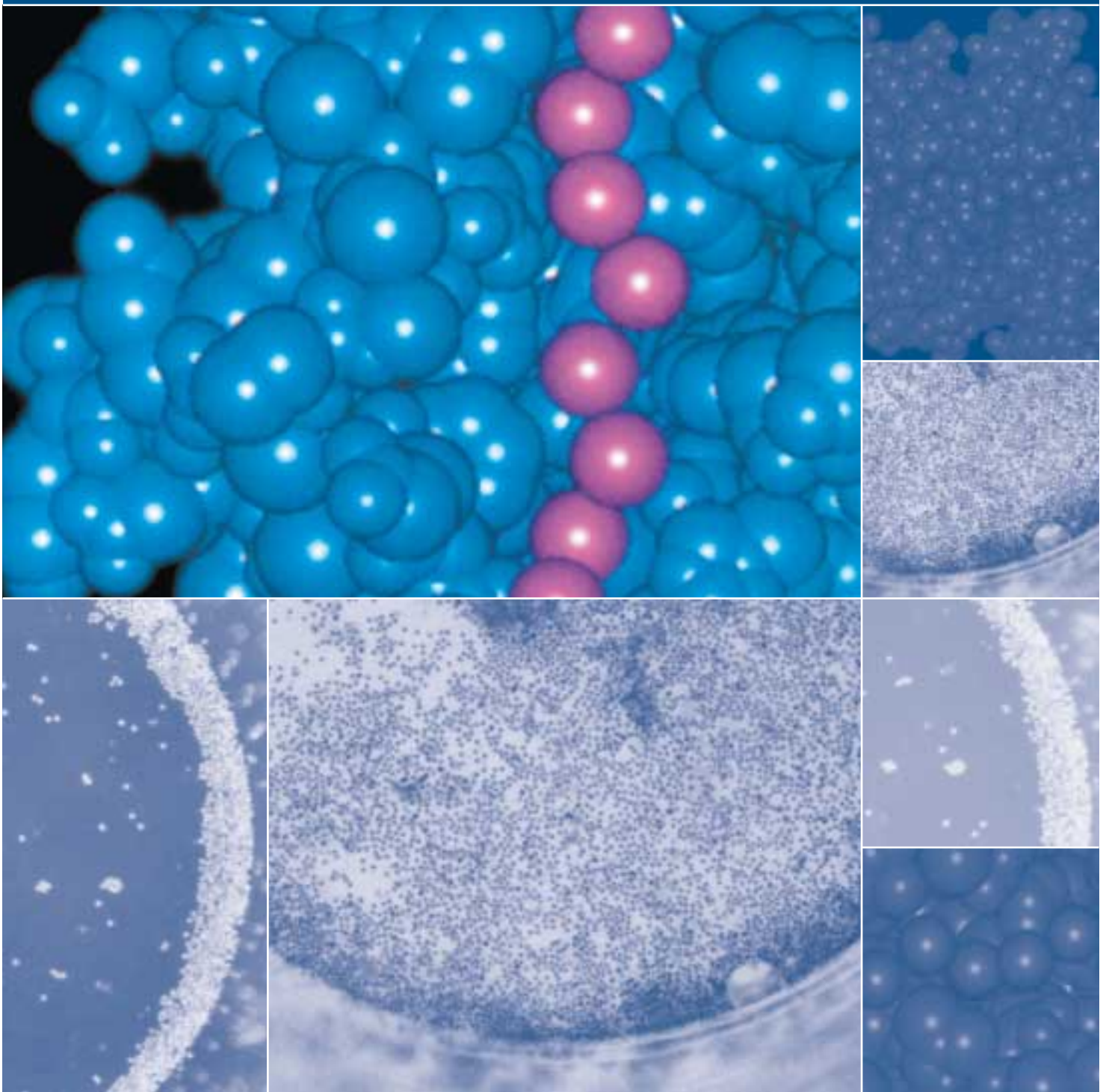


# Guidelines for the detection and characterisation of clinically relevant antibodies in solid organ transplantation

August 2004



# Guidelines for the detection and characterisation of clinically relevant antibodies in solid organ transplantation

A collaboration between: British Society for Histocompatibility and Immunogenetics and British Transplantation Society

The British Society for Histocompatibility and Immunogenetics is the professional body for Healthcare and Basic Scientists supporting clinical transplantation and transplantation research.  
[www.bshi.org.uk](http://www.bshi.org.uk)

The British Transplantation Society is the professional body for Surgeons, Physicians, Transplant Coordinators, Nurses, Healthcare Scientists, Allied Health Professionals and Basic Scientists actively working in clinical transplantation and transplantation research.  
[www.bts.org](http://www.bts.org)



Published by:

British Society for Histocompatibility & Immunogenetics,  
PO Box 134, Leeds, LS9 7WQ

and British Transplantation Society,  
Triangle House, Broomhill Road, London, SW18 4HX

ISBN: 0-9542221-6-4

Designed and produced by indigo 020 8858 5100

## Contents

<b>NEED FOR GUIDELINES</b>	<b>02</b>	<b>KIDNEY AND PANCREAS TRANSPLANTATION</b>	<b>14</b>
<b>WRITING COMMITTEE</b>	<b>03</b>	Pre-transplant Antibody Screening	14
Clinical reviewers	03	Definition of Unacceptable Mismatches	15
Commentators	03	The Clinical Relevance of Crossmatching	15
		– The CDC crossmatch	15
		– Flow cytometric crossmatching	16
		– Omitting the pre-transplant crossmatch	16
		– Reporting crossmatch results	17
<b>OVERVIEW, RECOMMENDATIONS AND BEST PRACTICE</b>	<b>04</b>	Development of HLA specific antibodies following renal transplantation	17
Immunological risk	04	Recommendations	18
Recommendations	04		
– Kidney and pancreas	04	<b>THORACIC ORGAN TRANSPLANTATION</b>	<b>19</b>
– Cardio-thoracic organs	05	HLA specific antibody screening and crossmatching prior to transplantation	19
– Liver and intestine	05	Post-transplant production of HLA specific antibodies	19
– Antibody removal	05	Non-HLA specific antibodies	20
		Recommendations	20
<b>INTRODUCTION</b>	<b>06</b>		
The Immune System	06	<b>LIVER TRANSPLANTATION</b>	<b>21</b>
– T cell recognition of foreign antigens	06	Recommendations	21
– Effector cell activation and functions	06		
– Control of Allorecognition	07	<b>INTESTINE TRANSPLANTATION</b>	<b>22</b>
Priming sources	07	Recommendations	22
Acute Antibody Mediated Rejection Mechanisms	07		
– Hyperacute rejection	07	<b>ANTIBODY REMOVAL</b>	<b>23</b>
– Acute rejection	07	Immunosuppressive drugs	23
		Plasmapheresis	23
<b>DEFINING RISK</b>	<b>08</b>	Immunoabsorption	24
		Intravenous immunoglobulin	24
<b>IDENTIFICATION OF HLA SPECIFIC ANTIBODIES</b>	<b>09</b>	Recommendations	25
Complement dependent cytotoxicity	09		
Enzyme-linked immunosorbent assays	09	<b>APPENDICES</b>	<b>27</b>
Flow Cytometry	09	1] Laboratory resources and relationship	27
Screening strategies	10	2] Glossary	28
Collection and storage of samples for antibody screening and donor crossmatching	10	3] References	37
– Pre-transplant	10	4] Suggested audit standards	47
– Post-transplant	11	5] Statements of potential conflicts of interests	48
– Sample storage	11		
Recommendations	11		
<b>CROSSMATCHING</b>	<b>12</b>		
Crossmatch Techniques	12		
Results	12		
Recommendations	13		

## Need for guidelines

A positive crossmatch test report is perceived as disastrous by the potential recipient since it vetoes transplantation. Understandably, patients and clinicians question the conditions which define a positive crossmatch. Histocompatibility laboratory staff are constantly re-defining crossmatch boundaries to allow successful transplantation of sensitised patients. These guidelines are published at a time when the ability of Histocompatibility laboratories to both detect and define sensitisation has seen significant improvements over long-established technologies. Current techniques of ELISA and flow cytometry provide the breakthrough in standardisation and quality control, which has been long awaited. The aim of this document is to place the output from these technologies in the clinical context to support transplant surgeons and physicians in making their assessment of the immunological risk should a transplant proceed. With donated organs being an increasingly rare resource their best use is paramount and the dramatic correlation of a positive crossmatch with hyperacute rejection must never be ignored. It follows that careful definition of the conditions defining a positive crossmatch is also of paramount importance.

02

## Writing committee

Executive bodies from the BSHI and BTS asked the persons below to compile these guidelines. Two meetings were chaired by Andrea Harmer, firstly a planning meeting to delegate writing tasks and secondly a review meeting to discuss the manuscripts. Andrea Harmer compiled the final draft. Three clinical colleagues made comments on the final draft. Comments from BSHI and BTS Members were invited by placing the final draft on the Society websites for a four week period. Finally, Phil Dyer incorporated comments and finalised the document for publication.

### Andrea Harmer PhD, MRCPATH

Consultant Clinical Scientist

Histocompatibility & Immunogenetics Laboratory,  
National Blood Service, Longley Lane, Sheffield S5 7JN

Writing Committee chairperson

### David Briggs PhD

Consultant Clinical Scientist

Histocompatibility & Immunogenetics Laboratory,  
National Blood Service, Vincent Drive, Edgbaston,  
Birmingham B15 2TT

### Philip Dyer PhD, FRCPath

Consultant Clinical Scientist and Honorary Reader  
in Transplantation Science

Transplantation Laboratory, Manchester Royal  
Infirmary, Manchester, M13 9WL

President of the British Transplantation Society

### Susan Fuggle DPhil, FRCPath

Consultant Clinical Scientist

Transplant Immunology Laboratory, Oxford  
Transplant Centre, Churchill Hospital, Oxford, OX3 7LJ

### Susan Martin PhD, FRCPath

Consultant Clinical Scientist and Honorary  
Senior Lecturer

Transplantation Laboratory, Manchester Royal  
Infirmary, Manchester, M13 9WL

### John Smith PhD

Principal Clinical Scientist

Tissue Typing Laboratory,  
Heart Science Centre, Harefield Hospital, Harefield,  
Middlesex UB9 6JH

### Craig Taylor PhD, FRCPath.

Consultant Clinical Scientist,

Addenbrooke's Hospital, Hills Road,  
Cambridge CB2 2QQ

Chairman of the British Society for  
Histocompatibility and Immunogenetics

### Robert Vaughan PhD, FRCPath

Consultant Clinical Scientist

Tissue Typing Laboratory, New Guy's House,  
Guy's Hospital, London SE1 9RT

## Clinical reviewers:

### Dr Charles Newstead

Consultant Renal Physician

Renal Unit, St James's University Hospital,  
Beckett St, Leeds LS9 7TF

Chairman BTS Standards Committee

### Dr Anthony Warrens

Consultant in Nephrology & Immunology

Immunology & Renal Medicine, Imperial College,  
Hammersmith Hospital, Du Cane Road,  
London W12 0NN

### Mr Christopher Watson

Consultant Transplant Surgeon

Addenbrooke's Hospital, Hills Road,  
Cambridge. CB2 2QQ

## Comments submitted by:

**Terry Horsburgh**

**Jane Matthews**

**Derek Middleton**

**Judith Worthington**

03

# Overview, recommendations and best practice

## Immunological risk

Gebel et al [10] have suggested three broad risk categories for a given donor-recipient combination (adapted below):

- 1) High immunological risk is indicated when there are high titre circulating antibodies specific for mismatched donor HLA antigens present at the time of transplantation. In most cases the high risk of hyperacute rejection would constitute a veto to transplantation. Some centres may advocate carefully planned pre-transplant desensitisation regimes together with close post-transplant immunological monitoring.
- 2) Intermediate immunological risk is indicated by the presence of risk factors such as prior donor reactive sensitisation (that is absent at the time of transplantation) and in selected donor-recipient combinations with only weak sensitisation to certain mismatched HLA specificities. In such cases, it may be justified to consider augmented immunosuppression and post-transplant immunological monitoring.
- 3) Low (or standard) immunological risk is indicated when non-sensitised patients or sensitised patients receive a 'favourably' HLA matched organ in absence of current or historical donor reactive antibodies.

04

## Recommendations

### Kidney and pancreas transplantation:

- Laboratories must have a strategy in place for the detection and characterisation of clinically relevant antibodies.
- The techniques adopted must have the capability of defining antibody class and specificity.
- As no single technique can provide complete information, a combination of methods is recommended. This may have cost implications for laboratory services.
- Laboratories should have a programme for investigating and evaluating newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments.
- Patient serum samples must be sent to the histocompatibility laboratory no less than three-monthly for routine antibody monitoring and at 14 days and 28 days following transfusion of any blood products.
- Post-transplant serum samples should be obtained monthly for the first three months, quarterly up to one year and annually thereafter.
- Serum samples must be stored indefinitely for potential use in future antibody screening and crossmatch tests.
- Crossmatch tests should be capable of distinguishing T cell and B cell populations and should discriminate between IgG and IgM antibodies.
- Serum samples used for crossmatching must include a current sample and, where HLA specific antibodies have been detected, samples that are representative of the patient's antibody profile, over time.
- The reporting of results to clinical teams should include appropriate advice on the clinical relevance of the result.

- Laboratories providing services for renal transplant programmes must have the capability of precisely defining HLA-A, -B, -C, -DR and -DQ antibody specificities in their patients so that donors who will be crossmatch negative can be identified.
- HLA antigens to which a patient has produced antibodies should be listed as 'unacceptable mismatches'.
- A pre-transplant crossmatch should be performed for all patients unless a programme exists for identifying those individuals who can confidently be defined as unsensitised.
- Sensitised patients should be crossmatched using flow cytometric techniques.
- Post-transplant antibody monitoring is recommended to help identify patients at increased risk of graft rejection/loss and to aid in determining the requirements for immunosuppression.
- It is the responsibility of the clinical team to inform the laboratory of potential allo-sensitising events.

### Cardio-thoracic organs:

- Laboratories must be able to precisely define antibodies specific for HLA -A, -B, -Cw, -DR and -DQ.
- Unacceptable mismatches should be defined as those HLA antigens to which antibodies have been detected.
- Patients should be screened for HLA specific antibodies at regular intervals, especially following sensitising events, both pre- and post-transplantation.
- Prospective crossmatching for all sensitised patients should be performed.
- Sensitised patients with fully defined HLA specific antibodies and with no residual reaction frequency may be transplanted without a prospective crossmatch provided the donor does not carry those HLA specificities to which the patient is sensitised.

- A retrospective crossmatch using serum collected within 24 hours prior to transplantation should be performed.
- Crossmatch techniques should discriminate between IgG and IgM as well as T and B cells as targets.
- Post transplant antibody monitoring is recommended to help identify patients at increased risk of graft rejection/graft loss.

### Liver and intestine:

- Prospective crossmatching is not indicated for recipient selection.
- Identification of donor-specific antibody can be used to identify patients at high risk of acute rejection and chronic graft loss and to aid post-transplant management, such as changes in immunosuppression regimen or antibody removal.

### Antibody removal:

- The HLA specificity and titre of antibodies should be fully determined prior to antibody removal.
- Antibody titre must be monitored regularly throughout the duration of treatment to determine its effectiveness.
- Antibody removal should only be undertaken following establishment of a clinical protocol.

05

## 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 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 cellular rejection, which is difficult to control using conventional immunosuppressive agents. Patient exposure to alloantigens of another individual is a common occurrence and takes place through pregnancies, blood transfusions and rejection of a previous transplant. An audit of the UK National Kidney Transplant List in 2003 showed that 20% of patients awaiting a first transplant and 77% of patients registered for a re-graft were sensitised. **A critical function of Histocompatibility Laboratories that support transplantation programmes is to select a suitable donor to which the recipient is not sensitised.**

## The Immune System

### T cell recognition of foreign antigens

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

Antigen specific T cell clones with T cell receptors (TCR) that recognise foreign peptide bound to self-HLA engage the antigen presenting cells (APC). In the presence of co-stimulatory molecules present on mature APC's (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.

### Effector cell activation and functions

The cytokine profile secreted by T helper cells directs 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, IL-13 induces activation and differentiation of antigen specific B cells. In the presence of these cytokines, naïve B cells that express cell surface IgM undergo immunoglobulin class switching so that high affinity IgG antibodies can be produced. Under T cell help, B cells differentiate into antibody producing plasma cells with the initial production of IgM antibodies and subsequently IgG antibodies due to class switching; and into memory B cells that respond rapidly upon repeat exposure to the same antigenic stimulus (figure 1). Antibody binding to its target antigen facilitates opsonisation by phagocytes, and chemotaxis and lysis via the classical complement pathway.

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

Helper T cells can initiate cellular immune responses by the activation of antigen specific

cytotoxic T cells and are also involved in the non-specific activation of macrophages and natural killer cells causing cell-mediated cytotoxicity. Currently there is no routine assay of T cell sensitisation.

### Control of Allorecognition

In kidney transplantation, attempts are made to reduce the immunogenicity and alloantigen load through HLA matching of donors to recipients. This is effective at reducing the number and severity of acute rejection episodes and is also translated into improved long-term graft survival [1]. 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.**

### Priming sources

Exposure of an individual's immune system to alloantigens of another individual by pregnancy, blood transfusion and transplantation can result in immunological priming. HLA specific antibodies have been observed in patients in the absence of the above priming sources. Idiopathic HLA specific antibodies are usually IgM and may arise through cross-reactivity with infectious micro-organisms. It has been suggested that the use of leucodepleted 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 [2]. **The risk of sensitisation after blood transfusion is highly variable and is influenced by recipient factors such as genetic control of the immune response, storage time of transfused blood and previous exposure to alloantigens.**

## Acute Antibody Mediated Rejection Mechanisms

### Hyperacute rejection

Circulating antibodies which bind to donor 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, 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 [3]. Perfusion of kidneys with plasma containing antibodies directed against HLA antigens present in the kidney has been shown to cause hyperacute rejection [4].

### Acute rejection

Whilst acute rejection is regarded as primarily a cell mediated process there is some evidence for antibody involvement. The onset of acute rejection may be accompanied by the appearance of antibodies specific for donor HLA antigens [5]. 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 [6] and the demonstration of plasma cells amongst infiltrating cells recovered from failed renal grafts [7] indicates that both cellular and humoral responses may be present in acute rejection. Further evidence comes from the identification of C4d in biopsies obtained from kidney and heart transplants undergoing acute rejection [8]. C4d is a product of complement activation indicating antibody dependent activation.

Antibodies may also initiate graft damage by the mechanism of antibody dependent cell-mediated cytotoxicity (ADCC). Graft infiltrating cells have been shown to mediate cellular lysis of antibody coated cells [9] and antibodies eluted from rejected grafts have been found to mediate ADCC activity to donor cells [6].

## Defining risk

**Recent advances in immunosuppressive therapy have enabled successful transplantation in some sensitised recipients and it is important for clinicians and histocompatibility scientists to identify the immunological risk and understand its clinical significance.** Gebel et al [10] have suggested three broad risk categories for a given donor-recipient combination (adapted below):

- 1) High immunological risk is attributed when there are high titre circulating antibodies specific for mismatched donor antigens present at the time of transplantation. In most cases the high risk of hyperacute rejection would constitute a veto to transplantation although some centres may advocate carefully planned pre-transplant desensitisation regimes together with close post-transplant immunological monitoring.
- 2) Intermediate immunological risk may be considered in the presence of risk factors such as prior donor reactive sensitisation (that is absent at the time of transplantation) and in selected donor-recipient combinations with only weak sensitisation to certain mismatched HLA specificities. In such cases, it may be justified to consider augmented immunosuppression and post-transplant immunological monitoring.
- 3) Low (or standard) immunological risk may be attributed to non-sensitised patients or to sensitised patients receiving a 'favourably' HLA matched organ in absence of known current or historical donor reactive antibodies.

## Identification of HLA specific antibodies

### Complement dependent cytotoxicity

The first established method for the detection and definition of HLA specific antibodies was the complement dependent cytotoxicity (CDC) test that employs lymphocyte targets to detect complement-fixing IgG and IgM antibodies. The CDC test is used widely in both antibody screening and crossmatching protocols. Despite its widespread use the CDC assay has several inherent problems [11]. An adequate cell panel can be difficult to obtain, test specificity and sensitivity are influenced by:

#### cell viability

#### the rabbit complement used

Furthermore, only complement-fixing antibodies are detected but these may not be HLA specific. Results from the CDC test are presented as the percentage of the cell panel with which a serum has reacted (%PRA). The "% PRA" is absolutely dependent on the composition of the cell panel. For example, a serum from a patient with a monospecific antibody to HLA-A2, that is present at high frequency in the population, may react with 50% of a random cell panel but with a much lower percentage of a carefully selected panel. **PRA results cannot therefore be compared between cell panels or between laboratories and do not necessarily reflect a patient's level of sensitisation to the potential donor pool.** This problem is compounded by false positive results due to the presence of autoreactive lymphocytotoxic antibodies which could give "100% PRA" but are probably irrelevant to transplant outcome [12]. Hence **patients should not be defined as sensitised simply on the basis of "%PRA"**. Firstly, autoantibodies must be removed by absorption with autologous lymphocytes and / or treatment of the serum with dithiothreitol to remove IgM antibodies. Secondly, **the HLA specificities of the antibodies must be defined.**

The detection of IgM antibodies in the CDC test is seen as a disadvantage and dithiothreitol is used to remove 'false' positive autoreactivity due to IgM. However, it will also remove reactivity due to IgM alloantibodies as well as autoantibodies. A number of developments in ELISA and flow cytometry assays have significantly improved the detection and

characterisation of not only IgG but also IgM alloantibodies in sensitised patients.

### Enzyme-linked immunosorbent assays

ELISA-based tests use soluble HLA antigens as targets and have advantages over CDC [13,14]:

**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**

There are two types of ELISA based assays which are commercially available. The first detects the presence or absence of HLA specific antibody. Large studies of this type of ELISA [15] have found that they reliably detect IgG HLA specific antibodies although they may be less reliable for IgM. The second type of ELISA defines antibody specificity. These tests have the advantage that when positive reactions are ranked according to optical density then specificities cluster. This facilitates analysis of sera from sensitised patients with a high reaction frequency [16,17]. **The sensitivity of ELISA test kits is higher than CDC but lower than flow cytometry.**

### Flow Cytometry

Flow cytometry screening tests were originally developed using cell pools designed to cover all major HLA specificities or serological cross reactive groups. Cells from chronic lymphocytic leukaemia (CLL) patients [18], Epstein Barr Virus (EBV) transformed lymphoblastoid cell line cells [19,20] and peripheral blood lymphocytes (PBL) have all been used [21,22].

Screening sera using flow cytometry with individual cell panels is cumbersome for large-scale use so the option of pooled cells offers advantages. It allows for a large number of sera to be tested over a short period and is still more sensitive than CDC. Antibody positive sera are then investigated to define specificity using flow cytometry against individual

## Identification of HLA specific antibodies

cell panels, ELISA and CDC. Flow cytometry does not detect IgM autoreactive antibodies. However, non-HLA specific IgG antibodies will be detected using a flow cytometry technique with cell targets and that must be considered in the interpretation of results.

A flow cytometry method using microparticles coated with soluble HLA antigens to detect alloantibodies has been described [23]. The presence of HLA class I specific and/or class II specific antibodies in a serum can be determined in a single test using this method [24]. Following the same principles, HLA antigen coated microparticles are available that enable the separate definition of antibodies to HLA-A, B, Cw and HLA-DR, DQ, DP. Studies have shown these microparticles to be more sensitive and more specific than CDC for the detection of HLA specific antibodies [16]. A further advance has been the development of microparticles coated with a single antigen. It is probable that these will facilitate antibody specificity definition and in particular will enable the identification of HLA specificities to which a highly sensitised patient is not sensitised termed “acceptable mismatches” [25,26].

Commercially available ELISA and flow cytometry assays are primarily designed to detect IgG antibodies specific for HLA antigens although a recently reported modification to the flow cytometry assay enables characterisation of IgM antibodies [27].

The development of an assay using multiplexed beads and flow cytometry [28] has also been applied to the detection and characterisation of HLA specific antibodies. This method allows simultaneous analysis of far greater numbers of HLA antigen coated microparticles than other current methods although only limited published data are currently available on the performance of this technique in the clinical setting [29].

Flow cytometry crossmatching (FCXM) is now widely used, particularly for sensitised and/or repeat transplant patients. **The increased sensitivity of the FCXM means that antibody results derived from screening by CDC are not necessarily predictive of the FCXM result. There is therefore a requirement for increased sensitivity in the screening test.**

### Screening strategies

**A comprehensive programme for antibody detection and characterisation is an essential component of the Histocompatibility Laboratory's support for solid organ transplantation.**

Fewer than half of the transplant candidates screened for HLA specific antibodies are likely to be positive [15]. A rapid screening test is required to determine whether a sample is antibody positive or negative. Effort can then be focused on antibody definition in the positive samples.

A number of laboratory techniques using CDC, flow cytometry and ELISA are now available for the definition of recipient sensitisation. These tests have often been considered as alternatives, but they each yield different information and have their individual advantages and limitations. It is possible to devise a strategy that employs a combination of ELISA, flow cytometry and CDC 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. It is important to bear in mind that the crossmatch is the final pre-transplant test. **For antibody screening to provide data predictive of the crossmatch result it must employ methods of equivalent specificity and sensitivity to the crossmatch test.**

### Collection and storage of samples for antibody screening and donor crossmatching

#### Pre-transplant

**The sensitisation status of a patient varies with time, and therefore regular monitoring of antibody levels is necessary from the time transplantation is considered as a treatment option.** In order to define an individual's sensitisation status and interpret antibody screening results it is essential to have accurate information about the timing and nature of potential priming events including transfusions,

pregnancies and infections. It is the responsibility of the clinical team to inform the Histocompatibility Laboratory of potential allo-sensitisation events and ensure that samples for antibody screening are sent to the laboratory at the agreed frequency. **Patient serum samples must be taken at 14 days and 28 days following transfusion of any blood products and no less than three-monthly for routine antibody monitoring.**

#### Post-transplant

De-novo synthesis of donor reactive antibodies after kidney transplantation is associated with acute rejection and chronic graft attrition. Following transplantation, it is recommended that recipients are monitored for the presence of donor reactive HLA specific antibodies and that the Histocompatibility Laboratory continues to receive serum samples for antibody screening. Failure to provide these samples may jeopardise a patient's future chances of transplantation. Local policy should stipulate the frequency of testing as well as testing following reduction/cessation of immunosuppression and/or transplant nephrectomy. **It is recommended that, post-transplant serum samples are obtained monthly for the first three months, quarterly up to one year and annually thereafter. Additional samples may be obtained at times of graft dysfunction when rejection is a likely cause.**

#### Sample storage

Use of selected historical patient serum samples for donor crossmatching is essential to avoid an anamnestic immune response towards a donor organ [30]. The use of only contemporary patient sera in the donor crossmatch test is correlated with sub-optimal graft survival [31]. Serum samples must be stored indefinitely for potential use in future antibody screening and crossmatch tests. For patients transferring to a different transplant centre, samples of all archived serum specimens, records and test results must be made available to the centre currently responsible for that patient.

### Recommendations

- Laboratories must have a strategy in place for the detection and characterisation of clinically relevant antibodies.
- The techniques adopted must have the capability of defining antibody class and specificity.
- As no single technique can provide complete information, a combination of methods is recommended. This may have cost implications for laboratory services.
- Laboratories should have a programme for investigating and evaluating newly emerging technologies so that the histocompatibility service supporting clinical transplant programmes develops in line with current treatments.
- Patient serum samples must be sent to the histocompatibility laboratory no less than three-monthly for routine antibody monitoring and at 14 days and 28 days following transfusion of any blood products.
- Post-transplant serum samples should be obtained monthly for the first three months, quarterly up to one year and annually thereafter.
- Serum samples must be stored indefinitely for potential use in future antibody screening and crossmatch tests.

# Crossmatching

The purpose of the crossmatch test is to determine whether a patient has antibodies which indicate sensitisation against the specific donor. Pre-formed antibodies present in recipient serum at the time of renal transplantation and directed against donor HLA antigens have been shown to cause hyperacute rejection [32]. **A crossmatch between donor and recipient which detects the presence of such antibodies is firmly established as an essential pre-requisite transplantation test to prevent hyperacute rejection.** The pre-transplant crossmatch can also indicate patients with an increased risk for graft loss.

## Crossmatch Techniques

The first technique to be developed to detect donor specific antibodies was based on the complement dependent lymphocytotoxicity test [33]. A positive donor specific lymphocytotoxic crossmatch (CDC) test was shown to be predictive of hyperacute rejection in 1969 [34] and since that time has become a universally accepted method of detecting pre-transplant donor HLA specific antibodies. The standard technique detects both HLA and non-HLA specific complement fixing antibodies. The flow cytometric crossmatch was later developed [35] and is also recognised as a reliable and highly sensitive method for the detection of donor HLA specific antibodies. Like the CDC test this technique detects HLA and non-HLA specific antibodies. The test can be adapted to detect different immunoglobulin classes although the majority of methods in routine use detect both complement fixing and non-complement fixing IgG subclasses. The flow cytometric crossmatch is therefore able to detect antibody classes which are not identified by the standard CDC test.

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

**The selection of patient serum samples is of great importance and will affect the quality of the information obtained in the crossmatch.** A sample taken immediately prior to the crossmatch test being performed is the most reliable means of determining the current status of donor specific sensitisation. In some cases a sample which has been collected within the last 3 months may be accepted as a current sample where it is known that the patient has had no potential sensitising events in the intervening period. In addition it is advisable to crossmatch a selection of historic serum samples which are representative of the patient's sensitisation status over time. This should include samples in which all the antibody specificities which have been detected are represented. The laboratory must inform the clinical team whenever more than three months has lapsed since a patient's serum sample has been received. A persistent delay may justify temporary suspension from the transplant list due to the increasing risk of unknown sensitisation.

## Results

**The results of a crossmatch test can be either positive or negative.** A negative crossmatch indicates absence of donor specific antibodies from the recipient serum. 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 of the antibodies causing the positive crossmatch is the most important factor in the interpretation of the result and where it can be demonstrated that the antibodies are not HLA specific the positive result is not generally regarded as a veto to transplantation. In addition the antibody class and the timing of samples giving a positive result (i.e. historic versus current) can be of relevance in determining the clinical relevance of the result. **It is essential that the interpretation of a positive crossmatch result is undertaken by experienced personnel who are able to determine and provide appropriate advice on the clinical relevance of the result obtained.**

## Recommendations

- Crossmatch tests should be capable of distinguishing T cell and B cell populations and should discriminate between IgG and IgM antibodies.
- Serum samples used for crossmatching must include a current sample and, where HLA specific antibodies have been detected, samples that are representative of the patient's antibody profile, over time.
- The reporting of results to clinical teams should include appropriate advice on the clinical relevance of the result.

# Kidney and Pancreas transplantation

Particular care is required when a donor kidney or pancreas is transplanted into a sensitised patient. Special consideration has to be given to the donor HLA antigen match grade and to avoid HLA mismatched specificities to which the patient is sensitised. The more stringent organ allocation criteria of a favourable donor HLA match and negative pre-transplant crossmatch means that many sensitised patients have longer than average waiting times. In highly sensitised patients who have HLA specific antibodies that react against >85% of potential donors, the increased immunological risk of transplant rejection may have to be balanced against clinical risks of remaining on dialysis. In some highly sensitised patients the likelihood of locating a suitable donor is remote and specific strategies for desensitisation should be considered.

**Within each HLA mismatch category, sensitised patients should be given priority over non-sensitised patients for a crossmatch negative donor organ and this is optimised by participation within organ exchange programmes.**

Registry data from a large number of transplant centres has shown that renal transplant outcome in sensitised patients and regrafts are inferior to that in non-sensitised patients [36]. However with detailed sensitisation history and careful donor selection facilitated through antibody screening and donor crossmatch strategies, survival rates equivalent to those of primary allografts in non-sensitised recipients can be achieved. Data from the UK shows that for transplants performed between 1997 and 2001 1 year graft survival in highly sensitised patients (85%) was not significantly different from that for all other patients (87%) [37].

## Pre-transplant Antibody Screening

Antibody characterisation aids the interpretation of crossmatch results and also contributes to the success of organ sharing schemes set up to facilitate successful transplantation of sensitised patients with well matched kidneys. Although the importance of HLA matching is well accepted, it is not the only factor to influence transplant outcome. Investigators have reported the

detrimental effect of prolonged cold storage times and delayed graft function on transplant outcome [38,39]. **It is important that transplant centres participating in organ sharing programmes ensure that laboratory processes are in place to minimise the chance of kidneys being shipped and then being crossmatch positive.**

If sera are carefully screened during patient work-up for transplantation then HLA specific antibodies can be defined and hence a patient's crossmatch reactivity against a particular donor of known HLA type predicted [40-42]. In the case of zero HLA-A, -B, -DR mismatched transplants, antibodies specific for these loci would not be expected to have a role. However, the immunological loss of some zero HLA-A, -B, -DR mismatched transplants suggests that antibodies specific for HLA-C or -DQ locus antigens could have a role in transplant failure [43,44]. Definition of antibodies to HLA-C and -DQ locus antigens in addition to -A, -B and -DR is therefore necessary in order to predict whether a crossmatch with a particular donor will be positive. After initial sample testing, sera must be screened following each sensitising event such as blood transfusion so that at the time of crossmatch against a potential organ donor each patient has a comprehensive antibody profile available. This avoids unnecessary crossmatching and facilitates interpretation of positive results. A significant influence of matching for HLA-DP in repeat patients also suggests a possible role for HLA-DP specific antibodies in transplant failure [45].

Similarly, the presence of IgM autoantibodies can be identified during patient work-up through antibody screening and auto-crossmatching. For patients known to have IgM autoantibodies, the crossmatch can be carried out in the presence of DTT and false positive crossmatch results can therefore be avoided.

## Definition of Unacceptable Mismatches

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

For patients with a functioning transplant in situ requiring transplantation of an additional organ (e.g. thoracic organ or liver transplant patients requiring a renal transplant, or renal transplant patients requiring a subsequent pancreas transplant) previous mismatched antigens should not be listed as unacceptable unless antibody specific for the mismatched antigens has been demonstrated. This position is based only on case reports.

Other HLA antigens may be listed as unacceptable where it is desirable to avoid sensitisation to these antigens. This would be the case for those patients who may be considered for living donor transplantation at a future time.

## 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 may also be of relevance.

## The CDC Crossmatch

It is generally accepted for renal transplantation that IgG antibodies directed against donor HLA-A or -B specificities and present at the time of transplant will cause hyperacute rejection [46,47]. Although less data are available, donor HLA-DR specific antibodies present in the recipient may also result in rejection [48]. The outcome will differ between individuals depending on antibody titre and level of expression of HLA-DR on the donor organ. Hyperacute rejection has been described in cases of positive B cell crossmatches due to HLA class II specific antibody and the elution of class II specific antibody from the rejected kidney provides strong evidence of a role for this antibody in the rejection process [49]. There is little information on the role of antibodies to HLA-Cw or HLA-DQ specificities in transplant failure. Renal transplant failure in a patient who had antibodies to HLA-Cw5 present in the donor has been reported [43] and acute humoral rejection has been associated with high titre IgG HLA-DQ specific antibodies [50]. If patients have antibodies to HLA-Cw or HLA-DQ, many centres now consider it advisable to avoid organ donors with those specificities just as they would when a patient has antibodies to HLA-A, B and DR.

IgM autoreactive antibodies react with autologous as well as allogeneic lymphocytes in the CDC crossmatch test and have been shown to be irrelevant to transplant outcome [51]. They therefore give rise to false positive results. The clinical relevance of IgM HLA specific antibodies is not clear and whilst in many cases they appear not to be detrimental in some circumstances there may be an association with rejection [30].

The clinical relevance of antibodies in non-current sera is also a point of debate. There have been reports of successful renal transplantation with a "peak positive, current negative" crossmatch [52] but again it is the specificity of the antibodies that is the crucial factor. IgG HLA-A or B specific antibodies present in historic sera are associated with accelerated rejection and decreased graft survival [46,53]. It has been suggested that the earlier antibody response might be associated with T cell activation which then later mediates acute graft rejection [54]. This hypothesis is supported by evidence of rejection associated with the presence

## Kidney and Pancreas transplantation

of cyclosporin resistant cytotoxic T lymphocytes specific for antigens to which antibody had previously been detected [55,56]. Rejection could also be antibody mediated as there has been a report of a renal transplant following a “peak positive, current negative” crossmatch due to an IgM alloantibody which resulted in a secondary antibody response within 5 days post-transplant, vascular rejection and transplant failure [57].

**Decisions regarding the transplantation of patients with antibodies in non-current sera should take into account the available immunosuppressive protocols.**

### Flow Cytometric Crossmatching

The first study of flow cytometric crossmatching showed the method to be more sensitive than conventional CDC crossmatches for the detection of antibody [35]. The greater sensitivity of flow cytometry and an association of a positive flow crossmatch with graft rejection have been confirmed [58]. The technique has also been shown to be more sensitive than the AHG augmented CDC crossmatch as well as the standard method [59].

The application of flow cytometry crossmatching (FCXM) to specific groups of potential recipients is a matter on which evidence varies. The first clear association between a positive flow crossmatch and graft failure in CDC crossmatch negative renal allograft recipients was shown in 1987 [60]. This association was significant only in sensitised recipients (those with previous failed grafts or with panel reactive antibodies). Other studies have demonstrated associations between positive FCXMs and complications in both first and re-grafts [61, 62]. In one of the largest studies of FCXM and primary kidney transplantation there was a significant association between a positive T cell FCXM and reduced graft survival at one year [63]. In contrast other studies have not found a significant association between a positive FCXM and graft function [64, 65] although the latter study did show a non-significant trend towards poor graft survival in the small number of re-grafts studied.

The above studies focused on the T cell FCXM. B cell FCXMs are also performed by increasing numbers of laboratories. A significant association between positive T and B cell FCXMs and graft failure has been described [66]. The demonstration

of antibody binding to both T and B cells suggests the antibody detected is likely to be directed at the HLA class I antigens. A T cell positive FCXM where there is no antibody binding with B cells suggests that the antibody may not be HLA specific. B cell positive FCXMs may occur when the T cell FCXM is negative. A strongly positive B cell FCXM was significantly associated with poorer graft survival at one year compared with those where negative, or weakly positive, B cell FCXMs occurred [67]. This association was found only in those patients receiving allografts mismatched for at least one HLA-DR antigen suggesting that the antibody detected may be specific for HLA class II.

Stratification of outcome according to the FCXM results has been shown with the highest survival in patients with T and B cell negative, intermediate survival with a B cell positive and poorest survival with T and B cell positive [68,69]. 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 [70].

**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 graft rejection and failure. In particular large multi-centre studies do indicate a significant association between FCXM and graft outcome [63, 69].

### Omitting the Pre-transplant Crossmatch test – “virtual crossmatching”

The purpose of the pre-transplant crossmatch is the detection of pre-formed donor-HLA specific antibodies. It therefore follows that if the recipient has never experienced any potential sensitising events and/or has never produced HLA specific antibodies the crossmatch is superfluous to requirements. The difficulty in translating this theoretical standpoint into practice is the uncertainty that sufficient information exists regarding potential sensitising events and the ability to prove definitively

that a patient has never, at any time, produced HLA specific antibodies. Some transplant units have speculated on the possibility of being able to define a sub-set of patients where the pre-transplant crossmatch could be omitted [71, 72] with the aim of reducing cold storage time. It has been demonstrated that this works in practice with a study omitting the pre-transplant crossmatch for a well defined group of patients [73]. Crossmatches which were performed retrospectively in this group were all negative indicating that prediction of a negative crossmatch was reliable in this carefully selected sub-set of patients. Regrettably, the cold storage time was not reduced indicating that other logistical factors come into play. Transplant teams considering this approach must proceed with caution.

### Reporting Crossmatch Results

**A positive crossmatch result is obtained when the patient’s serum sample contains antibody(s) which binds to the potential donor’s cells.** The evidence indicates that when this antibody is IgG with specificity for HLA there is a high risk of rejection and/or complications. Usually the risk is 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 clinically relevant and irrelevant positive reactions.

## Development of HLA specific antibodies following renal transplantation

A significant proportion of renal allograft recipients develop HLA specific antibodies following transplantation [57, 70, 74-87]. Antibodies have been identified either by specifically crossmatching against donor cells or by demonstrating HLA specific antibody reactivity in conventional antibody screening assays. The proportion of recipients reported to develop antibodies varies between 12 and 60% [88]. Clearly a number of factors could influence these figures including the type and sensitivity of assay used and clinical factors such as the degree of mismatching between donor

and recipient and immunosuppressive protocols. Modification of immunosuppressive treatment can affect antibody production.

**The development of HLA specific antibodies following renal transplantation has been shown to be associated with a poorer transplant outcome.**

Recipients developing HLA specific antibodies have an increased incidence of acute rejection and of chronic allograft nephropathy than those patients without antibodies. Many of the early reports demonstrated the presence of HLA specific antibodies using complement dependent cytotoxicity (CDC) assays, but with the availability of new technology donor-reactive antibodies have been more accurately defined using sensitive flow cytometry and enzyme linked immunosorbent assays. In order to identify de novo production of post transplant antibodies it is important to specify reactivity against mismatched donor antigens. In a recent report where donor HLA specificity was assigned to the antibody produced de novo following renal transplantation, antibodies directed against donor HLA-A, B, Cw, DR and DQ mismatches have been shown to be strongly predictive of transplant failure [57].

Whilst mismatched classical HLA antigens present targets for antibody responses, other polymorphic antigens may also be important in this context. Antibodies to the mismatched MICA antigens have recently been described in the sera of transplant recipients [89, 90]. These antibodies may be of particular interest because MICA expression has been described on renal tubular epithelia in rejecting allografts [91] and on endothelium in vitro, but not on lymphocytes [92, 93] and therefore pre-existing MICA antibodies would not be detected by current crossmatching tests.

While circulating donor-specific antibodies can be easily detected following transplantation, the histological detection of immunoglobulins bound to the endothelium in a transplant has proved difficult, because antibody is rapidly removed from the endothelial surface. However, after antibody-mediated activation of the classical complement pathway, the complement protein C4d is covalently bound to the endothelial surface leaving an imprint of antibody activity that persists after complement activation. Following the initial report from Feucht

## Kidney and Pancreas transplantation

and colleagues [94], the presence of C4d on peritubular capillaries of renal transplant biopsies has been shown to be a reliable marker for acute humoral rejection and as such an immunohistochemical marker of post-transplant donor reactive antibody responses. Studies of biopsies obtained during renal allograft dysfunction have revealed that C4d deposition in the peritubular capillaries is present in approximately 30% of acute rejection biopsies [95-99]. There is now very strong evidence that circulating donor reactive antibodies detected by post-transplant crossmatching and screening are significantly associated with C4d deposition [8, 95, 97-100]. Almost all studies show a significant association between C4d deposition in renal allograft biopsies obtained during graft dysfunction and poor graft outcome in the long-term. The Banff 97 classification of renal allograft rejection has been modified to include C4d deposition as part of the definition of acute humoral rejection together with the presence of donor-reactive antibodies and histological evidence of graft injury [101].

Since the production of donor HLA specific antibodies following transplantation is associated with poor outcome there is a potential benefit to monitoring patients for production of post transplant antibody. There is evidence to suggest that the introduction of agents such as mycophenolate mofetil into immunosuppressive regimens decreases antibody production [102-104].

### Recommendations

- Laboratories providing services for renal transplant programmes must have the capability of precisely defining HLA-A, -B, -C, -DR and -DQ antibody specificities in their patients so that donors who will be crossmatch negative can be identified.
- HLA antigens to which a patient has produced antibodies should be listed as “unacceptable mismatches”.
- A pre-transplant crossmatch should be performed for all patients unless a programme exists for identifying those individuals who can confidently be defined as unsensitised.
- Sensitised patients should be crossmatched using flow cytometric techniques.
- Post-transplant antibody monitoring is recommended to help identify patients at increased risk of graft rejection/loss and to aid in determining the requirements for immunosuppression.
- It is the responsibility of the clinical team to inform the laboratory of potential allo-sensitising events.

## Thoracic organ transplantation

### HLA specific antibody screening and crossmatching prior to transplantation

For many years thoracic organ transplants were performed without prospective donor specific crossmatching due largely to time constraints caused by the short acceptable cold ischaemic time of explanted thoracic organs. This has led to a number of transplants being performed in the face of a positive crossmatch. In most instances the donor specific crossmatch is performed retrospectively using cells isolated from donor spleen.

**The published evidence now demonstrates that pre-transplant donor HLA specific antibodies are strongly associated with hyperacute or accelerated rejection of thoracic organ allografts, usually leading to death of the recipient.** [105-108]. An early report showed that all 4 patients with a positive crossmatch had early graft failure and death contrasting with 7 of 28 (25%) recipients with a negative crossmatch [107]. When T and B cell crossmatches are analysed separately it can be seen that a positive IgG T cell crossmatch is associated with accelerated graft failure for both heart and heart-lung transplant recipients [105]. Of 7 patients transplanted with a positive T cell crossmatch, 5 (71%) had died within 2 weeks of transplantation contrasted with 31 of 258 (12%) patients transplanted with a negative T cell crossmatch. Recently, HLA class II reactive antibodies have also been associated with increased high-grade acute rejection in recipients of cardiac allografts [108]. Flow cytometry crossmatching is a more sensitive technique than conventional CDC crossmatching and has demonstrated a correlation with increased early acute rejection episodes in heart transplantation [109] and severe graft dysfunction in lung transplantation [110].

**Patients should be screened for HLA specific antibodies following sensitising events such as blood transfusions 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 so that collection of blood samples can be arranged.

**If a potential recipient of a thoracic organ transplant is shown to have produced HLA specific antibodies**

**a prospective crossmatch with donor lymphocytes should be performed.** 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 5 hours careful consideration should be given to the location of the donor hospital as to when prospective crossmatching is feasible. There may be occasions when avoidance of the sensitised HLA antigens in the donor phenotype may allow transplantation to proceed without waiting for a prospective crossmatch result. It is good practice that for all non-sensitised patients crossmatching is performed retrospectively using donor spleen lymphocytes. However, a lack of accurate information regarding potential sensitising events in these patients would mean that there will always be a degree of uncertainty as to whether some patients may have produced HLA specific antibodies at some point in their history e.g. following pregnancy.

The crossmatching techniques utilised, such as CDC or flow cytometry 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.

### Post-transplant production of HLA specific antibodies

HLA specific antibodies produced following thoracic organ transplantation have also been shown to have deleterious effects on graft outcome [111-118]. Recipients of cardiac allografts can produce lymphocytotoxic antibodies following transplantation some of which are directed against HLA specificities [112,113]. Antibodies specific for donor HLA antigens have been shown to convey increased risk of acute rejection [112,114], poor graft survival [111] and chronic rejection [117,118].

This is also true for lung transplantation with patients producing HLA specific antibodies at increased risk of developing chronic rejection manifesting as bronchiolitis obliterans syndrome (BOS). In one study, 66% of 15 patients with BOS produced HLA specific antibodies, whilst none of 12 patients free from BOS had formed antibodies [116]. Similarly, over 60% of lung recipients without demonstrable HLA specific antibodies lacked signs

## Thoracic organ transplantation

of chronic rejection whereas all patients with HLA specific antibodies had developed BOS within 2 years of transplantation [115].

**It is recommended that post-transplant monitoring of patients for the production of HLA specific antibodies is performed at regular intervals following transplantation.**

### Non-HLA specific antibodies

The chronic production of antibodies to non-HLA antigens, primarily endothelial cell antigens, has been associated with the development of transplant associated vasculopathy after cardiac transplantation [119-122]. Although many antigens are likely to be involved, one of these is known to be the intermediate filament vimentin [123]. A simple ELISA for the detection of anti-vimentin antibodies performed at regular intervals within the first year of transplant identifies patients at risk for developing transplant CAV [124].

### Recommendations

- Laboratories must be able to precisely define antibodies specific for HLA –A, –B, –Cw, –DR and –DQ.
- Unacceptable mismatches should be defined as those HLA antigens to which antibodies have been detected.
- Patients should be screened for HLA specific antibodies at regular intervals, especially following sensitising events, both pre- and post-transplantation.
- Prospective crossmatching for all sensitised patients should be performed.
- Sensitised patients with fully defined HLA specific antibodies and with no residual reaction frequency may be transplanted without a prospective crossmatch provided the donor does not carry those HLA specificities to which the patient is sensitised.
- A retrospective crossmatch using serum collected within 24 hours prior to transplantation should be performed.
- Crossmatch techniques should discriminate between IgG and IgM as well as T and B cells as targets.
- Post transplant antibody monitoring is recommended to help identify patients at increased risk of graft rejection/graft loss.

## Liver transplantation

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

Hyperacute rejection of livers is unusual [125]. 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. Ratner et al reported that the associated HLA specific antibodies had titres of between 1:16000 and 1:32000, which are high by any measure [125]. The recipient was a multiply transfused male, and transfused within a few days of his first transplant.

It is the practice of some units to perform a crossmatch, but often in retrospect and not for recipient selection. Studies from such centres show no association between a positive crossmatch and reduced graft survival in about half [126-133] and a significant association in the other half [134-142] of the transplant recipients. Where a statistically significant association between a positive crossmatch and reduced survival has been shown, the correlation is with early 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 [127,129,130].

In general, positive cytotoxic (CDC) T cell crossmatches have been shown to be a predictor of outcome contrasting with B cell or flow cytometry crossmatches. This implies that clinically significant specific antibodies may be limited to HLA Class I. The increased sensitivity of flow cytometry may detect antibodies at a level below clinical significance. Where survival data have been analysed in relation to flow cytometry crossmatches no association has been seen [131,132], although Scornik et al [132] found that high level HLA Class

I-specific antibodies were associated with steroid-resistant rejections.

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

The detrimental effects of pre-existing donor-specific antibodies are seen during the early post transplant period. Graft loss may be prevented by effective management [127,135] or be dependent on the HLA specific antibody titre. Persistence after transplantation has also been shown to be an important factor in the pathogenicity of donor-specific antibodies [127,137]. 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” [130]. Overall, the effect of a positive crossmatch is measurable and stands above the background of other pressures on outcome.

### Recommendations

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

## Intestine transplantation

Due to the current low numbers of intestinal transplantation there is insufficient evidence to indicate the value of a pre-transplant crossmatch for recipient selection. Rejection is the major cause of graft failure in intestinal transplantation. In contrast to renal transplantation, the risk of acute rejection of an intestine allograft may not be reduced with concomitant liver transplantation [143]. Vascular rejection resulting in reduced graft survival is seen following small bowel transplantation and this is associated with a positive crossmatch [144]. Post-transplant production of donor-specific antibodies has been described in one case with acute vascular rejection [144]. The incidence of exfoliative rejection however has been shown not to associate with a positive crossmatch [145].

Donor-specific antibodies are known to compromise all other forms of organ transplantation to varying degrees so it is reasonable to assume that intestinal transplantation is no exception. However the paucity of current data makes it difficult to determine the magnitude of the effect of a positive crossmatch in such transplants. In addition it may be that the general extreme immunogenicity of intestinal tissue obscures the effect of such a single factor.

### Recommendations

- A pretransplant crossmatch and HLA specific antibody screen can identify those at high risk of acute or vascular rejection.

## Antibody removal

Patients with antibodies directed at a broad spectrum of HLA antigens are likely to wait significantly longer for a transplant. Although some of these patients will modulate their antibodies naturally over a period of 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 [146]. 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 [147].

T cell cooperation is needed for B cells to develop into alloantibody producing plasma cells and the characterisation of the epitope(s) to which an alloantibody is directed can be used as a measure of the extent of T cell sensitisation. Although the original T and B cell antibody epitope 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 the original HLA epitope specific antibody may broaden to cover other specificities. One way of investigating this is to test a serum in dilution. When titred 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 containing these HLA mismatches.

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 [148]. Renal transplantation with a current negative, historic positive crossmatch has become more widely accepted, but is not always successful [52]. 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.

Various methods have been used:

### Immunosuppressive Drugs

Reports indicate that HLA specific antibody cannot be significantly down-regulated by the administration of immunosuppressive drugs. Mycophenolate Mofetil has been shown to inhibit antibody production by B cells in vitro [149] and has antibody lowering effects in vivo [104], it has not been as effective in the context of HLA specific antibody reduction in patients awaiting transplantation. A chimeric humanised monoclonal antibody (Rituximab) specific for the CD20 B cell surface antigen has been recently reported to reduce HLA specific alloantibody in five of the nine renal patients treated [150] Rituximab shows potential in modulating antibody in some auto-immune diseases [151] and may have a role in modulating alloantibody. Rituximab has recently been used in conjunction with plasmapheresis, IVIg and splenectomy for successful pre-transplant HLA specific antibody removal [152].

### Plasmapheresis

Plasmapheresis or plasma exchange (PP), where a patient's plasma is removed and replaced with fresh frozen plasma or another substitute, was one of the first methods to show success in alloantibody reduction. It has been used to reduce pre-transplant antibody levels with success [153-156] but it has the disadvantage that immunosuppressive drugs are needed to discourage antibody synthesis and prevent a rebound of the antibody level post plasma removal. Cyclophosphamide was the immunosuppressive drug of choice in early trials [153], although mycophenolate mofetil may be an alternative [156].

Another disadvantage of PP is that plasma proteins need replacement. It has been estimated that the removal of one gram (g) of IgG antibody by PP results in the loss of 150g of albumin together with other proteins and clotting factors [157]. The use of plasma as a replacement fluid can lead to adverse side effects, and may even lead to an increase in allosensitisation [155]. Albumin may normally be the best replacement fluid, however the use of plasma has been recommended within 48 hours

## Antibody removal

of a transplant to avoid complications such as haematoma [156].

PP has been used as therapy for acute vascular rejection [158-162] and hyperacute rejection of the lung [163]. A major reason for this use of PP in acute rejection would appear to be antibody modulation, but rarely have the donor HLA specific antibody levels been serially monitored. The reported endpoint is usually resolution, or otherwise, of the rejection episode so it is difficult to judge the efficacy of this procedure in antibody reduction post transplant.

## Immunoadsorption

Extra-corporeal immunoadsorption (IA) involves the removal of immunoglobulin by passage of plasma over an affinity matrix. Protein A is the most widely used affinity column in clinical practice and has been used to reduce alloantibody pre-transplant [164-169]. Protein A is effective at binding all subclasses of IgG except IgG3 which it binds poorly; Protein G removes all sub-classes of IgG [170]. Two columns are usually used alternately, by switching the extracorporeal blood plasma flow between the columns one column can be regenerated by acid elution of bound antibody. IA has advantages over PP, it does not require the replacement of plasma proteins and allows the treatment of higher plasma volumes when regeneration of the protein A columns is used. IA still requires immunosuppression to try to prevent antibody (re)synthesis post treatment and it is a more complex and expensive process. It has been estimated that a Protein A column is capable of adsorbing 50% of the IgG from a volume of plasma [171] and multiple passages can result in 90% depletion of plasma IgG levels [157,172].

An overview of the early results of a number of centres [173] indicates that about 50% of patients reduced their antibodies long-term while the rest need repeated treatment.

The short-term graft survival of patients transplanted after immunoadsorption of alloantibody has generally been lower than might be expected for unsensitised patients, particularly when the treated patients had a previous positive crossmatch [168]. A five year follow-up of a series of post IA renal transplant recipients indicated that there was no increase in chronic allograft nephropathy in these

patients, and argued that the five year graft survival (44%) was comparable with figures for highly sensitised, re-grafted patients transplanted in the same time period (1987-9) [174].

Although it has been reported that donor specific alloantibody returns immediately post transplant, this does not necessarily lead to graft loss [175] and specific modulation of donor specific antibody may occur. This phenomenon, known as accommodation, has been intensively studied in relation to xenograft rejection [176], but has important implications in the understanding of antibodies in human allotransplantation.

Acute immunoadsorption may lead to the unmasking or production of auto-antibodies [177] which need to be taken into account in the crossmatching procedure. A further practical consideration is the leaching of antibody from the extra-vascular space; this can convert a negative crossmatch to positive in a few hours. Prompt transplantation in these circumstances is important.

## Intravenous Immunoglobulin (IVIg)

IVIg was originally developed and used to provide protection to patients suffering from agammaglobulinaemia or hypogammaglobulinaemia. It was observed that administration of high doses of immunoglobulin caused an increase in platelet counts in patients suffering from autoimmune thrombocytopenic purpura [178]. This has led to the use of IVIg in a wide range of autoimmune conditions where antibody is implicated [179].

Commercial preparations of IVIg contain immunoglobulins purified from the serum of many thousands of healthy blood donors, thereby ensuring there will be representation of virtually all antigen-binding specificities. The process concentrates IgG molecules to about ten times the normal serum concentration but maintains a distribution of subclasses that corresponds to that found in normal serum. Very small amounts of IgA are found in most preparations, with some also containing trace amounts of IgM [180]. Other reported components include CD4, CD8, soluble HLA molecules [181] and cytokines [182], although

the potential importance of these ingredients in the mechanism of action of IVIg has to be tempered by their trace amounts. As IVIg preparations are made from pools the efficacy may vary between batches.

Being a complex mixture, the mechanism of IVIg action is obscure, but it is likely to be multifactorial and involve complement down-regulation, Fc receptors, and anti-idiotypic interactions. IVIg is well tolerated and a major advantage of IVIg over other methods of antibody removal is that it does not require the simultaneous administration of immunosuppressive drugs.

There are many reports of the use of IVIg to lower antibody levels prior to renal [183-188] and cardiac transplantation [189,190]. In some cases IVIg has been used in conjunction with PP [156,185,191,192]. The specific removal of IgG by IA and its replacement with IVIg would be a logical approach worthy of investigation.

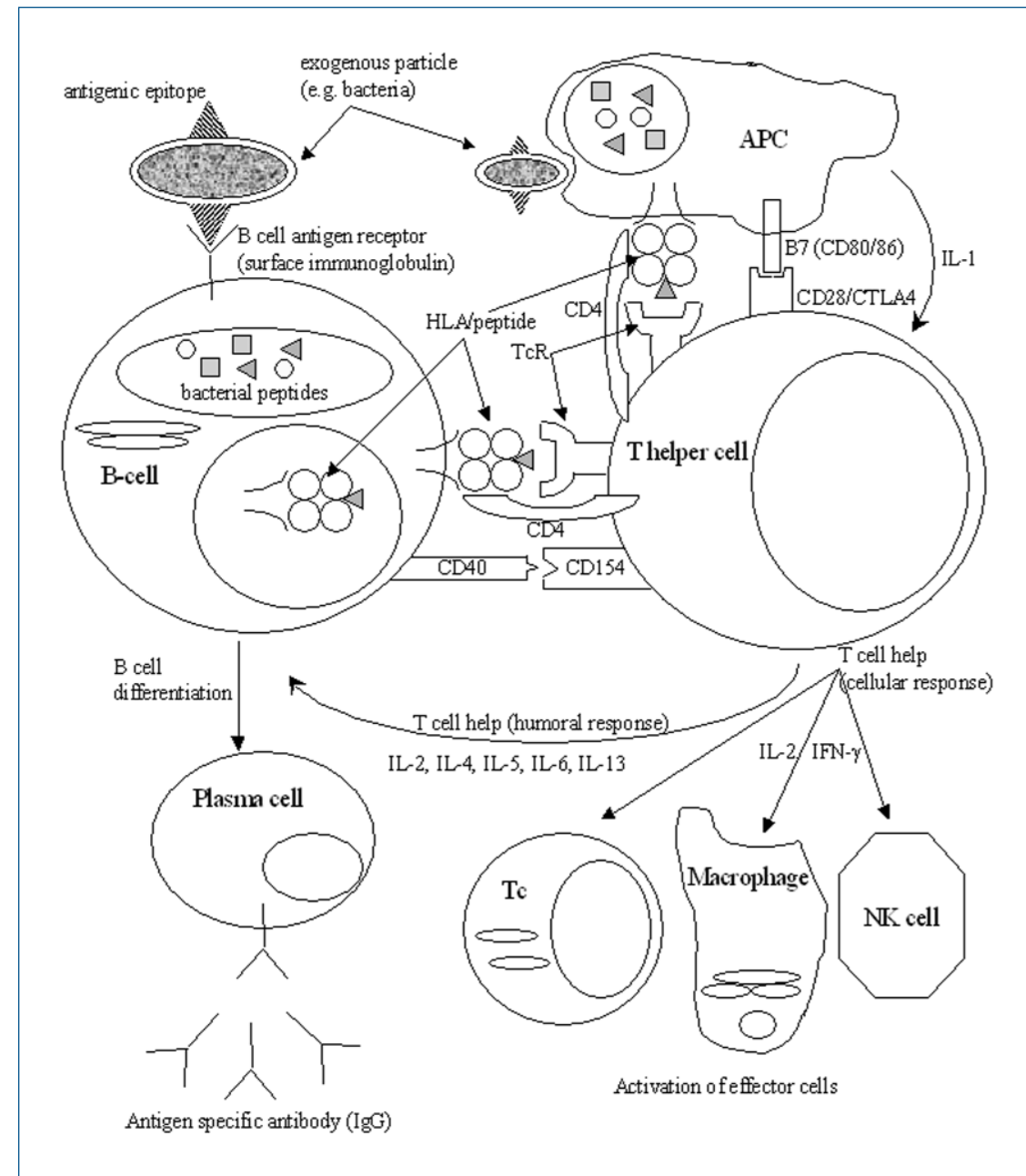
IVIg has been used as an effective rescue therapy for acute vascular rejection in renal [193] and cardiac patients [189]. IVIg has been used to reverse steroid resistant rejection [194], and in a randomised prospective study has been compared favourably with OKT3 as a treatment for rejection [195].

## Recommendations

- The HLA specificity and titre of antibodies should be fully determined prior to antibody removal.
- Antibody titre must be monitored regularly throughout the duration of treatment to determine its effectiveness.
- Antibody removal should only be undertaken following establishment of a clinical protocol.

The British Transplantation Society is currently developing clinical guidelines for antibody removal see [www.bts.org.uk](http://www.bts.org.uk).

**Figure 1: Schematic representation of antigen presentation to T cells and the activation of effector cells involved in the immune response.**



**Legend**

- APC** Antigen Presenting Cell
- Tc** Cytotoxic T cell
- NK cell** Natural Killer cell
- TcR** T cell Receptor

## Appendices

### 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 medical consultant or a substantive consultant Healthcare Scientist 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 Membership of the Royal College of Pathologists in Histocompatibility & Immunogenetics or evidence of at least an equivalent level of training in the subject.

Other Healthcare Scientist staff should have successfully completed a recognised training scheme in Histocompatibility & Immunogenetics (for example British Society for Histocompatibility & Immunogenetics Diploma) and have attained state registration with the Health Professions Council. 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.

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

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

# Appendices

## 2) Glossary (intended for non-professional readers)

<b>ABO blood group</b>	Sugar molecules carried by red blood cells (and other tissues) which function as antigens. There are four main ABO blood groups, O (46% of 616 heart-beating deceased kidney donors in the UK in 2003), A (41%), B (9%) and AB (4%). Group O patients have naturally occurring antibodies to groups A and B, group A to group B, group B to group A while group AB have no naturally occurring antibodies. Organ transplants and blood transfusions can be: ABO identical e.g. O to O, A to A etc ABO compatible e.g. O to A, B to AB etc ABO incompatible e.g. B to A ABO incompatibility constitutes a high risk (usually a veto).
<b>absorption</b>	Active process of binding to another substance e.g. binding of antibody to an affinity column.
<b>acceptable mismatch</b>	A non-self HLA antigen to which a recipient has no antibody reactivity, prior to transplantation.
<b>accommodation</b>	An incompatibility which is accepted by the recipient.
<b>acute rejection episode</b>	The recipient immune response (usually mediated by T cells) attempts to reject a transplanted organ. This usually occurs during the first three months after transplantation.
<b>affinity column</b>	A matrix, usually polymer beads in suspension, which acts as a carrier of biologically or chemically active molecules capable of binding another molecule.
<b>allele</b>	A genetic variant with a frequency of more than 1%. An allele can be at the DNA or protein level.
<b>allograft</b>	A transplant between members of the same species e.g. between humans.
<b>anamnestic response</b>	A response because of memory. The immune system responds aggressively when re-exposed to an antigen in a secondary response.
<b>antibody</b>	A serum protein produced by B-lymphocytes, which is capable of binding to a unique antigen. Antibodies are part of the natural defence mechanism and function to rid the body of harmful pathogens such as viruses and bacteria. In transplantation they can cause rapid destruction of the transplanted organ.
<b>antibody dependent cell mediated cytotoxicity (ADCC)</b>	Cell killing which has specificity (antibody) and is mediated by cytotoxic cells.
<b>antibody detection</b>	A method which identifies the presence of an antibody in a serum sample.
<b>antibody isotype</b>	Immunoglobulins occur as single molecules (IgG), pentamers (IgM),

	dimers (IgA) or in other forms (IgD, IgE) collectively termed isotypes.
<b>antibody removal</b>	An artificial process used to get rid of a usually harmful antibody from a patient's blood.
<b>antibody screening</b>	A laboratory test to identify the presence of HLA specific antibodies in a patient's serum (blood) sample. If antibodies are identified then they must be characterised to define their specificity i.e. to list the antigens to which they are directed.
<b>antigen presenting cell</b>	An immune system cell which can process antigens and expose them to other immune system cells to initiate an immune response.
<b>antigen</b>	A non-self molecule or part of a molecule, to which the body's immune system can manufacture an antibody or mount a cellular response. Antigens are usually harmful chemicals produced by viruses and bacteria. In transplantation they are molecules present on the donor organ but absent in the recipient.
<b>anti-idiotypic antibodies</b>	An antibody with specificity for that part of another antibody which binds antigen.
<b>audit</b>	A cyclic process of review of a laboratory or clinical systems with the aim of identifying improvements and implementing change.
<b>autoimmunity</b>	An immune response to self antigens, tissues and organs which can result in serious illness such as type 1 diabetes or rheumatoid arthritis.
<b>autoreactive lymphocytotoxic antibodies</b>	Antibodies which cause a positive reaction in a lymphocytotoxic assay when the serum and target cells are from the same individual. An important cause of a false-positive result. Are of no clinical relevance.
<b>B cell crossmatch (B-cell XM)</b>	A crossmatch test in which target cells are B lymphocytes.
<b>B lymphocytes</b>	White blood cells, which produce antibodies. They circulate in the blood and increase in number at the time of infection or in transplantation, at the time of rejection. They work with T-lymphocytes. Their action can be limited by immunosuppressive drugs.
<b>Banff 97 grades</b>	An internationally recognised system of grading pathology in biopsy specimens from a kidney. Used to diagnose and grade rejection.
<b>Biomedical Scientist</b>	A Healthcare Scientist qualified to practice following a specified training course leading to State Registration ( <a href="http://www.hpc.org.uk">www.hpc.org.uk</a> ) and attaining continuous professional development.
<b>British Renal Society</b>	<a href="http://www.britishrenal.org">www.britishrenal.org</a>
<b>British Society for Histocompatibility and Immunogenetics</b>	<a href="http://www.bshi.org.uk">www.bshi.org.uk</a>

<b>bronchiolitis obliterans syndrome</b>	Occlusion of the airways in a transplanted lung. A manifestation of rejection.
<b>C4d</b>	A component of the complement system which indicates an antibody mediated process has occurred. Detection is diagnostic of rejection.
<b>calcineurin inhibitor</b>	An immunosuppressive drug (ciclosporin, tacrolimus) which acts by blocking immune cell activation by the calcineurin pathway.
<b>CD-4, 8, 20, 28, 40, 80, 154</b>	Cell surface molecules (Cluster of Differentiation) defined and recognised by an international standardisation body <a href="http://ca.expasy.org/cgi-bin/lists?cdlist.txt">http://ca.expasy.org/cgi-bin/lists?cdlist.txt</a>
<b>cell viability</b>	The proportion of target cells alive before or after a CDC assay.
<b>characterisation of antibodies</b>	A process of testing for antibody(s) in serum in such a way as to identify the specific antigen(s) with which the antibody reacts.
<b>chemotaxis</b>	Attraction of a cell to a source mediated by secretion of a chemical attractant.
<b>chronic lymphocytic leukaemia (CLL) cells</b>	Chronic lymphocytic leukaemia is a cancer of a B lymphocyte lineage resulting in high numbers of B cells entering the blood system. These can be used as target cells in a CDC assay.
<b>chronic rejection</b>	Response to a transplanted organ characterised by agreed (Banff) criteria. Onset is usually at least one year after transplantation and progression is slow. E.g. chronic transplant nephropathy (kidney), bronchiolitis obliterans syndrome (lung) and coronary artery disease (heart).
<b>class switch</b>	The event when antibody in the circulation changes in isotype e.g. from IgM to IgG indicating specificity.
<b>clinical significance</b>	Of relevance to and influential of patient treatment.
<b>cold ischaemia time</b>	The time during which an organ for transplant is stored outside the body, usually in melting ice at 4C.
<b>complement</b>	A group of chemicals in the blood which react together to severely damage or kill body cells.
<b>complement dependent cytotoxicity (CDC)</b>	A laboratory test to identify presence of antibodies in a serum sample using lymphocytes bearing antigens as targets.
<b>Clinical Scientist</b>	A science graduate who has followed a recognised training scheme to achieve Registration with the Health Professions Council ( <a href="http://www.hpc.org.uk">www.hpc.org.uk</a> ). Consultant CSs have Membership of the Royal College of Pathologists, have extensive (more than 8 years) experience and have documented continuous professional development (CPD).
<b>co-stimulatory molecule</b>	A cell-surface molecule which enhances recognition of non-self antigens by binding to a ligand on a T cell in association with HLA antigens and the T cell receptor.

<b>CPD</b>	Continuous Professional Development schemes provide independent assessment that an individual maintains ongoing training.
<b>crossmatch test (XM)</b>	A test to identify antibody mediated reactivity to target antigens in a potential organ donor. The test report must be either positive or negative.
<b>cross-reactive</b>	An antibody which is able to bind to a series of structurally closely related antigens.
<b>cyclophosphamide</b>	A drug which is cytotoxic. A powerful, infrequently used immunoablative drug.
<b>cytokine</b>	A chemical secreted by an immune cell which may either enhance or suppress an immune response.
<b>desensitisation</b>	Removal of antibodies which are indicative of sensitisation.
<b>differentiation</b>	A process of specialisation of cells and tissues to become a functional organ or system. A one way step.
<b>dithiothriitol</b>	A chemical used in laboratory assays to dissociate the pentameric IgM molecule and abrogate its activity.
<b>donor / recipient (in)compatibility</b>	The degree to which a donor's tissues or organs differ from a recipient's. The reactivity of a recipient's immune response to donor antigens.
<b>endothelial cells</b>	Cells which line the blood vessels.
<b>enzyme linked immunosorbent assay (ELISA)</b>	A laboratory assay in which antibody can be detected. Known antigens are bound to a plastic plate and reacted with a patient's serum sample. 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.
<b>epitope</b>	That part of the antigen structure to which antibody binds.
<b>Epstein-Barr virus (EBV)</b>	A common virus causing glandular fever in healthy persons. In immunosuppressed transplant patients it can cause post-transplant lymphoproliferative disease, used to transform B cells into stable cell lines.
<b>extra corporeal</b>	Outside of the body.
<b>FC receptor</b>	A B cell surface molecule which binds to the constant sequence domain of an immunoglobulin molecule.
<b>false positive</b>	A laboratory assay reaction which gives a positive result which does not correlate with the clinical situation. An erroneous result.

<b>“favourable” mismatch</b>	A donor / recipient combination where there is no mismatch for HLA-DR and only one mismatch at HLA-A or –B or one mismatch at each of HLA-A and –B. ‘100’ or ‘010’ or ‘110’. A UK Transplant definition.
<b>flow cytometric crossmatch (FCXM)</b>	A test of donor/recipient compatibility, which uses a flow cytometer to measure binding of patient antibody to donor antigens. Sometimes, incorrectly this test is called a “FACS crossmatch”.
<b>flow cytometer</b>	Equipment using laser technology and a fluorescent stain coupled to a detection antibody and is a highly sensitive, semi-qualitative technique.
<b>guideline</b>	A statement intended to offer advice of how to proceed. Based on published evidence or established best practice.
<b>haematopoietic stem cell</b>	A cell which can differentiate into one of many possible blood lineage cells. Used to treat leukaemia by transplantation from a healthy donor.
<b>health professions council</b>	The UK regulatory body overseeing registration of Healthcare Scientists; www.hpc.org.uk
<b>highly sensitised patient (HSP)</b>	A patient who has developed HLA specific antibodies to 85% or more of a representative cell panel. This definition is set by UK Transplant.
<b>histocompatibility</b>	The degree of similarity between cells, tissues and organs of donors and recipients assessed by HLA antigen typing and matching.
<b>histocompatibility laboratory</b>	A highly specialised laboratory staffed by Healthcare Scientists who perform tests to facilitate effective organ, tissue and stem cell transplantation.
<b>historic serum sample</b>	A serum sample collected from a patient and stored for testing, usually at least six months prior to the current date.
<b>HLA antigens</b>	Human Leucocyte Antigens. Cell surface molecules coded on chromosome 6 which function as immune recognition antigens. There are several genes (HLA-A, -B, -Cw, -DR, -DQ, -DP) each coding for separate molecules and each gene locus is highly variable leading to a complex genetic system of fundamental importance in the immune response.
<b>HLA class I molecules</b>	HLA-A, -B and –Cw molecules with structural and functional similarity. Occur on almost all body cells.
<b>HLA class II molecules</b>	HLA-DR, -DQ and –DP molecules with structural and functional similarity. Restricted distribution on immune active cells.
<b>HLA specific antibodies</b>	Antibodies which bind to HLA antigens.
<b>humoral</b>	Of the blood. Usually used to indicate an antibody mediated response (cf cellular response).
<b>hyperacute rejection</b>	Rejection of a transplant within a very short time of operation (minutes) usually caused by antibodies present in the recipient which react with antigens on the transplanted donor organ. Usually results in immediate failure.

<b>IgG, IgM</b>	An immunoglobulin antibody isotype.
<b>immune response</b>	The reaction of the body’s immune system to non-self foreign substances e.g. viruses, bacteria.
<b>immunoabsorption</b>	Binding of immunoglobulins to a solid phase. A process which can remove immunoglobulin from the blood.
<b>immunogenicity</b>	The degree to which a substance can provoke the immune system to respond e.g. high or low.
<b>immunoglobulin</b>	A protein manufactured by B cells and which binds to an antigen.
<b>immunohistochemical</b>	A laboratory test which localises an antigen in a tissue section.
<b>immunosuppression</b>	Drug induced manipulation of the immune response to lessen its effectiveness.
<b>inflammatory response</b>	Cell and tissue swelling which accompanies an immune response.
<b>interleukin</b>	IL-2, -4, -5, -6, -13. Molecules manufactured by immune system cells which can up- or down-regulate immune cell activity.
<b>IVIg</b>	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.
<b>locus</b>	The positioning of a specified gene in the DNA sequence.
<b>lymph node</b>	Discrete, widely distributed body tissue at which lymphocytes reside.
<b>lymphocyte</b>	A white blood cell central to the actions of the immune system. There are many types and sub-types e.g. B lymphocytes, T lymphocytes, CD4 T lymphocytes.
<b>lymphoid cell line (LCL) cells</b>	White blood cells manipulated in the laboratory with EBV to grow rapidly to give large numbers of B cells of a specific type which can be used in some assays.
<b>macrophage</b>	A white blood cell specialised to engulf antigenic substances (viruses, bacteria) which can be presented to the immune system to induce an immune response.
<b>memory T cell</b>	A white blood cell T lymphocyte which is able to recall prior exposure to a specific antigen and respond immediately to kill the antigen carrying cell.
<b>MICA – MHC class I like molecules</b>	Molecules with close structural similarity to HLA molecules but with a different function. MICA interact with natural killer cells to regulate immune cell responses.
<b>mycophenolate mofetil</b>	An immunosuppressive drug with anti-proliferative properties.
<b>microparticles / beads</b>	Microscopic plastic beads which are commercially available as solid phase carriers of biological molecules such as antigens or antibodies.

<b>microthrombi</b>	Very small clumps of red blood cells which can cause blockage of capillary blood vessels.
<b>monospecific</b>	An antibody which reacts with a single structurally discrete antigen.
<b>natural killer cell</b>	White blood cells which function to moderate immune system responses. They prevent immune cells acting against the body's own cells and moderate production of cancer cells.
<b>negative crossmatch</b>	Result of a laboratory test which has not detected donor-specific antibody.
<b>non-self</b>	Substances which elicit an immune response, antigens or allo-antigens.
<b>OKT3</b>	Outdated name for the CD3 molecule. An antibody to OKT3 can be used as a highly potent immunosuppressive drug (Muromab-CD3, Orthoclone OKT3). Now used infrequently.
<b>organ exchange</b>	Agreed transport of a donated organ for transplantation into the most suitable recipient at another transplant centre. Facilitated by UK Transplant following exchange rules decided by agreement between transplant centres.
<b>panel reactive antibodies (PRA or % PRA)</b>	The calculated % of a panel of lymphocytes with which a patient's serum reacts. Now considered an outdated term, '%PRA' was a guide to the degree of sensitisation.
<b>peak positive / current negative crossmatch (PPCN XM)</b>	A crossmatch result where reactivity to donor target cells has been detected only in historic serum samples. There is no reactivity to donor target cells in recent serum.
<b>peptide</b>	A fragment of a protein molecule.
<b>peripheral blood</b>	A blood sample taken for clinical investigation from a vein.
<b>peritubular capillaries</b>	Small blood vessels located in the kidney adjacent to the structures (nephron) which filter the blood.
<b>phagocytes</b>	White blood cells which engulf antigenic substances.
<b>plasma cell</b>	A lymphocyte which actively manufactures antibodies, of B cell lineage.
<b>plasma exchange or plasmapheresis</b>	Removal of some of the plasma, usually about 0.5 litre at a time and replacement with plasma from a blood bank. This process can be used to lower the amount of an antibody in the blood.
<b>positive crossmatch</b>	Result of a laboratory test which has detected donor-specific antibody.
<b>post-transplant immunological monitoring</b>	Laboratory tests which may indicate the immune reactivity of a recipient to transplanted tissues and organs.
<b>pregnancy</b>	Any exposure to fetal immunogenetic tissues including miscarriages and live births.

<b>primary response</b>	The reaction of the immune system at the time of its first exposure to a novel antigen. Usually mild and non-specific.
<b>protein A</b>	A substance which non-specifically binds to immunoglobulins.
<b>rabbit complement</b>	Serum from rabbits. A laboratory reagent used as source of complement in the complement dependent cytotoxicity assay.
<b>reaction frequency</b>	The percentage of panel cells reacting with a serum sample.
<b>recommendation</b>	A guideline which should usually be adhered to.
<b>registry</b>	A database of patient records usually including outcome data facilitating survival analysis. e.g. <a href="http://www.renalreg.org">www.renalreg.org</a> , <a href="http://www.ctstransplant.org">www.ctstransplant.org</a>
<b>regraft</b>	Second and subsequent transplants.
<b>rescue therapy</b>	A treatment aimed to prevent failure of a transplanted organ in the face of an aggressive immune response. Usually uses an immunosuppressive drug which has not already been used in the patient's treatment.
<b>risk</b>	The degree to which a hazardous outcome is predicted.
<b>rituximab</b>	A monoclonal antibody modified to be mainly human protein. Specificity is for CD20. Used in transplantation to severely restrict the efficacy of the immune response.
<b>Royal College of Pathologists</b>	<a href="http://www.rcpath.org">www.rcpath.org</a>
<b>screening strategy</b>	A process to detect and define sensitisation.
<b>secondary response</b>	An aggressive immune response mounted on re-exposure to a previously recognised antigen.
<b>sensitisation</b>	An immune response to an antigen resulting in alloreactive T and B cells and antibody formation.
<b>sensitivity (of a patient)</b>	The ability to mount an immune response to an antigen.
<b>sensitivity (of an assay)</b>	An evaluation of the accuracy of the results of a laboratory test to predict an outcome. Usually quoted as a percentage.
<b>serum</b>	The straw-coloured fluid from the blood plasma in which body chemicals are suspended. Serum is used to test for the presence or absence and levels of these chemicals, including antibodies.
<b>solid phase assays</b>	A laboratory test to identify presence of antibodies in a serum sample 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 antigen target is usually cell free HLA antigens.
<b>specificity</b>	The defined reactivity of an antibody e.g. specific for an HLA molecule.
<b>spleen</b>	An organ located close to the liver which acts as a reservoir of immune active lymphocytes.

<b>steroid-resistant rejection</b>	A rejection episode which cannot be suppressed by administration of steroid drugs.
<b>T cell receptor</b>	A T cell surface molecule which interacts with HLA antigens on antigen presenting cells to initiate an immune response.
<b>T lymphocyte</b>	A white blood cell which gains maturity in the thymus. There are several types and sub-types reflecting the function of a specific group of T cells. Characterised by expression of specific CD markers.
<b>target cell</b>	A cell population used in an immune reactivity assay which carries antigenic molecules to which the test cell or serum responds.
<b>T-cell crossmatch (T-cell XM)</b>	A crossmatch test in which the target cells are T lymphocytes.
<b>thoracic organ</b>	An organ located in the chest cavity (thorax); the heart and lungs.
<b>titre</b>	The level at which an antibody occurs in blood. Measured by repeatedly diluting serum until activity disappears.
<b>transplant list</b>	A register of patients eligible to receive an organ transplant. May include patients temporarily unavailable due to ill health or absence on holiday.
<b>unacceptable antigen</b>	HLA specificities which must not be present on a transplanted organ (veto). They can be the result of sensitisation or prior exposure, as in a pregnancy.
<b>vascular rejection</b>	An immune response with activity detected in the blood vessels of the transplanted organ. Usually an aggressive process mediated by antibodies.
<b>vasculopathy</b>	Diseased blood vessels.
<b>veto</b>	Prohibition or contra-indication to a transplant proceeding due to a considered unacceptable high risk of early transplant failure e.g. ABO blood group incompatibility.
<b>“weak positive” crossmatch</b>	Redundant term. The report following a crossmatch test must either be ‘positive / incompatible’ or ‘negative / compatible’.
<b>xenograft</b>	Transplantation of an organ between different species e.g. baboon liver to a human.

## Appendices

### 3) References

- Taylor CJ, Welsh KI, Gray CM, et al. *Clinical and socio-economic benefits of serological HLA-DR matching for renal transplantation over three eras of immunosuppression regimens in a single unit.* Clinical Transplants 1993, 233-241
- Van de Watering L, Hermans J, Witvliet M, et al. *HLA and red blood cell immunisation after filtered and buffy coat-depleted blood transfusion in cardiac surgery: a randomised controlled trial.* Transfusion 2003, 43:765-771
- Williams GM, Hume DM, Hudson RP, Morris PJ, Kano K, Milgrom F. *Hyperacute renal-homograft rejection in man.* NEJM 1968, 279:611-618
- Cross DE, Whittier FC, Cuppage FE, Crouch T, Manuel EL, Grantham JJ. *Hyperacute rejection of renal allografts following pulsatile perfusion with a perfusate containing specific antibody.* Transplantation 1974, 17:626-629
- Porter KA. *The effects of antibodies on human renal allografts.* Transplant Proc. 1976, 8:189-197
- Tilney NL, Garovoy MR, Busch GJ, Strom TB, Graves MJ, Carpenter CB. *Rejected human renal allografts. Recovery and characteristics if infiltrating cells and antibody.* Transplantation 1979, 28:421-426
- Garovoy MR, Reddish MA, Busch GJ, Tilney NL. *Immunoglobulin-secreting cells recovered from rejected human renal allografts.* Transplantation 1982, 33:109-111
- Lederer SR, Kluth-Pepper B, Schneeberger H, Albert E, Land W, Feucht HE. *Impact of humoral alloreactivity early after transplantation on the long term survival of renal allografts.* Kidney Int. 2001, 59:334-342
- Strom TB, Tilney NL, Paradysz JM, Banciewicz J, Carpenter CB. *Cellular components of allograft rejection: identity, specificity and cytotoxic function of cells infiltrating acutely rejection allografts.* J. Immunology 1977, 118:2020-2026
- Gebel HM, Bray RA, Nickerson P. *Pre-transplant assessment of donor-reactive HLA-specific antibodies in renal transplantation: contraindication vs risk.* Am. J. Transplantation 2003, 3:1488-1500
- Zachary AA, Klingman L, Thorne N, et al. *Variations of the lymphocytotoxicity test. An evaluation of sensitivity and specificity.* Transplantation 1995, 60:498-503
- Ting A, Morris PJ. *Renal transplantation and B-cell cross-matches with autoantibodies and alloantibodies.* Lancet 1977, ii:1095-1097
- Buelow R, Chiang T, Monteiro F, et al. *Soluble HLA antigens and ELISA – a new technology for crossmatch testing.* Transplantation 1995, 60:1594-1599
- Kao KJ, Scornik JC, Small SJ. *Enzyme-linked immunoassay for anti-HLA antibodies – an alternative to panel studies by lymphocytotoxicity.* Transplantation 1993, 55:192-196
- Lucas DP, Paparounis ML, Myers L, et al. *Detection of HLA class I specific antibodies by the QuikScreen enzyme-linked immunosorbent assay.* Clin. Diagn. Lab. Immunol. 1997, 4:252-257
- Worthington JE, Robson AJ, Sheldon S, Langton A, Martin S. *A comparison of enzyme-linked immunosorbent assays and flow cytometry techniques for the detection of HLA specific antibodies.* Human Immunology 2001, 62:1178-1184
- Zachary AA, Delaney NL, Lucas DP, Leffell MS. *Characterisation of HLA class I specific antibodies by ELISA using solubilized antigen targets: evaluation of the GTI QuikID assay and analysis of antibody patterns.* Human Immunology 2001, 62:228-235
- Harmer AW, Sutton M, Bayne A, et al. *A highly sensitive, rapid screening method for the detection of antibodies directed against HLA class I and class II antigens.* Transpl. Int. 1993, 6:277-280
- Harmer AW, Heads AJ, Vaughan RW. *Detection of HLA class I and class II specific antibodies by flow cytometry and PRA-STAT screening in renal transplant recipients.* Transplantation 1997, 63:1828-1832

- 20 Lederer SR, Schneeberger H, Albert E, et al. *Early graft dysfunction: The role of preformed antibodies to DR-typed lymphoblastoid cell lines.* Transplantation 1996, 61:313-319
- 21 Ciccirelli J, Helstab K, Mendez R. *Flow cytometry PRA, a new test that is highly correlated with graft survival.* Clin. Transplantation 1992, 6:159-164
- 22 Shroyer TW, Deierhoi MH, Mink CA, et al. *A rapid flow cytometry assay for HLA antibody detection using a pooled cell panel covering 14 serological crossreacting groups.* Transplantation 1995, 59:626-630
- 23 Karuppan SS, Moller E. *The use of magnetic beads coated with soluble HLA class I or class II proteins in antibody screening and for specificity determination of donor-reactive antibodies.* Transplantation 1996, 61:1539-1545
- 24 Pei R, Wang C, Tarsitani C, et al. *Simultaneous HLA class I and class II antibodies screening with flow cytometry.* Human Immunology 1998, 59:313-322
- 25 Ellis TM, Gebel HM, Pierce KL, Bray RA. *Limitations to antibody detection using single antigen flow beads.* Human Immunology 2003, 64(suppl1):S14
- 26 El-Awar N, Terasaki PI, Lee J-H et al. *Analysis of mouse monoclonal antibodies with single antigen beads.* Human Immunology 2003, 64(suppl1):S14
- 27 Khan N, Robson AJ, Worthington JE, Martin S. *The detection and definition of IgM alloantibodies in the presence of IgM autoantibodies using flow PRA beads.* Human Immunology 2003, 64:593-599
- 28 Fulton RJ, McDade RC, Smith PL, Kienker LJ, Kettman JR. *Advanced multiplexed analysis with the FlowMetrix system.* Clin. Chem. 1997, 43:1749-1756
- 29 Chesterton KA, Pretl K, Sholander JT et al. *Rapid and reliable detection of HLA-specific antibodies with the luminex platform.* Human Immunology 2003, 64(suppl 1):S108
- 30 Taylor CJ, Chapman JR, Ting A, Morris PJ. *Characterization of lymphocytotoxic antibodies causing a positive crossmatch in renal transplantation. Relationship to primary and re-raft outcome.* Transplantation 1989, 48:953-958
- 31 Claas F. *Eurotransplant data (personal communication)*
- 32 Kissmeyer-Nielsen F, Olsen S, Petersen V.P, Fjeldborg O. *Hyperacute rejection of kidney allografts associates with pre-existing humoral antibodies against donor cells.* Lancet 1966, 1:662-665
- 33 Terasaki PI, McClelland JD. *Microdroplet assay of human serum cytotoxins.* Nature 1964, 204:998-1000
- 34 Patel R, Terasaki PI. *Significance of the positive crossmatch test in kidney transplantation.* NEJM 1969, 280:735-739
- 35 Garovoy MR, Rheinschmidt MA, Bigos M, Perkins H, Colombe B. *Flow cytometry analysis: a high technology crossmatch technique facilitating transplantation.* Transplant Proc. 1983, 15:1939-1944
- 36 Cecka JM. *The UNOS Transplant Registry. In: Clinical Transplants 2001 Eds: M Cecka & PI Terasaki.* UCLA Press, Los Angeles. 1-18
- 37 Fuggle SV, Martin S. *Towards transplantation of highly sensitised patients.* Transplantation 2004, 78:186-189
- 38 Connolly JK, Dyer PA, Martin S, et al. *Importance of minimising HLA-DR mismatch and cold preservation time in cadaveric renal transplantation.* Transplantation 1996, 61:709-714
- 39 Takemoto S, Carnahan E, Terasaki P. *Report on 604 six-antigen-matched transplants.* In Terasaki P (ed): Clinical Transplants 1990, Los Angeles, UCLA Tissue Typing Laboratory, 1991, 485-495
- 40 Braun WE. *Laboratory and clinical management of the highly sensitised organ transplant recipient.* Human Immunology 1989, 26:245-260
- 41 Duquesnoy RJ, White LT, Fierst JW, et al. *Multiscreen serum analysis of highly sensitised renal dialysis patients for antibodies toward public and private class I HLA determinants.* Transplantation 1990, 50:427-437
- 42 Rodey GE, Revels K, Fuller TC. *Epitope specificity of HLA class I alloantibodies: Stability of cross reactive group patterns over extended time periods.* Transplantation 1997, 63:885-893
- 43 Chapman JR, Taylor C, Ting A, Morris PJ. *Hyperacute rejection of a renal allograft in the presence of anti-HLA-Cw5 antibody.* Transplantation 1986, 42:91-93
- 44 Takemoto S, Cecka JM, Gjertson DW, et al. *Six-antigen-matched transplants: causes of failure.* Transplantation 1993, 55:1005-1008
- 45 Mytilineos J, Deufel A, Opelz G. *Clinical relevance of HLA-DPB locus matching for cadaver kidney transplantation: a report of the Collaborative Transplant Study.* Transplantation 1997, 63:1351-1354
- 46 Chapman JR, Taylor C, Ting A, et al. *Immunoglobulin class and specificity of antibodies causing positive T cell crossmatches: relationship with renal transplant outcome.* Transplantation 1986, 42:608-613
- 47 Fenoglio J, Ho E, Reed E, et al. *Anti-HLA antibodies and heart allograft survival.* Transplant Proc 1989, 21: 807-809
- 48 Mohanakumar T, Rhodes C, Mendez-Picon G, et al. *Renal allograft rejection associated with presensitization to HLA-DR antigens.* Transplantation 1981, 31:93-95
- 49 Scornik JC, LeFor WM, Ciccirelli JC, et al. *Hyperacute and acute kidney graft rejection due to antibodies against B cells.* Transplantation 1992, 54:61-64
- 50 Taylor CJ, Chapman JR, Fuggle SV, et al. *A positive B cell crossmatch due to IgG anti-HLA-DQ antibody present at the time of transplantation in a successful renal allograft.* Tissue Antigens 1987, 30:104-112
- 51 Ting A, Morris PJ. *Successful transplantation with a positive T and B cell crossmatch due to autoreactive antibodies.* Tissue Antigens 1983, 21:219-226
- 52 Cardella, CJ. *The use of the kidney with an historical positive, and current negative crossmatch.* Pediatr. Nephrol. 1991, 5:126-129
- 53 Ten Hoor GM, Coopmans M, Allebes WA. *Specificity and Ig class of preformed alloantibodies causing a positive crossmatch in renal transplantation.* Transplantation 1993, 56:298-304
- 54 Roelen DL, van Bree FPMJ, Witvliet MD. *IgG antibodies against HLA antigen are associated with activated cytotoxic T cells against this antigen. IgM are not.* Transplantation 1994, 57:1388-1392
- 55 Oostingh GJ, Davies HFS, Bradley JA, Taylor CJ. *Comparison of allogeneic and xenogeneic in vitro T cell proliferative responses of sensitised patients awaiting kidney transplantation.* Xenotransplantation 2003, 10:545-551
- 56 Van Kampen CA, Roelen DL, Versteeg-van der Voort Maarschalk MFJ, Hoitsma AJ, Allebes WA, et al. *Activated HLA class I-reactive cytotoxic T lymphocytes associated with a positive historical crossmatch predict early graft failure.* Transplantation 2002, 74:1114-1119
- 57 Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RWG. *Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome.* Transplantation 2003, 75:1034-1040
- 58 Chapman JR, Deierhoi MH, Carter NP, Ting A, Morris PJ. *Analysis of flow cytometry and cytotoxicity crossmatches in renal transplantation.* Transplant Proc. 1985, 17:2480-2481
- 59 Thistlethwaite JR, Buckingham MR, Stuart JK, Stuart FP. *Detection of presensitisation in renal allograft recipients using a flow cytometric immunofluorescence crossmatch.* Transplant Proc. 1986, 18:676-679
- 60 Cook DJ, Terasaki PI, Iwaki Y, Terashita GY, Lau M. *An approach to reducing early kidney transplant failure by flow cytometry crossmatching.* Clinical Transplantation 1987, 1:253-256
- 61 Lazda VA, Pollak R, Mozes MF, Joasson O. *The relationship between flow cytometer crossmatch results and subsequent rejection episodes in cadaver renal allograft recipients.* Transplantation 1988, 45:562-565
- 62 Talbot D, Givan AL, Shenton BK, et al. *The prospective value of the preoperative flow cytometric crossmatch assay in renal transplantation.* Transplantation 1990, 49:809-810

- 63** Ogura K, Terasaki PI, Johnson C, et al. *The significance of a positive flow cytometric crossmatch test in primary kidney transplantation.* Transplantation 1993, 56:294-298
- 64** Horsburgh T, Mistry N, Weston S, et al. *Relevance of positive crossmatches in renal transplantation.* Transplant Proc. 1992, 24:2508-2509
- 65** Evans PR, Lane AC, Lambert CM, et al. *Lack of correlation between IgG T-lymphocyte flow cytometric crossmatches with primary renal allograft outcome.* Transplant International 1992, 5:S609-S612
- 66** Martin S, Liggett H, Robson A, Connolly J, Johnson RWG. *The association between a positive T and B cell flow cytometry crossmatch and renal transplant failure.* Transplant Immunology 1993, 1:270-276
- 67** Lazda VA. *Identification of patients at risk for inferior renal allograft outcome by a strongly positive B cell flow cytometry crossmatch.* Transplantation 1994, 57:964-969
- 68** Kimball P, Rhodes C, King A, Fisher R, Ham J, Posner M. *Flow crossmatching identifies patients at risk of post operative elaboration of cytotoxic antibodies.* Transplantation 1998, 65:444-446
- 69** Cho YW, Cecka JM. *Crossmatch tests – an analysis of UNOS data from 1991-2000.* In Clinical Transplants 2001; Eds: M Cecka and PI Terasaki, UCLA Press, Los Angeles, 2002, 237-246
- 70** El Fettouh HA, Cook DJ, Bishay E, et al. *Association between a positive flow cytometry crossmatch and the development of chronic rejection in primary renal transplantation.* Urology 2000, 56:369-372
- 71** Matas AJ, Sutherland DER. *Kidney transplantation without a final crossmatch.* Transplantation 1998, 66:1835-1836
- 72** Kerman RH, Susskind B, Ruth J, Katz S, Van Buren CT, Kahan BD. *Can an immunologically nonreactive potential allograft recipient undergo transplantation without a donor-specific crossmatch?* Transplantation 1998, 66:1833-1845
- 73** Taylor CJ, Smith SI, Morgan CH, et al. *Selective omission of the donor crossmatch before renal transplantation.* Transplantation 2000, 69:719-723
- 74** Suciu-Foca N, Reed E, D'Agati VD, et al. *Soluble HLA antigens anti-HLA antibodies and antidiotypic antibodies in the circulation of renal transplant recipients.* Transplantation 1991, 51:593-601
- 75** Halloran PF, Schlaut J, Solez K, Srinivasa NS. *The significance of the anti-class I response. II. Clinical and pathologic features of renal transplants with anti-class I-like antibody.* Transplantation 1992, 53:550-555
- 76** Al-Hussein KA, Shenton BK, Bell A, et al. *Characterisation of donor-directed antibody class in the post-transplant period using flow cytometry.* Transplant Int. 1994, 7:182-189
- 77** Harmer AW, Koffman CG, Heads AJ, et al. *Sensitisation to HLA antigens occurs in 95% of primary renal transplant rejections.* Transplant Proc. 1995, 27:666-667
- 78** Trpkov K, Campbell P, Pazderka F, Cockfield S, Solez K, Halloran PF. *Pathologic features of acute renal allograft rejection associated with donor-specific antibody. Analysis using the Banff grading schema.* Transplantation 1996, 61:1586-1592
- 79** Martin S, Dyer PA, Mallick NP, Gokal R, Harris R, Johnson RW. *Post transplant antidonor lymphocytotoxic antibody production in relation to graft outcome.* Transplantation 1997, 44:50-53
- 80** Monteiro F, Buelow R, Mineiro C, Rodrigues H, Kalil J. *Identification of patients at high risk of graft loss by pre- and post-transplant monitoring of anti-HLA class I IgG antibodies by enzyme linked immunosorbent assay.* Transplantation 1997, 63:542-546
- 81** Abe M, Kawai T, Futatsuyama K, et al. *Postoperative production of anti-donor antibody and chronic rejection in renal transplantation.* Transplantation 1997, 63:1616-1619
- 82** Kerman RH, Orosz CG, Lober MI. *Clinical relevance of anti-HLA antibodies pre and post transplant.* Am. J. Med. Sci. 1997, 313:275-278

- 83** Scheonemann C, Groth J, Leveerez S, May G. *HLA class I and class II antibodies: monitoring before and after kidney transplantation and their clinical relevance.* Transplantation 1998, 65:1519-1523
- 84** Christiaans MH, Overhof-de Roos R, Nieman F, van Hoeff JP, van den Berg-Loonen EM. *Donor-specific antibodies after transplantation by flow cytometry: relative change in fluorescence ratio most sensitive risk factor for graft survival.* Transplantation 1998, 65:427-433
- 85** Muller-Steinhardt M, Fricke L, Kirchner H, Hoyer J, Kluter H. *Monitoring of anti-HLA class I and II antibodies by flow cytometry in patients after first cadaveric kidney transplantation.* Clinical Transplant 2000, 14:85-89
- 86** Piazza A, Borrelli L, Monaco PI, et al. *Post-transplant donor-specific antibody characterisation and kidney graft survival.* Transplant Int. 2000, 13:S439-S443
- 87** Piazza A, Poggi E, Borrelli L, et al. *Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: posttransplant analysis using flow cytometric techniques.* Transplantation 2001, 71:1106-1112
- 88** McKenna RM, Takemoto SK, Terasaki PI. *HLA antibodies after solid organ transplantation.* Transplantation 2000, 69:319-326
- 89** Zwirner NW, Marcos CY, Mirbaha F, Zou Y, Stasny P. *Identification of MICA as a new polymorphic alloantigen recognised by antibodies in sera of organ transplant recipients.* Human Immunology 2000, 61:917-924
- 90** Sumitran-Holgersson SS, Wilczek HE, Holgersson J, Soderstrom K. *Identification of the nonclassical HLA molecules, MICA, as targets for humoral immunity associated with irreversible rejection of kidney allografts.* Transplantation 2002, 74:269-277
- 91** Hankey KG, Deachenberg CB, Papadimitriou JC, et al. *MIC expression in renal and pancreatic allografts.* Transplantation 2002, 73:304-306
- 92** Zwirner NW, Fernandez-Vina MA, Stasny P. *MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes.* Immunogenetics 1998, 47:139-148
- 93** Zwirner NW, Dole K, Stasny P. *Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes and monocytes.* Human Immunology 1999, 60:323-330
- 94** Feucht HE, Schneeberger H, Hillebrand G, et al. *Capillary deposition of C4d complement fragment and early renal graft loss.* Kidney Int. 1993, 43:1333-1338
- 95** Crespo M, Pascuala M, Tolkoff-Rubin N, et al. *Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics.* Transplantation 2001, 71:652-658
- 96** Herzenberg AM, Gill JS, Djurdjev O, Magil AB. *C4d deposition in acute rejection: an independent long-term prognostic factor.* J. Am. Soc. Nephrol. 2002, 13:234-241
- 97** Mauiyedi S, Crespo M, Collins AB, et al. *Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification.* J. Am. Soc. Nephrol. 2002, 13:779-787
- 98** Nickleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. *Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications.* J. Am. Soc. Nephrol. 2002, 13:242-251
- 99** Bohmig GA, Exner M, Habicht A, et al. *Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury.* J. Am. Soc. Nephrol. 2002, 13:1091-1099
- 100** Collins AB, Schneeberger EE, Pascual MA, et al. *Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries.* J. Am. Soc. Nephrol. 1999, 10:2208-2214
- 101** Racusen LC, Colvin RB, Solez K, et al. *Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection.* Am. J. Transplant. 2003, 3:708-714
- 102** Theruvath TP, Saidman SL, Mauiyedi S, et al. *Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection.* Transplantation 2001, 72:77-83
- 103** Rose ML, Smith J, Dureau G, Keogh A, Kobashigowa J. *Mycophenolate mofetil decreases antibody production after cardiac transplantation. J. Heart Lung Transplant 2002, 21:282-285*

- 104** Ishida H, Tanabe K, Furusawa M, et al. *Mycophenolate mofetil suppresses the production of anti-blood type antibodies after renal transplantation across the ABO blood barrier.* Transplantation 2002, 74:1187-1189
- 105** Smith JD, Danskine AJ, Laylor RM, Rose ML, Yacoub MH. *The effect of panel reactive antibodies and the donor specific crossmatch on graft survival after heart and heart-lung transplantation.* Transplant Immunology 1993, 1:60-65
- 106** Weil R, Clarke DR, Iwaki Y, et al. *Hyperacute rejection of a transplanted human heart.* Transplantation 1981, 32:71-72
- 107** Singh G, Thompson M, Griffith B, et al. *Histocompatibility in cardiac transplantation with particular reference to immunopathology of positive serologic crossmatch.* Clinical Immunology and Immunopathology 1983, 28:56-66
- 108** Itescu S, Tung TC, et al. *Preformed IgG antibodies against major histocompatibility complex class II antigens are major risk factors for high-grade cellular rejection in recipients of heart transplantation.* Circulation 1998, 98:786-793
- 109** Przybylowski P, Balogna M, Radovancevic B, et al. *The role of flow cytometry-detected IgG and IgM anti-donor antibodies in cardiac allograft recipients.* Transplantation 1999, 67:258-262
- 110** Scornik JC, Zander DS, Baz MA, Donnelly WH, Staples ED. *Susceptibility of lung transplants to preformed donor-specific HLA antibodies as detected by flow cytometry.* Transplantation 1999, 68:1542-1546
- 111** Suciu-Foca N, Reed E, Marboe C, et al. *The role of anti-HLA antibodies in heart transplantation.* Transplantation 1991, 51:716-724
- 112** Smith JD, Danskine AJ, Rose ML, Yacoub MH. *Specificity of lymphocytotoxic antibodies formed after cardiac transplantation and correlation with rejection episodes.* Transplantation 1992, 53:1358-1362
- 113** George JF, Kirklin JK, Shroyer TW, et al. *Utility of posttransplantation panel-reactive antibody measurements for the prediction of rejection frequency and survival of heart transplant recipients.* Journal of Heart and Lung Transplantation 1995, 14:856-864
- 114** Leech SH, Mather PJ, Eisen HJ, et al. *Donor-specific HLA antibodies after transplantation are associated with deterioration in cardiac function.* Clinical Transplantation 1996, 10:639-645
- 115** Sundaresan S, Mohanakumar T, Smith MA, et al. *HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome.* Transplantation 1998, 65:648-653
- 116** Jaramillo A, Smith MA, Phelan D, et al. *Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation.* Transplantation 1999, 67:1155-1161
- 117** Rose EA, Pepino P, Barr ML, et al. *Relation of HLA antibodies and graft atherosclerosis in human cardiac allograft recipients.* Journal of Heart and Lung Transplantation 1992, 11(3 Pt 2):S120-S123
- 118** Reed EF, Hong B, Ho E, Harris PE, Weinberger J, Suciu-Foca N. *Monitoring of soluble HLA alloantigens and anti-HLA antibodies identifies heart allograft recipients at risk of transplant-associated coronary artery disease.* Transplantation 1996, 61:566-572
- 119** Dunn MJ, Crisp SJ, Rose ML, Taylor PM, Yacoub MH. *Anti-endothelial antibodies and coronary artery disease after cardiac transplantation.* Lancet 1992, 339:1566-1570
- 120** Faulk WP, Rose M, Meroni PL, et al. *Antibodies to endothelial cells identify myocardial damage and predict development of coronary artery disease in patients with transplanted hearts.* Human Immunology 1999, 60:826-832
- 121** Ferry BL, Welsh KI, Dunn MJ, et al. *Anti-cell surface endothelial antibodies in sera from cardiac and kidney transplant recipients: association with chronic rejection.* Transplant Immunology 1997, 5:17-24
- 122** Fredrich R, Toyoda M, Czer LS, et al. *The clinical significance of antibodies to human vascular endothelial cells after cardiac transplantation.* Transplantation 1999, 67: 385-391
- 123** Wheeler CH, Collins A, Dunn MJ, et al. *Characterization of endothelial antigens associated with transplant-associated coronary artery disease.* The Journal of Heart and Lung Transplantation 1995, 14:S188-197
- 124** Jurcevic S, Ainsworth ME, Pomerance A, et al. *Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation.* Transplantation 2001, 71:886-892
- 125** Ratner LE, Phelan D, Brunt EM, Mohanakumar T, Hanto DW. *Probable antibody-mediated failure of two sequential ABO-compatible hepatic allografts in a single recipient.* Transplantation 1993, 55:814-819
- 126** Freese DK, Snover DC, Sharp HL, Gross CR, Savick SK, Payne WD. *Chronic rejection after liver transplantation: a study of clinical, histopathological and immunological features.* Hepatology 1991, 13:882-891
- 127** Lobo PI, Spencer C, Douglas MT, Stevenson WC, Pruett TL. *The lack of long-term detrimental effects on liver allografts caused by donor-specific anti-HLA antibodies.* Transplantation 1993, 55:1063-1066
- 128** Donaldson PT, Thomson LJ, Heads A, et al. *IgG donor-specific crossmatches are not associated with graft rejection or poor graft survival after liver transplantation. An assessment by cytotoxicity and flow cytometry.* Transplantation 1995, 60:1016-1023
- 129** Goggins WC, Fisher RA, Kimball PM, et al. *The impact of a positive crossmatch upon outcome after liver transplantation.* Transplantation 1996, 62:1794-1798
- 130** Doyle HR, Marino IR, Morelli F, et al. *Assessing risk in liver transplantation. Special reference to the significance of a positive cytotoxic crossmatch.* Ann. Surg. 1996, 224:168-177
- 131** Fujita S, Rosen C, Reed A, et al. *Significance of preformed anti-donor antibodies in liver transplantation.* Transplantation 1997, 63:84-88
- 132** Scornik JC, Soldevilla-Pico C, Van der Werf WJ, et al. *Susceptibility of liver allografts to high or low concentrations of preformed antibodies as measured by flow cytometry.* Am J Transplant 2001, 1:152-156
- 133** Gordon RD, Fung JJ, Markus B, et al. *The antibody crossmatch in liver transplantation.* Surgery 1986, 100:705-715
- 134** Batts KP, Moore SB, Perkins JD, Wiesner RH, Grambsch PM, Krom RA. *Influence of positive lymphocyte crossmatch and HLA mismatching on vanishing bile duct syndrome in human liver allografts.* Transplantation 1988, 45:376-379
- 135** Takaya S, Bronsther O, Iwaki Y, et al. *The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors.* Transplantation 1992, 53:400-406
- 136** Nikaein A, Backman L, Jennings L, et al. *HLA compatibility and liver transplant outcome. Improved patient survival by HLA and cross-matching.* Transplantation 1994, 58:786-792
- 137** Manez R, Kelly RH, Kobayashi M, et al. *Immunoglobulin G lymphocytotoxic antibodies in clinical liver transplantation: studies toward further defining their significance.* Hepatology 1995, 21:1345-1352
- 138** Charco R, Vargas V, Balsells J, et al. *Influence of anti-HLA antibodies and positive T-lymphocytotoxic crossmatch on survival and graft rejection in human liver transplantation.* J. Hepatol 1996, 24: 452-459
- 139** Hathaway M, Gunson BK, Keogh AC, Briggs D, McMaster P, Neuberger JM. *A positive crossmatch in liver transplantation--no effect or inappropriate analysis? A prospective study.* Transplantation 1997, 64:54-59
- 140** Doran TJ, Geczy AF, Painter D, et al. *A large, single centre investigation of the immunogenetic factors affecting liver transplantation.* Transplantation 2000, 69:1491-1498
- 141** Bathgate AJ, McColl M, Garden OJ, Forsythe JL, Madhavan KK, Hayes PC. *The effect of a positive T-lymphocytotoxic crossmatch on hepatic allograft survival and rejection.* Liver Transpl. Surg. 1998, 4:280-284
- 142** Bishara A, Brautbar C, Eid A, Sherman L, Ilan Y, Safadi R. *Is presentization relevant to liver transplantation outcome?* Human Immunology 2002, 63:742-750
- 143** Grant D, on behalf of the Intestinal transplant registry. *Intestinal transplantation: 1997 report of the international registry.* Transplantation 1999, 67:1061-1064

- 144** Ruiz P, Garcia M, Berney T, et al. *Mucosal vascula alterations in isolated small-bowel allografts: relationship to humoral sensitisation.* Am. J. Transplantation 2003, 3:43-49.
- 145** Ishii T, Mazariegos GV, Bueno J, et al. *Exfoliative rejection after intestinal transplantation in children.* Pediatr Transplant 2003, 7:185-191
- 146** Heyman B. *Regulation of antibody responses via antibodies, complement and Fc receptors.* Ann. Rev. Immunol. 2000, 18:709-737
- 147** Cardella CJ, Falk JA, Nicholson MJ. *Successful renal transplantation in patients with T-cell reactivity to donor.* Lancet 1982, 2:1240-1243
- 148** Hack N, Angra S, Friedman E, McKnight T, Cardella CJ. *Anti-idiotypic antibodies from highly sensitized patients stimulate B cells to produce anti-HLA antibodies.* Transplantation 2002, 73:1853-1858
- 149** Eugui EM, Almquist SJ, Muller CD, et al. *Lymphocyte-selective and immunosuppressive effects of mycophenolic acid in vitro: role of deoxyguanosine nucleotide depletion.* Scand. J. Immunol. 1991, 33:161-173
- 150** Viera C, Agarwal A, Book B. *Rituximab for reduction of anti-HLA antibodies in patients awaiting renal transplantation.* In Transplant. Proc. XIX International Congress of the Transplant Society 2002.
- 151** Looney RJ. *Treating human autoimmune disease by depleting B cells.* Ann Rheum Dis 2002, 61:863-866
- 152** Gloor JM, DeGoey SR, Pineda AA, et al. *Overcoming a positive crossmatch in living-donor kidney transplantation.* Am. J. Transplantation 2003, 3:1017-1023
- 153** Taube DH, Welsh KI, Kennedy LA, et al. *Successful removal and prevention of resynthesis of anti-HLA antibody.* Transplantation 1984, 37:254-255
- 154** Raftery MJ, Malik ST, Tidman, N, et al. *Successful renal transplantation despite a positive fluorescence-activated cell sorter crossmatch following plasma exchange of donor-specific anti-HLA antibodies.* Transplantation 1986, 4:131-133
- 155** Reisaeter AV, Leivestad T, Albrechtsen D, et al. *Pretransplant plasma exchange or immunoadsorption facilitates renal transplantation in immunized patients.* Transplantation 1995, 6:242-248
- 156** Schweitzer EJ, Wilson JS, Fernandez-Vina M, et al. *A high panel-reactive antibody rescue protocol for cross-match-positive live donor kidney transplants.* Transplantation 2000, 70:1531-1536
- 157** Hakim RM, Milford E, Himmelfarb J, et al. *Extracorporeal removal of anti-HLA antibodies in transplant candidates.* Am. J. Kidney. Dis. 1990, 16:423-431
- 158** Rifle G, Chalopin JM, Turc JM, et al. *Plasmapheresis in the treatment of renal allograft rejections.* Transplant Proc. 1979, 11:20-26
- 159** Blake P, Sutton D, Cardella CJ. *Plasma exchange in acute renal transplant rejection.* Prog. Clin. Biol. Res. 1990, 337:249-252
- 160** Bonomini V, Vangelista A, Frasca G, et al. *Effects of plasmapheresis in renal transplant rejection. A controlled study.* Trans. Am. Soc. Artif. Intern. Organs 1985, 31:698-703
- 161** Frasca G, Vangelista A, DiFelice A, et al. *The rationale for plasmapheresis in renal graft rejection.* Life Support Syst. 1984, 2:131-136
- 162** Grandtnerova B, Javorsky P, Kalacay J, et al. *Treatment of acute humoral rejection in kidney transplantation with plasmapheresis.* Transplant Proc. 1995, 27:934-935
- 163** Bittner HB, Dunitz J, Hertz M, et al. *Hyperacute rejection in single lung transplantation--case report of successful management by means of plasmapheresis and antithymocyte globulin treatment.* Transplantation 2001, 71:649-651
- 164** Palmer A, Taube D, Welsh KI, et al. *Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation.* Lancet 1989, 1:10-12
- 165** Kupin WL, Venkat KK, Hagashi H, et al. *Removal of lymphocytotoxic antibodies by pretransplant immunoadsorption therapy in highly sensitized renal transplant recipients.* Transplantation 1991, 51:324-329
- 166** Hiesse C, Kriaa F, Rousseau P, et al. *Immunoadsorption of anti-HLA antibodies for highly sensitized patients awaiting renal transplantation.* Nephrol. Dial. Transplant. 1992, 7:944-951
- 167** Ross CN, Gaskin G, Gregor-Macgregor S, et al. *Renal transplantation following immunoadsorption in highly sensitised recipients.* Transplantation 1993, 55:785-789
- 168** Higgins RM, Bevan DJ, Carey BS, et al. *Prevention of hyperacute rejection by removal of antibodies to HLA immediately before renal transplantation.* Lancet 1996, 348:1208-1211
- 169** Hicksteina H, Karten G, Bast R, et al. *Immunoadsorption of sensitized kidney transplant candidates immediately prior to surgery.* Clin. Transplant. 2002, 16:97-101
- 170** Barocci S, Nocera A. *In vitro removal of anti-HLA IgG antibodies from highly sensitized transplant recipients by immunoadsorption with protein A and protein G sepharose columns: a comparison.* Transpl. Int. 1993, 6:29-33
- 171** Gjorstrup P. *Anti-HLA antibody removal in hyperimmunized ESRF patients to allow transplantation. The Collaborative Study Group on Anti-HLA Antibody Removal.* Transplant Proc. 1991, 23:392-395
- 172** Samuelsson G. *Extracorporeal immunoadsorption with immunosoba protein A.* In: Therapeutic Plasmapheresis, Eds: T Agishi, A Kawamura and M Minneshima. 1993. X11:843
- 173** Gjorstrup P, Watt R. *Therapeutic Protein A immunoadsorption. A review.* Transfusion Science 1990, 11:281-302
- 174** Higgins RM, Bevan DJ, Vaughan RW, et al. *5-year follow-up of patients successfully transplanted after immunoadsorption to remove anti-HLA antibodies.* Nephron. 1996, 74:53-57
- 175** Bevan DJ, Carey BS, Vaughan RW, et al. *Modulation of anti-HLA antibody production following renal transplantation in sensitised, immunoadsorbed patients.* Transplant Proc. 1997, 29:1448.
- 176** Soares MP, Lin Y, Sato K, et al. *Accommodation.* Immunol. Today 1999, 20:434-437
- 177** Bevan DJ, Carey BS, Vaughan RW, et al. *Antibody removal and subsequent transplantation of a highly sensitised paediatric renal patient.* Transpl. Int. 1996, 9:155-1560
- 178** Imbach P, Barandum S, d'Apayzo V, et al. *High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood.* Lancet 1981, 1:1228-1231
- 179** Kazatchkine MD, Kaveri SV. *Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin.* N. Engl. J. Med. 2001, 345:747-755
- 180** Wassmuth R, Hauser IA, Schuler K, et al. *Differential inhibitory effects of intravenous immunoglobulin preparations on HLA-alloantibodies in vitro.* Transplantation 2001, 71:1436-1442
- 181** Blasczyk R, Westhoff U, Grosse-Wilde H. *Soluble CD4, CD8, and HLA molecules in commercial immunoglobulin preparations.* Lancet 1993, 341:789-790
- 182** Lam L, Whitsett CF, McNicholl JM, et al. *Immunologically active proteins in intravenous immunoglobulin.* Lancet 1993, 342:678
- 183** Glotz D, Haymann JP, Samsonetti N, et al. *Suppression of HLA-specific alloantibodies by high-dose intravenous immunoglobulins (IVIg). A potential tool for transplantation of immunized patients.* Transplantation 1993, 56:335-337
- 184** Tyan DB, Li VA, Czer L, et al. *Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ.* Transplantation 1994, 57:553-562
- 185** Montgomery RA, Zachary AA, Racuseal LC, et al. *Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients.* Transplantation 2000, 70:887-895
- 186** Al-Uzri AY, Seltz B, Yurgin PD, et al. *Successful renal transplant outcome after intravenous gamma-globulin treatment of a highly sensitized pediatric recipient.* Pediatr Transplant 2002, 6:161-165
- 187** Glotz D, Antoine C, Julia P, et al. *Desensitisation and Subsequent Kidney Transplantation of Patients Using Intravenous Immunoglobulins (IVIg).* American Journal of Transplantation 2002, 2:758-760

- 188** Jordan SC, Vo A, Bunnapradist S, et al.  
*Intravenous immune globulin treatment inhibits crossmatch positivity and allows for successful transplantation of incompatible organs in living-donor and cadaver recipients.*  
Transplantation 2003, 76:631-636
- 189** McIntyre JA, Higgins RM, Britton R, et al.  
*Utilization of intravenous immunoglobulin to ameliorate alloantibodies in a highly sensitized patient with a cardiac assist device awaiting heart transplantation. Fluorescence-activated cell sorter analysis.*  
Transplantation 1996, 62:691-693
- 190** John R, Leitz K, Burke R, et al.  
*Intravenous immunoglobulin reduces anti-HLA alloreactivity and shortens waiting time to cardiac transplantation in highly sensitized left ventricular assist device recipients.*  
Circulation 1999, 100:11229-11235
- 191** Robinson JA, Radvany RM, Mullen MG et al.  
*Plasmapheresis followed by intravenous immunoglobulin in presensitized patients awaiting thoracic organ transplantation.*  
Ther Apher 1997, 1:147-151
- 192** Sonnenday CJ, Ratner LE, Zachary AA, et al.  
*Preemptive Therapy with Plasmapheresis/Intravenous Immunoglobulin Allows Successful Live Donor Renal Transplantation in Patients with a Positive Cross-Match.* Transplantation Proc. 2002, 34:614-616
- 193** Jordan SC, Quartel AW, Czer LS, et al.  
*Posttransplant therapy using high-dose human immunoglobulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action.*  
Transplantation 1998, 66:800-805
- 194** Luke PP, Scantlebury VP, Jordan ML, et al.  
*Reversal of steroid- and anti-lymphocyte antibody-resistant rejection using intravenous immunoglobulin (IVIg) in renal transplant recipients.*  
Transplantation 2001, 72:419-422
- 195** Casadei DH, Del C, Rial M, et al.  
*A randomized and prospective study comparing treatment with high-dose intravenous immunoglobulin with monoclonal antibodies for rescue of kidney grafts with steroid-resistant rejection.*  
Transplantation 2001, 71:53-58

## Appendices

### 4) Suggested audit standards

- 1) The percentage of patients shown to have developed HLA specific antibodies for:
  - Transplant list patients according to time waited and number of sensitising events
  - Transplanted recipients according to time after transplantation and by HLA mismatch
- 2) For antibody positive patients the frequency of:
  - a) de novo specific sensitisation
  - b) immunoglobulin class established
  - c) specificity for HLA class I, II and I+II
- 3) The percentage of transplant list patients from whom:
  - serum samples were received within the past three months
  - a positive crossmatch report was issued
- 4) Positive crossmatch rates according to patient gender, local or imported donor organ and HLA mismatch including specificity for HLA-Cw, -DQ and -DP mismatches
- 5) The percentage of transplanted patients from whom:
  - a crossmatch test was performed with a non-current serum sample, including timing
  - a flow cytometric crossmatch assay was performed
  - a retrospective crossmatch was performed including positive results
- 6) Occurrence of "high" and "intermediate" risk transplants as defined in these Guidelines. Include pre-transplant treatments and post-transplant management
- 7) The incidence and timing of post-transplant serum sample receipt and testing
- 8) The concurrence of post transplant biopsy C4d positivity and HLA specific antibody positivity with and without specificity for donor antigens

## Appendices

---

### 5) Statements of potential conflicts of interests

Philip Dyer has received honoraria from Novartis, One Lambda, Roche and Wyeth to attend advisory meetings and to give lectures. He has received financial support for registration fees, travel and accommodation to national and international conferences from Dynal Biotech, Fujisawa, Novartis, One Lambda, Roche and Wyeth. He co-supervise two trainee Clinical Scientists who are 50% funded by Dynal Biotech.

Andrea Harmer holds a patent which is subject to a licensing agreement with Dynal Biotech Ltd. She has received sponsorship from VHBio and One Lambda to attend the 2002 One Lambda European Histocompatibility Conference.

Susan Martin has received occasional grants to contribute towards the cost of attending the Transplantation Society and European Society for Organ Transplantation from Fujisawa and Novartis. She co-supervises two trainee Clinical Scientist posts which are 50% funded by Dynal Biotech.

Craig Taylor has received expenses for travel and accommodation to attend scientific meetings from Fujisawa. He has received honoraria for various lectures and contributions to books. His research and that of collaborators has been in part sponsored by Novartis and Addenbrooke's Hospital Trust Funds.

Robert Vaughan is holder of a patent which is subject to a licensing agreement with Dynal Biotech Ltd. He has received sponsorship from Novartis to attend the American Transplant Congress.

David Briggs, Susan Fuggle and John Smith made no declaration.



Published by:

British Society for Histocompatibility & Immunogenetics,  
PO Box 134, Leeds, LS9 7WQ

and British Transplantation Society,  
Triangle House, Broomhill Road, London, SW18 4HX

ISBN: 0-9542221-6-4

Designed and produced by indigo 020 8858 5100