying those individuals who can confidently be defined as unsensitised. Patients with no detectable HLA-specific antibodies can be transplanted on the basis of a negative virtual crossmatch (vXM) without waiting for a crossmatch test to be performed.
- Sensitised patients should be crossmatched using flow cytometric techniques.
• Post-transplant antibody monitoring should be performed at agreed regular intervals, at the time of biopsy and in cases of suspected rejection. Samples should also be tested at times of declining graft function when there is no other clinical cause.
• The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, pregnancy (including known miscarriage) and recent infection or vaccination.

10: ISLET TRANSPLANTATION

Islet transplantation is becoming established as a therapy for selected patients with type 1 diabetes mellitus. Patients may require more than one allograft from different donors in order to achieve metabolic success. The more limited pool of donors suitable for islet isolation and the small number of patients on the transplant list precludes significant HLA matching between donors and recipients and therefore recipients may be exposed to multiple mismatched HLA specificities during a course of treatment. Transplants may be performed as islet alone, islet after kidney (IAK) or as simultaneous islet and kidney transplants.

10.1 Pre-transplant antibody screening

There is evidence that pre-existing sensitisation to donor HLA is detrimental to survival of islet transplants [133, 204]. Prior to listing a patient for transplant, it is recommended that antibody screening and specificity analysis are performed on two separate samples. Once the patient is listed, samples for antibody analysis should be obtained no less than three monthly. Potential priming
events should be notified promptly to the laboratory and samples sent approximately 2-4 weeks after the event. It is recommended that antibody testing is performed by two different assays, including a highly sensitive technique to determine the specificity of the antibodies. Specificities detected against a kidney graft in an IAK patient should be considered as unacceptable.

10.2 Post-first transplant and pre-second transplant antibodies

The appearance of donor HLA-specific antibodies has been reported following successful islet transplantation and in a recent case study, humoral rejection occurred in a graft that was subsequently rescued with rituximab and IVIg therapy [134]. The incidence of donor specific antibodies following transplantation is difficult to determine from the literature as studies have used technologies that differ in sensitivity and some report data on relatively small cohorts of patients. In islet alone transplant recipients, 23% patients developed donor specific antibodies whilst on immunosuppression [135] and in combined kidney and islet transplants the incidence of sensitisation has been reported to be similar to that in kidney transplants alone [136]. The incidence of HLA-specific antibodies has been reported to rise significantly after failure of islet transplants and withdrawal of immunosuppression [137].

In order to monitor a patient’s antibody status after the first transplant it is recommended that samples are obtained for antibody screening and specificity analysis regularly until the next transplant. All donor-specific antibodies should be reported to the clinical team and should be used to inform decisions about selection of subsequent islet transplants. All HLA-specific antibodies detected in
the antibody screening programme may not necessarily be listed as unacceptable specificities and a patient may be crossmatched against a donor expressing such an antigen. If the crossmatches are negative and there is appropriate discussion with the Consultant Clinician responsible for the transplant programme, it is possible that a transplant may proceed in the presence of donor specific antibodies detected only by Luminex technology. It is recommended that samples are taken regularly following the first and any subsequent transplants.

10.3 Crossmatching

Both CDC and FC crossmatching are recommended, but proceeding to transplant on the basis of a virtual crossmatch may be acceptable if the laboratory and transplant centre have a validated policy.

It is necessary to distinguish between auto- and alloreactivity, either by performing autologous and allo-crossmatches in the acute on-call situation or by performing the auto-antibody testing at an earlier stage in the work up of the patient. A current sample should be included in the crossmatch and is usually defined as a sample obtained within one month of the transplant, providing there have been no sensitising events.

10.4 Recommendations

- Prior to listing a patient for transplant, it is recommended that antibody screening and specificity analysis are performed on two separate samples.
• Once the patient is listed, samples for antibody analysis should be obtained no less than three monthly.
• Potential priming events should be notified promptly to the laboratory and samples sent approximately 2-4 weeks after the event.
• Antibody testing should be performed by two different assays, including a highly sensitive technique to determine the specificity of the antibodies.
• Both cytotoxic and flow cytometry crossmatching are recommended.
• Proceeding to transplant on the basis of a virtual crossmatch may be acceptable if the laboratory and transplant centre have a validated policy.
• It is recommended that samples are taken regularly following the first and any subsequent transplants.

11: THORACIC ORGAN TRANSPLANTATION

The considerations of HLA-specific antibodies for cardiothoracic transplantation are similar to those required for deceased donor kidney transplantation. In the early era of cardiothoracic organ transplantation, patients were transplanted without regard to their HLA-specific antibody status and without prospective donor crossmatching. This led to a number of transplants being performed in the face of a positive crossmatch, often leading to accelerated graft failure. In most instances the donor-specific crossmatch was performed retrospectively, using cells isolated from donor spleen.
11.1 Pre-transplant HLA-specific antibodies

For many years the CDC assay was the only method available for the detection of HLA-specific antibodies and donor-specific crossmatching. Using CDC based assays, it was demonstrated that pre-transplant donor HLA-specific antibodies are strongly associated with hyperacute or accelerated rejection of thoracic organ allografts, usually leading to death of the recipient [138-140]. An early report showed that all 4 patients with a positive crossmatch had early graft failure and death, contrasting with 7 of 28 (25%) recipients with a negative crossmatch [139]. When T and B cell crossmatches are analysed separately it can be seen that a positive IgG T cell crossmatch is associated with accelerated graft failure for both heart and heart-lung transplant recipients [138]. Of 7 patients transplanted with a positive T cell crossmatch, 5 (71%) died within 2 weeks of transplantation, contrasting with 31 of 258 (12%) patients transplanted with a negative T cell crossmatch [138].

In recent years, methods with increased sensitivity have been introduced to detect and characterise HLA-specific antibodies. These assays utilise purified or recombinant HLA molecules coated onto fluorescent labelled microbeads (X-Map Luminex technology). The major problem facing cardiothoracic centres and laboratories using these solid phase assays is that although low levels of circulating antibodies can be detected, the clinical significance of these antibodies is unclear.

Recent evidence suggests that HLA-specific antibodies detected by the more sensitive solid phase assays are associated with increased acute rejection and decreased graft survival. Stastny et al have demonstrated that microbead-
based assay detected donor HLA-specific antibodies are associated with increased graft loss and increased acute rejection in cardiac transplant recipients [141]. Furthermore, a recent study of 565 cardiac recipients showed that patients with pre-formed donor HLA-specific antibodies had significantly decreased graft survival compared to patients with no antibodies and those with non donor-specific antibodies. In addition, a modification of the X-Map Luminex assay which enabled detection of complement fixation on the microspheres demonstrated that donor HLA-specific antibodies which activate the complement cascade had the poorest graft survival [142].

Prior to listing, patients must be screened for HLA-specific antibodies on at least two separate occasions preferably taken no less than 24 hours apart, although this may not be possible for urgent patients. Samples must also be collected following any sensitising events such as transfusion of blood products or pregnancy, and every 3 months whilst on the waiting list so that a complete antibody profile is available prior to transplantation. It is important therefore that the histocompatibility laboratory is informed of any sensitising events and that collection of blood samples is arranged.

FCXM is a more sensitive technique than conventional CDC crossmatching and has demonstrated a correlation with increased early acute rejection episodes in heart transplantation [143] and severe graft dysfunction in lung transplantation [144].

If a potential recipient of a thoracic organ transplant is known to have produced well defined HLA-specific antibodies with no undetermined reactivity, a vXM should be performed. However, a lack of accurate information regarding
potential sensitising events in these patients would mean that there will always be a degree of uncertainty as to whether some patients may have produced HLA-specific antibodies at some point in their history (e.g. following pregnancy).

Evidence in lung transplantation suggests that the vXM is an acceptable method for donor/recipient selection for sensitised patients, with comparable outcomes to patients with no detectable HLA-specific antibodies [145]. If however, the patient is highly sensitised, it may be necessary to perform a prospective crossmatch with donor lymphocytes. This requires blood to be sent from the donor hospital to the recipient’s histocompatibility laboratory. Given that the acceptable ischaemia time for thoracic organs is less than five hours, careful consideration should be given to the location of the donor hospital as to when prospective crossmatching is feasible. For those cases where a vXM is used, the crossmatch test should also be performed retrospectively using donor lymphocytes. Using X-Map Luminex assays it is now possible to define antibodies directed against HLA-DP molecules. At present deceased donors are not routinely typed for HLA-DP and for patients with HLA-DP specific antibodies prospective crossmatching should be performed.

The crossmatching techniques utilised should be able to determine the presence of antibodies reactive with T and/or B cells as well as immunoglobulin isotype which may have some relevance to graft outcome.

11.2 Post-transplant production of HLA-specific antibodies

HLA-specific antibodies produced following thoracic organ transplantation have also been shown to have deleterious effects on graft outcome [146-152].
Recipients of cardiac allografts produce HLA-specific antibodies following transplantation which, if specific for donor HLA antigens, have been shown to convey increased risk of acute rejection [147], poor graft survival [146] and chronic rejection [148,149,151]. A recent study utilising a solid phase assay (FlowPRA) has shown that de novo detected HLA-specific antibodies are associated with increased rejection, with HLA class II directed antibodies strongly associated with graft vasculopathy and graft failure [151]. This is also true for lung transplantation with patients producing HLA-specific antibodies at increased risk of developing chronic rejection manifesting as bronchiolitis obliterans syndrome (BOS). In one study, 66% of 15 patients with BOS produced HLA-specific antibodies, whilst none of 12 patients free from BOS had formed antibodies [149]. Similarly, over 60% of lung recipients without demonstrable HLA-specific antibodies lacked signs of chronic rejection whereas all patients with HLA-specific antibodies had developed BOS within 2 years of transplantation [148].

It is recommended that post-transplant monitoring of patients for the production of HLA-specific antibodies be performed at regular intervals following transplantation, preferably every 3 months within the first year, annually thereafter and if clinically necessary.

11.3  Non HLA-specific antibodies

There has been renewed interest in the production of antibodies to non-HLA antigens in thoracic organ transplantation. Antibodies directed against endothelial cell antigens have been associated with the development of
transplant associated vasculopathy after cardiac transplantation [153-156]. Although many antigens are likely to be involved, one of these is known to be the intermediate filament vimentin [157]. A simple ELISA for the detection of vimentin specific antibodies performed at regular intervals within the first year of transplant can identify patients at risk for developing transplant vasculopathy [158]. MICA is a functional gene of the MHC class I related chain family located within the MHC region on chromosome 6. MICA molecules are structurally similar to HLA class I molecules and are constitutively expressed on some epithelial cells and has been shown to have inducible expression on endothelial cells (25).

11.4   Recommendations

- Laboratories must be able to precisely define antibodies specific for HLA – A, -B, -Cw, -DR, -DQ and –DP using sensitive solid phase assays.
- HLA antigens to which a patient has produced antibodies should be listed as “unacceptable mismatches”.
- Before transplantation patients must be screened for HLA-specific antibodies on at least two occasions prior to listing, preferably on sera taken at least 24 hours apart, although this may not be possible for urgent patients.
- Samples must also be collected following any sensitising events such as transfusion of blood products or pregnancy, and every three months whilst on the waiting list so that a complete antibody profile is available prior to transplantation.
Patients with no detectable HLA-specific antibodies can be transplanted following virtual crossmatching by the histocompatibility laboratory.

Sensitised patients with fully defined HLA-specific antibodies and with no residual reactivity can also be transplanted without a prospective crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. Prospective crossmatching must be performed for all other sensitised patients.

For patients where a prospective crossmatch test is not performed a retrospective crossmatch using serum collected within 24-48 hours prior to transplantation must be performed.

Crossmatch techniques should distinguish between IgG and IgM as well as T and B cells as targets.

Following transplantation patients should be screened for HLA-specific antibodies at regular intervals and must be tested when clinically necessary.

12: LIVER TRANSPLANTATION.

Studies on HLA-specific antibodies in liver transplantation span transplants performed over many years during which survival rates continued to improve. This might reduce the value of comparisons between studies, which cover different eras. There is no consensus regarding the clinical significance of HLA-
specific antibodies, but there is sufficient published information to indicate where donor-specific antibodies constitute a risk and how this might be managed.

Hyperacute rejection in liver transplantation is unusual [159]. The fact that HLA (and ABO)-specific antibodies can mediate immediate and irreversible rejection of liver allografts demonstrates that the liver is not completely protected from humoral rejection. It has been suggested that high titre alloantibodies are necessary for hyperacute rejection, but without definition of “high titre” in this context. However, it has been reported that in a multiply-transfused male, (transfused within a few days of his first transplant), with failure of two sequential hepatic allografts, HLA donor-specific antibodies had titres between 1:16000 and 1:32000, which are high by any measure [159].

It is the practice of some units to perform a crossmatch, but often in retrospect and not for recipient selection. Some studies from such centres show no association between a positive crossmatch and reduced graft survival [160-167], while in others a significant association has been reported [168-176]. Where a statistically significant association between a positive crossmatch and reduced survival has been shown, the correlation is with graft loss within the first 12 months. Furthermore, an increased rate of early rejection has been found even in the absence of a high graft failure rate in crossmatch positive cases [161,163,164].

In general, CDC T cell positive crossmatches have been shown to be a better predictor of outcome contrasting with CDC B cell or FXCM. This implies that clinically significant donor-specific antibodies may be limited to HLA class I. The increased sensitivity of flow cytometry may detect antibodies at a level
below clinical significance. Where survival data have been analysed in relation to flow cytometry crossmatches no association has been seen [165,166], although it has been reported that high level HLA class I-specific antibodies were associated with steroid-resistant rejections [166].

Evidence shows that donor HLA-specific antibodies represent a risk to liver allografts, but in most cases this does not result in graft failure. There are considerable differences between centres reporting the effects of a positive crossmatch on outcome, but combining all cases shows one year graft survival is reduced by about 12%. In most centres the rate of positive crossmatch transplants is around 10% (range 7%-23%) and these were more likely for female recipients.

The detrimental effects of pre-existing donor-specific antibodies are seen during the early post transplant period. Graft loss may be prevented by effective management [161,169] or be dependent on the HLA-specific antibody titre. In addition, the size of the liver and tissue mass relative to the size of the patient together with the strength of donor-specific antibody may also be important factors that determine the resilience of a transplanted liver to antibody mediated rejection. This may be particularly important in adult and paediatric living donor liver transplantation and the use of ‘split’ livers that are smaller in size and less able to resist immune mediated damage caused by pre-transplant allosensitisation [177,178].

Persistence after transplantation has also been shown to be an important factor in the pathogenicity of donor-specific antibodies [161,169]. Long term outcome seems to be less dependent on a positive crossmatch at the time of
transplant. This could in part be due to selection for particularly resilient transplants, as well as the collective effect of all other influences that Doyle et al describe as “background noise” [164]. Overall, the effect of a positive crossmatch is measurable and stands above the background of other pressures on outcome.

### 12.1 Recommendations
- Prospective crossmatching is not indicated prior to liver transplantation.
- Identification of donor-specific antibody can be used to identify patients at high risk of acute rejection and can aid post-transplant management, such as changes in immunosuppression regimen or antibody removal.

### 13: INTESTINAL and MULTI-VISCERAL TRANSPLANTATION
Definitive evidence for a clinically important role of HLA allosensitisation and the value of ensuring a negative pre-transplant donor-recipient crossmatch before intestinal transplantation is lacking. The pool of potentially suitable deceased multi-organ donors for a given patient is limited and any additional requirement to avoid donor allosensitisation can be prohibitively difficult. For this reason, some centres do not undertake any histocompatibility testing before transplantation of intestinal organs and donor selection is often based solely on ABO blood group compatibility, donor age, size and anatomy. Nevertheless,
there is no reason to suppose that intestinal transplants behave differently from other solid organs (with the exception of the liver) and ignoring the presence of donor-specific antibodies in modified multivisceral and small bowel alone transplantation carries an inherent risk.

Rejection is the major cause of graft failure in intestinal transplantation. It is accepted that when intestinal organs are transplanted together with a liver obtained from the same donor, this will confer immediate protection from hyperacute rejection caused by HLA class I specific antibodies. In contrast to kidney transplantation, where a concomitant liver transplant from the same donor is thought to reduce the incidence of acute rejection and improve kidney graft survival, the risk of acute rejection of an intestinal allograft may not be reduced with concomitant liver transplantation [179]. Furthermore, there is now accumulating evidence, mainly in the form of individual case reports, of the clinical importance of pre-transplant recipient allosensitisation, donor HLA-specific antibodies and intestinal transplant rejection. Vascular rejection resulting in reduced graft survival is seen following small bowel transplantation and this is associated with a positive crossmatch [180-182]. A case of hyperacute rejection following isolated intestinal transplantation has also been reported [183], along with a case of ‘lethal hyperacute rejection’ following small bowel alone transplantation [184]. Following this experience, the latter group went on to investigate ‘second-set rejection’ following small bowel transplantation in rats that were immunologically primed by a previous skin graft and described mucosal necrosis, neutrophil infiltration and massive bleeding on day one, similar to that of severe necrotizing haemorrhagic enteritis [185]. Wu et al
studied a series of five adult isolated intestinal allografts undertaken with a strong positive crossmatch, all of which developed severe mucosal injury immediately post-reperfusion, and three recipients had focal haemorrhage within the first ten days, although this was successfully reversed with prompt treatment using OKT3 [186]. More recently a case of immediate antibody mediated hyperacute rejection in a small bowel transplant was recorded in the presence of donor-specific antibodies causing severe ischaemic injury and arteritis [187] but as noted above, after prompt intervention by intense immunosuppression and plasmapheresis the transplant recovered and the patient was clinically stable more than one year after transplant.

Post-transplant production of donor-specific antibodies has been described in one case with acute vascular rejection [180] and the development of HLA-specific antibodies after intestinal transplantation has been associated with acute rejection episodes [188]. However, the incidence of exfoliative rejection has been shown not to associate with a positive crossmatch [189]. Donor-specific antibodies are known to compromise all other forms of organ transplantation to varying degrees so it reasonable to assume that intestinal transplantation is no exception. However, the paucity of current data makes it difficult to determine the magnitude of the effect of a positive crossmatch in such transplants. In addition it may be that the general extreme immunogenicity of intestinal tissue obscures the effect of such a single factor.
13.1 Recommendations

- Pre-transplant HLA-specific antibody screening and pre-transplant donor lymphocyte crossmatch can identify those patients at high risk of acute cellular or vascular rejection.

- A positive donor cytotoxic crossmatch caused by IgG HLA class I specific antibodies is a relative contra-indication to isolated intestinal transplantation (in the absence of a liver transplant from the same donor) and the final decision to proceed with transplantation will depend on a careful evaluation of the relative risk of proceeding versus the risk of delayed transplantation.

14: HLA-SPECIFIC ANTIBODY INCOMPATIBLE TRANSPLANTATION (AiT)

Patients with HLA-specific antibodies, particularly those reacting with a broad spectrum of HLA antigens (e.g. with specificity for common HLA epitopes) are likely to wait significantly longer for a transplant. Although some of these patients will modulate their antibodies naturally over time, for others the antibodies will remain at high titre and of broad specificity, apparently without any reduction over many years. The factors which govern the natural down-regulation of antibody levels are not adequately understood [190]. Anti-idiotypic antibodies probably have a role to play in the natural decline of an antibody response, although in certain circumstances they may be stimulatory and act to sustain a response [191].

The main rationale for removing antibody from patients awaiting transplantation was provided by the observation that patients may be
successfully transplanted with a negative crossmatch with current sera, but a positive T cell crossmatch using historical sera [192]. Kidney transplantation with a current negative, historic positive crossmatch has become more widely accepted, but is not always successful [83,84]. Nonetheless, the extended waiting time for a transplant in highly sensitised patients and the less favourable life expectancy on dialysis has encouraged the use of antibody removal techniques to allow transplantation.

The recent enhanced assay sensitivities through technological developments in HLA-specific antibody detection and characterisation have shown that complete antibody removal is rarely achieved in desensitisation protocols. Antibody reduction to a level considered clinically manageable is recognised as the aim of antibody removal, and this generally means reduction to give a negative crossmatch by conventional leukocyte crossmatches (CDC or FXCM) [38, 193-197]. In most cases residual donor specific antibodies remains readily detectable using a bead-based assay [38]. CDC crossmatch test negative, bead positive (i.e. virtual crossmatch positive but CDC negative and FCXM negative such that the donor specific antibody is only detected in the solid-phase assay) transplants can give good outcome results for transplants following antibody removal. This is consistent with outcome results shown for CDC crossmatch negative cases without pre-transplant treatment where donor specific antibody was detected using bead assays either prospectively [198] or retrospectively [66].

There is currently no published methodology for precise quantitation of HLA-specific antibodies, but traditionally cytotoxic titre or relative antibody binding measured by flow cytometry have been used. More recently bead based assays are being used to determine relative antibody levels and these seem to correlate with clinical significance [199] and in HLA AIT rejection associates with the higher pre-treatment or pre-transplant bead binding values (MFI – median fluorescence intensity) [39,200]. Particularly where bead-based (or other solid-phase purified antigen) assays are used, there must be local validation to determine critical values [39,195,200].

Clearly the cytotoxic positive pre-treatment donor-specific antibody levels carry the highest risk of poor outcome but all three assays can be used to evaluate risk and if necessary guide or prescribe treatment. Measurement of pre-treatment donor-specific antibody levels by CDC, FCXM and bead assay allows prediction of how much antibody removal treatment is required as well as the risk of subsequent rejection [39,201]. There are numerous approaches to reducing humoral donor-specific reactivity, ranging from \textit{in vivo} agents such as IVIg, CD20 and other immunosuppressive agents to extracorporeal methods such as plasma exchange. Guidance on antibody removal protocols is beyond the scope of this document (although one of the factors used to choose which method will be the level and type of donor-specific antibody in question). For this the reader should consult the clinical guidelines for antibody removal developed by The British Transplantation Society (\url{www.bts.org.uk}).
The optimum frequency of post-transplant monitoring is yet to be established: the bead assays are relatively new and this is likely to vary across different programmes because of case-mix variation. Bead-based assays, as opposed to cell-based assays (i.e. CDC or FCXM), are also particularly useful for post transplant monitoring to track changes in donor-specific antibody levels [39,195,200]. These assays have been able to show early modulation or persistence of post-transplant donor-specific antibody [39] and where daily testing in the early post-transplant period has been used rapid and dramatic rises (and falls) in donor-specific antibody have been revealed [202] with significant variations seen within 24-hour periods. While such information is relevant to the diagnosis of rejection, the clinical consequences of such changes are yet to be fully understood. Cases of good graft function in the presence of even rising levels of donor-specific antibody have been described, although in general re-synthesis of donor-specific antibody and increasing donor specific antibody levels associate with rejection [39,200]. As such, the treatment of persisting or rising donor-specific antibody should depend on the developing clinical situation, any histological information and other risk factors, including the pre-transplant level of donor-specific antibody.

Bead-based, or other solid phase assays using purified HLA provide the most practical method of donor-specific antibody monitoring at the intervals required for antibody incompatible transplantation. Furthermore, these are more standardised than donor leukocyte-based assays and if widely used, help comparisons between centres using different transplant protocols. Cost may be an important factor to be balanced against the significant benefits of avoiding
unnecessary treatment and the detection of early immunological changes which can guide treatment. Whatever the frequency of testing, donor-specific antibodies must be discriminated from other third-party specificities, and for HLA this means single antigen assays must be used [203], since as the confounding effects of other antigens in the assay can give misleading results on both the specificity and, crucially, the level of a specific antibody [202].

14.1 Recommendations

- The HLA-specificity and level of donor-specific antibodies must be fully determined prior to antibody reduction.
- Antibody levels must be monitored regularly throughout the duration of treatment to determine its effectiveness using a method that can unequivocally distinguish between antibodies directed against donor HLA mismatches, other HLA-specific antibodies and non-HLA antibodies.
- The frequency of post-transplant donor-specific antibody monitoring should match the risk of adverse immunological events and this is advised for all HLA AiT cases whether or not pretransplant removal has been used.
- Antibody reduction should only be undertaken following establishment of a clinical and laboratory protocol.
### Table 1. Immunological pre-transplant risk assessment based on donor crossmatch and antibody screening results

<table>
<thead>
<tr>
<th>Donor crossmatch result</th>
<th>Crossmatch method</th>
<th>Current/ historical</th>
<th>Antibody screening results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive T &amp; B cell</td>
<td>CDC (DTT)</td>
<td>Current</td>
<td>IgG HLA class I DSA</td>
<td>Hyperacute rejection (veto to transplantation)</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (DTT)</td>
<td>Current</td>
<td>IgG HLA class II DSA</td>
<td>High immunological risk*</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (DTT)</td>
<td>Current</td>
<td>Weak IgG HLA class I DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive T &amp; B cell</td>
<td>FCXM (CDC neg)</td>
<td>Current</td>
<td>IgG HLA class I DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>FCXM (CDC neg)</td>
<td>Historical</td>
<td>IgG HLA class II DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive T &amp; B cell</td>
<td>CDC (DTT)</td>
<td>Historical</td>
<td>IgG HLA class I DSA</td>
<td>High immunological risk$</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (DTT)</td>
<td>Historical</td>
<td>IgG HLA class II DSA</td>
<td>High immunological risk$</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (DTT)</td>
<td>Historical</td>
<td>Weak IgG HLA class I DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive T &amp; B cell</td>
<td>FCXM (CDC neg)</td>
<td>Historical</td>
<td>IgG HLA class I DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>FCXM (CDC neg)</td>
<td>Historical</td>
<td>IgG HLA class II DSA</td>
<td>Intermediate immunological risk^</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (neg DTT)</td>
<td>C or H</td>
<td>IgM HLA class I DSA</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (neg DTT)</td>
<td>C or H</td>
<td>IgM HLA class II DSA</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (neg DTT)</td>
<td>C or H</td>
<td>IgM non-HLA (often autoreactive)</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Positive B cell</td>
<td>CDC (neg DTT)</td>
<td>C or H</td>
<td>IgM non-HLA (often autoreactive)</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Negative T &amp; B cell</td>
<td>FCXM</td>
<td>C or H</td>
<td>IgG HLA class I or II DSA (detected by Luminex SAB alone)</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Positive T &amp;/or B cell</td>
<td>CDC and/or FCXM</td>
<td>C or H</td>
<td>Negative (Luminex Ab detection and/or SAB)</td>
<td>Low immunological risk (IgM/IgG non-HLA, often showing in vitro autoreactivity)</td>
</tr>
<tr>
<td>Positive T; negative B cell</td>
<td>CDC and/or FCXM</td>
<td>C or H</td>
<td>Positive (Luminex SAB - not donor-specific) or negative</td>
<td>Low immunological risk (results suggest antibody is not HLA-specific)</td>
</tr>
<tr>
<td>Negative T &amp; B cell</td>
<td>FCXM</td>
<td>C or H</td>
<td>Positive (Luminex SAB) not donor HLA-specific</td>
<td>Low immunological risk</td>
</tr>
<tr>
<td>Negative T &amp; B cell</td>
<td>CDC and/or FCXM</td>
<td>C or H</td>
<td>Negative (Luminex Ab detection and/or SAB)</td>
<td>Low immunological risk</td>
</tr>
</tbody>
</table>

* high immunological risk: hyperacute rejection is unlikely (reported only in cases with very high titre HLA-DR antibodies) but donor-specific HLA class II antibodies are increasingly recognised as being associated with refractory humoral rejection and poor transplant prognosis.

^ intermediate immunological risk transplantation should be avoided if reasonably possible (i.e. short waiting time, easy to avoid unacceptable mismatches) but may be undertaken with appropriate clinical caution; consideration for enhanced immunosuppression, proactive use of clinical intervention strategies and post-transplant antibody monitoring.

$ risk of anamnestic secondary T and/or B cell response; need to consider high risk immunosuppression strategy, the duration, titre and priming source of antibody and repeat mismatches (pregnancy or regraft). Historical positive crossmatches caused by cross-reactive alloantibodies (avoiding the main specificity and priming stimulus) constitute intermediate immunological risk and are less likely to be associated with refractory T or B cell responses.
Figure 1: Schematic representation of antigen presentation to T cells and the activation of effector cells involved in the immune response.

Terms and abbreviations are explained in the glossary.
15: APPENDICES

15.1 Laboratory resources and relationship

Crucial to the provision of a quality service and the introduction of new developments are the staffing structure and personnel qualifications within the Histocompatibility & Immunogenetics laboratory. A Consultant healthcare scientist or Medical consultant who is in charge of the day-to-day laboratory activity and is available for contact outside normal working hours must direct the laboratory. The director of the laboratory must have experience of working in a Histocompatibility & Immunogenetics laboratory and must have either Fellowship of the Royal College of Pathologists in Histocompatibility & Immunogenetics or evidence of at least an equivalent level of training in the subject.

Other healthcare scientist staff should have successfully completed a recognised training scheme in Histocompatibility & Immunogenetics (for example British Society for Histocompatibility & Immunogenetics Diploma) and have attained registration with the Health Professions Council (HPC). Trainee healthcare scientists must participate in a recognised training scheme so it is therefore essential that training opportunities be provided within the laboratory for all personnel. The HPC has published guidance on the expectations of trainees (see www.hpc-uk.org).

Staffing levels and laboratory resources should be sufficient to meet the demands of the service, including staff training, annual leave, unforeseen absence and compliance with the European Working Time Regulations. Work activity levels and provision for laboratory resources required to meet this demand should be an integral part of the transplant centre Business Plan. Recommendations for staffing numbers, skills and competencies required are detailed in the British Renal Society Renal Workforce Planning document. All Histocompatibility & Immunogenetics laboratory staff should participate in appropriate CPD
activities, provided under the auspices of the BSHI, the Institute of Biomedical Sciences (IBMS) or the Royal College of Pathologists.

It is important that close liaison is maintained between the laboratory scientists and the clinical team. The Laboratory Director and other appropriate laboratory staff must therefore establish good professional relationships with the medical and professional staff in the transplant unit. Laboratory representation at relevant clinical and audit meetings is essential.

15.2 Glossary

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
</table>
| ABO blood group       | Genetic polymorphism of specific carbohydrates carried by red blood cells and other tissues. There are four main ABO blood groups, O (46% of 616 heart-beating deceased kidney donors in the UK in 2003), A (41%), B (9%) and AB (4%). Group O patients have naturally occurring antibodies to groups A and B, group A to group B, group B to group A while group AB have no naturally occurring antibodies against ABO. Organ transplants and blood transfusions can be:  
  ABO identical e.g. O to O, A to A etc  
  ABO compatible e.g. O to A, B to AB etc  
  ABO incompatible e.g. B to A  
  ABO incompatibility constitutes a high risk. |
<p>| Absorption            | Active process of binding to another substance e.g. binding of antibody to an affinity column. |
| Acceptable mismatch   | A non-self HLA antigen to which a recipient has no antibody reactivity, prior to transplantation. |
| Accommodation         | An incompatibility which is accepted by the recipient. |
| Acute rejection episode | Overt immunological response against a graft usually within the first three months after transplantation. |
| Affinity column       | A matrix, usually polymer beads in suspension, which acts as a carrier of biologically or chemically active molecules capable of binding another molecule. |
| <strong>Allele</strong> | A genetic variant with a population frequency of more than 1%. An allele can be defined at the DNA or protein level. |
| <strong>Allograft or allotransplant</strong> | A transplant between members of the same species e.g. between humans. |
| <strong>Anamnestic response</strong> | A response because of memory. The immune system responds more aggressively when re-exposed to an antigen in a secondary response. |
| <strong>Antibody</strong> | Serum immunoglobulin expressed by B cells and secreted by plasma cells that recognises a specific antigen. |
| <strong>Antibody dependent cell mediated cytotoxicity (ADCC)</strong> | Cytotoxic reaction whereby the effector activity is provided by Fc receptor expressing cells (e.g. macrophages, natural killer cells) that recognise antibody coated targets. |
| <strong>Antibody detection</strong> | A method which identifies the presence of an antibody in a serum sample. |
| <strong>Immunoglobulin isotype</strong> | Different structural, and therefore functionally different, forms due to the use of constant region alternatives of the heavy chain. Designated IgM, IgD, IgG (subclasses IgG1, IgG2, IgG3, and IgG4), IgE, and IgA. |
| <strong>Antibody removal</strong> | Artificial reduction in circulating donor-specific antibody to a level that allows transplantation (high immunological risk). |
| <strong>Antigen presenting cell (APC)</strong> | Specialised immune system cells which present degraded antigen in the form of peptides complexed with MHC molecules. |
| <strong>Antigen</strong> | Any substance that is recognised by an immune system. |
| <strong>Anti-idiotypic antibody</strong> | An antibody with specificity for that part of another antibody which binds antigen. |
| <strong>Audit</strong> | A cyclic process of review of a laboratory or clinical systems with the aim of identifying improvements and implementing change. |</p>
<table>
<thead>
<tr>
<th><strong>Autoimmunity</strong></th>
<th>An immune response to self antigens, tissues and organs which can result in serious illness such as type 1 diabetes or rheumatoid arthritis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoreactive lymphocytotoxic antibodies</strong></td>
<td>Antibodies which cause a positive reaction in a lymphocytotoxic assay when the serum and target cells are from the same individual. Often IgM and without obvious specificity these can cause of a false-positive donor crossmatch and not considered clinically relevant.</td>
</tr>
<tr>
<td><strong>B cell crossmatch (B cell XM)</strong></td>
<td>A crossmatch test in which target cells are B lymphocytes. Use to detect the presence of donor HLA Class I (A, B, Cw) and Class II (DR, DQ, DP) –specific antibodies.</td>
</tr>
<tr>
<td><strong>B lymphocytes</strong></td>
<td>Bone marrow matured lymphocytes that express membrane-bound immunoglobulin. In response to antigen contact these differentiate into antibody secreting plasma cells or memory B cells.</td>
</tr>
<tr>
<td><strong>Banff 97 grades</strong></td>
<td>An internationally recognised system of grading pathology in biopsy specimens from a kidney. Used to diagnose and grade rejection.</td>
</tr>
<tr>
<td><strong>Biomedical Scientist</strong></td>
<td>A Healthcare Scientist qualified to practice following a specified training course leading to Registration (<a href="http://www.hpc.org.uk">www.hpc.org.uk</a>) and participating in documented continuous professional development.</td>
</tr>
<tr>
<td><strong>British Renal Society</strong></td>
<td><a href="http://www.britishrenal.org">www.britishrenal.org</a></td>
</tr>
<tr>
<td><strong>British Society for Histocompatibility &amp; Immunogenetics</strong></td>
<td><a href="http://www.bshi.org.uk">www.bshi.org.uk</a></td>
</tr>
<tr>
<td><strong>British Transplantation Society</strong></td>
<td><a href="http://www.bts.org.uk">www.bts.org.uk</a></td>
</tr>
<tr>
<td><strong>Bronchiolitis obliterans syndrome</strong></td>
<td>A manifestation of rejection characterised clinically by a reduction in the forced expiratory volume in 1 second (FEV1), and histologically by narrowing or obliteration of the airway lumen.</td>
</tr>
<tr>
<td><strong>C4d</strong></td>
<td>A product of activation of the classical complement system.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Calcineurin inhibitor</td>
<td>An immunosuppressive drug (ciclosporin, tacrolimus) which acts by blocking immune cell activation by the calcineurin pathway.</td>
</tr>
<tr>
<td>Calculated reaction frequency</td>
<td>Calculated by NHSBT-ODT as the % incidence, among a pool of 10,000 ABO compatible organ donors, of HLA antigen incompatible donors with patient defined HLA-specific antibody(s).</td>
</tr>
<tr>
<td>CD4, 8, 20, 28, 40, 80, 154, etc.</td>
<td>Cell surface molecules defined by specific monoclonal antibodies (Cluster of Differentiation) and recognised by an international standardisation body <a href="http://ca.expasy.org/cgi-bin/lists?cdlist.txt">http://ca.expasy.org/cgi-bin/lists?cdlist.txt</a></td>
</tr>
<tr>
<td>Cell viability</td>
<td>The proportion of target cells alive before or after a CDC assay.</td>
</tr>
<tr>
<td>Characterisation of antibodies</td>
<td>A process of testing for antibody(s) in serum in such a way as to identify the specific antigen(s) with which the antibody reacts.</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>Modified movement of cells due to a concentration gradient of a secreted substance.</td>
</tr>
<tr>
<td>Chronic rejection</td>
<td>Process of graft failure occurring months or years post transplantation. Progression is usually slow; e.g. chronic transplant nephropathy (kidney), bronchiolitis obliterans syndrome (lung) and coronary artery disease (heart).</td>
</tr>
<tr>
<td>Class switch</td>
<td>Antigen driven process by which a B cell actively and irreversibly changes the isotype but not the specificity of the antibody it expresses.</td>
</tr>
<tr>
<td>Clinical Scientist</td>
<td>A science graduate who has followed a recognised training scheme to achieve Registration (<a href="http://www.hpc.org.uk">www.hpc.org.uk</a>) and have documented continuous professional development (CPD).</td>
</tr>
<tr>
<td>Cold ischaemia time</td>
<td>The time during which an organ for transplant is stored outside the body in the cold between cold perfusion in the donor and removal for implantation in the recipient.</td>
</tr>
<tr>
<td>Complement</td>
<td>A group of serum proteins which react in a regulated enzymatic cascade. The classical pathway is initiated by antibodies thus providing a cytotoxic effector mechanism.</td>
</tr>
</tbody>
</table>
### Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement dependent cytotoxicity (CDC)</td>
<td>A laboratory test to identify presence of antibodies in a serum sample using lymphocytes as targets and cell viability as the read-out.</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Of red blood cells to form a thrombus (clot) mediated by antibodies</td>
</tr>
<tr>
<td>Co-stimulatory molecule</td>
<td>Cell surface ligand or receptor providing a non-specific signal which is necessary for an antigen-specific response by T or B cells.</td>
</tr>
<tr>
<td>CPD</td>
<td>Continuous Professional Development schemes provide independent assessment that an individual maintains ongoing training.</td>
</tr>
<tr>
<td>Crossmatch test (XM)</td>
<td>A test to identify antibody mediated reactivity to target antigens in a potential organ donor. The test report must be either positive or negative.</td>
</tr>
<tr>
<td>Cross-reactive</td>
<td>An antibody which is able to bind to a series of structurally closely related antigens.</td>
</tr>
<tr>
<td>Cytokine</td>
<td>A chemical secreted by an immune cell which may either enhance or suppress an immune response.</td>
</tr>
<tr>
<td>Desensitisation</td>
<td>Removal of antibodies which are indicative of sensitisation.</td>
</tr>
<tr>
<td>Differentiation</td>
<td>A process of specialisation of cells and tissues to become a functional organ or system. A one way step.</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>A chemical used in laboratory assays to dissociate the pentameric IgM molecule and abrogate its activity.</td>
</tr>
<tr>
<td>Donor-Specific Antibody</td>
<td>An antibody with specificity for a mismatched antigen (HLA) present in an organ donor.</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Cells which line the blood vessels.</td>
</tr>
<tr>
<td>Enzyme linked immunosorbent assay (ELISA)</td>
<td>A laboratory assay in which specific antibody can be detected. Known antigens are bound to a plastic plate and reacted with a patient’s serum. If antibody is present it will bind to the immobilised antigen and can be detected by activation of an enzyme resulting in coloration of the reaction. An instrument is used to measure the colour change.</td>
</tr>
<tr>
<td>Epitope</td>
<td>That part of the antigen structure to which antibody binds.</td>
</tr>
<tr>
<td><strong>Epstein Barr virus (EBV)</strong></td>
<td>A common virus that can transform human B cells into stable cell lines. A causative agent of glandular fever and certain lymphomas. In immunosuppressed transplant patients it can cause post-transplant lymphoproliferative disease.</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Extracorporeal</strong></td>
<td>Outside of the body.</td>
</tr>
<tr>
<td><strong>Fc receptor</strong></td>
<td>A cell surface molecule specific for the heavy chain of certain immunoglobulin classes. Various forms found on lymphocytes, macrophages, natural killer cells and mast cells.</td>
</tr>
<tr>
<td><strong>Favourable match</strong></td>
<td>UK Transplant definition of a donor / recipient combination where there is no mismatch for HLA-DR and only one mismatch at HLA-A or –B or one mismatch at each of HLA-A and –B. ‘100’ or ‘010’ or ‘110’. Not used in the national kidney allocation algorithm since 2008.</td>
</tr>
<tr>
<td><strong>Flow cytometric crossmatch (FCXM)</strong></td>
<td>Use of a flow cytometer to measure binding of patient antibody to donor cells.</td>
</tr>
<tr>
<td><strong>Flow cytometer</strong></td>
<td>Equipment using laser technology and a fluorescent stain coupled to a detection antibody and is a highly sensitive, semi-qualitative technique.</td>
</tr>
<tr>
<td><strong>Guideline</strong></td>
<td>A statement intended to offer advice of how to proceed. Based on published evidence or established best practice.</td>
</tr>
<tr>
<td><strong>Haematopoietic stem cell</strong></td>
<td>A cell which can potentially differentiate into all blood lineage cells.</td>
</tr>
<tr>
<td><strong>Health Professions Council</strong></td>
<td>The UK regulatory body overseeing registration of Healthcare Scientists; <a href="http://www.hpc.org.uk">www.hpc.org.uk</a>.</td>
</tr>
<tr>
<td><strong>Highly sensitised patient (HSP)</strong></td>
<td>NHSBT-ODT definition of a patient who has developed HLA-specific antibodies against 85% of a pool of 10,000 blood group matched donors.</td>
</tr>
<tr>
<td><strong>Histocompatibility</strong></td>
<td>The degree of similarity between cells, tissues and organs of donors and recipients assessed by HLA antigen typing and matching.</td>
</tr>
<tr>
<td><strong>Histocompatibility laboratory</strong></td>
<td>A highly specialised laboratory staffed by Healthcare Scientists who perform tests to facilitate effective organ, tissue and stem cell transplantation.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Historic serum sample</td>
<td>A previously collected serum sample from a patient.</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte Antigen. Cell surface molecules determined by highly polymorphic linked genes on chromosome 6 (HLA-A, -B, -Cw, -DR, -DQ, -DP: the major histocompatibility complex or MHC). Biologically these function to present protein fragments to T cells.</td>
</tr>
<tr>
<td>HLA class I molecules</td>
<td>HLA-A, -B and -Cw molecules with structural and functional similarity. Expressed by almost all nucleated cells.</td>
</tr>
<tr>
<td>HLA class II molecules</td>
<td>HLA-DR, -DQ and -DP molecules with structural and functional similarity. Constitutively expressed only on specialised antigen presenting cells but may be inducible.</td>
</tr>
<tr>
<td>HLA Matchmaker</td>
<td>A computer software programme used to predict the likelihood of HLA epitope (in)compatibility with a specified HLA antigen</td>
</tr>
<tr>
<td>Human Tissue Authority</td>
<td><a href="http://www.hta.gov.uk">www.hta.gov.uk</a></td>
</tr>
<tr>
<td>Humoral</td>
<td>Of the blood. Usually used to indicate an antibody mediated response (c.f. cellular response).</td>
</tr>
<tr>
<td>Hyperacute rejection</td>
<td>Rejection of a transplant within a very short time of transplantation (minutes) typically caused by pre-existing donor-specific antibodies Usually results in irreversible failure.</td>
</tr>
<tr>
<td>Immunoadsorption</td>
<td>Binding of immunoglobulins to a solid phase. A process which can remove immunoglobulin from the blood.</td>
</tr>
<tr>
<td>Immunogenicity</td>
<td>The degree to which a substance can provoke the immune system to respond e.g. high or low.</td>
</tr>
<tr>
<td>Institute of Biomedical Scientists</td>
<td>The professional body for Biomedical Scientists <a href="http://www.ibms.org">www.ibms.org</a></td>
</tr>
<tr>
<td>Interleukin (IL)</td>
<td>Leukocyte secreted cytokine that affects the growth or development immune system cells. E.g. IL-2, -4, -5, -6.</td>
</tr>
<tr>
<td>Islets</td>
<td>Cluster of cells within the pancreas containing the beta cells which secrete insulin.</td>
</tr>
</tbody>
</table>
### IVIg
Intravenous immunoglobulin. A commercial preparation of serum from a large number of blood donors which has high levels of immunoglobulin with a wide range of antibody specificities. Used to enhance, or suppress the immune response.

### Locus
The position of a specified gene on a chromosome.

### Lymphocyte
Mononuclear leukocytes of various lineages (B cell, T cell, NK cell).

### Lymphoid cell line (LCL) cells
Stable B lymphocyte line transformed with EBV in vitro.

### Macrophage
Mononuclear phagocytic leukocyte.

### MICA – MHC class I - related chain A molecules
Molecules with close structural similarity to HLA molecules but with a different function. MICA interact with natural killer cells to regulate immune cell responses.

### Microbeads
Microscopic polystyrene beads to which antigens or DNA probes can be bound. A vehicle for a solid-phase assay.

### Median fluorescence intensity
A semi-quantitative readout of the degree of binding of antibody bound to (HLA) antigen on a microbead.

### Mycophenolate mofetil
An immunosuppressive drug with anti-proliferative properties.

### Monoclonal antibody
An antibody secreted by a non-human cell line with specificity for a single antigenic epitope. May be produced commercially for in vivo therapeutic use. May be "humanised" by engineering recombination of the functional antibody binding domain with a major part of a human immunoglobulin molecule to minimise immunogenicity.

### Natural killer (NK) cell
Mononuclear leukocyte with innate ability to kill certain tumours and virally infected cells.

### Multivisceral transplant
Transplantation of the liver and small bowel, possibly including other organs such as the pancreas.

### Modified multivisceral transplant
Transplantation of the small bowel and other organs such as the pancreas, but excluding the liver.

### Negative crossmatch
Result of a laboratory test that has not detected donor-specific antibody.
### Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Panel reactive antibodies (PRA or % PRA)</strong></td>
<td>The calculated % of a panel of lymphocytes with which a patient’s serum reacts.</td>
</tr>
<tr>
<td><strong>Peak positive / current negative crossmatch (PPCN XM)</strong></td>
<td>A crossmatch result where reactivity to donor target cells has been detected only in historic serum samples. There is no reactivity to donor target cells in recent serum.</td>
</tr>
<tr>
<td><strong>Peritubular capillaries</strong></td>
<td>Small blood vessels located in the kidney adjacent to the structures (nephron) which filter the blood.</td>
</tr>
<tr>
<td><strong>Plasma cell</strong></td>
<td>End stage cell of B lymphocyte lineage that secretes immunoglobulin.</td>
</tr>
<tr>
<td><strong>Plasma exchange or plasmapheresis</strong></td>
<td>Removal or dilution of plasma to lower the amount of circulating antibody.</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>Small, irregular blood borne anucleate cells which are an important component of a thrombus (clot).</td>
</tr>
<tr>
<td><strong>Positive crossmatch</strong></td>
<td>Result of a laboratory test that has detected donor-reactive antibody.</td>
</tr>
<tr>
<td><strong>Post-transplant immunological monitoring</strong></td>
<td>Laboratory tests to indicate the immune reactivity of a recipient to transplanted tissues and organs.</td>
</tr>
<tr>
<td><strong>Primary response</strong></td>
<td>The reaction of the immune system at the time of its first exposure to a novel antigen.</td>
</tr>
<tr>
<td><strong>Protein A</strong></td>
<td>A substance which non-specifically binds to immunoglobulins.</td>
</tr>
<tr>
<td><strong>Reaction frequency</strong></td>
<td>Originally the percentage of panel cells reacting with a serum sample. Now defined as the proportion of a pool of 10,000 blood group matched donors against which a recipient has HLA antibodies.</td>
</tr>
<tr>
<td><strong>Recommendation</strong></td>
<td>A guideline which should usually be adhered to.</td>
</tr>
<tr>
<td><strong>Rescue therapy</strong></td>
<td>A treatment aimed to prevent failure of a transplanted organ in the face of an aggressive immune response.</td>
</tr>
<tr>
<td><strong>Risk</strong></td>
<td>The degree to which a hazardous outcome is predicted.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Rituximab</td>
<td>Brand of therapeutic chimeric CD20-specific monoclonal antibody.</td>
</tr>
<tr>
<td>Royal College of Pathologists</td>
<td><a href="http://www.rcpath.org">www.rcpath.org</a></td>
</tr>
<tr>
<td>Screening strategy</td>
<td>A process to detect and define sensitisation.</td>
</tr>
<tr>
<td>Secondary response</td>
<td>An enhanced immune response mounted on re-exposure to a previously recognised antigen.</td>
</tr>
<tr>
<td>Sensitisation</td>
<td>An immune response to an antigen resulting in T and/or B cell memory.</td>
</tr>
<tr>
<td>Sensitivity (of a patient)</td>
<td>The ability to mount an immune response to an antigen.</td>
</tr>
<tr>
<td>Sensitivity (of an assay)</td>
<td>An evaluation of the accuracy of the results of a laboratory test to predict an outcome. Usually quoted as a percentage.</td>
</tr>
<tr>
<td>Single antigen beads</td>
<td>A multiplex of microbeads each identifiable group being loaded with a single HLA antigen</td>
</tr>
<tr>
<td>Solid phase assays</td>
<td>A laboratory test to detect antibodies using antigen targets immobilised to a plastic tray or microparticle. These assays are performed as ELISA or fluid phase assays using a flow cytometer. The target antigen can be cell-free HLA molecules.</td>
</tr>
<tr>
<td>Specificity</td>
<td>The defined reactivity of an antibody e.g. specific for an HLA molecule.</td>
</tr>
<tr>
<td>T lymphocyte</td>
<td>Mononuclear leukocyte having developed in the thymus. Expresses a receptor specific for MHC plus processed antigen.</td>
</tr>
<tr>
<td>T cell crossmatch (T cell XM)</td>
<td>A crossmatch test in which the target cells are T lymphocytes.</td>
</tr>
<tr>
<td>Titre</td>
<td>Reciprocal of the last dilution of a serum giving a detectable reaction.</td>
</tr>
<tr>
<td>UK Transplant</td>
<td>The immediate predecessor of NHSBT-ODT.</td>
</tr>
<tr>
<td>Unacceptable antigen</td>
<td>Antigen which due to prior exposure and specific sensitisation (due to pregnancy or a transplant) excludes a transplant if present in the donor’s HLA type.</td>
</tr>
<tr>
<td>Vascular rejection</td>
<td>An aggressive antibody mediated immune response with activity detected in the blood vessels of the transplant.</td>
</tr>
</tbody>
</table>
Virtual Crossmatch | A crossmatch which is not based on an immediate pre-
transplant laboratory test. Usually, a Consultant Clinical
Scientist reviews a potential recipient’s pre-transplant
HLA-specific antibody status and recommends the
transplant proceeds without a CDC or FC crossmatch. A
virtual crossmatch must be based on a pre-agreed policy.

Xenograft | Transplantation of an organ between different species.

X-Map Luminex | www.luminexcorp.com

15.3 References


allografts. Recovery and characteristics if infiltrating cells and antibody. Transplantation 1979; 28: 421-426


20 Ting A, Morris PJ. Renal transplantation and B-cell cross-matches with autoantibodies and alloantibodies. Lancet 1977; ii: 1095-1097


26 Cicciarelli J, Helstab K, Mendez R. Flow cytometry PRA, a new test that is highly correlated with graft survival. Clin Transplantation 1992; 6: 159-164


31 Zachary AA, Delaney NL, Lucas DP, Leffell MS. Characterisation of HLA class I specific antibodies by ELISA using solubilized antigen targets: evaluation of the GTI QuikID assay and analysis of


33 Karuppan SS, Moller E. The use of magnetic beads coated with soluble HLA class I or class II proteins in antibody screening and for specificity determination of donor-reactive antibodies. Transplantation 1996; 61: 1539-1545


35 Ellis TM, Gebel HM, Pierce KL, Bray RA. Limitations to antibody detection using single antigen flow beads. Human Immunol 2003; 64(suppl1):S14


38 Vaidya S, Partlow D, Susskind B, Noor M, Barnes T, Gugliuzza K. Prediction of crossmatch outcome of highly sensitized patients by single and/or multiple antigen bead luminex assay. Transplantation 2006; 82: 1524-1528


44 Kosmoliaptsis V, Bradley JA, Sharples LD, Chaudhry A, Key T, Goodman RS, Taylor CJ. Predicting the immunogenicity of human leukocyte antigen class I alloantigens using structural epitope analysis determined by HLAMatchmaker. Transplantation 2008; 85: 1817-1825


46 Deng CT, El-Awar N, Ozawa M, Cai J, Lachmann N, Terasaki PI. Human leukocyte antigen class II DQ alpha and beta epitopes identified from sera of kidney allograft recipients. Transplantation 2008; 86: 452-459


48 Kosmoliaptsis V, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ. Detection of IgG HLA specific alloantibodies in renal transplant patients using single antigen beads is compromised by the presence of IgM HLA specific alloantibodies. Transplantation 2009; 87: 813-820


Khan N, Robson AJ, Worthington JE, Martin S. The detection and definition of IgM alloantibodies in the presence of IgM autoantibodies using flow PRA beads. Hum Immunol 2003; 64: 593-599


Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. NEJM 1969; 280: 735-739


Cecka JM. The UNOS Transplant Registry. Clinical Transplants. 2001:1-18

Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation


Braun WE: Laboratory and clinical management of the highly sensitised organ transplant recipient. Hum Immunol 1989; 26: 245-260


Caskey FJ, Johnson RJ, Fuggle SV. Renal after cardiothoracic transplant: the effect of repeat mismatches on outcome. Transplantation 2009; 87: 1727-1732


Taylor CJ, Chapman JR, Fuggle SV et al A positive B cell crossmatch due to IgG anti-HLA-DQ antibody present at the time of transplantation in a successful renal allograft. Tissue Antigens 1987; 30: 104-112


Ten Hoor G.M, Coopmans M, Allebes W.A. Specificity and Ig class of preformed alloantibodies causing a positive crossmatch in renal transplantation. Transplantation 1993; 56: 298-304

Roelen DL, van Bree FPMJ, Witvliet MD. IgG antibodies against HLA antigen are associated with activated cytotoxic T cells against this antigen. IgM are not. Transplantation 1994; 57: 1388-1392.


Van Kampen CA, Roelen DL, Versteeg-van der Voort Maarschalk MFJ, Hoitsma AJ, Allebes WA, Claas FHJ. Activated HLA class I-reactive cytotoxic T lymphocytes associated with a positive
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

historical crossmatch predict early graft failure. Transplantation 2002; 74: 1114-1119


97 Evans PR, Lane AC, Lambert CM et al. Lack of correlation between IgG T-lymphocyte flow cytometric crossmatches with primary renal allograft outcome. Transplant Int 1992; 5: S609-S612


99 Lazda VA. Identification of patients at risk for inferior renal allograft outcome by a strongly positive B cell flow cytometry crossmatch. Transplantation 1994; 57: 964-969


103 Suciu-Foca N, Reed E, D’Agati VD et al. Soluble HLA antigens anti-HLA antibodies and antidiotypic antibodies in the circulation of renal transplant recipients. Transplantation 1991; 51: 593-601

104 Halloran PF, Schlaut J, Solez K, Srinivasa NS. The significance of the anti-class I response. II. Clinical and pathologic features of renal transplants with anti-class I-like antibody. Transplantation 1992; 53: 550-555


Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation


Abe M, Kawai T, Futatsuyama K et al Postoperative production of anti-donor antibody and chronic rejection in renal transplantation. Transplantation 1997; 63: 1616-1619


McKenna RM, Takemoto SK, Terasaki PI HLA antibodies after solid organ transplantation. Transplantation 2000; 69: 319-326


Zwirner NW, Fernandez-Vina MA, Stastny P. MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes. Immunogenetics 1998; 47: 139-148


Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

127 Koo DH, Roberts ISD, Quiroga et al. C4d deposition in early renal allograft protocol biopsies. Transplantation 2004; 78: 398-403


133 Mohanakumar T, Narayanan K, Desai, N. A significant role for histocompatibility in human islet transplantation. Transplantation 2006; 82: 180-187


140 Itescu S, Tung TC, Burke EM et al. Preformed IgG antibodies against major histocompatibility complex class II antigens are major risk factors for high-grade cellular rejection in recipients of heart transplantation. Circulation 1998; 98: 786-793

141 Stastny P, Lavingia B, Fixler DE, Yancy CW, Ring WS. Antibodies against donor human leukocyte antigens and the outcome of cardiac allografts in adults and children. Transplantation 2007; 84: 738-745

142 Smith JD, Hamour IM, Banner NR, Rose ML. C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. American Journal of Transplantation. 2007; 7: 2809-2815

143 Przybrowski P, Baloga M, Radovancevic B et al. The role of flow cytometry-detected IgG and IgM anti-donor antibodies in cardiac allograft recipients. Transplantation 1999; 67: 258-262


145 Appel JZ 3rd, Hartwig MG, Cantu E 3rd, Palmer SM, Reinsmoen NL, Davis RD. Role of flow cytometry to define unacceptable HLA antigens in lung transplant recipients with HLA-specific antibodies. Transplantation 2006; 81: 1049-1057
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation

146 Suciu-Foca N, Reed E, Marboe C et al. The role of anti-HLA antibodies in heart transplantation. Transplantation 1991; 51: 716-724


148 Sundaressan S, Mohanakumar T, Smith MA. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. Transplantation 1998; 65: 648-653


150 Rose ML. De novo production of antibodies after heart or lung transplantation should be regarded as an early warning system. Journal of Heart Lung Transplant 2004; 23: 385-395

151 Tambur AR, Pamboukian SV, Costanzo MR. The presence of HLA-directed antibodies after heart transplantation is associated with poor allograft outcome. Transplantation 2005; 80: 1019-1025


158 Jurcevic S, Ainsworth ME, Pomerance A. Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. Transplantation 2001; 71: 886-892

159 Ratner LE, Phelan D, Brunt EM, Mohanakumar T, Hanto DW. Probable antibody-mediated failure of two sequential ABO-compatible hepatic allografts in a single recipient. Transplantation 1993; Apr;55(4): 814-819


166 Scornik JC, Soldevilla-Pico C, Van der Werf WJ. Susceptibility of liver allografts to high or low concentrations of preformed antibodies as measured by flow cytometry. Am J Transplant 2001;1: 152-156


168 Batts KP, Moore SB, Perkins JD, Wiesner RH, Grambsch PM, Krom RA. Influence of positive lymphocyte crossmatch and HLA mismatching on vanishing bile duct syndrome in human liver allografts. Transplantation 1988; 45:376-379


170 Nikaein A, Backman L, Jennings L et al. HLA compatibility and liver transplant outcome. Improved patient survival by HLA and cross-matching. Transplantation 1994; 58: 786-792


Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation


192 Hack N, Angra S, Friedman E, McKnight T, Cardella CJ. Anti-idiotypic antibodies from highly sensitized patients stimulate B cells to produce anti-HLA antibodies. Transplantation 2002; 73: 1853-1858


194 Montgomery RA, Zachary AA, Racusen LC et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation 2000; 70: 887-895


200 Burns JM, Cornell LD, Perry DK et al. Alloantibody levels and acute humoral rejection early after positive crossmatch kidney transplantation. Am J Transplant 2008; 8: 2684-2694
Guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation


204 Campbell PM, Salam A, Ryan EA. et al. Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. Am J Transplant. 2007; 7:1242-1248

15.4 Statements of potential conflicts of interests

Susan Fuggle has received support from Absorber for research and conference travel expenses.

Andrea Harmer holds a patent which is subject to licensing agreement with Invitrogen and has received support from Absorber towards conference attendance.

Martin Howell has received support from VH Bio and One Lambda to attend the 2008 One Lambda European Clinical Histocompatibility Conference.

Paul Sinnott has received support from One Lambda to attend conferences.

Robert Vaughan holds a patent which is subject to licensing agreement with Invitrogen.

David Briggs, Phil Dyer, Susan Martin, John Smith and Craig Taylor declared no conflicts of interest.