BSHI
British Society for Histocompatibility and Immunogenetics

BTS
British Transplantation Society

The detection & characterisation of clinically relevant antibodies in allotransplantation

Compiled by a Working Party of
The British Transplantation Society
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British Transplantation Society Guidelines

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British Society for Histocompatibility & Immunogenetics
Executive Business Support Ltd, City Wharf, Davidson Road, Lichfield, Staffordshire WS14 9DZ
Company No: 6078396 Registered in England and Wales, Registered Charity No: 1123760
www.bshi.org.uk

and

British Transplantation Society
South Park Road, Macclesfield, Cheshire SK11 6SH
Company No: 4691176 Registered in England and Wales, Registered Charity No: 1098584
www.bts.org.uk

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The British Society for Histocompatibility & Immunogenetics (BSHI) is the professional body for healthcare and academic scientists supporting clinical transplantation and transplantation research.

The British Transplantation Society (BTS) is the professional body for surgeons, physicians, specialist nurses for organ donation, nurses, healthcare scientists, allied health professionals and 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. The opinions presented in the Guidelines are subject to change and should not be considered to be a treatment recommendation for any individual patient.

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1 ABBREVIATIONS

AMR	Antibody Mediated Rejection
BSHI	British Society for Histocompatibility & Immunogenetics
BOS	Bronchiolitis Obliterans Syndrome
BTS	British Transplantation Society
CAV	Cardiac Allograft Vasculopathy
CBU	Cord Blood Unit
CDC	Complement Dependent Cytotoxicity
cRF	Calculated Reaction Frequency
DBD	Donation After Brain Stem Death
DCD	Donation After Circulatory Death
DSA	Donor-specific HLA antibodies
DTT	Dithiothreitol
ELISA	Enzyme Linked Immunosorbent Assay
FCXM	Flow Cytometry Crossmatch
HCPC	Health and Care Professions Council
MFI	Mean Fluorescence Intensity
NHSBT-ODT	NHS Blood and Transplant – Organ Donation and Transplantation
PRA	Panel Reactive Antibodies
TCR	T Cell Receptor
PAK	Pancreas After Kidney
PTA	Pancreas Transplant Alone
SPK	Simultaneous Pancreas and Kidney

2 PROCESS OF GUIDELINE DEVELOPMENT

The histocompatibility laboratory is now able to define the presence of HLA specific antibodies with a high degree of sensitivity, resulting in on-going re-definition of the crossmatch boundary to allow the successful transplantation of allosensitised patients. The introduction of solid phase assays led to the publication of the 2004 and later the 2010 Guidelines. Subsequently, technological developments continued allowing relative quantification 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. Recent developments and experience have prompted a further update of these Guidelines, so that optimum approaches can be applied to maximise safe and effective use of the donor organ pool.

These Guidelines are written from the standpoint of ABO blood group compatible transplantation and focus upon HLA-specific antibodies in both deceased and living donor allotransplantation. ABO and HLA antibody incompatible kidney transplantation are covered in a separate document: (http://www.bts.org.uk/Documents/Guidelines/Active/AiT%20guidelines%20Jan%202011%20FINAL.pdf). This revision of the Guidelines includes a new chapter describing the role of HLA antibodies in haematopoietic stem cell transplantation.

3 THE EVIDENCE BASE

The authors drew the evidence to support their recommendations from peer-reviewed publications up to December 2013. 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 frequent, but these have been referenced when possible. Furthermore, recent changes in technology to detect and define HLA specific sensitisation have made many historic publications less relevant or obsolete.

The recommendations are graded according to three levels following the principles outlined in the Consensus Guidelines on the Testing and Clinical Management Issues Associated with HLA and Non HLA Antibodies in Transplantation (Tait B *et al*, Transplantation 2013; 95: 19-47).

Level 1 Indicates guidance based on conclusions from peer-reviewed published data and currently proven practice

Level 2 Indicates guidance based on a consensus opinion of the authors but where there is insufficient published evidence to support practice.

Level 3 Indicates a recommendation in the absence of direct evidence but where the authors agree there may be benefit.

4 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 version 2 of the Guidelines in May 2010. A similar process has been followed during the preparation of this current revision, with Kay Poulton as Chair of the writing committee. Revisions have been agreed by the authors and comments were invited from all BSHI and BTS Members, by placing the draft Guidelines on both Society websites for a four week period. Comments were reviewed and the Guidelines amended by Kay Poulton, who also formatted this publication. There was no specific request for non-professional comment.

4.1 Writing Committee

Chair of Writing Committee for 2014 Guidelines
Kay Poulton PhD FRCPath, Consultant Clinical Scientist Transplantation Laboratory, Manchester Royal Infirmary M13 9WL

Chair of Writing Committee for 2010 Guidelines
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

4.2 Members

Martin Barnardo PhD FRCPath, Consultant Clinical Scientist Transplant Immunology and Immunogenetics Laboratory, Churchill Hospital, Oxford OX3 7LJ

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

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

Brendan Clark PhD FRCPath, Consultant Clinical Scientist Histocompatibility & Immunogenetics Laboratory, St James' University Hospital, Leeds LS9 7TF

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

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

John Harvey FIBMS, DBMS, Principal Clinical Scientist, NHS Blood & Transplant, Filton, Bristol BS34 7QH

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

Cristina Navarrete PhD FRCPath, Consultant Clinical Scientist, NHS Blood & Transplant, Colindale, London NW9 5BG

John Smith PhD FRCPath, 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

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

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

4.3 Clinical Reviewers

Peter Andrews MD FRCP, Chair of BTS Standards Committee, Consultant Nephrologist, SW Thames Renal & Transplantation Unit, Surrey SM5 1AA

Lisa Burnapp, Lead Nurse, Living Donation, Organ Donation and Transplantation, NHS Blood and Transplant
Consultant Nurse, Living Donor Kidney Transplantation, Guy's & St. Thomas' NHS Foundation Trust

Dr Martin Carby
Consultant Respiratory Physician
Royal Brompton & Harefield NHS Foundation Trust.

Adam Mclean, Consultant Nephrologist, Dialysis and Transplantation, Royal Free Hospital, London NW3 2QG

Dr Jayan Parameshwar, Consultant Cardiologist
Papworth Hospital NHS Foundation Trust

Chris Watson, Professor of Transplantation, Department of Surgery, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, CB2 0SP

4.4 Comments Received from BSHI and BTS Members

All comments received from all reviewers have been addressed where possible. Most comments received were discussion points which centred on either the level of risk assigned, or the use of the words “should” and “must” in the recommendations. Where all authors agreed on the response, I have included a comment below which outlines the sentiment behind the queried recommendations. Where the authors did not agree, the point will be considered more fully within the next revision.

Specific Comments:

1. 5.1.8 Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}
   Reviewer Comment – Nothing on HLA typing capability, especially with respect to timely typing of deceased donors?
   Authors’ Response – The authors considered that timing of HLA typing in deceased donation is not within the scope of this Antibody Guidelines document.
2. 5.2.6 In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling should be agreed with the local transplant unit. {1}
   Reviewer Comment : “should or “must”
   Authors’ Response: This is a very strong recommendation for high risk cases. We agree with the reviewers that this is a “must”.
3. 5.3.3 If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}
   Reviewer Comment: 1 or 2?
   Authors’ Response : There is insufficient published evidence base to justify a 1 at present. This may alter in time as our experience is documented.
4. 5.4.4 Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
   Reviewer Comment: “must”?
   Authors’ Response: This has to remain a “should” to leave open the possibility of doing alternative appropriate testing such as Single Antigen Bead testing on a marginal pancreas where the patient would benefit from a shorter ischaemia time and being able to proceed straight to theatre upon receipt of the organ, rather than waiting for a crossmatch result.
   An additional comment was received and agreed, that a complex antibody profile is considerably different to an incompletely defined profile and that these scenarios should be addressed independently. As our experience in this area is still growing, it was felt that this must be expanded in the next revision of the Guidelines.
5. 5.4.5 If a virtual crossmatch is performed, a retrospective laboratory crossmatch test must be performed using serum collected within 24-48 hours prior to transplantation. {3}

Reviewer Comment: “Should” or “must”, if level 3

Authors’ Response: It has been agreed that for now this will remain a “should”. It may be acceptable for the absence of donor-directed antibodies to be determined in a “current” sample in some circumstances.

6. 5.4.6 Laboratory crossmatch tests should distinguish donor T cell and B cell populations and between IgG and IgM antibodies. {1}

Reviewer Comment: “must” ?

Authors’ Response: Policies differ between centres on this recommendation and not all reviewers agreed. If the Unit has a policy which does not allow transplantation across Class I or Class II antibodies, there is no need to distinguish between the two cell populations. However, authors agreed that the test must be able to distinguish between clinically relevant IgG HLA specific antibodies that are donor-directed and IgM, so the wording has been clarified to reinfoce this requirement.

7. 5.5.2 The laboratory must inform the clinical team whenever more than three months has lapsed since a patient’s serum sample has been received. {2}

Reviewer Comment: “must/should” ?

Authors’ Response: The authors agreed generally that this was a “must” for patients who are active on the list for deceased donor transplants, but that it may not always be relevant for others. It was agreed to leave this as a stronger recommendation for this revision.

8. 5.9.2 For patients with pre-transplant DSA, the following risk stratification must be applied {3}:

Reviewer Comment: “must/should” ?

Authors’ Response: This recommendation came directly from the Bowel Advisory Group of NHSBT, and as such, it remains a “must” until this group chooses to modify it.

5 RECOMMENDATIONS AND BEST PRACTICE

Each chapter within this document concludes with recommendations for HLA antibody testing relevant to the area of transplantation covered within it. While some general principles of antibody testing can be applied to all aspects of transplantation, other recommendations remain organ-specific. This summary of the major recommendations of this document has been arranged into general recommendations (5.1 to 5.4), which apply to all branches of transplantation, and specific recommendations which apply only where indicated. The recommendation grades outlined in Chapter 2 are shown in parenthesis: e.g. {1} following the recommendation.

5.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

5.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}
2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}

6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

5.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

5.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}
6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

5.5 Kidney and Pancreas Transplantation

1. For patients awaiting transplantation, samples should be obtained and tested at intervals of no longer than three months and after known sensitising events. {2}

2. The laboratory must inform the clinical team whenever more than three months has lapsed since a patient's serum sample has been received, if the patient is active on the transplant waiting list. {2}

5.6 Islet Transplantation

1. Once the patient is listed, samples for antibody analysis should be obtained no less than three monthly. {3}
2. Both cytotoxic and flow cytometry crossmatching are recommended. {3}
3. Samples should be taken regularly following the first and any subsequent transplants. {2}

5.7 Thoracic Organ Transplantation

1. Prior to listing, antibody screening and, if appropriate, specificity analysis must be performed. {1} Testing two separate samples obtained at different time points is recommended. {2}
2. Samples should be sent from patients on the waiting list for antibody testing at regular intervals; at least three-monthly for previously sensitised patients, and six-monthly for patients who have consistently been negative for HLA specific antibodies. {2}
3. Each positive HLA specificity should be assigned a risk based on its MFI level {2}:

Risk Level	MFI	Description
I	No detectable HLA antibody	Standard
II	<2,000	Minimum risk of hyperacute rejection due to low level donor HLA specific antibodies but greater than standard risk of rejection
III	2,000 - 5,000	Low risk of hyperacute rejection but significant risk of early rejection and antibody mediated graft damage. Immediate pre-transplant antibody reduction may be considered when feasible.
IV	> 5,000	Transplant veto apart from exceptional cases. Further testing such as CDC tests, or complement fixation in Luminex assays (C1q, C3d or C4d) should be considered in these cases to further refine risk profiles

4. The retrospective crossmatch techniques used for sensitised patients above standard risk should include flow cytometry. {3}
5. Following transplantation, patients above standard risk should be tested for HLA-specific antibodies at 7 and 28 days; 3, 6, 9 and 12 months; and then as required. {2}

5.8 Liver Transplantation

1. Prospective crossmatching is not indicated prior to liver transplantation. {2}
2. HLA antibody testing should be considered at the time of transplant to identify patients at high risk of acute rejection and aid post-transplant management. {2}
3. Patients likely to have simultaneous liver and kidney transplantation should be tested for HLA specific antibodies pre-transplantation. Those with HLA class II specific antibodies are at a significantly higher risk of rejection of both kidney and liver. {2}

5.9 Intestinal and Multi-Visceral Transplantation

9. Each positive HLA specific antibody should be assigned immunological risk based on its MFI level. {2}
10. For patients with pre-transplant DSA, the following risk stratification must be applied {3}:

Risk Level	MFI	Description
I	No detectable HLA antibody	Standard
II	<2,000	Minimum risk of hyperacute rejection due to low level donor HLA specific antibodies but greater than standard risk of rejection
III	2,000 - 8,000	Flow Cytometric donor crossmatch likely to be positive, conferring an intermediate risk of humoral rejection.
IV	>8,000	Lymphocytotoxic donor crossmatch (CDC) likely to be positive, conferring a high risk of humoral rejection.

11. A positive donor cytotoxic crossmatch caused by IgG HLA class I specific antibodies indicates a higher risk in intestinal transplantation (in the absence of a liver transplant from the same donor). The final decision to proceed with transplantation will depend on evaluation of the relative risk of proceeding versus the risk of delayed transplantation. {2}

5.10 HLA Antibody Incompatible Transplantation

1. The HLA-specificity and level of DSA must be fully determined prior to antibody reduction. {1}
2. Crossmatching by CDC must be used to identify the immunological risk of the transplant. {1}
3. DSA levels must be monitored regularly throughout the duration of treatment to determine its effectiveness. {1}

5.11 Haematopoietic Progenitor Cell Transplantation

1. In selecting HLA mismatched donors HLA antibody testing of the recipient should be performed at the time of donor selection and at the time of transplantation if there is a significant time lapse. {3}
2. The clinical team must be made aware of any HLA antibody incompatibility detected in the recipient. {3}
3. It is recommended that HLA antibody testing is performed in cases of non-engraftment. {3}

6 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. allografts, 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 can be 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 transplantation. 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 reaction frequency cRF >10%).

A critical function of the histocompatibility laboratory is to identify sensitisation in patients to reduce the immunological risks of allotransplantation.

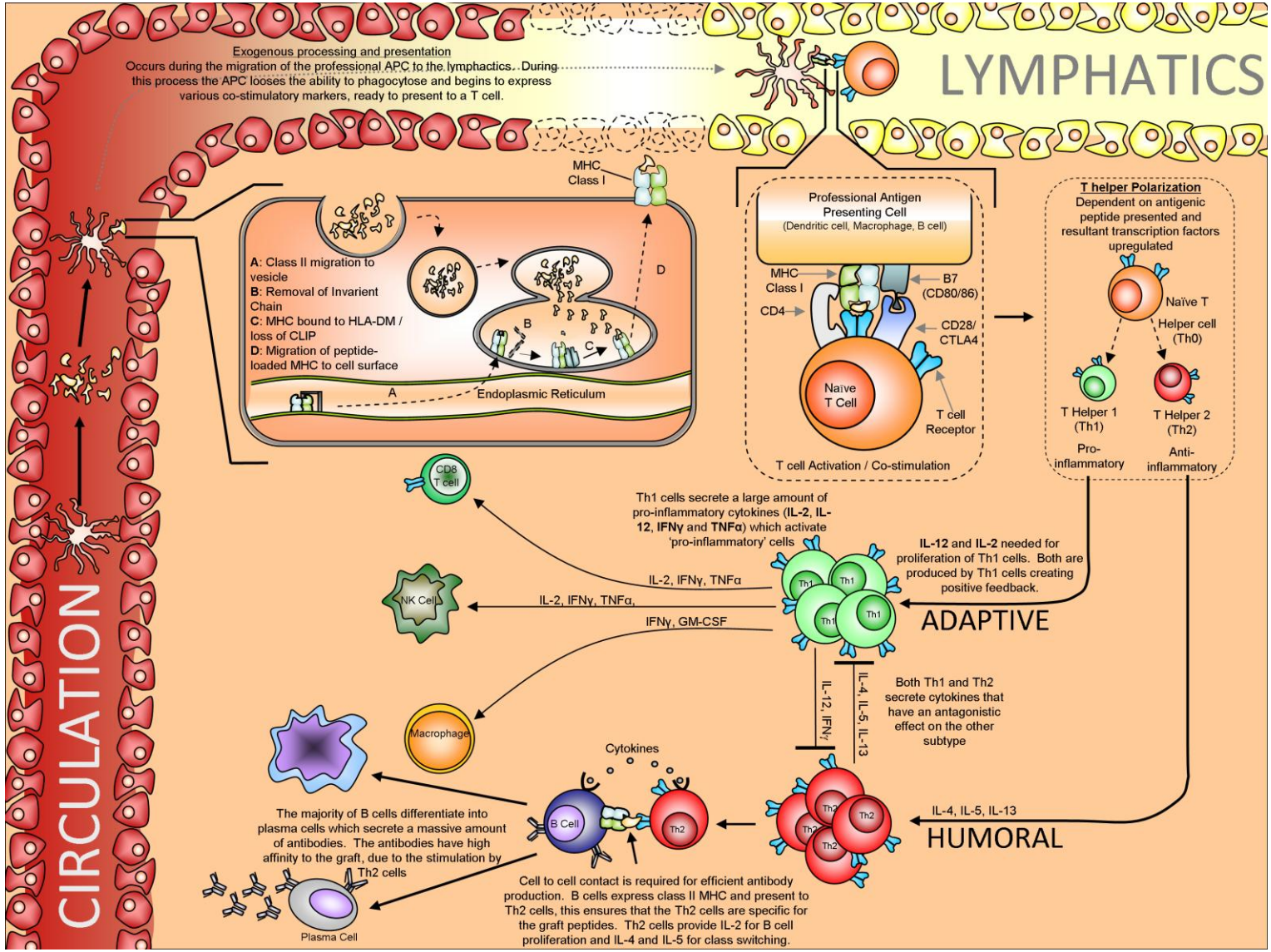
6.1 The Immune System

6.1.1 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-HLA, in which antigens are bound and presented 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.

Figure 1: Antigen presentation to T cells and the activation of effector cells involved in the immune response



6.1.2 Allorecognition

In transplantation a unique process of T cell activation is observed. When the immune system is exposed to transplanted non-self HLA there is a vigorous T cell response. T cells recognise non-self HLA via two distinct pathways known as direct and indirect allorecognition. It is estimated that 1-5% of an individual's T cell repertoire can react to foreign HLA through direct activation, a process where the recipient's T cells interact directly with the foreign HLA molecules expressed by donor APC. It is still not clear how such a large proportion of the T cell repertoire is activated by direct interaction with foreign HLA, with debate as to the relative contribution of the TCR interaction with the foreign HLA molecule and presented peptide. As well as direct T cell allorecognition, which is thought to occur early after transplantation as passenger leukocytes migrate to the lymph nodes, foreign HLA can also trigger T cell activation via indirect allorecognition. This latter process is analogous to the activation of T cells against any non-self protein and occurs following the uptake and presentation of donor proteins, including HLA, by the recipient APC. Indirect T cell allorecognition is a process that can continue throughout the lifetime of the allograft and may therefore contribute more to long-term rejection events.

6.1.3 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. T cells provide help to B cells which differentiate into antibody-producing plasma cells with the initial production of IgM antibodies and subsequently IgG antibodies 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. Antibodies can also directly activate the endothelium [1].

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 co-operation 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 to evaluate T cell sensitisation.

6.1.4 Control of allorecognition

In kidney, pancreas and islet cell transplantation, immunogenicity and alloantigen load can be reduced through minimising 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 [2-5]. 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.

6.2 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. Highly sensitive solid phase binding assays have identified IgG antibodies with apparent HLA specificity in the sera of normal healthy individuals with no history of allosensitisation events, although there is evidence that these are not clinically relevant [6-7].

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 [8]. 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.

6.3 Acute Antibody Mediated Rejection Mechanisms

6.3.1 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 [9]. Perfusion of kidneys with plasma containing antibodies directed against HLA antigens present in the kidney has been shown to cause hyperacute rejection [10].

6.3.2 Accelerated rejection

Accelerated rejection is a form of rejection that has similar features to, but a distinct identity from that of, Hyperacute and cell-mediated acute rejection. Accelerated rejection is an amnesic form of rejection where the graft functions normally in the first 24-48 hours, with subsequent rapid deterioration in function. Previous exposure to donor antigen leaves the recipient immune system “primed” for a secondary response. Re exposure to antigen induces donor-specific antibodies and memory T-cells which rapidly proliferate and differentiate on second exposure. Exposure of antigen to memory donor specific T and B-cells to graft antigens induces rapid activation and differentiation of T-cells and production of DSA leading to graft dysfunction and destruction.

6.3.3 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 [11,12]. 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 [13] and the demonstration of plasma cells amongst infiltrating cells recovered from failed kidney grafts [14] indicate that both cellular and humoral responses may be present in acute rejection. Further evidence comes from the identification of *de novo* DSA in patient sera and C4d in biopsies obtained from kidney and heart transplants undergoing acute or chronic rejection [15,16]. 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. Graft infiltrating cells have been shown to mediate cellular lysis of antibody coated cells [17] and antibodies eluted from rejected grafts have been found to mediate antibody-dependent cell-mediated cytotoxicity activity to donor cells [13].

6.4 References for Introduction

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7 DEFINING RISK

The concept of defining risk categories was first described by Gebel et al [1] in the context of renal transplantation. The model has recently been extended and modified for use in cardiothoracic and intestinal transplantation. The recommendations of this review panel are summarised in Chapter 5.

The principles of risk assessment are:

1) **High immunological risk:**

- High levels of circulating antibodies specific for mismatched donor HLA present at the time of transplantation

  The high risk of hyperacute rejection would normally constitute a veto to transplantation, but pre-transplant desensitisation regimens can ameliorate this risk.

2) **Intermediate immunological risk:**

- Low level DSA present at the time of transplantation
- Historic DSA not detectable at the time of transplantation

  In such cases, it may be justified to consider augmented immunosuppression and post-transplant immunological monitoring.

3) **Standard immunological risk**:

- The absence of donor directed sensitisation to HLA

A summary of immunological pre-transplant risk assessment in renal transplantation, based on donor crossmatch and antibody screening results [2] is given in Table 1 [3].

7.1 References for Defining Risk

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Table1. Immunological pre-transplant risk assessment based on donor crossmatch and antibody screening results

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

* 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.

8 IDENTIFICATION OF HLA SPECIFIC ANTIBODIES

There have been significant advances in recent years in the ability of the histocompatibility laboratory to precisely detect and characterise HLA antibodies. Since the first use of cytotoxicity testing to define HLA antibodies many methods have been developed which vary in their target, configuration, sensitivity and specificity. Some methods, such as CDC, are still used because of their clear clinical correlation. In contrast, other tests have been useful for a period but have now mostly been superseded by current solid phase assays, such as Luminex based techniques. Methods available for HLA-specific antibody testing have been comprehensively reviewed by Tait et al [1], and are outlined as follows:

8.1 Cell based assays

8.1.1 Complement dependent cytotoxicity

The first established method for the detection and definition of HLA-specific antibodies was the CDC test that employs lymphocyte targets to detect complement-fixing IgG and IgM antibodies. The CDC test is still used for both antibody screening and crossmatching protocols, usually in addition to other, more sensitive methods. The CDC assay for HLA-specific antibody detection has several inherent problems; 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 and these may not be HLA-specific. Results derived from CDC testing used to be presented as the percentage of the cell panel with which a serum had reacted (%PRA). The “% PRA” was dependent on the composition of the cell panel and was therefore of limited value. PRA results could not be compared between cell panels or between laboratories and did not necessarily reflect the proportion of the donor pool to which a patient was sensitised. False positive results due to the presence of autoreactive lymphocytotoxic antibodies could also give “100% PRA” despite being irrelevant to transplant outcome. Hence sensitisation in UK patients is no longer defined in terms of “%PRA”, but has been replaced with a calculated reaction frequency (cRF) defined by NHS Blood & Transplant - Organ Donation and Transplantation Directorate (NHSBT–ODT) when patients are listed for transplantation (described later). In the CDC assay, autoantibodies can be removed by absorption with autologous lymphocytes and/or abrogated by treatment of the serum with dithiothreitol (DTT). However, DTT will also remove reactivity due to IgM *allo*antibodies as well as autoantibodies.

A number of developments beyond CDC testing improved the detection and characterisation of alloantibodies in sensitised patients. A method was described that employs the non-HLA expressing cell line K562, transfected with cDNA encoding single HLA class I alleles [2]. These cells could be used in cytotoxicity and flow cytometry assays

and because they express only a single HLA antigen their use facilitated the characterisation of HLA antibody specificity, particularly in highly sensitised patients.

8.1.2 Flow Cytometry

As flow cytometry crossmatching (FCXM) became more widely used, the increased sensitivity of the FCXM meant that antibody results derived from screening by CDC were not 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 patients, Epstein Barr Virus (EBV) transformed lymphoblastoid cell line cells and peripheral blood lymphocytes have all been used.

Testing sera using flow cytometry with individual cell panels is cumbersome for large-scale use, so the option of pooled cells allowed for a large number of sera to be screened over a short period and was more sensitive than CDC. Antibody positive sera could then be investigated to define specificity using other techniques. The use of cell lines expressing a single HLA antigen in a flow cytometry assay enabled the identification of HLA antigens to which sensitised patients did and did not have antibodies [2]. 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.

8.2 Solid phase assays

The development of solid phase enzyme linked immunosorbent assays (ELISA) and bead 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:

- 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

8.2.1 Enzyme-linked immunosorbent assays

ELISA-based tests were the first solid phase assays to be introduced in the 1990s [3] and allowed screening for the detection of HLA-specific antibody and antibody specificity definition. The tests were advantageous as positive reactions could be ranked according to optical density in the test readout and antibody specificities clustered to aid interpretation [4]. As with other newer manifestations of solid phase assays discrepancies were 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 [5].

8.2.2 Flow Cytometry

Flow cytometry solid phase assays using microparticles coated with soluble HLA antigens to detect alloantibodies were developed in the mid-1990s [6,7]. As with the ELISA, kits were developed that enabled both screening for the presence of HLA class I or II specific antibodies or the separate definition of antibodies to HLA-A, -B, -C and HLA-DR, -DQ, -DP. Studies showed these microparticles to be more sensitive and more specific than CDC for the detection of HLA-specific antibodies [4]. A further advance was the development of microparticles coated with a single antigen [5] These facilitated antibody specificity definition and in particular enabled the identification of HLA specificities to which a highly sensitised patient is not sensitised, termed “acceptable mismatches” [8].

8.2.3 X-Map (Luminex)

This now commonly used assay utilises multiplexed beads (or microparticles) and flow cytometry [9]. X-Map (Luminex) technology utilises microbeads which are coloured with a combination of two dyes. 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, thus allowing the simultaneous analysis of a far greater number of HLA antigen coated microbeads than other methods. HLA-specific antibody binding to the microbeads is detected using R-phycoerythrin conjugated anti-human immunoglobulin and a flow analyser.

Assays are available for HLA-specific antibody screening using pooled antigen panels comprising beads coated with multiple class I (HLA-A, -B and –C) or class II (HLA-DR, -DQ, -DP) antigens. There are also phenotype panels for specificity definition in which each bead population is coated with either HLA class I or class II proteins from a single cell line. The availability of HLA class I and II recombinant single antigens from transfected cell lines has also allowed the production of microparticles coated with single HLA antigens which has enabled clear identification of antibody specificities in highly reactive sera [10]. These bead array assays provide a semi-quantitative numeric fluorescence value and so can be applied to monitoring patient antibody profiles for particular specificities, such as in monitoring antibody removal pre-transplantation [11] and the identification of DSA post-transplantation [12]. However, it is important to note that the MFI value represents the amount of antibody bound to an individual bead and not the titre of the antibody. Another advantage of this solid phase ‘single antigen bead’ technology is that the data can be used to define target epitopes more accurately and hence understand patterns of antibody reactivity [13].

The development and use of single antigen beads has enabled the identification of antibodies to previously hidden targets, such as HLA-DQA1 and HLA-DPB1 epitopes [14] and allele-specific antibodies [15]. The widespread use of Luminex technology has led to

investigations of the clinical relevance of antibodies detected by this sensitive method which are not detected by other assays such as CDC. Although this is currently debated there is good evidence in renal transplantation that the presence of HLA DSA that do not cause a CDC positive crossmatch are still a risk factor for the development of antibody mediated rejection (AMR) and graft failure [16,17]. A meta-analysis of outcome in renal transplantation has even shown that the presence of DSA in CDC and FCXM negative patients associates with a doubling in the risk of AMR and an increased risk of graft failure [18].

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. The observation that high level antibodies can be 'blocked' in the Luminex assay led to reports that IgM HLA-specific antibodies could block binding of IgG HLA-specific antibodies to single antigen beads and give a misleadingly low or negative assessment of alloantibody levels [19,20]. More recently evidence has emerged that this blocking effect is most likely due to complement fixation by HLA-specific antibodies [21]. The binding of complement reduces the ability of secondary IgG detection antibodies to bind and leads to misleadingly low values in the assay. A number of test modifications can be used to overcome this problem where it is suspected, including heat inactivation of sera, EDTA treatment and DTT treatment. This is becoming increasingly widely used, especially in highly sensitised patients. Clearly when using single antigen beads for monitoring IgG HLA-specific antibodies in sensitised patients, it is important to consider pre-treatment of sera to reveal potentially clinically relevant HLA class I and class II antibody specificities that may otherwise be masked or otherwise only suspected to be present at low levels.

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 [22] and similar modifications to Luminex based bead array assays have been reported. In a study using a modified IgM Luminex assay, 9/34 patients with no IgG DSA at the time of transplant were defined as having IgM DSA, the presence of which was associated with immunological rejection [23].

Another modification to the bead array enables detection of only those antibodies that fix complement, by detecting the complement fragments C4d or C1q [24]. The aim of this modification is to detect only those antibodies that fix complement (which may highlight a greater risk for AMR), to provide a tool for better risk stratification through the transplant process. Indeed, the presence of C1q binding antibodies after kidney transplantation has been shown to associate with a much increased risk of graft loss [25]. However, there is evidence that this C1q-SAB assay may not directly represent the ability of complement fixing IgG1 and IgG3 donor HLA-specific antibodies to bind C1q, and as such may not provide useful clinical information over and above that provided by the standard IgG-SAB assay [26].

8.3 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 was 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, the cRF is determined at 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 cRF is expressed as a percentage 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. NHSBT-ODT have also developed a tool named the 'Relative Chance of Kidney Transplant Calculator' (http://www.odt.nhs.uk/doc/chance_of_transplant.xls), which uses a number of patient variables, including HLA data, to estimate the chance of a patient receiving a transplant within five years of listing. For patients registered within the Paired/Pooled scheme, a different profile of unacceptable antigens may be registered without affecting the profile listed within the deceased donor scheme.

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 reports of naturally occurring HLA-specific antibodies in sera from healthy males with no history of alloimmunisation. Many of these antibodies react with dissociated antigens and have been reported to be irrelevant to transplant outcome [27]. Nevertheless, the possibility of their existence in patient sera must be considered when the results of bead based assays are being interpreted.

The main purpose of characterising a patient's HLA-specific antibody profile is to define donor HLA antigens that are unacceptable for a 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 without a prospective laboratory crossmatch test (termed a virtual crossmatch) [28]. Critical to the success of this approach is an understanding of the relationship between antibodies detected in a solid phase assay and the crossmatch test result. One of the reasons it is not possible to directly correlate

the MFI for the bead to which antibody has bound and the crossmatch result is because the amount of HLA protein bound to each bead varies between beads within a batch and between batches. Attempts have been made to correct for this variation by testing each lot of beads with monoclonal antibodies to determine the antigen density and indeed some manufacturers provide reports that take into account this variation. When using data from single antigen bead testing to define unacceptable antigens, it is also important to note that these assays now allow the identification of allele-specific antibodies [15]. 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 possible to identify potentially graft damaging antibodies that might previously have been dismissed as self (HLA-A68) reactive and therefore not graft damaging.

Bearing in mind the problems outlined above (blocking of antibody detection, denatured antigen on the beads, variable antigen density, allelic antibodies, DQA1/DPA1 directed specificities etc), 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.

8.4 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 positive samples.

As outlined above, 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 advisable for laboratories 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 laboratory 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.

8.5 Sample collection and storage for antibody screening and donor crossmatching

8.5.1 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. It is the responsibility of the clinical team to inform the histocompatibility laboratory of all potential allo-sensitisation events, including transfusions, pregnancies, transplantation and infections as well as vaccination and treatment with therapeutic antibodies e.g. rituximab. The clinical team must also 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. Samples will be obtained at intervals of no more than three months for routine antibody monitoring.

8.5.2 Post-transplant

Patients who undergo a desensitisation regimen will be at high risk of AMR and require regular monitoring of DSA levels post-transplant. Those patients, who have been transplanted with a negative crossmatch, but in the presence of a DSA defined by Luminex, may be at a higher risk of AMR and regular monitoring in these cases is also recommended. Even in those patients considered as low risk, with no evidence of DSA at the time of transplant, it is recommended that DSA testing should be performed at least once in the first year post-transplant and when antibody production may be suspected (change in function, modification to immunosuppression, suspected non-adherence). Irrespective of whether post-transplant samples require immediate testing for HLA antibodies, it is important that the histocompatibility laboratory continues to receive serum samples post-transplant at least annually. Failure to provide these samples may jeopardise a patient's future chances of transplantation as the testing of these samples at the time of any graft loss will help to define those antigens that should be listed as unacceptable for future transplantation. Local policy should stipulate the frequency of testing post-transplant in different risk groups as well as testing following reduction or cessation of immunosuppression and or graft removal. Serum samples should also be obtained and tested at times of biopsy for graft dysfunction to support the diagnosis of antibody-mediated rejection.

8.5.3 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. 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.

8.6 Recommendations

8.6.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

8.6.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}
2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is

suspected. {2}

6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

8.6.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

8.7 References for Identification of HLA Specific Antibodies

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9 CROSSMATCHING

The purpose of the crossmatch test is to determine whether a patient has antibodies that react with antigens expressed by a given donor and to inform the immunological risk assessment for that patient/donor combination. Preformed 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 [1]. A crossmatch between donor and recipient is the definitive pre-transplantation test to avoid hyperacute rejection due to donor-specific HLA antibodies. 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.

9.1 Crossmatch Techniques

The first technique to be developed to detect donor-specific antibodies was based on the CDC test [2]. A positive donor-specific cytotoxic crossmatch test has been shown to be predictive of hyperacute rejection [3]. The standard CDC technique detects both HLA and non HLA-specific complement fixing antibodies of IgG/IgM classes. Variant methods have been described that increase specificity and sensitivity of the standard technique [4,5].

The FCXM was subsequently developed [6] 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 FCXM is therefore able to detect some antibody classes which are not identified by the standard CDC test.

The target cells used for the crossmatch test are donor leukocytes. 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.

More recently solid phase crossmatch tests have been developed employing synthetic beads coated with donor HLA antigens. However, the take up of solid phase crossmatch tests has been low and there are few data to support their use at present. An advantage of these tests is that any reactivity demonstrated within them can be confidently attributed to HLA antibody. The greatest utility of such methods may exist in post-transplant follow-up of patients receiving treatments that could compromise valid interpretation of cell based methods.

It is generally considered that FCXM and bead based assay systems provide greater sensitivity than the CDC method although the significance of this for transplant outcome remains a matter of debate.

Whilst significant developments in crossmatch methodology have occurred since the original description of the CDC crossmatch, the ideal crossmatch test capable of detecting all clinically relevant DSA and of excluding clinically irrelevant auto- and alloantibodies has still not been found [7].

9.2 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. 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 laboratory crossmatch test could be omitted with the aim of reducing organ cold storage time [8,9]. It was first demonstrated that this works in practice with a study omitting the pre-transplant laboratory crossmatch test for a well-defined group of patients [10]. Crossmatch tests 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 [11]. This large study also demonstrated that the omission of the crossmatch did result in reduced cold storage time, a finding now supported by national audit data, and additionally that there may be a reduction in delayed graft function for these patients.

The term virtual crossmatch 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 laboratory crossmatch 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 [12].

9.3 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, in the deceased donor situation commonly referred to as the 'time of offer' sample, 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.

For living donation, a preliminary crossmatch may be performed either as a virtual crossmatch after extensive testing for HLA-specific antibodies, or as a laboratory crossmatch test using a current sample from the potential recipient. The crossmatch must then be repeated immediately prior to transplantation (within one week of the planned donation) to ensure that any potential sensitising events since the preliminary crossmatch have not induced a donor-directed antibody response.

It is the responsibility of the referring centre to provide the patient sensitisation history and appropriate screening samples to the laboratory, as it is not possible to assess a patient's potential immunological risk to an allograft without a comprehensive review. It is therefore essential to eliminate any risk of unknown sensitisation and minimize governance issues associated with failure to screen potential recipients.

9.4 Results and Interpretation

For each serum sample tested the result of a crossmatch is either positive or negative. A negative crossmatch indicates that DSA are absent from the recipient serum or are not detectable by the test, either being below the threshold for detection or due to technical reasons. 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 confidently demonstrated that the antibodies are not HLA-specific the positive result is not a veto to transplantation. In all other circumstances 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 can 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 clinical relevance of the results obtained is of paramount importance in the correct interpretation of crossmatch results and in assigning the potential risk associated with a transplant. As outlined in the following sections of these guidelines, whilst the clinical relevance of a positive cytotoxic crossmatch due to DSA 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, while at the same time immunosuppression and other interventions have led to a greater ability to transplant in the face of DSA. 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 positive virtual crossmatch is not always associated with a positive laboratory crossmatch test. Studies have shown associations between HLA antibodies detected by bead-based assays only and acute rejection episodes [13] and with long term but not short term graft outcome [14]. Also, it has been shown that antibodies reacting in bead-based assays are found in some non-transfused, non-transplanted males [15]**.** It is now accepted that many of these antibodies are directed against epitopes exposed on the surface of denatured antigen and are not clinically relevant [16,17]. A negative virtual crossmatch is therefore a reliable indicator of a negative laboratory crossmatch, whereas the interpretation of a positive virtual crossmatch is less straightforward [18]. Detailed analysis of the strength, specificity and, where known, the patient's exposure to potential sensitising events, is important in the risk analysis in such cases.

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.

9.5 Recommendations

9.5.1 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}
6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

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10 KIDNEY and PANCREAS TRANSPLANTATION

Kidney transplantation is the treatment of choice for patients with end stage kidney disease. Over the last 20 years, better immunosuppression, donor selection, HLA matching and antibody screening have all contributed to improved graft survival in kidney transplant recipients. There is no single reliable tool to predict whether an allogeneic graft will undergo immunological rejection but there is a clear benefit of HLA matching if a 000 HLA mismatched donor (i.e. no mismatched antigens at HLA-A, B or DRB1 loci) is available. If a fully matched donor is not available it is important to ensure there are no HLA antibodies in the potential recipient directed against mismatched donor antigens. . Kidney transplantation can be performed from either deceased donors or live donors with live donor transplants generally having better outcomes. Although the immunological principles that apply to deceased donor transplantation also apply to live donor transplantation, the ability to plan a transplant date and the availability of the donor may allow the patient to have preconditioning, or to consider entering a kidney exchange programme.

Immunological graft loss has decreased in all types of pancreas transplantation over the last 20 years. The one year immunological pancreas graft loss has decreased from 38% to 6% in pancreas transplantation alone (PTA), from 28% to 3.7% in pancreas after kidney transplantation (PAK), and from 7% to 1.8% in simultaneous pancreas and kidney (SPK) transplantation [1]. This said, donor/patient factors and surgical complication rates have the greatest impact on pancreas function [2].

There are few specific pancreas only data, and the recent “Consensus Guidelines on the Testing and Clinical Management Issues Associated with HLA and non-HLA antibodies in Transplantation” contains only one pancreas alone case study [3]. In this document the authors suggest that recommendations for kidney transplantation should apply to the pancreas for SPK transplantation. Therefore in this section, where appropriate, kidney and pancreas transplantation are considered together in terms of pre-transplant workup, crossmatching and follow up.

The benefits of HLA matching in deceased donor kidney transplantation have been recognised for a long time, and these benefits were formalised in the 1998 National Kidney Allocation Scheme (NKAS). This scheme involved allocation of well-matched kidneys from heart beating donors (now referred to as ‘donation after brain stem death’ or ‘DBD’ donors) nationally by segregating allocated organs into different tiers based mainly on HLA match grade. This scheme also accounted for DR homozygous patients and favoured those with antibody sensitisation. The scheme was redefined in 2006 to address observed inequities in access to transplantation, but still gave absolute priority to 000 HLA-A, B and DR mismatched grafts and gave points for age and HLA mismatch to ensure well matched grafts for young patients. The scheme also continued to recognise the problems of highly sensitised patients. Although the benefits of HLA matching are not so apparent in live donor

kidney transplantation, there are clear benefits for re-transplantation, this is particularly evident in patients who are transplanted at an early age.

In 2010, NHSBT-ODT introduced a national pancreas allocation scheme for the allocation of SPK, PTA and PAK and pancreatic islets. This included HLA matching between recipient and donor and sensitisation points for HLA sensitisation as part of the allocation process. There is evidence that HLA matching has an effect on pancreas outcome and this is supported by recent data showing that formation of DSA post-transplant has a negative impact on pancreas survival post-transplantation [4].
In sensitised patients, special consideration has to be given to the donor HLA mismatch and to avoid HLA mismatched specificities to which the patient is sensitised. However, a more stringent allocation criterion 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 rejection may have to be balanced against the risk of not receiving a transplant, and this is taken into consideration in national pancreas allocation schemes.

Registry data from a large number of transplant centres have shown that kidney transplant outcome in sensitised patients and re-grafts is inferior to that in non-sensitised patients [5], and in the recent antibody consensus guidelines it is accepted that both kidney and pancreas are at risk for AMR and that pre-transplant DSA should be avoided wherever possible [3]. Pre-sensitised kidney patients wait longer to get a suitable offer, particularly those with multiple HLA antibodies. To help address these issues for patients with a potential live donor NHSBT-ODT introduced the paired/pooled Kidney sharing scheme in 2007 and more recently included non-directed altruistic donors into the scheme.

Analysis of a large group of recipients showed that for first transplants and re-transplants performed between 2000-08, 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; re-transplants 75-78% vs 82%, p=0.002).

10.1 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 the transplantation of sensitised pancreas transplant candidates with poorly matched but compatible organs. Although the importance of HLA matching in transplantation is well understood, it is only one of multiple factors that influence transplant outcome. There is a clear detrimental effect of prolonged cold storage times and delayed graft function on transplant outcome. Therefore it is important that transplant centres ensure that processes are in place to minimise the chance of kidneys and/or pancreases being shipped and then being crossmatch positive. This includes appropriate sample collection and pre-transplant testing.

If sera are regularly collected and screened during patient work-up for transplantation, HLA-specific antibodies can be defined and a patient's crossmatch reactivity against a particular donor of known HLA type predicted [6]. This allows the UK national allocation scheme to perform a virtual crossmatch prior to allocation, which should in turn predict a negative laboratory crossmatch. In the case of zero HLA-A, B, DR mismatched transplants, antibodies specific for these loci would not be expected to be pathogenic. 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 [7].Therefore, definition of antibodies to HLA-Cw, DQ and DP antigens is also necessary in order to prevent positive crossmatches. 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. 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 [8].

There is contradictory literature on the role of the recently introduced Luminex assays for the detection of complement fixing antibodies [9,10], with some studies reporting correlation between Luminex MFI and complement fixation assays when compared to other techniques. However, most studies show that MFI values on IgG assays are not a suitable marker for the prediction of complement binding [11,12]. 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 positive crossmatch results caused by irrelevant non-HLA antibodies can therefore be avoided or predicted.

HLA-specific antibodies that are not generated by exposure to alloantigen have been detected with the latest sensitive screening techniques. Their origin and clinical relevance is still undetermined [13].

10.2 Definition of Unacceptable Mismatches

Regular antibody screening and identification should be used to define unacceptable mismatches as antibodies identified pre transplant which are directed against the donor have been shown to be associated with poor long term survival [14]. These will include HLA antigens for which the patient has been shown to develop specific HLA antibodies. Other antigens may be considered as unacceptable mismatches; these can include mismatched antigens on previous failed transplants to which specific antibody has not been demonstrated, particularly if there is an incomplete sample history. This is because there may be immunological memory even if there is no antibody currently detectable.

Mismatches which do not elicit an antibody response may be repeated with no detrimental effect, 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 failure. Where 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.

In previously transplanted PAK patients with a functioning kidney requiring transplantation of a pancreas, previously 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 [15].

Other HLA antigens may be listed as unacceptable where it is desirable to avoid sensitisation to these antigens, for example when living donor transplantation from a known donor may be considered at a future date. This should be balanced against the clinical need of the patient and the likelihood of another offer. In certain situations, even when there are no barriers to transplantation, for example a poorly matched pair with a young recipient with no antibodies, it may be beneficial to include donors and recipients into kidney sharing schemes to increase the chance of finding a more suitable donor

10.3 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.

10.3.1 The cytotoxic crossmatch

It is generally accepted for kidney and pancreas transplantation that IgG antibodies directed against donor HLA-A or -B specificities and present at the time of transplant can cause hyperacute rejection. Although fewer data are available, donor class II specific antibodies present in the recipient may also result in rejection and are associated with worse long term outcome [16]. The outcome will differ between individuals depending on the level of antibody at the time of transplant and level of expression of antigen 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 [17]. There is little information on the role of antibodies to HLA-Cw, -DQ or –DP specificities in transplant failure. There are reports of transplant failure in a patient with HLA-Cw specific antibodies, and acute humoral rejection has been associated with HLA-DQ antibodies. If

patients have antibodies to HLA-Cw or HLA-DQ, many centres now avoid crossing these 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 [18]. They therefore give rise to misleading positive results. The clinical relevance of IgM HLA-specific antibodies is not clear and whilst in many cases they appear not to be detrimental, and they could be a marker for an early immune response.

The clinical relevance of antibodies in non-current sera is also not fully elucidated. There have been reports of successful kidney transplantation with a “peak positive, current negative” crossmatch. But IgG HLA-A or -B specific antibodies present in historic sera are associated with accelerated rejection and decreased graft survival [19]. Decisions regarding the transplantation of patients with antibodies in non-current sera should include the requirement for effective post-transplant management.

10.3.2 Flow cytometric crossmatching

Early studies of FCXM showed the method to be more sensitive than conventional CDC crossmatches for the detection of antibody, and the greater sensitivity of flow cytometry and an association of a positive flow crossmatch with graft rejection have been confirmed [20]. The technique has also been shown to be more sensitive than the anti-human globulin augmented CDC crossmatch.

The application of FCXM to specific groups of potential recipients is a matter on which evidence varies. The first clear association between a positive FCXM and graft failure in CDC crossmatch negative kidney allograft recipients was shown in 1987 [21]. This association was significant only in sensitised recipients (those with previous failed grafts or with panel reactive antibodies). In kidney transplantation, positive FCXMs have been associated with complications in both primary and re-grafts. If antibody binds to both T and B cells in the FCXM, it suggests the antibodies detected are likely to be directed at HLA class I antigens or that there is a mixture of class I and II antibodies. A T cell positive FCXM where there is no antibody binding with B cells suggests that the antibody may not be HLA-specific. A T cell negative B cell positive FCXM may be caused by HLA class II antibodies or by antibodies to antigens other than HLA.

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. 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. 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 indicate a significant association between FCXM and graft outcome [22].

10.3.3 Virtual crossmatching (See also 8.3)

The purpose of the pre-transplant crossmatch is to determine the immunological suitability of a donor organ for transplant into a selected recipient. Therefore, a crossmatch should not be required if the recipient has never experienced any potential sensitising events and/or has never produced HLA-specific antibodies. 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, been sensitised to the donor HLA antigens. Some transplant units have allowed a sub-set of patients to be transplanted using a replacement virtual crossmatch to replace the pre-transplant crossmatch with the aim of reducing time to transplant, a practise which has been utilised in cardiothoracic transplantation since the 1980s. This approach has been shown to significantly lower the cold ischaemic time in kidney transplantation [23]. Where a virtual crossmatch strategy is implemented for selected patients, close liaison between the transplant team and the histocompatibility laboratory is essential.

10.3.4 Reporting crossmatch results

When a positive CDC or FCXM is caused by antibody which is IgG, with specificity for HLA, there is a high risk of rejection and/or complications and the risk usually 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.

10.4 Development of HLA-specific antibodies after Kidney/Pancreas transplantation

Following transplantation, *de-novo* HLA-specific antibodies have been identified in both kidney and pancreas allograft recipients [4, 24-25]. Antibodies have been identified either by specifically crossmatching against donor cells or by demonstrating HLA-specific antibody reactivity in antibody screening assays. The proportion of recipients reported to develop antibodies varies between 12% and 60% [26]. A number of factors can 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 or compliance issues with immunosuppressive treatment affect antibody production.

The development of HLA-specific antibodies following kidney transplantation has been shown to be associated with a poorer transplant outcome, and in pancreas transplantation, should be considered in the differential diagnosis of early graft thrombosis and graft

dysfunction [3]. Recipients developing HLA-specific antibodies have a higher incidence of acute rejection and of chronic graft dysfunction than those patients without. Many of the early reports demonstrated the presence of HLA-specific antibodies using CDC assays, but with the availability of new technology, donor-reactive HLA antibodies are more accurately defined. In order to identify *de-novo* production of potentially deleterious antibodies post-transplant it is important to specify reactivity against mismatched donor antigens. Post-transplant 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. Antibodies to mismatched MHC class I related chain A (MICA) antigens have been described in the sera of transplant recipients. MICA molecules have close structural similarity to HLA molecules, but have a different immunological role in that they interact with natural killer cells to regulate immune cell responses. These antibodies may be of particular interest because MICA expression has been demonstrated on kidney tubular epithelia in rejecting allografts [27] and on endothelium *in vitro*, but not on lymphocytes [27,28]. Therefore pre-existing MICA antibodies would not be detected by current crossmatching tests.

While circulating DSA can easily be detected following transplantation, the histological detection of immunoglobulin bound to the endothelium in a transplant is difficult due to several factors. 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. Following the initial report from Feucht and colleagues [29], the presence of C4d on peritubular capillaries of kidney transplant biopsies has been shown to be a marker of humoral rejection and as such an immunohistochemical marker of post-transplant donor reactive antibody responses. However, the sensitivity of the test and the long duration of staining after an initial antibody response need to be taken into account when using this test diagnostically. In-keeping with kidney transplantation C4d positive staining in inter-acinar capillaries has been shown to correlate with AMR, graft damage and a return to insulin therapy due to a loss of C-peptide in pancreas transplant patients [30], and pancreas transplant outcomes have been shown to be worse in the presence of DSA [31]. 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 [32]**.** Circulating donor reactive antibodies detected by post-transplant crossmatching and screening are significantly associated with C4d deposition.

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 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. Since the 2007 report, antibody mediated rejection has gained momentum with the major focus of discussions in the Banff 2011 meeting being about categories of AMR [33].

Since the production of DSA following transplantation is associated with poor outcome, there is a potential benefit to monitoring patients for production of antibody post-transplant. There is some evidence to suggest that the introduction of agents such as mycophenolate mofetil into immunosuppressive regimens decreases antibody production [34], and newer therapies such as Bortezomib and Eculizumab may have a role to play in the future management of antibody positive transplants.

10.5 Recommendations

10.5.1 Kidney and Pancreas Transplantation

1. For patients awaiting transplantation, samples should be obtained and tested at intervals of no longer than three months and after known sensitising events. {2}
2. The laboratory must inform the clinical team whenever more than three months has lapsed since a patient's serum sample has been received, if the patient is active on the transplant waiting list. {2}

10.6 References for Kidney and Pancreas Transplantation

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11 ISLET TRANSPLANTATION

Islet transplantation is 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 or as simultaneous islet and kidney transplants.

11.1 Pre-transplant antibody screening

There is evidence that pre-existing sensitisation to donor HLA is detrimental to survival of islet transplants [1,2]. 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 islet after kidney patient should be considered as unacceptable.

11.2 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 [3]. The incidence of DSA 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 DSA whilst on immunosuppression [4] and in combined kidney and islet transplants the incidence of sensitisation has been reported to be similar to that in kidney transplants alone [5]. The incidence of HLA-specific antibodies has been reported to rise significantly after failure of islet transplants and withdrawal of immunosuppression [6].

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 on a monthly basis until the next transplant. All DSA 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 between the laboratory and the clinical team, it is possible that a transplant may proceed in

the presence of DSA detected only by Luminex technology. It is recommended that samples are taken regularly following the first and any subsequent transplants.

11.3 Crossmatching

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. Antibody-based therapies may cause positivity in the CDC and flow crossmatches leading to complexity in interpretation of a crossmatch result. This is of particular relevance in the case of islet transplants as patients will normally have more than one transplant within a short period. Under these circumstances, virtual crossmatching may not be an option, and a laboratory crossmatch test may be indicated prior to transplantation.

11.4 Recommendations

11.4.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

11.4.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}

2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}
6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

11.4.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

11.4.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}

6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

11.4.5 Islet Transplantation Specific Recommendations

1. Once the patient is listed, samples for antibody analysis should be obtained no less than three monthly. {3}
2. Both cytotoxic and flow cytometry crossmatching are recommended. {3}
3. Samples should be taken regularly following the first and any subsequent transplants. {2}

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12 THORACIC ORGAN TRANSPLANTATION

When assessing the importance of HLA-specific antibodies in cardiothoracic transplantation, similar factors to those required for deceased donor kidney transplantation apply. Prior to the introduction of solid phase assays, particularly bead based assays such as those utilising Luminex X-map technology, access to transplant for sensitised patients was often limited as it was necessary to perform a prospective crossmatch. Given the relatively short cold ischaemia tolerance of the thoracic organs, some 4-5 hours, and prospective crossmatching was not always logistically possible. Since the introduction of Luminex assays, virtual crossmatching has superseded the need for a prospective crossmatch for the majority of sensitised patients awaiting thoracic organ transplantation. However, as a result of the increased sensitivity of the Luminex assays the number of sensitised patients listed for thoracic transplantation has increased such that today approximately 40% of patients on UK thoracic transplant waiting lists are considered to be sensitised.

12.1 Pre-transplant HLA-specific antibodies

Historically the CDC assay was the sole method available for the detection and identification of HLA-specific antibodies and donor-specific crossmatching. Pre-transplant donor HLA-specific antibodies detected by CDC based assays were found to be strongly associated with hyperacute or accelerated rejection of thoracic organ allografts, usually leading to death of the recipient [1,2]. A positive IgG T cell crossmatch is associated with accelerated graft failure for both heart and heart-lung transplant recipients [1]. 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 [1].

Following the introduction of Luminex assays it has become clear that HLA-specific antibodies are present in much higher frequency than was seen with CDC assays. When these assays were introduced the major problem facing cardiothoracic centres and laboratories using these assays was that although low levels of circulating antibodies could be detected, the clinical significance was unclear.

There is also increasing evidence suggesting that HLA-specific antibodies detected by the more sensitive solid phase assays are associated with rejection and decreased graft survival [3-7] after thoracic organ transplantation. Stastny et al have demonstrated that microbead based assay detected DSA are associated with increased graft loss and increased acute rejection in cardiac transplant recipients [4]. Furthermore, a study of 565 cardiac recipients has shown that patients with pre-formed DSA had significantly decreased 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 led to the poorest patient survival [3]. There is some evidence in lung transplantation that pre-existing DSA are associated with poor graft survival following transplantation [8;9]. Smith et al have shown that one year patient survival was significantly reduced in patients with pre-formed DSA.

Following the introduction of Luminex X-map assays for the detection of HLA-specific antibodies, the number of patients on cardiothoracic transplant waiting lists considered to be sensitised has increased [10;11]. Under the auspices of NHSBT-ODT Cardiothoracic Advisory Group a working group was set up to try and establish proposals which would encourage and improve access to transplantation for sensitised patients awaiting cardiothoracic transplantation. The working group has suggested a procedure of risk stratification for sensitised patients based on the MFI levels of detectable HLA antibody specificities. Cumulative MFI values of below 2,000 (i.e. the sum of MFI values of each defined antibody specificity corresponding to donor HLA mismatches) could be considered to confer an additional, although manageable risk of early rejection but with minimal risk of hyperacute rejection. It was felt that the immunological risk from such low levels of DSA could be managed by enhanced immunosuppression with early routine post-transplant HLA-specific antibody monitoring.

For patients with pre-formed DSA in the MFI range 2,000 – 5,000 the risk of hyperacute rejection was considered to be low. Pre-transplant antibody reduction with enhanced immunosuppression and post-transplant antibody monitoring could be used to manage the risk associated with this level of antibody. Above 5,000 the risk of hyperacute rejection would be sufficiently high for this to be a contraindication to transplantation in all but exceptional cases where further testing such as prospective crossmatching and/or complement fixing activity in Luminex assays could be considered.

Prior to listing, patients must be screened for HLA-specific antibodies on at least two independent samples 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 pregnancy, transfusion of blood products, and every 3 months whilst on the waiting list for sensitised patients 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. For non-sensitised patients, samples should be collected every 6 months whilst on the waiting list.

If a potential recipient of a thoracic organ transplant is known to have produced well defined HLA-specific antibodies with no undetermined reactivity, a virtual crossmatch should be performed. However, a lack of accurate information regarding potential sensitising events in these patients means 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 suggests that the virtual crossmatch is an acceptable method for donor/recipient selection for sensitised patients, with comparable outcomes to

patients with no detectable HLA-specific antibodies [11,12]. 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 whether prospective crossmatching is feasible. For those cases where a virtual crossmatch 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 wherever possible.

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. FCXM is a more sensitive technique than conventional CDC crossmatching and has demonstrated a correlation with increased early acute rejection episodes in heart transplantation [13] and severe graft dysfunction in lung transplantation [14]. Flow cytometric crossmatching should be performed for sensitised patients.

12.2 Post-transplant production of HLA-specific antibodies

HLA-specific antibodies, particularly donor HLA-specific antibodies produced following thoracic organ transplantation have deleterious effects on graft outcome [15-19].

It is well established that *de novo* production of DSA after cardiac transplantation is associated with development of AMR [17,20,21]. Zhang et al [21] have shown in a series of 168 cardiac transplant recipients that DSA were detectable in 22 (60%) of 37 patients diagnosed with AMR compared to 6 of 131 (4.6%) patients with no evidence of AMR. Similarly, Ho et al have reported an association between DSA and AMR [15]. In 23 patients with AMR, 21 were found to have DSA detectable by CDC. Furthermore DSA associated with AMR are likely to be complement fixing [17,20].

A consensus report from the International Society for Heart and Lung Transplantation on antibody mediated rejection in cardiac transplantation [22] has recommended that post-transplant monitoring for HLA-specific antibodies be performed at 2 weeks, 1, 3, 6 and 12 months, and annually thereafter, as well as when AMR is suspected.

There is also increasing evidence that *de novo* DSA are associated with the development of cardiac allograft vasculopathy (CAV) [23,24] and bronchiolitis obliterans syndrome after lung transplantation (BOS) [19]. Frank et al have recently shown that patients with DSA directed against class II donor antigens were at increased risk for developing CAV [22]. In lung transplantation Snyder et al have shown in a large single centre study that de novo

HLA-specific antibodies were an independent predictor for development of BOS [19]. Furthermore, Safavi et al have shown that de novo DSA are an independent predictor for earlier development of BOS and that the hazard ratio increases with increasing severity of the disease [25]. Hachem et al treated patients with *de novo* DSA with IVIg and/or rituximab and found improved survival and increased freedom from BOS in patients where DSA had been cleared suggesting that *de novo* DSA are implicated in the development of BOS [26]. *De novo* DSA have also been found to be independent predictors for poor survival following both heart [15] and lung transplantation [19;25].

It is recommended that post-transplant monitoring of patients for the production of HLA-specific antibodies be performed at regular intervals following transplantation, preferably at 1 month, 3, 6, 9, 12 months, and annually thereafter as well as if clinically necessary.

12.3 Non HLA-specific antibodies

Antibodies to a number of non-HLA targets have been associated with adverse outcomes following thoracic organ transplantation. In cardiac transplantation, antibodies directed against endothelial cell antigens have been associated with the development of CAV including vimentin [27], k-alpha-1 tubulin [28] and myosin [29]. Following lung transplantation, antibodies directed against collagen V [30] and k-alpha-a-tubulin [30] have been implicated in rejection and the development of BOS.

It has also been suggested that antibodies to MICA, both pre-existing and produced after transplant, are associated with poor outcomes following heart transplantation [21,31] although it has also been reported that antibodies to MICA have no effect on the outcomes of heart transplant recipients [32].

12.4 Recommendations

12.4.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile

is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}

7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

12.4.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}
2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}
6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

12.4.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

12.4.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual

crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}

4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}
6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

12.4.5 Thoracic Organ Transplantation Specific

1. Prior to listing, antibody screening and, if appropriate, specificity analysis must be performed. {1} Testing two separate samples obtained at different time points is recommended. {2}
2. Samples should be sent from patients on the waiting list for antibody testing at regular intervals; at least three-monthly for previously sensitised patients, and six-monthly for patients who have consistently been negative for HLA specific antibodies. {2}
3. Each positive HLA specificity should be assigned a risk based on its MFI level {2}:

Risk Level	MFI	Description
I	No detectable HLA antibody	Standard
II	<2,000	Minimum risk of hyperacute rejection due to low level donor HLA specific antibodies but greater than standard risk of rejection
III	2,000 - 5,000	Low risk of hyperacute rejection but significant risk of early rejection and antibody mediated graft damage. Immediate pre-transplant antibody reduction may be considered when feasible.

IV	> 5,000	Transplant veto apart from exceptional cases. Further testing such as CDC tests, or complement fixation in Luminex assays (C1q, C3d or C4d) should be considered in these cases to further refine risk profiles

4. The retrospective crossmatch techniques used for sensitised patients above standard risk should include flow cytometry. {3}
5. Following transplantation, patients above standard risk should be tested for HLA-specific antibodies at 7 and 28 days; 3, 6, 9 and 12 months; and then as required. {2}

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13 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. The more recent studies tend to confirm the general resistance of liver grafts to preformed DSA and there is sufficient published information to indicate where pre-existing DSA constitute a risk and how this might be managed. More studies are revealing post-transplant, *de novo* DSA, and while these antibodies can be associated with rejection, their direct or indirect pathogenicity against the liver remains to be proven.

Hyperacute rejection in liver transplantation is unusual [1]. 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 DSA had titres between 1:16000 and 1:32000, which are high by any measure [1].

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 [2,3], while in others a significant association has been reported [4-12]. 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 [13-15].

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 DSA 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 the FCXM no association has been seen [16,17], although it has been reported that high level HLA class I-specific antibodies were associated with steroid-resistant rejections [17].

Evidence shows that HLA class I DSA represent a low risk to liver allografts, and in most cases these do not cause 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 DSA are seen during the early post-transplant period. A recent and importantly, prospective study showed a statistically significant [18] association between even very low pre-transplant DSA levels and clinically significant acute cellular rejection (ACR). This contrasts with the observations from Taner et al [19], again a prospective study, who found no higher rate of ACR in cases of pre-existing DSA. A difference in ACR diagnosis may account for the difference; subclinical ACR may not be associated dependent on prior HLA humoral sensitisation. Graft loss may be prevented by effective management [13,15] 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 DSA 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 [20,21].

Persistence after transplantation has also been shown to be an important factor in the pathogenicity of DSA [13,5]. 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" [15]. Overall, the effect of a positive crossmatch is measurable and stands above the background of other pressures on outcome. DSA is more prevalent and DSA levels (i.e. MFI levels) tend to be higher in patients with chronic rejection (CR) [22] but a high frequency of DSA negative CR cases means that this factor alone cannot be used to guide treatment for potential CR.

Data on the prevalence and clinical significance of *de novo* DSA are limited. Kaneku et al [23] tested one year post transplant sera from a fairly large cohort of liver transplant cases although the significance of their observations is limited due to a large selection bias. They found about 8% of recipients developed *de novo* DSA (mostly against HLA DQ) and this associated with a significantly lower rate of graft survival. Despite this it should be noted that 80% of grafts exposed to DSA detected at about twelve months survived to at least year five. It is likely that the prevalence of *de novo* DSA will increase with time and is dependent on the sensitivity of detection. Further studies including these two parameters are needed before the value of post-transplant antibody can be assessed.

Antibody mediated rejection in liver transplantation is relatively rare, particularly compared to most other forms of organ transplantation. C4d deposition is associated with AMR but can also result from non-alloantibody causes. The coincidence of rejection with DSA and C4d might be taken as substantiation that these antibodies contribute to rejection [24].

Musat et al [25] also suggest C4d deposition in cases with DSA provides evidence for antibody mediated pathology and further show that the greater risk of acute cellular rejection (ACR) is with the combination of DSA positivity and diffuse tubular C4d staining. The association of humoral with cellular immune features is expected given that physiologically these are not due to isolated pathways. O'Leary et al [22] showed improved graft survival patients with pre-formed DSA who received anti-T cell induction therapy.

Simultaneous liver-kidney transplantation is also undertaken in patients with preformed DSA. The received wisdom is that the liver protects the kidney from AMR by removing DSA from the circulation (*simultaneous* being slightly inaccurate; the liver must be connected to the circulation first for this to work). Two recent studies have each shown that while donor class I HLA-specific antibodies are rapidly adsorbed by the donor liver this is not the case for class II-specific antibodies to the extent that there is a high risk of humoral rejection of the kidney and poor outcome in cases with antibodies specific for donor class II HLA [26,27]. The data from O'Leary et al [27] also show that rejection and graft loss of the liver, as well as the kidney, was associated with class II DSA.

13.1 Recommendations

13.1.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

13.1.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}

2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}
6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

13.1.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

13.1.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}

6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

13.1.5 Liver Transplantation Specific

1. Prospective crossmatching is not indicated prior to liver transplantation. {2}
2. HLA antibody testing should be considered at the time of transplant to identify patients at high risk of acute rejection and aid post-transplant management. {2}
3. Patients likely to have simultaneous liver and kidney transplantation should be tested for HLA specific antibodies pre-transplantation. Those with HLA class II specific antibodies are at a significantly higher risk of rejection of both kidney and liver. {2}

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14 INTESTINAL and MULTI-VISCERAL TRANSPLANTATION

There is no definitive evidence for a clinically important role of HLA allosensitisation or the value of ensuring a negative pre-transplant donor-recipient crossmatch before intestinal transplantation. 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 DSA 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. However, 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 [1]. 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 is associated with a positive crossmatch [2-4]. A case of hyperacute rejection following isolated intestinal transplantation has also been reported [5], along with a case of ‘lethal hyperacute rejection’ following small bowel alone transplantation [6]. 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 [7]**.** 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 [8]. More recently a case of immediate antibody mediated hyperacute rejection in a small bowel transplant was recorded in the presence of DSA causing severe ischaemic injury and arteritis [9] but, as noted above, the transplant recovered after prompt intervention with intense immunosuppression and plasmapheresis and the patient was clinically stable more than one year after transplantation.

Post-transplant production of DSA has been described in one case with acute vascular

rejection [2] and the development of HLA-specific antibodies after intestinal transplantation has been associated with acute rejection episodes [10]. However, the incidence of exfoliative rejection has been shown not to associate with a positive crossmatch [11]. Where bowel is transplanted in the absence of a liver, antibodies against all HLA specificities should be considered equally. The risks of transplanting against known DSA should be balanced against the risk to the patient of not transplanting, and the likelihood of the patient receiving an alternative donor with a lower immunological risk within a clinically acceptable time-frame.

14.1 Recommendations

14.1.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

14.1.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}
2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}

5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}
6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

14.1.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

14.1.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}
6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}

9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

14.1.5 Intestinal and Multi-Visceral Transplantation Specific

1. Each positive HLA specific antibody should be assigned immunological risk based on its MFI level. {2}
2. For patients with pre-transplant DSA, the following risk stratification must be applied {3}:

Risk Level	MFI	Description
I	No detectable HLA antibody	Standard
II	<2,000	Minimum risk of hyperacute rejection due to low level donor HLA specific antibodies but greater than standard risk of rejection
III	2,000 - 8,000	Flow Cytometric donor crossmatch likely to be positive, conferring an intermediate risk of humoral rejection.
IV	>8,000	Lymphocytotoxic donor crossmatch (CDC) likely to be positive, conferring a high risk of humoral rejection.

3. A positive donor cytotoxic crossmatch caused by IgG HLA class I specific antibodies indicates a higher risk in intestinal transplantation (in the absence of a liver transplant from the same donor). The final decision to proceed with transplantation will depend on evaluation of the relative risk of proceeding versus the risk of delayed transplantation. {2}

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15 HLA-SPECIFIC ANTIBODY INCOMPATIBLE TRANSPLANTATION

HLA incompatible transplantation, coupled with antibody removal, is mainly applied to live donor transplantation, where a given patient has a potential live donor for which she/he has donor directed antibodies.

In the deceased donor setting, 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 [1]. 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 [2].

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 [3]. Kidney transplantation with a current negative, historic positive crossmatch has become more widely accepted, but is not always successful [4,5]. 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.

Bead based assays for HLA-specific antibody detection 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) [6,7-11]. In most cases residual DSA remain readily detectable using a bead-based assay [6]. CDC crossmatch test negative, bead positive (i.e. virtual crossmatch positive but CDC negative and FCXM negative such that the DSA is only detected in the solid-phase assay) transplants can give good outcome results. This is consistent with outcome results shown for CDC crossmatch negative cases without pre-transplant treatment where DSA was detected using bead assays either prospectively [12] or retrospectively [13].

There is currently no published methodology for quantification of HLA–specific antibodies, but traditionally cytotoxic titre or relative antibody binding measured by flow cytometry have been used. Bead based assays are now used to determine relative antibody levels and these seem to correlate with clinical significance [14]. In HLA antibody incompatible transplantation, higher MFI values pre-treatment or pre-transplant have been associated with rejection [15, 16].

Cytotoxic positive pre-treatment DSA 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 as there is no definite correspondence between these different assays [17]. Measurement of pre-treatment DSA 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 [15,18]. There are numerous approaches to reducing humoral donor-specific reactivity, ranging from *in vivo* agents such as IVIg, anti-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 DSA in question). For this the reader should consult the clinical guidelines for antibody removal developed by The British Transplantation Society (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 DSA levels [9,15,16]. These assays have been able to show early modulation or persistence of post-transplant DSA [15] and where daily testing in the early post-transplant period has been used rapid and dramatic rises (and falls) in DSA have been revealed [19] 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 DSA have been described, although in general re-synthesis of donor-specific antibody and increasing DSA levels associate with rejection [15,17]. As such, the treatment of persisting or rising DSA should depend on the developing clinical situation, any histological information and other risk factors, including the pre-transplant level of DSA.

Bead-based, or other solid phase assays using purified HLA provide the most practical method of DSA 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. In assays with multiple HLA antigen targets, changes in individual antibody specificities can be difficult to determine [19]. Therefore, DSA must be discriminated from other third-party specificities using single antigen assays [20].

15.1 Recommendations

15.1.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

15.1.2 Frequency and Timing of Testing

1. For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. {1}
2. The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). {1}
3. Samples must be sent to the laboratory two weeks following a transfusion. {1}
4. The clinical team must inform the laboratory of other factors that may influence the HLA antibody test results. These include infection, vaccination, and treatment with therapeutic antibodies. {1}
5. Post-transplant samples should be sent to the laboratory when graft rejection is suspected. {2}
6. In higher risk transplants (e.g. donor-specific antibody present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. {1}
7. Serum samples must be stored for potential use in future antibody screening and crossmatch tests. {1}
8. Before transplantation, it is recommended that antibody screening and specificity analysis is performed on two separate samples obtained at different time points. {2}

15.1.3 Interpretation of Data

1. HLA data must be assessed and reported by an appropriately qualified Health and Care Professions Council (HCPC) registered scientist. {1}
2. A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable. {1}
3. If single antigen bead mean fluorescence intensity (MFI) values are used to determine risk, cumulative values for all DSA must be calculated. Where a donor is homozygous for a mismatch the corresponding MFI must be doubled. {2}

15.1.4 Crossmatching

1. A prospective crossmatch must be performed (except for liver transplants). {1}
2. The crossmatch may be undertaken by carrying out a laboratory crossmatch test {1} or, in selected cases, by performing a virtual crossmatch. {2}
3. Patients with no antibodies, or those with fully defined HLA-specific antibodies can be transplanted without a prospective laboratory crossmatch test provided the virtual crossmatch is negative i.e. the donor does not carry those HLA specificities to which the patient is sensitised. {1}
4. Patients with a complex antibody profile or incompletely defined antibody profile should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). {1}
5. If a virtual crossmatch is performed, a retrospective laboratory crossmatch test should be performed using serum collected within 24-48 hours prior to transplantation. {3}
6. Laboratory crossmatch tests should distinguish between donor T cell and B cell populations; they must detect clinically relevant IgG HLA class I and class II donor specific antibodies, and distinguish these from IgM. {1}
7. Serum samples used for crossmatching must include the current sample and consider samples or results from the patient's serological history. {1}
8. All crossmatches must be assessed and reported by an appropriately qualified HCPC registered scientist. {1}
9. The report must include appropriate advice on the crossmatch results in the context of the patient's antibody profile. {3}

5.10 HLA Antibody Incompatible Transplantation

1. The HLA-specificity and level of DSA must be fully determined prior to antibody reduction. {1}
2. Crossmatching by CDC must be used to identify the immunological risk of the transplant. {1}
3. DSA levels must be monitored regularly throughout the duration of treatment to determine its effectiveness. {1}

15.2 References for HLA Antibody Incompatible Transplantation

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16 HAEMATOPOIETIC PROGENITOR CELL TRANSPLANTATION

Until recently the impact of HLA antibodies on haematopoietic progenitor (stem) cell engraftment has been unclear. Opinion was formed from contradictory case study reports in the literature with few cases available for analysis because of the matching criteria inherent in HLA matched related and unrelated donor transplants. The use of HLA mismatched cord blood and related haplo-identical donors has led to more transplants being performed where the patient has antibodies directed against HLA specificities present in the donor. Recent studies indicate that DSA in the recipient is a significant risk factor for transplant non-engraftment [1,2].

In a Japanese study of 374 cord blood transplants, 16.4% (41/250) of patients aged between 16 and 74 years transplanted for malignancies had HLA antibodies [3]. Of those patients, eight had antibodies against HLA antigens present in the transplanted cord blood. Engraftment for patients with HLA antibodies but not against antigens present in the transplanted cord blood unit (CBU) was 93.6% with a median time to engraftment of 21 days. However when the HLA antibody was directed at donor antigen, engraftment fell to 58% (p=0.017) with a median time to engraftment of 46 days.

A National Marrow Donor Program study looking at failed haematopoietic stem cell transplants, found that the presence of recipient HLA antibodies reactive to donor HLA antigens was associated with an increased risk of non-engraftment (OR 22.8, p=0.0002) [4].

A further American study of 73 double cord blood transplants revealed that 18 of the patients had DSA [5]. Nine patients had DSA directed at the first infused CBU and two had DSA against the second infused CBU. Seven patients had DSA against both cord units infused and four patients had multiple DSA antibodies also reactive with both cords infused. The study links important clinical consequences to DSA.

The clinical associated complications were:

- An increased incidence of graft failure
  - (5.5 vs 18.2 vs 57.1% for none, single or dual DSA positivity, p=0.0001)
- prolongation of the time to neutrophil engraftment
  - (21 vs 29 days for none vs. any DSA, p=0.04)
- excess 100-day mortality or relapse
  - (23.6 vs 36.4 vs 71.4% for none, single or dual DSA positivity, p=0.01)
- The intensity of DSA reactivity was correlated with graft failure
  - (median of mean fluorescent intensity 17650 vs 1850, p=0.039)

These studies indicate that in HLA mismatched progenitor cell transplants, HLA-DSA in the recipient should be considered as a significant risk factor for non-engraftment and that HLA

antibodies screening of the patients prior and following transplantation may be a useful tool to follow up the outcome of these transplants.

16.1 Recommendations

16.1.1 Policies and Strategy

1. Laboratories must have procedures in place for the detection and characterisation of HLA specific antibodies. {1}
2. Laboratories must be able to define HLA-A, B, Cw, DR, DQ and DP antibody specificities. {1}
3. The techniques adopted must be able to differentiate IgG from IgM antibodies and define antibody specificity. {1}
4. Laboratories must employ methods to abrogate known causes of false positive or negative results. {1}
5. At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. {2}
6. HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g. following a sensitising event or following a change in the antibody screening test results. {1}
7. A combination of tests should be considered in order to fully resolve complex antibody profiles. {2}
8. Laboratories should be aware of newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments. {3}

16.1.2 Haematopoietic Progenitor Cell Transplantation

1. In selecting HLA mismatched donors HLA antibody testing of the recipient should be performed at the time of donor selection and at the time of transplantation if there is a significant time lapse. {3}
2. The clinical team must be made aware of any HLA antibody incompatibility detected in the recipient. {3}
3. It is recommended that HLA antibody testing is performed in cases of non-engraftment. {3}

16.2 References for Haematopoietic Progenitor Cell Transplantation

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17 APPENDICES

17.1 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 BSHI Diploma) and have attained registration with the HCPC. 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 HCPC 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 Directive. 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 continuing education activities.

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.

17.2 Glossary

ABO	Genetically-determined polymorphism of specific carbohydrates carried by red blood cells and other tissues.
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.
Allele	A genetic variant that can be defined at the DNA or protein level.
Allograft or allotransplant	A transplant between members of the same species e.g. between humans.
Antibody	Serum immunoglobulin expressed by B cells and secreted by plasma cells that recognises a specific antigen.
Antibody dependent cell mediated cytotoxicity (ADCC)	Cytotoxic reaction whereby the effector activity is provided by Fc receptor expressing cells (e.g. macrophages, natural killer cells) that recognise antibody coated targets.
Antibody removal	An intervention to reduce circulating donor-specific antibody to a level that allows transplantation (high immunological risk).
Antigen presenting cell (APC)	Specialised immune system cells which present degraded antigen in the form of peptides complexed with MHC molecules.
Antigen	Any substance that is recognised by an immune system.
Anti-idiotypic antibody	An antibody with specificity for that part of another antibody which binds antigen.
Autoimmunity	An immune response to self antigens, tissues and organs which can result in serious illness such as type 1 diabetes or rheumatoid arthritis.

Autoreactive lymphocytotoxic antibodies	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.
B cells	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.
Banff criteria	An internationally recognised system of grading pathology in biopsy specimens from a kidney. Used to diagnose and grade rejection.
British Renal Society	www.britishrenal.org
British Society for Histocompatibility & Immunogenetics	www.bshi.org.uk
British Transplantation Society	www.bts.org.uk
Bronchiolitis obliterans syndrome	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.
C4d	A product of activation of the classical complement system.
Calcineurin inhibitor	An immunosuppressive drug (ciclosporin, tacrolimus) which acts by blocking immune cell activation by the calcineurin pathway.
Calculated reaction frequency	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).
CD4, 8, 20, 28, 40, 80, 154, etc.	Cell surface molecules defined by specific monoclonal antibodies (Cluster of Differentiation) and recognised by an international standardisation body http://ca.expasy.org/cgi-bin/lists?cdlist.txt
Chemotaxis	Modified movement of cells due to a concentration gradient of a secreted substance.
Chronic rejection	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).
Class switch	Antigen driven process by which a B cell actively and irreversibly changes the isotype but not the specificity of the antibody it expresses.

Cold ischaemia time	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.
Complement	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.
Complement dependent cytotoxicity (CDC)	A laboratory test to identify presence of antibodies in a serum sample using lymphocytes as targets and cell viability as the read-out.
Coagulation	Of red blood cells to form a thrombus (clot) mediated by antibodies.
Co-stimulatory molecule	Cell surface ligand or receptor providing a non-specific signal which is necessary for an antigen-specific response by T or B cells.
Crossmatch test (XM)	A test to identify antibody mediated reactivity to target antigens in a potential organ donor. The test report must be either positive or negative.
Cross-reactive	An antibody which is able to bind to a series of structurally related antigens.
Cytokine	A chemical secreted by an immune cell which may either enhance or suppress an immune response.
Desensitisation	Removal of antibodies which are indicative of sensitisation.
Differentiation	A process of specialisation of cells and tissues to become a functional organ or system. A one way step.
Dithiothreitol	A chemical used in laboratory assays to dissociate the pentameric IgM molecule and abrogate its activity.
Endothelial cells	Cells which line the blood vessels.
Enzyme linked immunosorbent assay (ELISA)	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.
Epitope	That part of the antigen structure to which antibody binds.
Epstein Barr virus (EBV)	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.
Fc receptor	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.

Flow cytometer	Equipment using laser technology and a fluorescent stain coupled to a detection antibody and is a highly sensitive, semi-qualitative technique.
Guideline	A statement intended to offer advice of how to proceed. Based on published evidence or established best practice.
Haematopoietic stem cell	A cell which can potentially differentiate into all blood lineage cells.
Health and Care Professions Council	The UK regulatory body overseeing registration of Healthcare Scientists; www.hpc.org.uk.
Highly sensitised patient (HSP)	NHSBT-ODT definition of a patient who has developed HLA-specific antibodies against 85% of a pool of 10,000 blood group matched donors.
Histocompatibility	The degree of similarity between cells, tissues and organs of donors and recipients assessed by HLA antigen typing and matching.
Histocompatibility laboratory	A highly specialised laboratory staffed by Healthcare Scientists who perform tests to facilitate effective organ, tissue and stem cell transplantation.
Historic serum sample	A previously collected serum sample from a patient.
HLA	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.
HLA class I molecules	HLA-A, -B and –Cw molecules with structural and functional similarity. Expressed by almost all nucleated cells.
HLA class II molecules	HLA-DR, -DQ and –DP molecules with structural and functional similarity. Constitutively expressed only on specialised antigen presenting cells but may be inducible.
Humoral	Of the blood. Usually used to indicate an antibody mediated response (c.f. cellular response).
Hyperacute rejection	Rejection of a transplant within a very short time of transplantation (minutes) typically caused by pre-exisitng donor-specific antibodies Usually results in irreversible failure.
Immunogenicity	The degree to which a substance can provoke the immune system to respond e.g. high or low.
Immunoglobulin isotype	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.
Institute of Biomedical Scientists	The professional body for Biomedical Scientists www.ibms.org

Interleukin (IL)	Leukocyte secreted cytokine that affects the growth or development immune system cells. E.g. IL-2, -4, -5, -6.
Islets	Cluster of cells within the pancreas containing the beta cells which secrete insulin
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).
Lymphoblastoid 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.
Mean Fluorescence Intensity (MFI)	A semi-quantitative readout of the degree of antibody binding, indirectly measured by a fluorescent label.
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.
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.
Natural killer (NK) cell	Mononuclear leukocyte with innate ability to kill certain tumours and virally infected cells.
NHSBT-ODT	NHS Blood and Transplant – Directorate of Organ Donation and Transplant www.nhsbt.nhs.uk, www.organdonation.nhs.uk
Panel reactive antibodies (PRA or % PRA)	The calculated % of a panel of lymphocytes with which a patient's serum reacts. This has now been replaced in the UK by the calculated reaction frequency.
Peritubular capillaries	Small blood vessels located in the kidney adjacent to the structures (nephron) which filter the blood.

Plasma cell	End stage cell of B lymphocyte lineage that secretes immunoglobulin.
Plasma exchange or plasmapheresis	Removal or dilution of plasma to lower the amount of circulating antibody.
Platelets	Small, irregular blood borne anucleate cells which are an important component of a thrombus (clot).
Primary response	The reaction of the immune system at the time of its first exposure to a novel antigen.
Recommendation	A guideline which should usually be adhered to.
Rescue therapy	A treatment aimed to prevent failure of a transplanted organ in the face of an aggressive immune response.
Rituximab	Brand of therapeutic chimeric CD20-specific monoclonal antibody.
Royal College of Pathologists	www.rcpath.org
Secondary response	An enhanced immune response mounted on re-exposure to a previously recognised antigen.
Sensitisation	An immune response to an antigen resulting in T and/or B cell memory.
Sensitivity (of a patient)	The ability to mount an immune response to an antigen.
Sensitivity (of an assay)	An evaluation of the accuracy of the results of a laboratory test to predict an outcome. Usually quoted as a percentage.
Single antigen beads	A multiplex of microbeads each identifiable group being loaded with a single HLA antigen
Solid phase assays	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.
Specificity	The defined reactivity of an antibody e.g. specific for an HLA molecule.
T cell	Mononuclear leukocyte having developed in the thymus.
Titre	Reciprocal of the last dilution of a serum giving a detectable reaction.
Unacceptable antigen	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.

Vascular rejection	An aggressive antibody mediated immune response with activity detected in the blood vessels of the transplant.
Virtual Crossmatch	A comparison of a donor's HLA type with the patient's known HLA antibody profile.
xMAP Technology	Luminex xMAP technology uses a series of microspheres which can be individually identified by up to 500 unique dye mixtures. The microspheres are flexible and can be labelled with antigen, to detect the presence of antibody, or coupled to nucleic acids for use in probing assays. Test results are analysed using a Luminex 100/200 instrument, or a Luminex FlexMAP 3D. www.luminexcorp.com