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1.0. INTRODUCTION AND PRE-BSHI EDUCATION, TRAINING AND EMPLOYMENT

1.1 INTRODUCTION:

I have worked in the Histocompatibility and Immunogenetics Laboratory within XXXXXXXXX, since 1997. I originally was appointed as a CPSM (now Health Professions Council) state-registered Biomedical Scientist (BMS) Grade 1, where my responsibilities included HLA antibody screening of solid organ transplant recipients using Complement Dependent Cytotoxicity to determine the presence and specificity of antibodies encoded by the Human Leucocyte Antigen (HLA) system. Following a three-month training period in molecular HLA typing and flow cytometric and CDC crossmatching techniques, I became an active member of the on-call team in January 1999.

I was successfully appointed as a Grade B Trainee Clinical Scientist in January 2002, undertaking an established Department on Health training scheme run by the British Society of Histocompatibility and Immunogenetics (BSHI). On completion of the training scheme, a BSHI Diploma was awarded and I began Clinical Scientist Grade B pre-registration training to progress towards HPC State registration. Since completing the training scheme I have continued to gain experience within the field and have taken on increasing levels of responsibility as my knowledge base has both deepened and expanded.

This portfolio will demonstrate the competences I have gained and use regularly as part of my role within the laboratory. I will initially outline the BSHI training scheme followed by a summary of my MSc and details of my role within the laboratory as a pre-registrant Clinical Scientist. In addition I have completed three case studies to demonstrate the type of work that I am involved in on a day-to-day basis. Appendix 1 cross-references the portfolio with specific competences.

1.2 PRE-H&I SCIENTIST EDUCATION:

1.2.1 1991-1994 Bachelor of Biomedical Science (with Honours) (BSc. Hons) (1994). XXXXXXXXXX

This was a three year full-time modular degree and gave a good grounding in a number of scientific disciplines. Honours modules studied included cell biology and physiology; immunopathology, infection and cancer, haematology and immunology; molecular aspects of disease; medical microbiology and molecular biology. 40% percent of each module was dedicated to written and practical assessments, giving me an introduction to many of the concepts used in clinical and research laboratories with experience in laboratory skills, report writing and data handling.

During the final year of my degree I completed a thesis entitled “the Epidemiology of Cervical Cancer” The thesis concentrated on the pathology of cervical carcinomas and the differences in diagnostic and screening procedures employed Worldwide, in the detection and management of cervical cancer.

I graduated from the University of XXXXXXXX with a 2:1 BSc (Hons) in Biomedical Science. A copy of the degree certificate is included in Section 8 of this portfolio.

1.3 PRE-TRAINEE CLINICAL SCIENTIST LABORATORY EXPERIENCE

Prior to being appointed into the post of Trainee Clinical Scientist in the H&I laboratory, I spent four years working as a BMS Grade 1 responsible for the HLA antibody screening of solid organ transplant recipients using Complement Dependent Cytotoxicity (CDC). Responsibilities included the recruitment of new HLA typed volunteer panel donors and management and selection of existing donors to cover as many HLA Class I and Class II antibody specificities as possible, training as a phlebotomist to obtain fresh peripheral blood samples from volunteer panel donors, extraction of T and B cell lymphocytes using density gradient, dextran/ carbonyl ion, dynabeads and sheep cell rosette separation techniques, DTT treatment of patient sera and preparation of high quality frozen sera panel trays with both patient sera and controls and maintaining adequate stocks of frozen lymphocytes from HLA typed volunteer blood and bone marrow donors and deceased donor organ donors.

I also trained in specimen reception covering internal and external regulations for specimen transport, handling and storage, booking in procedures for all samples received into the laboratory, serological Class I HLA typing of all transplant recipients and disease association studies to provide assistance to other laboratory staff members in times of operational difficulty. Liaison with the Bone Marrow Panel Administrator to recall potential Bone Marrow Donors selected from the British Bone Marrow Registry (BBMR) for further testing, preparation of sample kits and organisation of the collection of blood samples for testing.

Whilst undergoing training to participate in the on-call rota I trained in DNA extraction methods using magnetic silica beads and salting out procedures, selection of PCR-SSP primers for assembly of in-house HLA typing trays and using commercial PCR-SSP typing kits for HLA typing and platelet genotyping, selection of patient sera for crossmatching, crossmatching of transplant recipients and donors by flow cytometry and CDC methodologies and ELISA based assays for HLA and HPA antibody screening.

I also compiled and reviewed many Standard Operating Procedures (SOPs), participated in risk assessments and COSHH forms and was involved in preparation of the department for audit by CPA and MHRA (Medicines for Health Regulatory Authority) for accreditation purposes.

2.0. THE BSHI DIPLOMA: COMPETENCIES SUCCESSFULLY ACHIEVED DURING TRAINING FOR THE BSHI DIPLOMA

The BSHI Diploma is a training scheme for Trainee Clinical Scientists and Trainee Biomedical Scientists working in H&I laboratories. The aim of the scheme is to provide practical and theoretical training to undertake work in an H&I laboratory. A log-book is provided and trainees must complete the five mandatory sections and a minimum of 75% of the remainder of the log-book. Section 3 lists the competences attained as part of the BSHI log-book. In addition to a log book, trainees also must fulfil the following requirements listed below:

2.1 ESSAYS AND CASEBOOK:

Two essays and a casebook comprising three case studies were submitted. All required a detailed literature review and critical analysis of the subjects being researched. The essays and case studies demonstrated my ability to read and critically appraise literature and to understand and analyse results and information. The two essays I submitted for assessment had the following titles:

- Essay one: "Discuss the advantages and disadvantages of replacing serological HLA typing with DNA techniques in a routine H&I laboratory". *This was submitted in March 2005 and awarded a pass.*
- Essay two: "Describe the structure of MHC antigens and their role in presentation of peptide to T cells". *This was submitted in February 2006 and awarded a pass.*

The set of three case studies I submitted were:

- Group 1: Deceased Donor Transplant- *Management of an ABO compatible but non-identical Heart- Lung Transplant*
- Group 2: Sibling Haematopoietic Stem Cell Transplant- *Successful Allogeneic Bone Marrow Transplant in a patient with Acute Myeloid Leukaemia*
- Group 3: Serum Screening- *Passive maternal HLA antibody transfer*

The case studies were submitted in April 2006 and awarded a pass.

2.2 BSHI PROJECT:

Completion of an MSc project granted an exemption from submitting a BSHI project. The project was submitted as partial fulfilment for the BSHI Diploma and an abstract of the project can be found in Section 1.2.2.

2.3 HOST LABORATORY VISIT:

To fulfil one of the requirements of the BSHI Diploma, I spent two days at XXXXXXXXXXXXX where I was able to gain experience of sequence based typing (SBT) techniques, which were not carried out in XXXXX at the time. This included theoretical and practical knowledge of sequence based typing and comparisons of current analysis software.

Day 1: I was given an informal presentation of SBT, covering both enzymatic and dideoxy-mediated chain termination techniques, development of SBT for Class I and Class II, selection of sequencing primers, slab gel and automated SBT, role of SBT in the laboratory and future directions of SBT. This was followed by a practical demonstration, setting up a series of reactions for a sample which had an unresolved HLA-B locus. A low-resolution PCR-SSP was initially performed to establish as much HLA typing information about the sample and aid selection of sequencing primers to maximise analysis. The amplified PCR product was then treated with EXOSAP-IT™. The addition of this enzymatic reagent ensured that any unincorporated dNTP's (deoxynucleotides) and primers in the amplified PCR product were removed and would therefore not interfere with the sequencing reaction. Primers were added each PCR product. A set of forward and reverse primers for both exon 2 and exon 3 were used. Each sequencing reaction contained quantities of the freeze-dried sequencing primer, the EXOSAP-IT™ PCR product, sequencing buffer, Big Dye Terminator (contains dNTP's, ddNTP's (di-deoxynucleotides) and DNA polymerase) and molecular grade water. Following PCR cycle sequencing, the sequencing reactions were purified using magnetic beads (CleanSEQ®) and a series of 80% ethanol washes to remove unincorporated dyes, nucleotides, salts and contaminants. Samples were loaded onto an ABI 3100 sequencer for analysis using a 36cm capillary, which can handle up to 500 bases in 1 hour. There were 4 sets of sequencing reactions for each HLA-B locus; therefore our sequencing would take 8 hours. A brief overview of the instrument and the software was given and the results would be analysed the following day.

Day 2: The sequences were collected from the sequencing reactions set up on the previous day and quickly scanned to check for areas at the start and end of the sequence which may not be clean and could affect interpretation. These areas can be

cut from the rest of the sequence before being imported into the analysis software. The sequences were lined up to a reference sequence in the analysis software for each primer and both forward and reverse primers were visualised simultaneously as well as multiple exons. It is important that the HLA allele libraries within the analysis software are updated regularly to include newly discovered and variant alleles. Any positions which were ambiguous are checked to determine whether the position is a heterozygous position or not. True heterozygous positions displayed two peaks; the second peak is 40% of the height of the first peak. Results were interpreted using Matchtools™ and Assign™ analysis software and the advantages and disadvantages of each were discussed.

2.4 ORAL PRESENTATION:

An oral presentation is a requirement of the BSHI Diploma. I presented on "Living organ donation- addressing the organ shortage" to XXXXX based H&I staff. This focused on why there is an organ shortage, comparison of numbers of patients on the waiting list versus actual transplants, statistics for UK and USA HLA matching in living donor transplantation, ethics of living donor transplantation, unrelated living donor transplantation, comparisons of renal graft survival for live-related, live-unrelated and deceased donor categories, the impact of HLA mismatching on survival and paired kidney donation.

I have also given report backs on meetings or lectures that I have attended and presented the following transplant-related topics at laboratory Departmental Seminars:

- Haemachromatosis
- Platelet Refractoriness and the role of the H&I laboratory

2.5 BSHI TRAINING DAYS:

These training days are run by the BSHI training executive and provide scientific education and allow trainees from across the UK to meet and exchange ideas. Trainees must attend a minimum of a two day workshop and a one day meeting. I attended a two day training workshop at Chancellors Residential Conference Centre, University of Manchester in November 2002 covering theoretical aspects of antibody detection and recognition, HLA typing, automation in the laboratory, new approaches to immunosuppression, post-transplant monitoring and NK polymorphism. We also participated a series of team building exercises. I also attended a one day training course at Royal Free Hospital, London in June 2004, covering theoretical aspects of pattern recognition receptors, complement system, NK cells and their role in the immune response, development of T cells in the thymus and initiation, progression and roles in different antibody classes.

2.6 EXTERNAL ASSESSMENTS:

Regular assessments are carried out to ensure that training is progressing and identifies any problems at an early stage. My log book was assessed in September 2003, March 2005 and March 2006. My mid-term assessment was carried out in March 2005 by two external members of the BSHI Training Executive.

2.7 VIVA VOCE:

My viva voce was successfully completed in April 2006.

2.8 MEETINGS, SEMINARS AND COURSES ATTENDED:

Meetings: During my BSHI training, I attended many meetings including:
The American Society of Histocompatibility and Immunogenetics (ASHI) in San Francisco 2001

- BSHI AGM, Cambridge (2001)
- HLA in Transplant and Transfusion, H&I Sheffield (2002)
- Regional Transplant study day, XXXXXX Hospital (2002)
- British Blood Transfusion Society (BBTS), Edinburgh (2002)
- Symposium on Organ Transplantation, Chepstow (2003)
- BSHI AGM, Newcastle (2003)
- BSHI AGM, Dublin (2004)
- BSHI AGM, Sheffield (2006)

Courses:
I have attended the following NBS/ NHSBT training courses:

- Orcid writers course (for SOP writing in approved NBS format) (2000)
- Good Manufacturing Practice (GMP)
- Fire Marshall
- Risk Assessment Course (2008)
- Cryogenic gases- practical decanting of liquid nitrogen (2009)
- Diversity Awareness (2009)
- Key Pulse Trainer Course (2009)

3.0. COMPETENCES ATTAINED AS PART OF BSHI LOG BOOK

BSHI LOG BOOK- SECTIONS COVERED (THESE WILL BE CROSS-REFERENCED WITH APPENDIX 1)

3.1. GOOD LABORATORY PRACTICE (mandatory section)

3.1.1 Statutes, regulations and guidance

3.1.1.1 Is aware of relevant Acts/Regulations/Guidance and their implications.

3.1.2 Health and safety

3.1.2.1 Understands the local health and safety policy
3.1.2.2 Understands the institution fire policy
3.1.2.3 Understands the hazards of biological material
3.1.2.4 Understands chemical hazards
3.1.2.5 Understands the nature of mechanical hazards

3.1.3 Confidentiality

3.1.3.1 Competently follows the local procedures to maintain confidentiality

3.1.4 Specimen handling

3.1.4.1 Understands the local procedures for sample reception and distribution
3.1.4.2 Competently follows Post Office Regulations on specimen transport
3.1.4.3 Competently follows the local procedures for the handling, storage and disposal of specimens
3.1.4.4 Competently follows the local procedures for the handling, storage and disposal of high risk specimens

3.1.5 Laboratory instrumentation

3.1.5.1 Competently uses laboratory equipment
3.1.5.2 Competently follows the local procedures for the maintenance, cleaning and decontamination of equipment

3.2. QUALITY ASSURANCE (mandatory section)

3.2.1 Quality standards

3.2.1.1 Understands the difference between quality assurance and quality control
3.2.1.2 Is aware of the national and international standards relating to quality management systems
3.2.1.3 Is aware of local documentation available to support a quality management system.

3.2.2 Quality control systems

3.2.2.1 Understands the use of local sample processing controls
3.2.2.2 Is aware of local procedures for the calibration of equipment and the necessary documentation.
3.2.2.3 Is aware of local procedures for sample identification and systems in place to allow audit

3.2.3 Quality assurance schemes

3.2.3.1 Understands the local involvement in national and international quality assurance schemes.

3.2.3.2 Understands the necessity of internal and external audit of services provide

3.3. MAJOR HISTOCOMPATIBILITY COMPLEX (Mandatory section)

3.3.1 History and Histocompatibility Workshops

3.3.1.1 Is aware of the important historical developments in Histocompatibility and Immunogenetics

3.3.1.2 Is aware of the role of Histocompatibility Workshops

3.3.2 Genes within the MHC

3.3.2.1 Knows the location and relationship of the clinically relevant HLA genes

3.3.2.2 Is aware of the other genes within the MHC

3.3.2.3 Understands the relationship between genes and proteins

3.3.2.4 Knows the basic structure of HLA genes

3.3.3 Nomenclature

3.3.3.1 Knows the current WHO HLA nomenclature

3.3.4 Structure, function and distribution of MHC antigens

3.3.4.1 Knows the structure and function of HLA class I antigens

3.3.4.2 Knows the structure and function of HLA class II antigens

3.3.4.3 Is aware of the distribution of MHC antigens

3.3.5 Genetics

3.3.5.1 Knows the mode of inheritance of HLA genes

3.3.5.2 Is aware of linkage disequilibrium and its implications

3.3.5.3 Understands the meaning of phenotype, genotype and haplotype

3.3.5.4 Is aware of the phenomenon of recombination and its implications

3.3.5.5 Is aware of statistical methods used in population genetics

3.3.5.6 Is aware of the likely mechanisms that generate HLA polymorphism

3.4. IMMUNOLOGY (mandatory section)

3.4.1 Basic Immunology

3.4.1.1 Is aware of the cells involved in the immune response, their development, primary functions and characteristics.

3.4.1.2 Is aware of the role of the lymphatics in lymphocyte recirculation

3.4.1.3 Knows the morphology of lymph nodes, MALT, PALs and Peyer's patches

3.4.1.4 Understands the terms antigen, superantigen, antibody, epitope, and idiotope

3.4.1.5 Is aware of the concepts of self and non-self

3.4.1.6 Is aware of the role of cytokines in the immune response

3.4.1.7 Is aware of other molecules of the immune system and cell killing mechanisms

3.4.1.8 Knows the classes and structure of immunoglobulins

3.4.1.9 Is aware of the structure of the T cell receptor

3.4.1.10 Understands the concepts of the generation of antibody and T cell receptor diversity and their implications

3.4.2 Antigen Presentation and Processing

3.4.2.1 Knows the role of professional and non-professional antigen presenting cells

3.4.2.2 Is aware of the current hypotheses of antigen presentation
3.4.2.3 Is aware of the significance of HLA and TAP polymorphism in antigen presentation

3.4.3 Transplant Immunology

3.4.3.1 Is aware of the immunological processes in graft rejection
3.4.3.2 Is aware of the different categories of rejection and understands the basic theoretical principles of each
3.4.3.3 Is aware of different immunosuppressive therapies and their mode of action
3.4.3.4 Is aware of the current theories of T & B cell tolerance
3.4.3.5 Is aware of the proposed mechanisms of graft versus host disease

3.5. ASSAY PRINCIPLES (mandatory section)

3.5.1 Serological and cellular methods

3.5.1.1 Understands the principles involved in lymphocytes isolation methods
3.5.1.2 Understands the alternative procedures available for T and B cell isolation
3.5.1.3 Understands the principles of lymphocytoxicity
3.5.1.4 Understands the principles of the various techniques employed for cross-matching and antibody detection
3.5.1.5 Understands the principles of the MLC test
3.5.1.6 Understands the principles of the CTLP and HTLP frequency assays

3.5.2 Molecular methods

3.5.2.1 Is aware of the various techniques available for DNA extraction and their principles
3.5.2.2 Understands the principles of RFLP analysis
3.5.2.3 Understands the principles of PCR-SSP, -SSO and -SSCP

3.6. MANAGEMENT SKILLS

3.6.1 Principles of personnel management

3.6.1.1 Is aware of recruitment procedures
3.6.1.2 Understands the significance of contracts of employment and job descriptions
3.6.1.3 Is aware of disciplinary and grievance procedures
3.6.1.4 Understands the implications of Individual Performance Review
3.6.1.5 Is aware of the role of trade unions
3.6.1.6 Is aware of the Management process

3.6.2 Training

3.6.2.1 Understands the role of the Training Officer
3.6.2.2 Is aware of the role of professional bodies and other organisations
3.6.2.3 Understands the concept of continued professional development.

3.6.3 Financial management

3.6.3.1 Is aware of the role and implications of the laboratory budget.

3.6.4 Communication skills and liaison

3.6.4.1 Understands how information is communicated within the organisation
3.6.4.2 Understands the structure of the organisation and inter-departmental relationships
3.6.4.3 Understands how the laboratory interacts with external organisations
3.6.4.4 Understands the principles of quality assurance

3.7. COMPUTATION AND DATA PROCESSING

3.7.1 Computation

3.7.1.1 Understands the use of local computer systems
3.7.1.2 Is aware of local procedures for file backup and archiving
3.7.1.3 Competently uses local word processing equipment and software
3.7.1.4 Is aware of the local facilities for computerised literature searches
3.7.1.5 Is aware of basic computer hardware, systems terminology and software applications

3.7.2 Data processing

3.7.2.1 Is aware of local procedures for reporting laboratory findings
3.7.2.2 Understands how laboratory results are interpreted and reported
3.7.2.3 Is aware of local procedures for the collection and processing of laboratory statistics
3.7.2.4 Knows the local systems for data protection and patient data confidentiality

3.8. SEROLOGICAL (AND BIOCHEMICAL) IDENTIFICATION OF HLA ANTIGENS

3.8.1 Sample reception

3.8.1.1 Competently follows the local procedures for sample reception and distribution

3.8.2 Lymphocyte isolation

3.8.2.1 Competently prepares lymphocytes suitable for typing using local procedures
3.8.2.2 Is aware of the various anticoagulants which may be used
3.8.2.3 Competently isolates T and B cells suitable for typing using local method
3.8.2.4 Competently assesses cell viability and standardises preparations for typing

3.8.3 Lymphocytotoxicity

3.8.3.1 Understands the local criteria for typing tray selection
3.8.3.2 Competently follows the procedure for tray preparation
3.8.3.3 Competently uses micro-dispensing equipment
3.8.3.4 Competently follows the local procedure for the lymphocytotoxic test, e.g. incubation times, cell visualisation
3.8.3.5 Competently assesses cell death using a scoring system
3.8.3.6 Is aware of alternative procedures for lymphocytoxicity testing

3.8.4 Interpretation of HLA phenotypes

3.8.4.1 Knows the current WHO HLA nomenclature for antigen specificities
3.8.4.2 Understands the concepts of "public" and "private" epitopes
3.8.4.3 Is aware of racial differences in antigen frequency and associations
3.8.4.4 Competently follows the procedures for phenotype validation

3.8.5 Preparation of typing trays

3.8.5.1 Understands the principle of reagent antisera selection
3.8.5.2 Understands the local procedure for storage, labelling and stock control
3.8.5.3 Understands the selection and use of control reagents
3.8.5.4 Competently uses equipment for dispensing reagents

3.9. SEROLOGICAL TESTING FOR HLA AND OTHER RELEVANT ANTIBODIES

3.9.1 Sample reception

3.9.1.1 Competently follows the local procedure for sample reception and distribution

3.9.2 Cell panels

3.9.2.1 Understands the principles of panel cell selection
3.9.2.2 Competently prepares panel cells suitable for antibody testing
3.9.2.3 Competently follows the local procedure for cell storage

3.9.3 Antibody identification

3.9.3.1.Competently follows the local procedures for antibody detection and specification
3.9.3.2 Understands and can interpret antiserum specificity analysis
3.9.3.3 Understands the application of titration, absorption and blocking techniques to aid antibody identification
3.9.3.4 Understands the methods available for the differentiation of allo-, auto- and non-HLA antibodies
3.9.3.5 Is aware of methods for increasing sensitivity of antibody detection
3.9.3.6 Is aware of alternative methods of antibody detection
3.9.2.7 Knows the potential sources of HLA typing reagents
3.9.3.9 Is aware of the production and use of monoclonal antibodies
3.9.3.10 Understands the significance of HLA and non- HLA antibodies in transplantation

3.10. DEFINITION AND ANALYSIS OF HLA GENE POLYMORPHISM BY DNA TECHNIQUES

3.10.1 Sample Reception

3.10.1.1Competently follows the local procedure for sample reception and distribution
3.10.1.2 Understands the importance of archiving sample

3.10.2 Basic DNA techniques

3.10.2.1 Competently follows local procedures to extract DNA from peripheral blood or tissue samples
3.10.2.2 Understands the principles of DNA quantitation and competently quantitates and adjusts the concentration of DNA samples using local procedures
3.10.2.3 Competently prepares and loads agarose gels and performs electrophoresis
3.10.2.4 Understands the use of molecular weight markers such as plasmid digests
3.10.2.5 Competently photodocuments and records the results of agarose gel electrophoresis
3.10.2.6 Understands the advantages and disadvantages of DNA-based typing systems, and their implications

3.10.3 HLA allogenotyping using RFLP analysis

3.10.3.1 Is aware of the actions of restriction enzymes and their applications
3.10.3.2 Is aware of different labelling techniques
3.10.3.4 Understands the advantages and disadvantages of alternative labelling techniques

3.10.3.6 Is aware of the Human Organ Transplants (1989) Act and later amendments, and its implications for the tester
3.10.3.7 Is aware of the advantages and disadvantages of RFLP typing

3.10.4 HLA typing using PCR techniques

3.10.4.1 Understands the principles of PCR including the concept of primer design
3.10.4.2 Understands the need for high quality DNA
3.10.4.3 Is aware of the major problems likely to be encountered relating to DNA extraction
3.10.4.4 Understands how varying parameters affect PCR
3.10.4.5 Is aware of procedures used to optimise PCR
3.10.4.6 Competently performs PCR-SSP following local procedures
3.10.4.7 Competently interprets PCR-SSP data
3.10.4.8 Competently performs PCR-SSO/reverse-SSO following local procedures
3.10.4.9 Competently interprets PCR-SSO/reverse-SSO data
3.10.4.10 Is aware of other PCR applications to HLA typing
PCR-RFLP
PCR-RSCA
3.10.4.11 Is aware of the different applications of various PCR-based typing systems
3.10.4.12 Is aware of the advantages and disadvantages of PCR based typing systems

3.10.5 PCR conformational analysis techniques for HLA matching

3.10.5.1 Is aware of DNA/PCR based matching techniques
3.10.5.2 Understands the principles of heteroduplex formation and analysis
3.10.5.3 Understands the principles of SSCP formation and analysis
3.10.5.4 Is aware of the applications of PCR based matching techniques
3.10.5.5 Is aware of the advantages and disadvantages of these conformational techniques in relation to other typing systems

3.10.6 Reagent preparation

3.10.6.1 Understands the principles and applications of the reagents used in this subject of study
3.10.6.2 Understands the basic principles of probe and primer design

3.10.7 Laboratory management specific to DNA techniques

3.10.7.1 Understands the potential problems of PCR contamination and the main steps to avoid such problems
3.10.7.2 Understands the quality control procedures used in this subject of study

3.11. RENAL (AND OTHER SOLID ORGAN) TRANSPLANTATION

3.11.1 Cadaveric transplantation

3.11.1.1 Is aware of the local involvement in solid organ transplant programmes
3.11.1.2 Is aware of the clinical criteria for suitability as a transplant recipient
3.11.1.3 Understands the importance of ABO blood group compatibility
3.11.1.4 Understands the importance of HLA-A, B and DR matching
3.11.1.5 Is aware of the implications of matching for graft survival
3.11.1.6 Understands the relevance of patient antibody profiles
3.11.1.7 Understands the significance of HLA and non- HLA antibodies
3.11.1.8 Understands the selection criteria of antisera for cross-matching

3.11.1.9 Is aware of the arrangements governing the placement of donor kidneys from UKTSSA

3.11.1.10 Competently follows the local procedures for recipient selection.

3.11.1.11 Is aware of the importance and significance of virology screening of potential organ donors

3.11.2 Living related transplantation

3.11.2.1 Understands the importance of family studies

3.11.2.2 Knows the importance of blood group compatibility

3.11.2.3 Is aware of the implications of the "Human Organ Transplants Act - 1989" and any amendments to the Act, Statutes or Regulations

3.11.2.4 Is aware of the role of the Unrelated Living Transplant Regulatory Authority (ULTRA)

3.12 HAEMOPOIETIC PROGENITOR CELL TRANSPLANTATION

3.12.1 Clinical aspects

3.12.1.1 Is aware of the various diseases for which stem cell transplantation is an appropriate treatment

3.12.1.2 Is aware of the possible sources of stem cells

3.12.1.3 Is aware of the pre-transplant treatment of patient and donor

3.12.1.4 Is aware of post transplant complications

3.12.1.5 Is aware of the processing of stem cells

3.12.1.6 Knows the factors affecting clinical outcome following stem cell transplantation

3.12.1.7 Is aware of the importance and significance of virology screening

3.12.2 Allo and auto-grafts

3.12.2.1 Understands the requirement for blood grouping, HLA typing and antibody screening of the patient, siblings and other family members

3.12.2.2 Understands the matching criteria and selection of a related donor

3.12.3 Volunteer unrelated bone marrow donor panels

3.12.3.1 Understands the principles of volunteer bone marrow donor panels

3.12.3.2 Knows the local involvement with volunteer bone marrow donor panels

3.12.3.3 Is aware of the various donor panels (including Cord Blood Banks)

3.12.3.4. Understands the principles of donor selection

3.12.4 Final Compatibility Tests

3.12.4.1 Understands the need for confirmatory HLA typing

3.12.4.2 Is aware of the use of cellular compatibility tests

3.12.4.3 Is aware of further tests of compatibility

3.12.5 Post-transplant monitoring

3.12.5.1 Is aware of the need for post transplant monitoring of BMT patients

4.0. MASTER OF BIOMEDICAL SCIENCE

4.1 OVERVIEW OF THE MSc

I completed a part-time MSc in Biomedical Science whilst undertaking the BSHI Diploma. The MSc took place at the University of XXXXXX and was designed as a three year part-time degree course consisting of taught lectures, seminars, tutorials and practical sessions as well as the submission of a research project thesis. The course is oriented towards learner-centred study programmes, with guided reading and assignment work in order to develop a sound basis of knowledge and analytical skills appropriate to understanding present and future aspects of biomedical sciences and the execution of biomedical research. The course is accredited by the Institute of Biomedical Sciences (IBMS) allowing successful students to apply for Fellowship. The course comprised 12 modules and the award of the MSc degree was based on performance of a combination of oral presentation, critical review, written examinations, written essay papers, two pieces of independent study (6,000 words each) and a research project (6,000 to 10,000 words). Each module is designed to allow the student to achieve an extensive knowledge of the aetiology, pathogenesis and treatment of diseases and disorders of major importance to human health and to foster an understanding and critical evaluation of the role and responsibilities of the pathology laboratory services in the diagnosis and monitoring of the disease.

4.2 BREAKDOWN OF THE MODULES STUDIED:

4.2.1. Lung and Cardiovascular Disease

Haematology/ Transfusion Science: aspects covered included the role of blood vessels and platelets in haemostasis; the structure, function and role of endothelial cells in the mechanisms of haemostasis; platelet morphology; biochemistry and kinetics; laboratory investigation of vascular and platelet disorders.
Clinical Biochemistry: biochemical markers of myocardial diseases and the causes, consequences and laboratory investigation of hyperlipidaemias.
Medical Microbiology: streptococcal infections and streptococcal antigens in heart disease; natural defence mechanisms of the respiratory tract and their circumvention by respiratory pathogens.
Cellular Pathology: histological investigation of cardiovascular disease and cytological investigation of bronchial lavage and brushings.

4.2.2. Cancer

Haematology & Transfusion Science: pathophysiology and clinical manifestations of chronic and acute haematopoietic stem cell disorders; laboratory investigations and the use of cytochemical, cell surface and cytogenetic markers for chronic and acute haematopoietic stem cell disorders; plasma cell dyscrasias.
Clinical Biochemistry: biochemical consequences of malignancy; role of the biochemistry laboratory in the diagnosis and treatment of cancer; ectopic hormone production and endocrine tumours.
Medical Microbiology: the role of micro-organisms in the development of gastric cancer and oncogeneic viruses.
Cellular Pathology: oncology and oncogenes; paraneoplastic changes; the use of tumour markers and prognostic factors in cancer; immunocytochemistry; ISH and PCR in diagnosis, interpretation and significance of non-neoplastic, pre-malignant and malignant changes in cytological preparations of epithelial surfaces; secretions and parenchymal tissue.

4.2.3. Transplantation

Haematology and Transfusion Science: uses of cell separation and plasmapheresis; indications of problems of organ transplantation; the HLA system and histocompatibility testing, including cytotoxicity testing, lymphocyte typing, MLR, RFLP and PCR.
Clinical Biochemistry: causes of liver, renal and heart failure; the role of the biochemistry laboratory in organ transplantation including assessment and monitoring of rejection.
Cellular Pathology: sequelae of heart, liver and renal failure; laboratory protocols and staining regimes used in assessment and monitoring of heart, lung, kidney and liver transplantation; cytological monitoring of bone marrow transplants.

4.2.4. Paediatric Pathology

Haematology and Transfusion Science: clinical, laboratory and therapeutic aspects of congenital immunodeficiency states; haemolytic disease of the newborn.
Clinical Biochemistry: antenatal diagnosis and biochemical assessment of foetal development by analysis of amniotic fluid and CVS; genetic defects and screening programmes for their detection; pituitary functions and growth hormone disorders.
Medical Microbiology; incidence, significance and pathogenesis of the principal infections of childhood including respiratory infections, cystic fibrosis, tuberculosis, viral childhood fevers, urinary tract infections, haemolytic uraemic syndrome; emerging pathogens; immunisation.
Cellular Pathology: neuropathological disorders in childhood; diagnosis of inherited metabolic disease; karyotyping and diagnosis of cytogenetic disorders; the use of electron microscopy and in-situ hybridisation.

4.2.5. Advances in Biomedical Analysis

This module was designed to introduce students to existing and new informative analytical methods that are having a major impact both in the diagnosis of disease and in fundamental research into disease processes. Teaching covered the use of separation techniques of biomolecules using differing types of chromatography (IEC, HIC, GPC, AC) and the use of high-performance liquid chromatography (HPLC) in enzymatic analysis of liquids, capillary electrophoresis and its application to separation and analysis of DNA. An introduction to biosensors which lead onto the different types of biosensors and current and potential future uses of biosensors; nuclear magnetic resonance (NMR) imaging and high resolution studies in the analysis of biological material; GC-mass spectroscopy; pyrolysis-mass spectrometry; fluorescence and chemiluminescence techniques.

4.2.6. Advances in Molecular Genetics

This module considered the methodologies, applications and future prospects of molecular genetics in modern medicine. Initially we were given a review of molecular genetics covering the cellular and molecular biology of RNA and DNA and recombinant DNA techniques. Learning progressed to the application of molecular genetics in genetic engineering; DNA cloning; applications, limitations and new developments involving PCR and DNA fingerprinting and current research involving genetic factors in human disease. We looked at the Human Genome Project (HGP) in detail, covering techniques used both past and present in the

construction of HGP and the implications for diagnosis, analysis and treatment of disease and the ethical, social and moral issues created.

4.2.7. Immunology

This module dealt with a range of fundamental aspects of immunology, including methods employed to attempt to control the immune response such as immunosuppression, immunopotentiation with drugs and various forms of immunotherapies to a range of disease states; the application of immunoassays using radioisotopic, enzymatic, fluorescent, spin and chemiluminescent labels; immunocytochemistry and data transformation in ligand-binding assays; immunological aspects of human tumours and the role of the immune system in both protection and enhancement of tumour immunity; possible mechanisms for the breakdown of self-tolerance and development of autoimmune conditions looking in detail at the aetiology, clinical manifestations, laboratory investigations, diagnosis and potential therapies of a range of non-organ specific autoimmune conditions.

4.2.8. Research Methods in Biomedical Science

This module provided opportunities for students to develop literature-based study skills. We received guidance on finding and using information; the use of electronic databases; planning and preparation; note-taking, analysis and interpretation; critique and reviewing techniques; avoidance of plagiarism and rules and regulations governing style and citation of references.

4.2.9. Laboratory Management

This module was designed to analyse and clarify how laboratory practice interacts with clinical decision making in the diagnosis of disease and patient care and to identify the success of operational management of the laboratory governed by Acts, statutes, regulations and guidelines at International, National and local levels. Major topics covered included quality control and quality assurance; measuring laboratory performance using internal and external assessment; standardisation and use and purpose of controls; accreditation; clinical governance, audit, litigation and negligence.

4.2.10. Project Preparation and experimental design

This module provided the opportunity to develop skills in scientific methodology, experimental design and production of a project proposal document as a preparation for an MSc research thesis in Biomedical Sciences. Topics covered included the use of hypotheses and controls in both observational and experimental science; replication, sampling, error and variability; applications and limitations of correlation and regression; hypothesis-testing statistics and the applications and limitations of parametric and non-parametric tests; requirements and regulations governing project proposal documentation, work-based supervisor and ethical approval.

4.2.11. Independent study (2 modules)

Students were required to choose a topic within their specialist discipline, not covered by the formally taught programme and carry out an in-depth literature study, culminating in presentation of this work in both the form of a dissertation and an oral presentation at the end of semesters 1 and 2. I chose to present on Haemochromatosis in semester 1 and Xenotransplantation in semester 2.

In September 2004 I was awarded the MSc in Biomedical Science with commendation. My dissertation was entitled "The detection of donor-specific IgG antibodies in post-transplant renal patients by Flow Cytometry and their relevance to kidney rejection". This project was also submitted as partial fulfilment for the BSHI Diploma and an abstract of the project is detailed below:

3.3 MSc PROJECT ABSTRACT:

BACKGROUND: Flow cytometric crossmatching (FCXM) was developed as a more sensitive assay than the standard complement-dependent cytotoxicity crossmatch (CDC) for detection of anti-donor HLA (Human Leucocyte Antigen) antibodies that are capable of mediating hyperacute rejection and graft loss in the early post- transplant period in renal transplant recipients.
The purpose of this study was to evaluate the role of post-transplant donor-specific HLA antibodies (DSA) detected by Flow Cytometry and their relevance to chronic rejection in renal patients.
METHODS: Serum samples from 30 renal transplant recipients, who were transplanted during the period of 1999-2001, were crossmatched using post-transplant sera against corresponding deceased donor splenic lymphocytes, using Flow Cytometry.
RESULTS: Flow cytometric crossmatch (FCXM) monitoring identified donor-specific antibodies in 2 (8%) of 25 transplanted patients, who both subsequently lost their grafts due to chronic rejection. Statistical analysis failed to indicate a higher incidence of acute rejection in patients with DSA than among negative patients (P=0.115). 13/25 (52%) patients experienced one or more episodes of rejection. 9/13 (69%) of patients who experienced acute rejection episodes had positive post-transplant FCXM results, compared to 4/12 (33%) of patients who experienced no acute rejection episodes.
CONCLUSIONS: Due to small numbers of patients there was no significance between a positive FCXM and patients undergoing an acute rejection episode (P=0.115). However, although not statistically significant, due to the small numbers of patients in each group, acute rejection episodes were 3.2 times more frequent in patients with a positive FCXM than with a negative crossmatch (likelihood ratio=3.293).

5.0. EXPERIENCE AS A PRE-REGISTRANT GRADE B CLINICAL SCIENTIST:

Following completion of the BSHI Diploma, a minimum period of one year of pre-registrant training is required. This enables scientists to develop fully into the role of a state registered Clinical Scientist, gaining depth of scientific and clinical knowledge and undertaking increased responsibilities within the Department.

5.1 PROVISION OF HLA/HPA MATCHED PLATELETS

Since 2006, under the direction of the Head of Department, I have been responsible for daily provision of HLA matched platelets to patients who are refractory to random platelets. This service is provided to a number of Hospital Trusts in the region. Previously, this work was undertaken by Medical staff in the Centre until 2003 and then for the period 2003-2006, was covered by the Serology/ Antibody screening Section.

Patients have a variety of clinical conditions and require varying numbers of platelets, so workload is often variable from day to day. This involves identification of the best units available for each patient, creation of the stock orders, collation of paperwork, liaison with Issues and Transport Departments and updating patient files and local and National databases. Transfusion planning for each patient often requires liaison with Haematology clinicians and hospital blood transfusion staff to ensure patients are receiving optimal provision. Advice is often given on samples needed by the laboratory or processes that must be followed to enable a diagnosis of refractoriness. Clinical platelet review meetings are held and are outlined in section 5.8.

I have completed a case study to illustrate this topic (Case Study One).

5.2 CARDIOTHORACIC/ PANCREATIC PATIENT ADMINISTRATION:

As a pre-registrant Clinical Scientist, I have become familiar with some of the administration aspects of transplantation. I manage the monthly Cardiothoracic and Pancreas/ Islet cell patient transplant waiting lists from both the Cardiothoracic Transplant Unit and Renal Transplant Units respectively. I ensure that all patient information is kept up-to-date so that the waiting list, Departmental database and patient records are all consistent.

As patient HLA antibody profiles are updated, discrepancies with the list are reported to the relevant Transplant Unit and I have designed local spreadsheets of all active patients, to ensure samples are being received into the laboratory for screening for the presence of HLA antibodies at regular intervals according to Departmental policy. I amend and update patient information on the Departmental database as is appropriate.

5.3 SEQUENCE BASED TYPING:

Patients receiving haematopoietic stem cell transplants and potential stem cell donors are HLA typed to determine suitability as potential stem cell donors. Where we do not identify a suitable family member we proceed to HLA type the patient to high resolution and initiate matched unrelated donor (MUD) searches. High resolution typing of patients and donors is currently performed using PCR-SSP; however the laboratory is keen to move towards providing Sequenced Based Typing for HLA typing of patients and donors. I have been involved in validating and setting up a routine service, which has required the sourcing of reagents and primers and validating each one with known reference DNA to ensure we have a substantial panel of primers for testing.

This has involved assistance from other H&I laboratories within the organisation who already provide SBT as a routine service to identify the primer sequences needed to develop the primer mixes and to write several local SOPs, forms, datasheets, to use for training other staff members. In parallel with this, we have a significant number of British Bone Marrow Registry panel donors with novel and rare HLA alleles which I am currently investigating using Sequence Based Typing to enable their submission to the HLA Nomenclature Committee as confirmed sequences.

5.4 ON CALL SERVICE:

The laboratory provides a 24 hour, 365 day a year service for typing of deceased solid organ donors, crossmatching renal, pancreatic, liver and cardiothoracic patients and carrying out urgent post-transplant work and urgent antibody screening for cardiothoracic patients requiring urgent transplant listing. Virtual crossmatching in sensitised cardiothoracic patients, who have clearly defined HLA antibodies, is also performed by the on call staff. I have been a member of the on-call team since 1999. Aspects of the on call work involve carrying out HLA typing and crossmatching, often in the middle of the night or at weekends. Checking of results, reporting of urgent results i.e. deceased donor HLA types, crossmatching and antibody screening and identification results are also performed by staff. Communication with Consultant Clinical Scientists, Transplant co-ordinators, surgeons and ODT (Organ Donation and Transplantation) staff is an essential part of this work. Case study two is a good representation of this.

5.5 PARTICIPATION IN EXTERNAL AND INTERNAL QUALITY ASSURANCE SCHEMES:

I participate in the following UK National External Quality Assurance (NEQAS) Schemes:
Scheme 1A Serological HLA Phenotyping (until 2007, when the laboratory ceased to participate in the scheme)
Scheme 2A Cytotoxic Crossmatching
Scheme 2B Flow Cytometric Crossmatching
Scheme 4 High Resolution HLA Class I and Class II typing (using SBT)

In addition, I also participate in the following internal quality exercises: Molecular typing (4 samples per year), Antibody identification exercise (4 samples per year), Antibody Rapid Screening (4 samples per year), Cytotoxic Crossmatching (4 samples per year)

5.6 RESEARCH AND DEVELOPMENT:

I have been involved in development of new techniques in the department, such as new PCR-SSO typing techniques for HLA typing of volunteer British Bone Marrow Registry (BBMR) donors.
Initially donors were typed for HLA-A, B and DRB1 and following a validation of an HLA-Cw typing kit in 2003, additional testing for HLA- Cw was incorporated into the workload.
As previously mentioned in 5.3, I have been involved in the validation of Sequence Based Typing to enable the laboratory to provide this as a clinical service in the near future.

I am also a trained risk assessor for the department and since completion of training; I have written a number of risk assessments for the department, covering equipment, processes and rooms. All risk assessments are to be reviewed at 12 monthly intervals or if significant change to a process has occurred.
I am a Key Pulse Trainer and this enables the training of staff within the department, in Pulse modules that I have access to. Pulse is a blood donor management system used by the organisation to manage a blood product through both donor and donation life cycles. One of the main applications the department uses Pulse for is the creation of specialist stock orders for the provision of HLA matched platelets.

5.7 DEPARTMENTAL MEETINGS:

I am a participant in and contribute to the following regular Departmental meetings:

- Monthly Departmental seminars involving both laboratory staff and external scientific speakers informing on a range of topics pertinent to Histocompatibility and Immunogenetics. These seminars provide contact with clinicians and experts in the field of H&I and the opportunity to gain insight into current research issues and specialist areas of H&I. Trainees are also encouraged to give oral presentations on a regular rotational basis to develop their presentation skills and broaden their scientific knowledge. I have previously given presentations on topics such as, Xenotransplantation, Hereditary Haemochromatosis and Platelet Refractoriness.
- Platelet review meetings are outlined in section 5.8.
- Regular H&I Management Meetings involving Section Heads and the Deputy Head and Head of Department. These meetings are held to discuss issues affecting the entire department and can include development and implementation of new procedures, training and education, Health and Safety issues, quality and accreditation issues, staffing issues as well as section-specific issues.
- Departmental laboratory meetings. I also prepare and present statistical data from Donor Section activity at monthly laboratory meetings. The opportunity to present information and data from the Donor Section of the laboratory is a useful tool to develop presentation skills and to share my working and theoretical knowledge of the section, giving other laboratory members an insight into how the Section functions within the whole H&I department.
- Donor Section meetings. These are held two-monthly and are used to discuss any issues which relate directly to the section.

5.8 CLINICAL LIAISON:

I have attended several liaison meetings including the following:
Paediatric Renal Transplant Review Meetings: These meetings are attended by Paediatric nephrologists, surgeons, transplant co-ordinators, nurses and H&I scientists. The meetings discuss all paediatric patients listed for kidney transplants, with possible live-related donors and also review the progress of transplanted patients. Other aspects of transplantation which are discussed include sensitised patients, non-compliance with immunosuppression and paediatric patients who are in transition to adult clinics.
Regional Renal Transplant Review Meeting: These meetings are attended by nephrologists from the regional hospitals, surgeons, transplant co-ordinators, nurses and H&I scientists. The meetings are used to review issues such as transplant activity, number of transplanted patients in the region, live-related and deceased donor transplants, desensitisation programmes for highly sensitised patients,

immunosuppression protocols, new developments, balance of organ exchange and also highlight any difficult cases.

HLA Matched Platelet Review Meetings: these review meetings are held fortnightly with Consultant and Senior Clinical Scientists, Medical Consultants, Apheresis ward staff and Donor Section staff to discuss management of provision of HLA-matched platelets, future transfusion planning and organising targeted donations for individual patients receiving HLA-matched platelet support.

Cardiac/ Pancreas/ Islet Cell Liaison: This involves discussions with Transplant co-ordinators with regard to specific patients, such as highly sensitised patients with a limited life expectancy, patients who may require prospective crossmatching and to ensure the patient HLA antibody screening profiles are kept up-to-date in accordance with Departmental policy, as discussed in section 5.2.

Out of hours clinical liaison: Reporting of results when on-call. This includes reporting HLA antibody screening and identification results, HLA typing and crossmatching (including virtual crossmatching) results to Transplant coordinators, consultants and other senior hospital staff and UK Transplant duty office (HLA typing of deceased donors), as discussed in section 5.4.

5.9 QUALITY MANAGEMENT:

I ensure that work is performed according to guidelines, policies, MPDs and SOPs and that staff are competently trained to current procedures. I undertake or supervise validations as part of quality assurance in the section. This includes new methodology, new equipment, software upgrades, new batch validations, quality control of equipment and equipment calibrations both routinely and following servicing. I have been involved in writing new local SOPs and risk assessments for processes within the section and managing their regular review.

Currently, together with other heads of sections in H&I laboratories, I am involved in the creation and design of National documentation for Sequenced Based Typing and HLA Matched Platelet provision, to standardise working practices across the organisation, which will replace the current local versions.

5.10 CPD POINTS:

I participate in BSHI CPD scheme and on completion of cycle 1 (April 2000- March 2005). I had a total of 547 CPD points: 248 Educational, 29 Professional, 224 Competency and 46 Managerial points.

Currently during cycle 2 (April 2005 to March 2010), I have a total of 547.25 CPD points: 100 Educational, 91.75 Professional, 168 Competency and 187.5 Managerial points at the end of March 2009.

5.11 ADDITIONAL SKILLS:

Personnel management: I coordinate the daily running of the section to ensure adequate staffing is provided over the course of the working day.

Training of visitors to the Department: For training purposes, work experience students, visitors from other organisations and trusts are regularly invited into the Centre and I am involved in delivering training to these visitors, with regard to activities that take place within the Donor section.

I am a platelet specialist trainer for Hematos, which is an Information Management and Technology system for the management, operation and administration of

activities within H&I and Red Cell Immunohaematology. This system was introduced into the H&I function in February 2009. I have been involved in co-ordinating training to other H&I staff with the department as well as to H&I staff from other H&I laboratories within the organisation.

I have also been heavily involved in the creation of a series of National documents for HLA Matched platelet provision and assisted other H&I staff with the creation, formatting, editing of National documentation for other specialisms within H&I.

6.0. SUMMARY OF MY ROLE WITHIN THE LABORATORY- CURRENT DUTIES:

6.1. I have managerial and scientific responsibility for the Donor Section in H&I, including day to day operational management of the section. This requires organisational skills to undertake the provision of HLA/HPA selected platelets to patients from Regional Hospital Trusts who are refractory to random platelets. Workload involves identification of the most suitable units in stock and transfusion planning with matched targeted donors, liaising with Haematology unit clinicians and hospital blood bank staff to ensure patients are receiving optimal provision and attending clinical review meetings to discuss ongoing platelet provision.

6.2. I ensure that work is performed according to guidelines, policies, MPDs and SOPs.

6.3. I have been an active member of the on-call team since 1999 and work unsupervised out-of hours with responsibility for the organisation, interpretation and release of results for matching/ selection of donors and recipients for transplantation and the review of HLA antibody results for sensitised Cardiothoracic patients.

6.4. I am responsible for the development, validation and implementation of new procedures within the Donor Section. At present, I am involved in the validation of a Sequenced Based Typing service, which the Department would like to use in conjunction with PCR-SSP to enable high resolution typing of Haematopoietic stem cell patients and donors.

6.5. I am currently involved in the creation, design and implementation of National Documentation for Sequence Based Typing and HLA Matched Platelet provision, meeting with other senior H&I scientists to consolidate the current local documentation into a streamlined process that will incorporate the requirements of all H&I laboratories within the organisation.

6.6. I ensure that staff are competently trained and training records are kept updated.

6.7. I am involved in delivering training to visiting students and scientist/ clinicians.

6.8. I have responsibility for quality monitoring within the section and manage corrective and preventative measures, should they occur.

6.9. I have responsibility for Risk Assessment management within the section and have produced risk assessments for a variety of processes and equipment, which are reviewed regularly.

6.10. I participate in UK National External Quality Assurance (NEQAS) and Internal Quality Assurance schemes to demonstrate continuing competency in working practices.

6.11. I am involved in clinical liaison with Regional Transplant Coordinators, clinicians and senior nursing staff both during and outside of working hours to report results, discuss patient management and discuss sample requirements according to departmental policy

7.0. CASE STUDIES:

7.1 Case 1: ALLOGENEIC STEM CELL TRANSPLANTATION IN AN AML PATIENT REQUIRING REGULAR HLA-MATCHED PLATELETS.

This case illustrates a clinical scenario and how it was managed:

- Platelet refractoriness, the investigations required and the management and follow-up of the case.
- The role of the H&I laboratory in this scenario.

INTRODUCTION: PATIENT CLINICAL HISTORY

The patient was a 42 year old female diagnosed with acute myeloid leukaemia (AML-M4). Initial presenting signs were bruising and low haemoglobin (anaemia). Cytogenetic abnormality was confirmed in 54% of cells and a low incidence variant t(16:16) chromosomal translocation was identified. This results in the fusion of the CBFβ (Core Binding Factor) gene at 16q22 to the MYH11 (smooth muscle myosin heavy chain) gene at 16p13, blocking the differentiation of myeloid cells (Mrozek & Bloomfield; 2008). This type of chromosomal abnormality is usually associated with a good prognosis (Pulsoni *et al*; 2008). As part of the supportive treatment whilst undergoing induction therapy, the patient was given both red cell and platelet transfusions, which increase the risk of alloimmunisation.

The patient was given four cycles of ADE induction therapy, comprising Cytabarine, Daunorubicin and Etoposide together with a monoclonal antibody called Myelotarg and achieved complete remission. During the first cycle she became unresponsive to random (non-HLA matched) platelets, which was shown to be due to the presence of HLA antibodies and required regular transfusions of HLA-matched platelets. Random platelets can either be pools derived from several donors or single donor apheresis units. The patient remained in remission for a period of four months before the initial disease returned. The patient was subsequently treated with a two courses of FLAG chemotherapy, comprising Flutarabine, Ara-C (cytabarine) and Granulocyte Colony Stimulating Factor (GCSF) and required HLA matched platelet support following both courses of chemotherapy. The patient achieved her second complete remission after the first course of this treatment and it was decided that stem cell transplantation would be the best long-term curative option and the search for a suitable donor was initiated.

HLA MATCHED PLATELET ALGORITHM

HLA antibodies account for the majority of cases of immunological refractoriness to platelet transfusions. Immunological refractoriness is defined as a failure to achieve an increment of $>10x10^9$/L between 1 and 24 hours after transfusion on at least 2 occasions. Patients who fail to increment with random apheresis platelets on at least two separate occasions are investigated for the presence of HLA Class I antibodies by Luminex technology in the H&I laboratory.

HLA Class I Typing of the patient by PCR-SSP or Luminex PCR-SSOP would also be performed to enable selection of appropriately matched platelets. If the presence of HLA antibodies is demonstrated and non-immune causes of refractoriness are

absent, authorisation of provision of HLA matched platelets by a Consultant Clinical Scientist in H&I or nominated deputy would be given.
Following the transfusion of HLA matched platelets, if the patient is demonstrating a poor response to well-matched platelets, investigation into the presence of HPA (Human Platelet Antigen) antibodies is performed. Samples are sent to the H&I laboratory for investigation where a serum screen for platelet antibodies (Indirect Platelet Immunofluorescence Test PIFT) and a serum screen for platelet-glycoprotein-specific antibodies (Monoclonal Antibody Immobilisation of Platelet Antigen MAIPA) are performed. If HPA antibodies are identified, HPA and HLA matched platelets would be required. HPA genotyping of the patient would also be performed. The most common HPA antibodies detected in patients are HPA-1a and HPA-5b; stocks of these platelets are managed by the H&I laboratory at XXXXX.

LABORATORY TESTS

HLA TYPING

HLA DNA typing was performed by low resolution Class I typing by PCR-SSP (2-digit allele assignment). Class I typing must, as a minimum, be at a resolution which allows definition of all serologically defined antigens.
Additionally, Class II typing at allelic level (4-digit assignment) was performed using high-resolution PCR-SSP for DRB1, DQB1 and DPB1, in accordance with H&I policy for work-up of stem cell transplant recipients.

ANTIBODY SCREENING

The local policy for investigation of platelet refractoriness states that a current serum sample is tested for the presence of HLA Class I antibodies by CDC, ELISA and Luminex methodologies. It is important when the platelet donor is not fully matched, to ensure that the patient does not have antibodies to the mismatched donor antigens.
Initial HLA Class I antibody screening was performed by Luminex LabScreen kit and ELISA LAT-M kit (One Lambda Inc). Antibody identification was then performed by Luminex using LifeMatch (Tepnel Inc) Class I kits. The sample was also subsequently tested by CDC for Class I complement fixing Class I antibodies using a selected panel of 40 cells.
The following tests were performed in other laboratories:

PLATELET ANTIBODY TESTING

Following poor increments with 9 packs of HLA-matched platelets over a period of 1 month, a sample was sent for investigation to the H&I laboratory in XXXX to determine the presence of antibodies to HPA using PIFT and MAIPA assays.

ABO/ RHD BLOOD GROUPING

ABO and Rh D grouping was performed by the Red Cell Immunohaematology (RCI) laboratory in the Centre, on fresh blood samples obtained from the patient and donor, using the Diaclon ABD confirmation gel cards (DiaMed). This is a serological reverse grouping method and uses monoclonal anti-A, B and D to detect the presence of A/B and D antigens on red cells. This can allow blood group matching of blood products and/ or platelets to prevent sensitising events, but an ABO/ Rh D mismatch is not a contraindication to transplant.

SUMMARY OF RESULTS FOR INVESTIGATION OF IMMUNOLOGICAL PLATELET REFRACTORINESS

INITIAL ANTIBODY SCREENING AND IDENTIFICATION

Initial testing by ELISA (GTI) showed a PRA of 100%. Testing by CDC using T-cells also showed a PRA of 100% (both with and without DTT) and no specific antibodies could be identified by these methods. Luminex testing gave a PRA of 96% and identified the strongest specificities against HLA-B7, B27, B40, and B41 which belong to the 7C CREG. Repeated antibody screening by CDC on a subsequent sample did identify B40, B7, B27 and B41 specificities.

PLATELET ANTIBODY SCREENING

PIFT testing was positive for the presence of IgG antibodies and negative for IgM, using donor panel cells. MAIPA testing was negative for platelet antibodies to the major platelet glycoproteins, but positive for the presence of HLA Class I IgG antibodies.
The results are shown in Table 1 below and indicated that the platelet refractoriness was not due to platelet specific antibodies, but supported the presence of HLA Class I antibodies, previously detected by our laboratory as the cause of refractoriness. The reason for the poor increments from initial HLA matched platelet transfusions is unclear and a review of the data reveals incomplete incremental data supplied from the hospital.

TABLE 1: PLATELET ANTIBODY TEST RESULTS

METHOD	CONJUGATE	MONO Ab/GP	RESULT	FREQUENCY
Indirect PIFT	IgG		Positive	3/3 panel cells
Indirect PIFT	IgM		Negative	0/3 panel cells
Indirect MAIPA	IgG HRP	IIb/IIIa	Negative	
Indirect MAIPA	IgG HRP	Ia/IIa	Negative	
Indirect MAIPA	IgG HRP	Ib/IX	Negative	
Indirect MAIPA	IgG HRP	HLA Class 1	Positive	

MANAGEMENT OF PLATELET REFRACTORINESS

Prior to transplant, the patient became refractory to random platelets with a platelet count persistently below $10x10^9$/L, following regular transfusions and the first cycle of ADE induction therapy. Consultation between the patient's doctor and H&I staff managing HLA-matched platelet provision, initiated an investigation into alloimmunisation causing refractoriness to platelets. HLA antibody screening demonstrated that the patient had antibodies to HLA antigens and a trial of HLA-matched platelets was initiated. This was in accordance with organisational policy on the provision of HLA/HPA selected platelets, which states that HLA/HPA selected platelets are warranted if poor responses to random donor platelets on two or more occasions occur and if the patient has defined HLA antibodies.
Platelets are matched according to both the HLA-A and HLA-B loci of the patient, with Grades A, B1, B2, B3 and B4. Grade A platelets are fully matched at HLA-Ax, HLA-Ay, HLA-Bx and HLA-By. Grade B1 match is a one antigen mismatch at either one of the HLA-A or HLA-B loci, Grade B2 is a two antigen mismatch, Grade B3 is a three antigen mismatch and B4 is a complete mismatch for HLA-Ax, HLA-Ay, HLA-Bx and HLA-By.
Following transfusion of 11 packs of HLA matched platelets of A and B1 grades shown in Table 2, the patient was reported have demonstrated a poor response to HLA-matched platelets and was investigated for the presence of antibodies to Human Platelet Antigens (HPA). The results in Table 1 show that platelet refractoriness was attributed to Class I HLA antibodies and not to HPA. Matching for HLA-C locus antigens is not required, as HLA-C antigens are expressed at very low levels on platelets and are not considered to play a role in refractoriness.

TABLE 2: PLATELET INCREMENT FOLLOW-UP DATA AFTER 1ST COURSE OF ADE INDUCTION THERAPY (PRIOR TO HPA TESTING)

Date of transfusion	Number of packs transfused	Mismatched Antigen(s)	Pre-transfusion platelet count	Post-transfusion platelet count
01/01/2006	2	NONE	4	21
28/01/2006	1	NONE		
30/01/2006	1	B37	12	
31/01/2006	2	B50	6	7
02/02/2006	1	A28	5	
02/02/2006	1	NONE		
03/02/2006	1	B13		28
05/02/2006	1	B13		29
06/02/2006	1	A24		42

Note: the patient HLA type is displayed in Table 6

The patient continued to receive HLA-matched platelets (Grades A, B1, B2) following subsequent courses of ADE induction therapy and platelet transfusions of a particular HLA type which resulted in poor increments were subsequently avoided in future transfusion planning. The patient incremented fairly well with A grade matched platelets and some B1 grade platelets, particularly involving A24, A29, B51 and B52 mismatches, which are found in a different cross reactive group (CREG) to the patient's antibodies, which are part of the 7C CREG. Results from these platelet transfusions are displayed in Tables 3 and 4 below.

TABLE 3: PLATELET INCREMENT FOLLOW-UP DATA FOLLOWING 2ND, 3RD AND 4TH COURSES OF ADE INDUCTION THERAPY

Date of Transfusion	Packs Given	Mismatched Antigens	Pre-Transfusion	Post-Transfusion
07/03/2006	1	B52		25
10/03/2006	2	A24		22
11/03/2006	1	NONE		12
13/03/2006	1	NONE		18
15/03/2006	1	B13		
17/03/2006	1	NONE		
19/03/2006	1	B75		
13/04/2006	1	B55		
15/04/2006	1	B55		
17/04/2006	1	NONE		17
18/04/2006	2	A26, B49	3	4
19/04/2006	1	B57	4	10
20/04/2006	1	B57	10	16
21/04/2006	1	A24		
22/04/2006	1	B37	19	22
23/04/2006	1	B37		
25/04/2006	1	B51	13	
27/04/2006	1	NONE	12	
29/04/2006	1	NONE		

TABLE 4: PLATELET INCREMENT FOLLOW-UP DATA FOLLOWING FLAG CHEMOTHERAPY

Date of Transfusion	Number of Packs Given	Mismatched Antigens	Pre-Transfusion	Post-Transfusion
10/10/2006	2	B50		
13/10/2006	1	A24		
12/10/2006	1	B27		
13/10/2006	2	B40		
16/10/2006	1	B51		
17/10/2006	1	A26, B49		
18/10/2006	1	B75		
20/10/2006	1	B51		
21/10/2006	2	NONE	4	27
23/10/2006	2	A29		
25/10/2006	2	A30		
27/10/2006	2	NONE		
01/11/2006	1	NONE		
03/11/2006	2	NONE		
26/11/2006	1	NONE		
29/11/2006	1	A30		
30/11/2006	1	A24, B15		
01/12/2006	1	A29, B57		
02/12/2006	2	B75		
03/12/2006	1	NONE	28	46
04/12/2006	1	NONE		38
06/12/2006	1	A29		43
09/12/2006	1	NONE		
11/12/2006	1	A29	15	33
12/12/2006	1	B51	30	48
14/12/2006	1	B51		
16/12/2006	1	B51		
18/12/2006	1	A24		
20/12/2006	1	A24		
22/12/2006	1	A30	22	
24/12/2006	1	A30	22	

Complete increment data were only obtained for 14/71 packs, with partial data for a further 17/71 packs. Table 5 below displays all the match grades of platelets transfused prior to transplant.

TABLE 5: NUMBER AND MATCH GRADE OF HLA-MATCHED PLATELETS TRANSFUSED

A MATCH (fully matched)	21
B1 MATCH (1 antigen mismatch)	45
B2 MATCH (2 antigen mismatch)	5
TOTAL PACKS	71

SUMMARY OF RESULTS PRIOR TO STEM CELL TRANSPLANTATION

HLA TYPE

The patient was HLA typed as described previously. One sibling was initially typed for DRB1 at 2-digit level and found to be matched. Further testing of the sibling showed that the sibling and patient were an identical match for HLA-A, B, Cw at 2-digit level and HLA-DRB1, DQB1 and DPB1 at 4-digit allele level. The results are displayed in Table 6 below.

TABLE 6: HLA TYPE OF THE PATIENT AND RELATED DONOR

	A*	B*	Cw*	DRB1*	DQB1*	DPB1*
Patient	02, 25	18, 44	05, 12	1301, 1501	0603, 0616	0201, 0401
Sibling	02, 25	18, 44	05, 12	1301, 1501	0603, 0616	0201, 0401

ABO/ RHD BLOOD GROUPING

Both the patient and selected stem cell donor were group A RhD positive.

PATIENT BIOCHEMISTRY, MICROBIOLOGY, ECG, ECHOCARDIOGRAM, X-RAY RESULTS AND DONOR VIROLOGY RESULTS

All biochemical tests were within normal ranges. Chest x-ray, EEG, ECG, GFR and PFR were also within normal ranges. All virology and microbiology tests were negative for both the patient and donor.

TRANSPLANTATION AND ENGRAFTMENT

Following allogeneic transplant workup and a conditioning regime of Fludarabine, Melphan Methylprednisolone and Campath, donor stem cells were harvested from the HLA identical sibling donor and transfused into the patient. The patient was given G-CSF at the time of transplant and Cyclosporin A maintenance therapy was commenced. 33 days post-transplant, the patient demonstrated good marrow engraftment with no sign of GvHD. 68 days post-transplant, the patient was generally well with no signs of GvHD and no recurrence of the leukaemia. After 100 days, Cyclosporin A therapy was terminated and patient T-cell graft chimerism had improved. 153 days post-transplant, the patient was given a dose $1x10^6$/kg of donor lymphocyte infusion (DLI). One month later, following a major drop in donor chimerism, the patient was given a second dose $5x10^6$/kg of DLI. Despite the second infusion, the myeloid chimerism counts continued to fall and leukaemia blasts were detected in the peripheral blood. The patient was told of the relapse of disease and informed that the disease was now incurable.

DISCUSSION

The patient described in this case study was diagnosed with AML. The patient became refractory to random platelets due to alloimmunisation, possibly due to regular transfusions and required HLA-matched platelet support prior to and following stem cell transplantation.

The patient subsequently became thrombocytopenic and unresponsive to random platelet transfusions. For patients who become sensitised and refractory to random platelets, HLA-matched platelets may be the only course of therapy.

Platelet refractoriness is characterised by the failure to show adequate increments of at least $10x10^9$/L after 1 hour post-transfusion following two random donor platelet transfusions (normal range is 140-400 $x10^9$/L). Refractoriness can be due to either immunological or non-immunological causes. Non-immunological causes include disseminated intravascular coagulopathy (DIC), splenomegaly, sepsis, fever, active bleeding and the use of certain drugs, e.g. amphotericin (Sherrill *et al*, 2005).

Alloimmunisation is due to HLA, HPA and/ or high-titre ABO alloantibodies (Eduardo & Mintz, 2000). Studies document alloimmunisation in 50-90% of multi-transfused patients and there is a high correlation between the development of lymphocytotoxic anti-HLA antibodies in the recipient and refractoriness to random donor platelets (Herzig *et al*; 1977).

In this case, the patient demonstrated the presence of HLA Class I antibodies. Over 90% of cases of platelet refractoriness are reported to be due to HLA Class I antibodies alone, 5% are due to the presence of both HLA and HPA and <5% are due to HPA alone.

Platelet destruction is thought to occur via the monocyte/ macrophage system. The Fab portion of an IgG antibody will recognise and bind to the incompatible platelet, leaving the Fc portion exposed. Monocytes are able to bind the Fc portion of the antibody via their Fc receptor, leading to phagocytosis and destruction of the platelets (Harrison & Navarrete; 2000)

Refractory patients are not necessarily given ABO matched platelets. Group O can be given to a group A or B patient and group A can be given to a group B patient (and vice versa), providing they are anti-A and anti-B high-titre negative (MacClennan; 2006). ABO is secondary to HLA for platelet matching. Platelets are matched for HLA-A and B loci and the patient received a combination of grade A (full match), grade B1 (1 antigen mismatch at A/B) and grade B2 (2 antigen mismatches at A/B) platelets.

HLA-C typing was previously performed by serological typing techniques using sera which defined a small number of HLA-C antigens and due to the molecular typing techniques, HLA-C antigens are now fully characterised and one study suggested that matching for HLA-C may be beneficial in some patients (Saito *et al*; 2002).

When mismatched platelets are given the HLA antibody profile of the patient is considered, to avoid mismatched antigens to which the patient has developed antibodies (Pamphillon; 2002). Selective mismatching can also be performed by consideration of cross-reactive groups of antigens (CREGs) which share a public epitope, however some studies report unsatisfactory increments in up to 40% of CREG-matched transfusions (Nambiar et al; 2006) and the probability of finding CREG-matched products for patients with uncommon HLA phenotypes is low.

The use of HLA Matchmaker, which is a computer based algorithm that determines matching at the amino acid level is a more recent development which may help identify suitably matched platelets. Initially, each HLA antigen is viewed as a

string of short linear sequences (triplets) involving polymorphic amino acid residues in antibody-accessible positions; however the algorithm has been updated to consider patches of antibody-accessible polymorphic residues called eplets, as essential components of HLA epitopes. This can determine which HLA alleles have eplets that do not react with the patients antibodies and so can be considered acceptable mismatches (Duquesnoy; 2008). Up to 5 eplet mismatches are considered to be acceptable.

Two months after receiving HLA-matched platelets, the hospital requested that the patient was investigated for HPA antibodies, due to poor increments with HLA-matched platelets. Laboratory testing was negative for their presence and the reason for testing, based on the incremental data (displayed in Table 3) is unclear. Hospital transfusion staff are required to take a pre-transfusion platelet count, followed by a post-transfusion count at 1 hour or 24 hours following transfusion. The lack of pre and post transfusion platelet counts and the duration in which they were taken highlights the importance of obtaining this clinical information, in order to provide suitable on-going HLA-matched platelet support, however obtaining this information may not always possible due to logistical reasons.

The patient received a total of 71 packs of HLA matched platelets over the course of 1 year, prior to receiving an HLA identical sibling stem cell transplant. An identical HLA match was found within the family and on this occasion it was not necessary to search for a donor from the bone marrow registries. HLA identical sibling stem cell transplants provide the best survival rates at 5 years (74%) and matching of HLA-A, B, C and DRB1 is associated with lower GvHD and higher rates of survival (Ottinger *et al*; 2003).

The transplant demonstrated good marrow engraftment with no signs of GvHD and no recurrence of leukaemia and the patient continued to receive HLA-matched platelet support for 6 weeks after transplant. Platelet counts were seen to increase to normal levels over the next 5 months. 100 days post-transplant and cessation of cyclosporin, the patient T cell graft chimerism was seen to be improving and no evidence of GvHD was detected. 153 days post-transplant, the patient was given a dose of $1x10^6$/kg of donor lymphocyte infusion (DLI). The purpose of DLI is to induce a GvL (graft versus leukaemia) effect in patients to prevent relapse of the disease and although much success has been achieved with CML, response rates to DLI in patients with AML are between 15-30% (Porter; 2003).

One month later, following a major drop in donor chimerism, the patient was given a second dose of $5x10^6$/kg of DLI. Despite the second infusion, myeloid chimerism counts continued to fall and leukaemia blasts were found in the peripheral blood. The patient was informed that a second allogeneic stem cell transplant would not help because the leukaemia had survived the first transplant. Further induction chemotherapy would be harmful and unlikely to prolong her life or restore remission. The disease was very progressive and life expectancy was anticipated as several months. The only care the hospital could offer was supportive care with blood and platelet transfusions as necessary and prompt treatment of infection to prolong the life of the patient.

The patient continued to receive twice weekly blood and HLA-matched platelet transfusions for a further 10 months, until she sadly died 18 months post-transplant.

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7.2. Case 2: LIVING-RELATED RENAL AND SUBSEQUENT DECEASED DONOR PANCREATIC TRANSPLANTATION- REJECTION OF PANCREAS WITH FUNCTIONING KIDNEY

This case was chosen as it illustrates:

- The H&I laboratory work-up involved in a live-related renal transplant and how this to selection of a recipient for a deceased donor pancreas
- The joint role of the hospital and the H&I laboratory in post-transplant monitoring and the management of rejection

INTRODUCTION: PATIENT CLINICAL HISTORY

The patient was a 40 year old male who was diagnosed with Type I Insulin Dependent Diabetes Mellitus (IDDM) in 1974, aged 16. Diabetes Mellitus is the most common endocrine disease worldwide (Rayhill *et al*; 2000) and despite the success of exogenous insulin therapy, is the leading cause of end-stage renal failure (ESRF) in Western countries (Odoni & Ritz; 1999).
Despite daily insulin injections, frequent blood glucose monitoring and adherence to a controlled diet, 24 years later (1998), the patient developed ESRF.
Type I diabetic patients with renal failure have several treatment options; they can remain on dialysis and daily insulin injections, but with 5 year survival rates at 21% (Rayhill *et al*; 2000), this is not an option usually considered. Patients can undergo various transplant scenarios, such as kidney transplant alone (KTA) and remain on insulin; kidney transplantation followed by subsequent pancreas transplant (PAKT) using a living-related kidney donor and deceased pancreas donor or simultaneous kidney and pancreas transplantation (SPKT) from either a deceased or a living related donor (Stratta; 2003).
The patient was placed on dialysis and in order for the patient to be assessed prior to listing for a renal transplant with United Kingdom Transplant (now Organ Donation Transplantation, a directorate of NHSBT), blood samples were sent for HLA typing and antibody screening. The median waiting time for adult patients registered with ODT for a kidney transplant in XXXXXXX between 2002-2005 was 429 days (www.uktransplant.org.uk), so family members were approached as potential live-related donors and bloods were also sent for HLA typing and antibody screening to identify a suitable match.
The patient underwent a fully HLA-matched live-related kidney transplant in 1999 from his brother. The transplant was successful with good graft function and the patient continued on insulin therapy for eight years until receiving a deceased donor pancreas transplant in 2007.

LABORATORY TESTS

HLA TYPING

HLA typing of the patient and potential live-related kidney donors was performed using in-house serological typing trays for HLA-A and B and PCR-SSP using a commercial kit for HLA-DRB1. Five siblings were assessed as potential live donors for this patient. Low resolution typing by serology for HLA-A and B and medium resolution for HLA-DRB1 were performed initially for the patient and all

donors. As part of the final work-up before transplant, high resolution HLA Class II typing (4 digits) was performed for the patient and selected donor to establish the claimed genetic relationship, as was required by the Human Organ Transplant Act regulations at the time of work-up (1998-1999).
HLA typing of the deceased pancreas donor was initially performed at the Transplant Centre of donor origin and once the organ had been allocated to our patient and we received donor spleen and lymph nodes, confirmatory medium resolution HLA Class I and II typing (2 digits) by PCR-SSP using a commercial kit was performed by our laboratory at the same time crossmatching between donor and recipient took place.

HLA ANTIBODY SCREENING

As part of the transplant work-up, screening for HLA antibodies was initially performed using complement dependent cytotoxicity (CDC) assay, a modified version of that described by Terasaki and McClelland (1964). Cell donors for the CDC panel are routinely selected to cover most of the known HLA specificities and a panel of 30 cells are used. Dithiothreitol (DTT) treated serum is used in addition to untreated serum to enable differentiation of IgM and IgG complement-fixing HLA antibodies. Subsequent serum samples from this patient were screened using a combination of CDC, Enzyme-linked ImmunoSorbent Assay (ELISA) using a GTI kit (Quest Biomedical) and Luminex technology using LabScreen (One Lambda) and LifeMatch ID (Tepnel Lifecodes) commercial kits.
British Transplantation Society (BTS) Standards for Solid Organ Transplantation in the United Kingdom, British Society for Histocompatibility and Immunogenetics (BSHI) Guidelines and Departmental policy state that any antibody-specific HLA antigen should be listed as an unacceptable antigen and an organ with the same specificity would not usually be considered for transplant.

CROSSMATCHING

Lymphocytotoxic crossmatching was performed using peripheral donor lymphocytes and patient sera between each potential live-related kidney donor and recipient at the time of work-up.
Lymphocytotoxic crossmatching and flow cytometric crossmatching of the selected sera were performed immediately prior to transplant. T and B cell flow crossmatching was performed using peripheral blood lymphocytes from the live-related donor and the most recent serum sample from the patient, whilst current and historical serum samples were used for lymphocytotoxic crossmatching.
Immediately prior to the deceased donor pancreas transplant, flow cytometric and lymphocytotoxic crossmatching were performed using splenic deceased donor lymphocytes.

ABO BLOOD GROUPING

ABO and Rh D grouping of the patient, all potential live-related kidney donors and the deceased pancreas donor were performed. This was carried out by the Red Cell Immunohaematology (RCI) laboratory in the Centre, on fresh blood samples obtained from the patient and donor, using the Diaclon ABD confirmation

gel cards (DiaMed). This is a serological reverse grouping method and uses monoclonal anti-A, B and D to detect the presence of A/B and D antigens on red cells.

LABORATORY TEST RESULTS FOR LIVE-RELATED KIDNEY TRANSPLANT

PATIENT AND DONOR HLA TYPES

The patient and 5 potential live-related kidney donors were initially typed for HLA-A, B and DRB1. Donor 4 was selected for further work-up as the potential live donor.
The HLA typing results are shown below in Table 1.

TABLE 1: PATIENT AND LIVE-RELATED DONORS HLA TYPES
(Red=mismatched antigens)

	A	B	DRB1*	DQB1*
PATIENT	1, 2	8	0301	0201
DONOR 1 (SISTER)	1, 2	8	0301/06/08	Not tested
DONOR 2 (BROTHER)	**3, 23**	**14, 44**	**07, 13**	Not tested
DONOR 3 (SISTER)	1, 2	8	0301/06/08	Not tested
DONOR 4 (BROTHER)	1, 2	8	0301	0201
DONOR 5 (BROTHER)	**3, 23**	**14, 44**	**07, 13**	Not tested

HLA ANTIBODY SCREENING RESULTS

Pre-transplant screening for HLA antibodies on the initial samples as part of live-related kidney pre-transplant work-up were performed using CDC only. These results showed the patient to be negative for the presence of both HLA Class I and Class II antibodies.

CROSSMATCHING RESULTS

Pre-transplant work-up CDC crossmatching of all 5 potential live-related kidney donors with the recipient were both T cell and B cell negative with the current and historical sera.
The immediate pre-transplant live-related CDC and flow cytometric crossmatches between the recipient and his brother were both T cell and B cell negative.

ABO/ RHD BLOOD GROUPING

The patient and live-related kidney donor were ABO compatible and were both group O positive.

LABORATORY TEST RESULTS FOR DECEASED DONOR PANCREAS TRANSPLANT

PATIENT AND DONOR HLA TYPE

Work-up of the patient for a deceased donor pancreas transplant involved HLA typing of the patient for HLA Class I and II using PCR-SSP, as departmental policy dictates that all renal and pancreas transplant recipients must have been HLA typed for Class I and II on two separate occasions.
Confirmatory typing of the pancreas donor was also performed using PCR-SSP for HLA Class I and II in our laboratory, prior to transplantation and was in agreement with the original HLA type performed by another H&I laboratory. The patient and donor were 111 HLA-mismatched (for HLA-A, B, DRB1) as shown in Table 2, with A3, B44, Cw5, Cw12, DR4 and DQ3 mismatches at antigen level.

TABLE 2: PATIENT AND DECEASED DONOR HLA TYPES
(Red=mismatched antigens)

	A*	**B***	**Cw***	**DRB1***	**DQB1***
PATIENT	0101 0201	0801	0701	0301	0201
DONOR	0201 **0301**	**4402**	**0501** **1203**	**0401** **0403**	**0301** **0304**

HLA ANTIBODY SCREENING RESULTS

HLA antibody screening following kidney transplantation was performed by CDC and then additionally by ELISA and Luminex methodologies. The patient maintained a negative HLA antibody profile following his living-related kidney transplant from 1999 to 2005. In 2006, during work-up for a deceased donor pancreas transplant, B63 and B72 antibody specificities were demonstrated by Luminex technology, but CDC results were negative. These antigens are low frequency within the population and are not normally covered by our local CDC panel.
Luminex technology is a more sensitive technique than CDC and can also detect non-complement fixing HLA antibodies. The ELISA screening results using LAT-M were also positive for HLA Class I in sera screened during pancreas transplant work-up.

CROSSMATCHING RESULTS

The CDC and flow cytometric crossmatches between the recipient and deceased donor pancreas donor were both T cell and B cell negative, immediately prior to transplant.

ABO BLOOD GROUPING

The patient and deceased donor pancreas donor were ABO compatible and were both group O positive.

PANCREAS TRANSPLANTATION AND SUBSEQUENT REJECTION

Cold and warm ischaemia time was 14 hours. The patient was placed on steroid therapy (methylprednisolone) at reperfusion and during surgery. A midline incision on the left hand side of the recipient was made and the transplant procedure and immediate post-operative course were uneventful. After transplantation, the patient was placed on an immunosuppression treatment regime of MMF (mycophenolate mofetil), prednisolone and azathioprine and 1 week later, azathioprine was substituted for tacrolimus.

Two weeks post-transplant, the patient complained of significant abdominal back pain on the left side and in his left testis with elevated temperature and little relief was obtained from analgesia. A CT scan showed a well perfused pancreas with normal graft arteria, but some venous thrombosis distal to the portal vein. The patient was placed on intravenous hydration and therapeutic anti-coagulants.

Two days later the patient developed chronic diarrhoea and was found to have a positive culture of *clostridium difficile*. A GI colonoscopy was performed and there appeared to be no bacterial contamination of the small bowel. Metronidazole anti-microbials were administered for two weeks, followed by two weeks of vancomycin. 29 days post-transplant the patient was well and was discharged from hospital.

Two days later the patient was re-admitted with another bout of diarrhoea and the vancomycin and MMF were stopped and anti-coagulant therapy was adjusted.

The patient was admitted several times with sudden onset of diarrhoea and abdominal pain persisted over the next four weeks and a further CT scan following a hyperglycaemic episode which required administration of insulin revealed significant infarction of the pancreas with occlusion of one of the two arterial limbs. Renal function was stable with a serum creatinine of 100μm/L.

Four weeks later the patient again admitted himself to hospital suffering with rectal pain, night sweats and shivers and intractable diarrhoea. Serum creatinine was stable at 119μm/L, but inflammatory markers were raised. C-reactive protein (CRP) levels were 64mg/L, leucocyte count was 9.1, potassium levels were 46mmol/L and erythrocyte sedimentation rate (ESR) was 94mm/hr. A CT scan showed deterioration in the appearance of the pancreas, suggestive of pancreatitis and chronic rejection with surrounding inflammatory change with poor arterial flow to the graft. Histological staining for C4d was positive and a sample was sent to the H&I laboratory to investigate whether the likely cause of rejection was antibody-

mediated. HLA antibody screening by Luminex using LabScreen single antigen beads (One Lambda) revealed the presence of both HLA Class I and Class II donor-specific IgG HLA antibodies to all the mismatched antigens of the pancreas graft, i.e. A3, B44, Cw5, Cw12, DR4, DR53 and DQ3. In addition a large number of other Class I and Class II specificities were detected and are shown in Table 3.
The pancreas was removed (143 days post-transplant) and found to be highly infarcted with vessel thrombosis and subsequent drainage of a peritoneal abscess revealed the presence of lactose fermenting coliforms. The patient was placed back onto exogenous insulin therapy and three months later began showing signs of diabetic neuropathy. An islet cell transplant was considered, but due to the high levels of HLA antibodies this would not be a feasible option. A solitary pancreas transplant using Campath immunosuppression and bladder drainage of exocrine pancreas secretions is currently under consideration, but at present the diabetes is under control and the renal function of the patient is excellent with a serum creatinine of 104µm/L.

TABLE 3: LIST OF ANTIBODY DEFINED UNACCEPTABLE HLA ANTIGENS FOLLOWING REMOVAL OF PANCREAS
(Donor-specific antibodies are in red)

HLA-LOCUS	UNACCEPTABLE HLA ANTIGENS
HLA-A	**A3**, A9, A11, A19, A25, A32, A34, A80
HLA-B	**Bw4**, B14, B18, B22, B35, B39, B40, B41, **B44**, B45, B46, B48, B50, B59, B62, B67, B71, B72, B73, B75, B76, B78, B82
HLA-Cw	Cw1, Cw2, Cw3, Cw4, **Cw5**, Cw6, Cw*0702, Cw8, **Cw12**, Cw14, Cw15, Cw16, Cw17, Cw18
HLA-DR	DR1, DR103, DR2, **DR4**, DR5, DR6, DR7, DR8, DR9, DR10, DR51, **DR53**
HLA-DQ	DQ1, **DQ3**, DQ4

DISCUSSION

The patient described here was diagnosed with chronic renal failure due to diabetic nephropathy. With 5 year survival rates of 21% (Rayhill *et al*; 2000) for patients undergoing exogenous insulin therapy and haemodialysis, transplantation offers an improvement in long- term survival and quality of life.
The main advantage of choosing PAKT over SKPT is that due to improved immunosuppression, refined surgical techniques, excellent antimicrobial prophylaxis, good HLA-matching and close post-transplant monitoring for rejection, PAKT recipients can enjoy >80% 1 year post-transplant graft survival (Gruessner *et al*; 2001).
Many insulin-dependant diabetic patients with renal failure decide to initially undergo a living-related donor kidney transplant to reduce waiting time on dialysis with elective surgery. Living-related allografts have less immunological disparity, comparatively minimal preservation injury and 5 year post-transplant graft survival at 85% is superior to deceased donor transplantation at 70% in the same period (Rayhill *et al*; 2000).

The patient was transplanted with a 000mm kidney from a living-related donor. Human Organ Transplant Act (HOTA) regulations (1989) were in force at that time and required establishment of a genetic relationship (in cases of claimed relationship) prior to a living related transplant taking place. In 2004, the Government introduced new legislation to regulate the removal, storage and use of Human Organs and Tissue. The Human Tissue Act 2004 replaced the Human Tissue Act 1961, Anatomy Act 1986 and the Human Organ Transplant Act 1989. The new legislation does not require establishment of a genetic relationship in living related donations, but involves assessment by independent assessors who review each case individually to ascertain that the donation is ethical (www.hta.gov.uk).

The transplant was uneventful and the patient was given the renal unit standard triple therapy of cyclosporine, prednisolone and azathioprine. Following successful transplantation, the patient remained on insulin therapy for a further six years until his diabetes worsened and he was listed for a pancreas transplant.

Eighteen months after being listed, an offer of a 111mm pancreas was received and prospective CDC and flow crossmatching of the patient sera with deceased donor splenic lymphocytes were negative, resulting in transplantation with cold ischaemia time of 14 hours.

143 days post-transplant, the pancreas allograft was removed due to chronic rejection which was identified as being antibody-mediated due to the formation of donor-specific HLA antibodies to the allograft.

This was supported by the detection of C4d deposition which occurs following an antibody-mediated immune response and coupled with the presence of donor-specific HLA antibodies is diagnostic for antibody-mediated rejection (Gaber; 2007). Chronic rejection is the most common cause of graft loss after an initially successful pancreas transplant; with PAKT and pancreas alone transplants, recipients are reported to have a significantly higher risk of chronic rejection than SKPT recipient at 1 year post-transplant (Carbajal *et al*; 2007). Graft loss due to chronic rejection is usually characterised by a gradual deterioration in pancreas graft function beginning at least two months post-transplant coupled with falling amylase levels and hyperglycaemic episodes (Humar *et al*; 2003).

In addition to the formation of donor-specific antibodies, a large number of Class I and II antibody specificities were also detected by single antigen bead analysis using Luminex. These specificities could be accounted for when HLA Matchmaker analysis was performed. HLA Matchmaker is a computer-based algorithm that considers patches of antibody-accessible polymorphic residues called eplets, which are surrounded in close proximity by residues in discontinuous sequence positions. These eplets are considered to be essential components of HLA epitopes (Duquesnoy & Marrari; 2009).

The antibody defined unacceptable antigens detected in the patient are listed in Table 3. All A locus specificities were accounted for by the mismatched 66RNQ eplet (A*0301) except for A9, A25, and A32 specificities which could be accounted for by exposure to the B*4402 epitopes 77TEN and 82LR. All of the B locus specificities were accounted for by 131S or 32L/41T (B*4402), although several had other mismatches as well. The Cw antigens which gave positive results all had the 65QKR epitope, including Cw*0702. The 65QKR epitope is not present in Cw*0701 (patient). All DRB specificities could be accounted for by the mismatched 76ADT epitope, except DR7 which has a 4Q mismatched eplet shared by DR53 associated antigens. (www.HLAMatchmaker.net). There were several potential targets in DQ but this was difficult to analyse due to polymorphism in the alpha and beta chain.

In this case a 111 mismatched pancreas resulted in exposure to a large number of mismatched epitopes shared by a limited number of HLA molecules, leading to antibody formation against numerous HLA specificities. This occurred despite the patient remaining on standard immunosuppression for his kidney graft. The patient remains on insulin therapy with a functioning kidney graft (10 years post-transplant), however is almost untransplantable due to his complex antibody profile.

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7.3 Case 3: PLASMA EXCHANGE PRIOR TO HEART TRANSPLANTION IN A HIGHLY SENSITISED RECIPIENT

This case was chosen because:

- It demonstrates the sequence of laboratory investigations preceding cardiothoracic transplantation and how this differs from other solid organ transplants, such as renal transplants.
- It highlights the importance of collaboration between scientific and clinical teams in the continual monitoring of HLA antibody levels in a highly sensitised recipient and how novel therapies can provide an opportunity for transplantation to proceed.

INTRODUCTION: PATIENT CLINICAL HISTORY

The case described here is of a 32 year old multiparous female patient diagnosed with dilated cardiomyopathy and requiring a heart transplant.
Dilated cardiomyopathy is a disease of the heart muscle that impairs its ability to circulate the blood. Causes are varied and can be due to infection, misuse of drugs and alcohol, endocrine problems, neuromuscular diseases, familial/ genetic or in some individuals the cause is undetermined. Pre-transplant, the patient had five children and smoked moderately, but drank very little alcohol. Her parents and grandfather all suffered a myocardial infarction and her aunt and two of her aunts' sisters all suffered an aortic aneurysm.
The patient self-presented with chest pain radiating into her back, upper abdomen and shoulders. Other symptoms were shortness of breath, palpitations and swollen ankles. An electrocardiogram (ECG) showed a t-wave abnormality (indicative of a problem with the ventricles of the heart) with sinus tachycardia and a soft tissue mass in the mediastinum and fluid in the horizontal fissure was detected by x-ray. Admission bloods showed a raised d-dimer, alkaline phosphatase and ALT, but troponin t was negative. An echocardiogram (EEG) showed severely impaired left ventricle function with an ejection fraction of 10-15% and the right ventricle was mildly dilated with moderate/ severe decrease in function. The patient was prescribed milrinone and frusomide and subsequently discharged, but one week later her condition worsened with shortness of breath with minimal exertion and palpitations and was re-admitted to hospital. The patient underwent right heart catheterisation and the cardiac unit submitted blood for an antibody screen. Antibody screening revealed a number of HLA Class I and Class II IgG antibodies and the patient was listed for a heart transplant. One month later, the patient underwent further antibody screening and additional antibodies were detected including a number of DP antibodies. Due to the specificities of these antibodies (without considering DP), the chances of finding a suitably matched heart were extremely low as approximately 90% of donors would be excluded. Since patient life expectancy was only 1-2 months without a transplant, prospective antibody removal was initiated, which involved constant monitoring by the laboratory. A desensitisation programme using plasma exchange (PE) in conjunction with IVIg was agreed as a method of removal or reduction in antibody levels to increase the chances of the patient receiving a transplant. Individual antibody specificities were monitored and as the antibody titres became negative following rounds of PE, donors expressing these antigens would be considered and a prospective crossmatch by flow cytometry and CDC performed to determine suitability of the heart for transplantation.

LABORATORY TESTS

PROTOCOL USED FOR CARDIOTHORACIC TRANSPLANT PATIENTS

The policy of the laboratory is to initially screen all cardiothoracic patients for HLA antibodies. Before a patient is listed for transplant, a clotted peripheral blood sample is submitted to the laboratory for antibody screening. Initially a positive/ negative screen is performed by ELISA and Luminex screening kits for HLA Class I and Class II antibodies. The sample is also batch-tested by CDC using a panel of 30 cells for Class I complement-fixing antibodies. If negative for HLA antibodies, the patient can be transplanted based on ABO compatibility and a retrospective CDC crossmatch is performed. While awaiting transplant, current policy dictates that antibody negative patients require HLA antibody screening every year, unless there are sensitising events, in which case a repeat antibody screen is performed. If a positive reaction is found in the initial screen by ELISA or Luminex, the samples are further tested for Class I and II specificities using Luminex identification kits. Any HLA antibodies identified by any technique are listed as unacceptable antigens for that patient. Also, any patient listed with unacceptable antigens requires HLA typing to confirm the antibodies are alloantibodies. Sensitised patients are currently screened every six months or following a sensitising event, such as a blood transfusion (policy agreed with the local cardiothoracic transplant unit). If HLA antibodies are clearly identified, a virtual crossmatch is performed and a retrospective crossmatch is performed as a guide to clinical management post-transplant. Patients with highly complex antibody profiles are listed for a prospective crossmatch by CDC, flow cytometry or both. The recent introduction of a LabScreen single antigen bead identification kit into routine use within the laboratory has enabled the characterisation of all HLA antibody specificities in most patients with highly complex antibody profiles, so for the majority of our patients removes the requirement for a prospective crossmatch.

ANTIBODY SCREENING

All samples were tested by LAT-M ELISA and Luminex screening kits (LabScreen, One Lambda Inc) for HLA Class I and Class II antibodies. The samples were further tested for Class I and II specificities using LifeMatch (Tepnel Inc) Luminex identification kits. The samples were also tested by CDC for Class I complement-fixing antibodies using a panel of 30 cells. DTT treated serum in the CDC assay was used to enable differentiation of IgM and IgG antibodies. The initial screening sample and the sample prior to commencing PE were also tested by Luminex using a LabScreen single antigen bead identification kit, due to the LifeMatch identification kit being unable to determine all specificities. Testing of these two samples also established a baseline value to measure the effectiveness of each round of PE in the patient. HLA antibody levels of pre-PE and post-PE samples were also monitored using flow cytometry to determine the strength of the antibodies present.

CROSSMATCHING

It is laboratory policy to retrospectively crossmatch transplanted heart patients within 24 hours of transplant using CDC as described above. Due to the large

number of HLA antibodies in this patient, prospective crossmatching of potential donors by both flow cytometry and CDC were performed to determine whether the heart was suitable for transplant. Flow cytometric crossmatching for cardiothoracic patients is not routinely performed in this laboratory, but was suggested for this patient due to the nature of the antibody profile and the deteriorating condition of the patient. Flow cytometric crossmatching is more sensitive than CDC and was used to monitor donor-specific reactivity.
Prior to transplant, seven other donors were prospectively crossmatched by CDC and flow cytometry. The patient was transplanted with donor 8. The crossmatching results can be seen in Table 1 below.

HLA TYPE BY PCR-SSP

Since the patient was sensitised, HLA typing was performed prior to transplant by PCR-SSP using a commercial kit. Medium resolution HLA typing (2 digits) for Class I and Class II was performed. The donors were all from outside the local region and were initially HLA typed in other laboratories by PCR-SSP. Table 2 displays the HLA type of the patient and Table 3 displays the HLA types of the 8 donors crossmatched with the patient.

ABO BLOOD GROUPING

Blood grouping is routinely carried out to enable the suitable allocation of organs. This was performed by the Red Cell Immunohaematology (RCI) laboratory in the Centre, on fresh blood samples obtained from the patient and donor, using the Diaclon ABD confirmation gel cards (DiaMed). This is a serological reverse grouping method and uses monoclonal anti-A, B and D to detect the presence of A/B and D antigens on red cells.

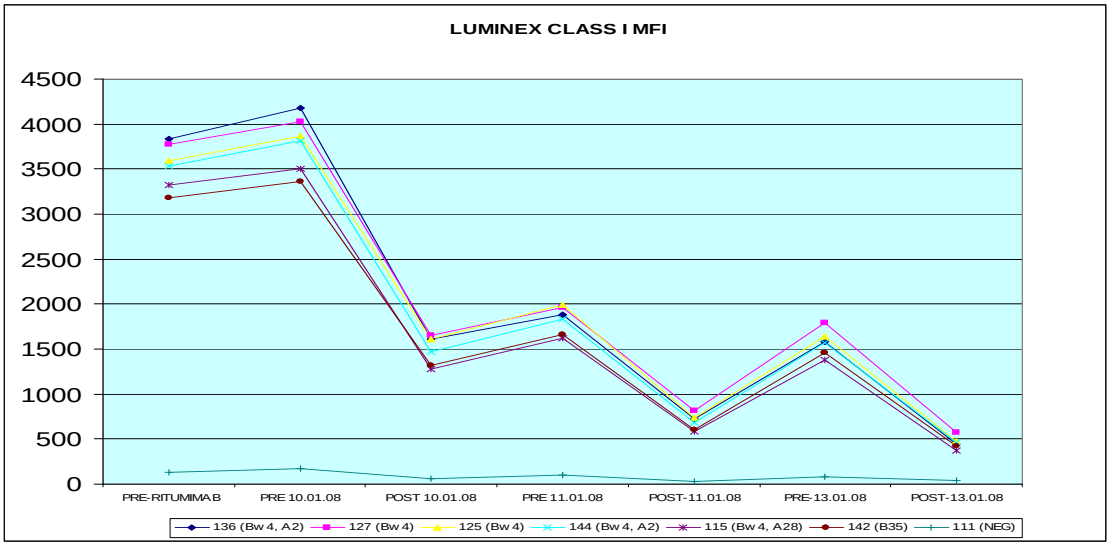
RESULTS

ANTIBODY SCREENING AND IDENTIFICATION

The initial sample prior to the patient being listed for a heart transplant was positive for HLA Class I and II antibodies by LAT-M ELISA and a Luminex screening kit (LabScreen). The sample was then tested using a Luminex antibody identification kit (LifeMatch) and resulted in 98% PRA for Class I with only Bw4 and B35 specificities discernable. Class II PRA was 60% with identifiable specificities for DR5, DR7, DR8 and DR9. Single antigen bead testing (LabScreen) using Luminex was necessary to determine Class I additional antibody specificities, masked using the LifeMatch kit. The following additional antibody specificities were identified; A2, A11, A28, A30, B45, B76, Cw1, Cw2, Cw4, Cw5, Cw6, Cw8, Cw14, Cw15, Cw17 and Cw18. A sample screened a month later contained additional specificities of B59, DQ4, DP1, DP3, DP5, DP9, DP10, DP11, DP13, DP14 and DP17.
Even without considering the DP antibodies, the specificities identified in the patient would exclude a large percentage of heart donors, based on antigen frequency in a panel of 10,000 local BBMR donors. As antibody specificities became negative, they were removed from the list of unacceptable antigens. The patient was placed on the national priority heart transplant waiting list and prospective crossmatching of a potential heart donor was to be performed using peripheral donor lymphocytes.

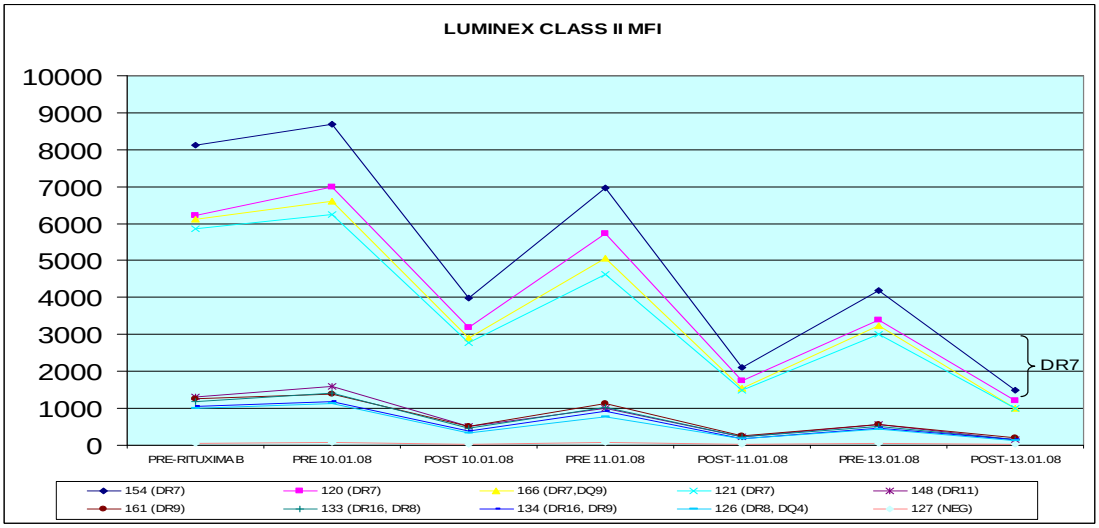
The patient underwent 8 rounds of PE over a period of 16 days and was successfully transplanted. The patient received a further 3 rounds of PE immediately following transplant and then two sets of 3 rounds of PE following two episodes of acute rejection.
Graph 1 below demonstrates the positive effects of the first 3 rounds of PE after the on the Class I antibody levels of the patient, prior to transplant.

GRAPH 1: CLASS I ANTIBODY TITRES FOLLOWING THE FIRST THREE SESSIONS OF PLASMA EXCHANGE PRIOR TO TRANSPLANT



Antibody levels for A2, A28, Bw4 and B35 have significantly decreased from MFI (mean fluorescence intensity) values of between 3183-3836 to between 370-576. Values below an MFI of 500 are considered negative. After three rounds of PE, only Bw4 appears to be weakly positive.
Graph 2 below demonstrates the positive effects of the first 3 rounds of PE after the on the Class II antibody levels of the patient, prior to transplant.

GRAPH 2: CLASS II ANTIBODY TITRES FOLLOWING THE FIRST THREE SESSIONS OF PLASMA EXCHANGE PRIOR TO TRANSPLANT



Antibody levels for DR11, DR9, DR8, DR7, DR16 and DQ9 significantly decreased from MFI (mean fluorescence intensity) values of between 1003-1305 pre-PE to 126-204, following three rounds of PE and these values are considered to be negative. The only class II antibody still present at this time was DR7, although MFI values have decreased significantly from 5861-8132 to 990-1500. Further rounds of PE may have resulted in lowering the titre even further to a negative value.

CROSSMATCHING

TABLE 1: PROSPECTIVE CROSSMATCHING RESULTS

	CYTOTOXIC CROSSMATCH				**FLOW CROSSMATCH**	
	T cell-DTT	T cell +DTT	B cell –DTT	B cell +DTT	T cell	B cell
Donor 1 (before PE)	POS	**NEG**	POS	POS	POS	POS
Donor 2 (before PE)	POS	**NEG**	Unreadable	Unreadable	POS	POS
Donor 3 (after 4xPE)	POS	POS	POS	POS	POS	POS
Donor 4 (after 6xPE)	Weak POS	Weak POS	Weak POS	Weak POS	**NEG**	Unreadable
Donor 5 (after 7xPE)	POS	POS	POS	POS	POS	NOT TESTED
Donor 6 (after 7xPE)	POS	POS	POS	POS	**NEG**	NOT TESTED
Donor 7 (after 7xPE)	POS	POS	NOT TESTED	NOT TESTED	**NEG**	POS
Donor 8 (after 7xPE)	Weak POS	Weak POS	Weak POS	Weak POS	**NEG**	NOT TESTED

The HLA types of all the prospective donors are shown in Table 3. Unacceptable antigens identified prior to initiating PE are highlighted in red.
A positive flow T cell crossmatch is a contraindication to transplant and therefore donors 1, 2, 3 and 5 were not considered suitable. Rituximab is a chimeric monoclonal antibody which selectively depletes B lymphocytes from the circulation, by binding to the CD20 receptor on the cell surface. Due to the administration of this drug, B cell crossmatch results are invalid as circulating B lymphocytes have anti-CD20 bound to their surface, which in turn will bind the reagents used in flow cytometric crossmatching. Although the cytotoxic crossmatch results for donor 4 in the current sample were weakly positive, possibly due to the presence of a mismatched A2 donor antigen, the heart was not transplanted. The hearts from donors 6 and 7 were diseased and not suitable for transplant. Donor 8 produced weak positive cytotoxic crossmatch results (same strength as donor 4). Five sera were tested by flow cytometry and the four most recent sera were all negative. The sample which was positive was dated 6 days prior to transplant and the patient had undergone two rounds of PE since the positive sample was taken. A further round of PE was given and the donor was transplanted with donor 8.

HLA TYPE

The HLA type of the patient is displayed in Table 2 below. Tissue typing of cardiothoracic patients is performed in our laboratory at low to medium resolution, following identification of HLA antibodies. If patients have a negative antibody profile, then HLA typing is not usually performed until retrospective crossmatching is done after transplant. Due to the presence of DP antibodies in this patient, DP typing was also performed using a commercial kit.

TABLE 2: PATIENT HLA TYPE

HLA-A*	HLA-B*	HLA-Cw*	HLA-DRB1*	HLA-DQB1*	HLA-DPB1*
01	07, 55	0303, 07	0301, 04	02, 0301	0201, 0401

The donors were from outside the local region and were HLA typed in other H&I laboratories. Table 3 displays each donor that a prospective crossmatch was performed with the patient. Mismatched antigens (patient doesn't have antibodies to) are in blue and mismatched antigens to which the patient has antibodies to are in red.

TABLE 3: COMPARISON OF HLA TYPES OF PROSPECTIVE DONORS

	HLA-A*	HLA-B*	HLA-Cw*	HLA-DRB1**	HLA-DQB1*
DONOR 1	**02**, 03	1402, **44**	**05**, **08**	13	06
DONOR 2	**02**	18, **44**	07, **08**	0301, 13	02, 06
DONOR 3	**02**, 25	18, **44**	**05**, 12	04, 13	0301, 06
DONOR 4	**02**, 03	07, **35**	**04**, 07	**11**, 15	0301, 06
DONOR 5	**02**, 66	72, 53	**02, 04**	**11, 12**	06, 0301
DONOR 6	24, 32	07, 1401	N/T	04, 15	0301, 06
DONOR 7	**02**	07, 08	07	0301, 0103	02, 05
DONOR 8	01, 29	08, **44**	07, 16	0301 ,04	02, 0301

BLOOD GROUP

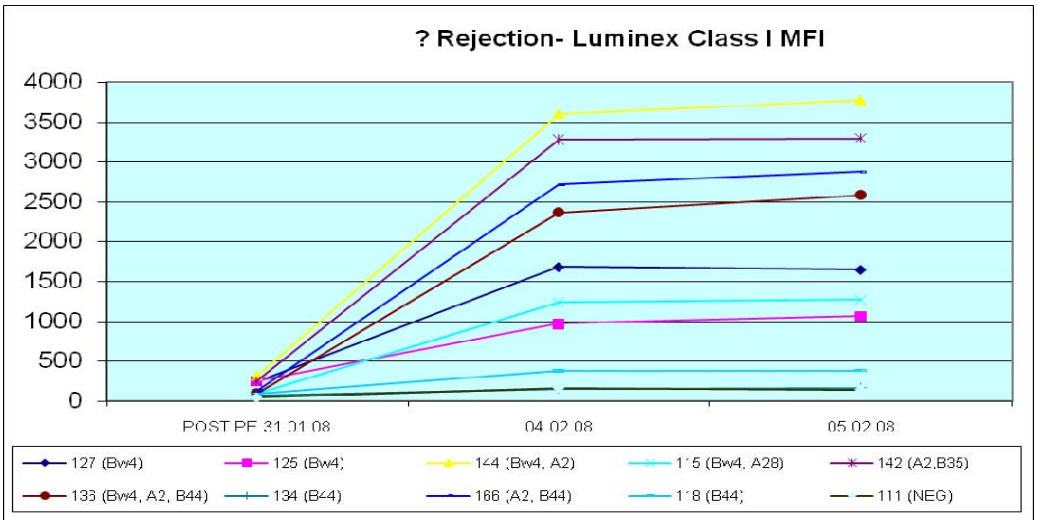
The blood group of the patient was tested as group B RhD positive. The donor was tested as group O RhD positive. It is not unusual to transplant group B patients with a group O donor, due to the low frequency of group B in the population (8%).

TRANSPLANTATION AND POST-TRANSPLANT MONITORING

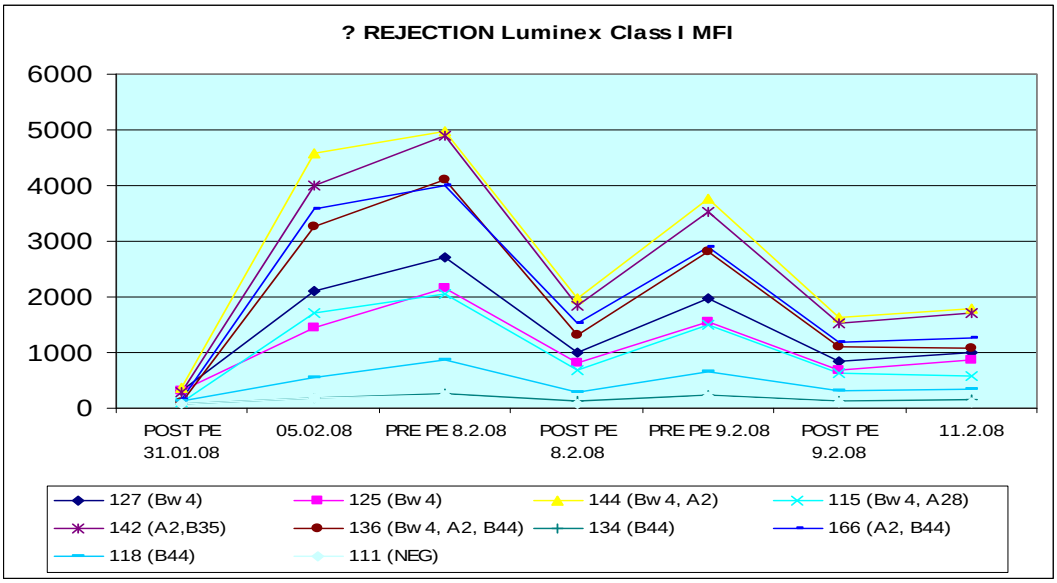
The patient and donor were prospectively crossmatched by flow cytometry and CDC on peripheral blood from the donor to determine suitability. The patient was subsequently transplanted using a median sternotomy incision with a cold ischaemia time of 192 minutes and placed on Tacrolimus and Prednisolone therapy. Flow cytometric and CDC crossmatching were repeated retrospectively using deceased donor splenic lymphocytes as per laboratory protocol. Following transplant, the patient underwent four sessions of PE on days 0, 1, 3 and 5 post-transplant and samples were sent to the laboratory to monitor antibody levels in the patient. The patient only demonstrated HLA antibodies to DR7 (non-donor-specific), which had been present at similar levels prior to transplant.

11 days after transplant and echocardiogram showed oedema and thickening of the ventricle walls. A cardiac biopsy showed C4d expression in the endothelial cells of some of the small vessels and in less than 50% of the intermytotic capillaries with ISHLT grade 1A rejection. A sample was sent for HLA antibody screening to determine whether the rejection was antibody-mediated. Results displayed in graphs 3 and 4 showed a significant rise in the levels of A2, B35, Bw4 antibodies. The donor was mismatched for A29, B8, B44 and CW16 antigen specificities as shown in Table 3. HLA antibodies to A29, B8, Cw16 and B44 donor-specific antigens were not demonstrated in the patient sera; however donor-specific antibodies to the Bw4 epitope expressed on B44 molecules were demonstrated.

GRAPH 3: DEMONSTRATION OF THE RETURN OF CLASS I ANTIBODIES 10 DAYS POST-TRANSPLANT

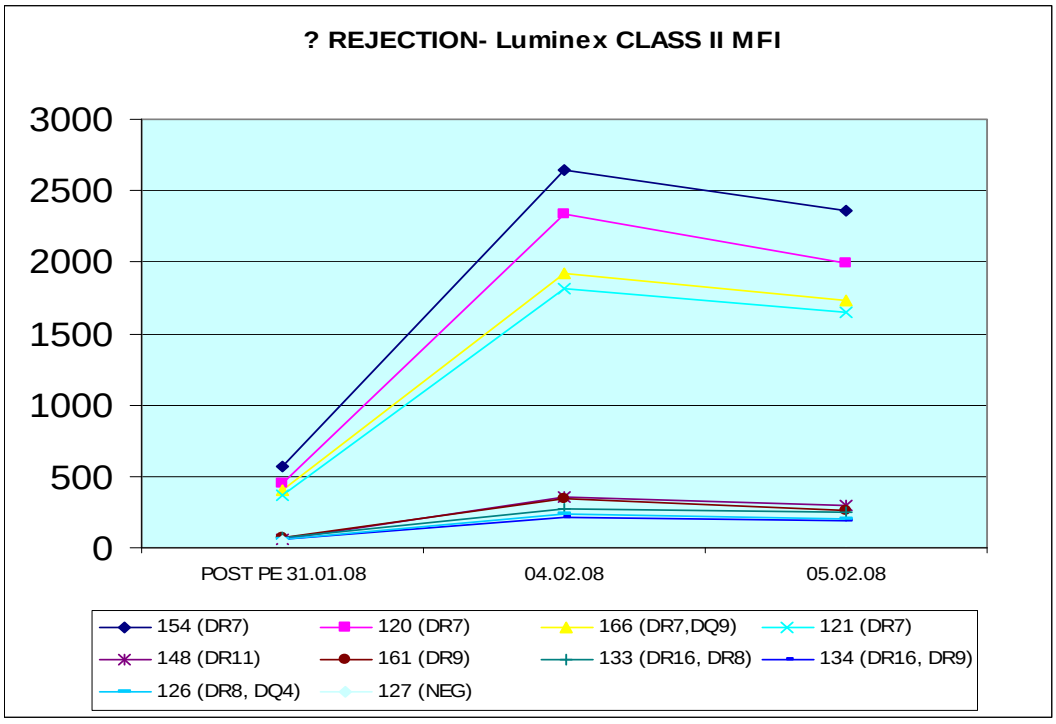


GRAPH 4: DEMONSTRATION OF THE RETURN OF CLASS I ANTIBODIES 10 DAYS POST-TRANSPLANT

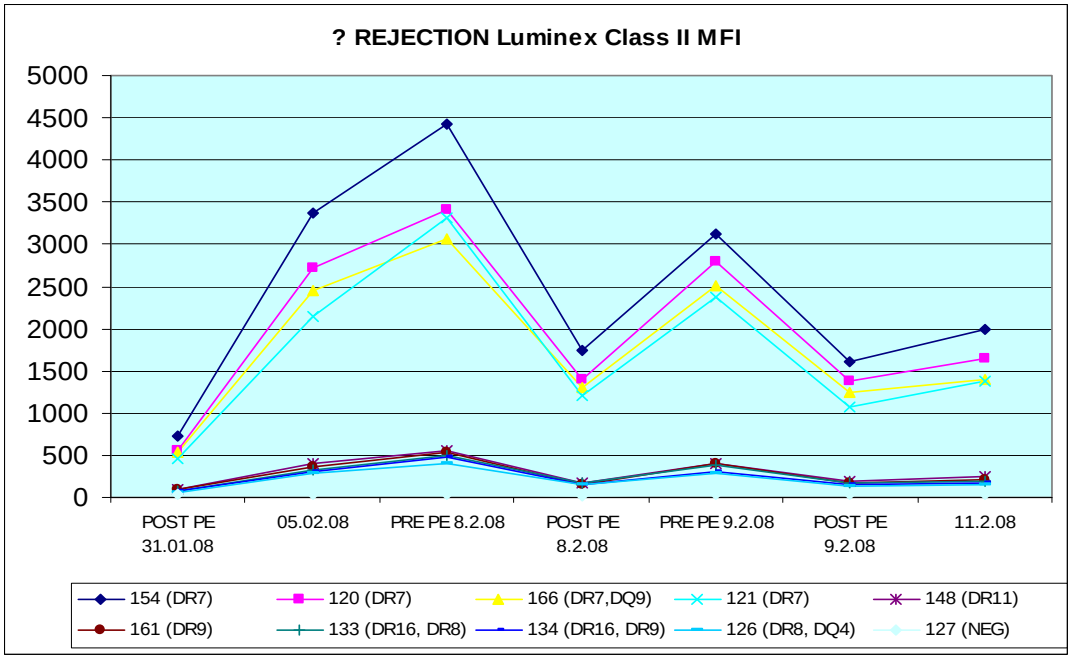


Results in graphs 5 and 6, show a significant rise in the level of DR7 antibody, which had successfully been reduced down to MFI levels of between 672-1028 (weakly positive) from pre-PE levels of 4044-5116, immediately prior to transplant. Antibody titres for DR7 had continued to fall and were at their lowest levels following PE 5 days post-transplant.

GRAPH 5: DEMONSTRATION OF THE INCREASE IN TITRE OF DR7



GRAPH 6: DEMONSTRATION OF THE INCREASE IN TITRE OF DR7



The patient underwent a further 3 rounds of PE on days 12, 13 and 15 post-transplant and was given increased steroid therapy. The patient stabilised and was subsequently discharged.
56 days after transplant the patient was re-admitted to hospital with increased weight and loin and back pain. Cardiac biopsy results were as those at day 11 post-transplant and further steroid therapy and PE were given. Following extensive investigation, no clear cause for the pain could be found. The patient subsequently became septic whilst in hospital and was treated with antimicrobials.
To date, the patient is currently well and although A2, B35, Bw4 and DR7 antibodies continue to be detected in the patient's sera, she has not demonstrated donor-specific antibodies to her graft and 15months post-transplant, the transplant is deemed to be a success. If this strategy of HLA antibody removal had not been initiated, due to the complex antibody profile of this patient, she would almost certainly have died without ever being transplanted.

DISCUSSION

Antibody monitoring prior to transplantation is vital and this case demonstrates the joint efforts of both the clinical team and laboratory staff in enabling this patient to receive a heart transplant and improve and extend her quality of life.
The patient described in this case was diagnosed with dilated cardiomyopathy of unknown aetiology. Cardiomyopathy is a general term for diseases of the heart muscle which loses its ability to pump blood and in some instances, arrhythmia can occur. With <50% survival at 5 years the disease is one of the leading causes for heart transplantation (www.cardiomyopathy.org/).
Cardiothoracic transplant recipients are not usually prospectively crossmatched in contrast to renal transplant recipients. Due to limited ischaemia time of 4-6 hours, the HLA antibody status of heart and lung transplant patients must be established prior to transplantation; patients who have a negative HLA antibody profile are retrospectively crossmatched following transplantation, whereas a virtual crossmatch is performed for sensitised patients with well-defined HLA antibody specificities. Cardiothoracic transplantation in sensitised recipients can be challenging as highly sensitised patients or those with highly complex HLA antibody profiles may require prospective crossmatching, which can be time-consuming and restrict the number of potential donors available.
The clinical significance of HLA antibodies in cardiothoracic transplantation is less well studied than in renal transplantation, but their importance is now well publicised. Studies have shown that cardiothoracic patients transplanted in the presence of donor-specific HLA antibodies are at an increased risk of early acute rejection and have lower graft survival (Smith *et al*; 2007, (Stastny *et al*; 2007).
Knowledge of the presence of HLA antibodies, their antigen specificities and their strength (titre) is crucial to understanding a patient's state of immunological sensitisation and reactivity. New technologies, such as Luminex have resulted in the ability to characterise the presence of multiple HLA specificities in patients with highly complex antibody profiles and in this case, provided a useful aid to monitoring the titres of antibodies present during plasma exchange. Prior exposure to antigen can result in antibody-mediated rejection, even if the antibody titre has decreased to an undetectable level, due to the presence of memory B-lymphocytes. Studies in the 1980s using plasmapheresis to reverse antibody-mediated rejection were largely unsuccessful, mainly due to a limited number of sessions being used for

fear of initiating infection and haemorrhage. The introduction of intravenous immunoglobulin (IvIG), followed by Rituximab and antithymocyte globulin have had a dramatic impact on success, with reversal of antibody-mediated rejection reported in 95% of patients (Crew and Ratner; 2007).

Antibody removal techniques such as plasma exchange and immunoadsorption are routinely used in highly immunised renal transplant recipients, but more recently their success has been seen in cardiothoracic transplantation (Pollock-BarZiv *et al*; 2007).

One session of plasma exchange would remove 30−40 ml/kg of plasma (1−1.5 plasma volumes) and be replaced with albumin and normal saline. The procedure must be controlled to ensure that the patient is kept in fluid balance, maintaining a stable, normal plasma volume. A single exchange removes approximately 75% of the patient's own plasma and the abnormal constituent in the plasma (McClelland; 2007). As shown in graphs 1 and 2, three sessions of plasma exchange were sufficient to reduce all circulating HLA antibodies to negative MFI values, except for Bw4 and DR7, which were weakly positive. The early results achieved with plasma exchange now enabled the Transplant unit to consider prospectively crossmatching deceased donors with all of these antigen specificities that were previously positive in the patients sera. The patient received 8 sessions of plasma exchange over a period of 16 days prior to transplant and was prospectively crossmatched with 6 deceased donors in that period. The patient initially endured a rocky course following transplantation with an increase in titres of both Class I and II antibodies which had been successfully removed following plasmapheresis and a significant increase in Bw4 and DR7 specificities coupled with C4d deposition on endothelial cells, indicative of an antibody-mediated acute rejection episode. A second acute rejection episode occurred four weeks later, with biopsy results similar to the first episode and an increase in antibody titres of both Class I and II specificities. On both occasions, the patient was aggressively treated with steroids and further immunosuppression and underwent further sessions of plasma exchange which has been reported to be effective in reversing antibody-mediated rejection (Beimler *et al*; 2009). In order to avoid early rebound of donor-specific antibodies it seems necessary to perform several post-transplant sessions of plasma exchange to achieve stable graft function.

Due to routine use of Luminex antibody technology we are now characterising DP antibodies in several solid organ transplant recipients and it is possible that the presence of donor-specific DP antibodies can adversely affect survival of the allograft, although matching for HLA-DP may have no direct impact on graft survival (Youngs; 2004).

In this case, pregnancy is reported to be the most likely reason to induce the formation of DP antibodies. The patient had five children, some of whom were born to different fathers and it is possible that the patient became sensitised during her pregnancies. The DP antibodies detected in the patient all have the DEAV motif at position 84-87, which is a major polymorphic site for DPB1. The role of DP antibodies in transplant graft survival is unclear. Currently, we do not perform routine HLA-DP typing on transplant recipients and donors, though it is likely that this will become routine practice in the near future. ODT (Organ Donation Transplantation) may require DP typing of donors and patients would also require DP typing if they have DP antibodies.

This experience of plasma exchange in a highly sensitised cardiothoracic patient shows that heart transplantation was possible following removal/ reduction in titre of

the HLA antibodies present and should increase the probability of transplantation in other alloimmunised cardiothoracic patients who have highly complex HLA antibody profiles.

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APPENDIX 1

This set of documents must be completed and returned in your portfolio.
Please complete the three header sections above on each page.
Refer to the Specific Competences document for guidance in completing this document.
Use typescript or black ink and block capitals for all sections.

EXPERIENCE: The candidate should be able to demonstrate that he/she has worked in an environment that has enabled the individual to receive training and gain experience relevant to the competences set out below.

1-SCIENTIFIC		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
3a.1p	• understanding the science that underpins the specialty (modality) and the broader aspects of medicine and clinical practice	1.2.1, 1.3, 2.5, 3.3.1, 3,3,2, 3.3.3, 3.3.4, 3.3.5, 3.4.1, 3.4.2, 3.4.3, 3.5.1, 3.5.2, 4.1, 4.2, 4.3, 5.6, 5.8, 7.1, 7.2, 7.3
3a.1g	• demonstrating a strong base of knowledge appropriate to the specialty and to the investigations and therapeutic options available	1.3, 2.1, 2.2, 3.3.1-3.5.2, 3.8.1-3.8.5, 3.9.1-3.9.3, 3.10.1-3.10.7, 6.1-6.11, 7.1, 7.2, 7.3
2b.1g 2b.1p	• experience of searching for knowledge, critical appraisal of information and integration into the knowledge base	1.2.1, 1.3, 4.1-4.3, 5.6, 7.1, 7.2, 7.3
2b.1g	• ability to apply knowledge to problems associated with the routine provision, and development, of the service	5.1-5.5, 5.8, 5.9, 6.1-6.11, 7.1, 7.2, 7.3
2a.1p	• ability to identify the clinical decision which the test/intervention will inform	1.3, 5.1, 5.2, 5.3, 5.4, 5.8, 6.1-6.11, 7.1, 7.2, 7.3
2c.1p	• ability to make judgements on the effectiveness of procedures	3.2.1-3.2.3, 3.5.1-3.5.2, 5.8, 5.9, 6.3, 7.1, 7.2, 7.3
3a.2g	• application of the knowledge base to the specialty (modality) and to the range of procedures/investigations available	1.3, 3.8, 3.9, 3.10, 3.11, 3.12, 5.1, 5.2, 5.3, 5.4, 6.3, 7.1, 7.2, 7.3

2-CLINICAL		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
2b.1p	• ability to provide interpretation of data and a diagnostic (therapeutic) opinion, including any further action to be taken by the individual directly responsible for the care of the patient	1.3, 5.1, 5.2, 5.3, 5.4, 5.8, 6.11, 7.1, 7.2, 7.3
3a.1p	• understanding of the wider clinical situation relevant to the patients presenting to his/her specialty	1.3, 2.1, 3.9.3, 3.10.4, 3.11.1, 3.11.2, 3.12.1-3.12.5, 5.4, 5.8, 6.3, 7.1, 7.2, 7.3
2b.3p	• ability to develop/devise an investigation strategy taking into account the complete clinical picture	1.2.1, 1.2.2, 1.3, 2.2, 4.3, 5.1, 5.2, 5.3, 5.4, 5.8, 7.1, 7.2, 7.3
3a.2p	• understanding of the clinical applications of his/her specialty and the consequences of decisions made upon his/her actions/advice	1.3, 3.8.3, 3.8.4, 3.9.3, 3.10.4, 3.11.1, 5.1, 5.2, 5.3, 5.4, 5.8, 6.3, 6.11, 7.1, 7.2, 7.3
3a.2p	• awareness of the evidence base that underpins the use of the procedures employed by the service	3.5.1, 3.5.2, 3.8, 3.9.2, 3.9.3, 3.10.4, 3.11.1, 3.11.2

3-TECHNICAL		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
3a.2p	• understanding of the principles associated with a range of techniques employed in the modality	3.3.4, 3.4, 3.5.1-3.5.3, 3.8.1, 3.8.2, 3.8.4, 3.8.5, 3.8.8, 3.9.1-3.9.3, 3.10.2, 3.10.4, 3.10.6, 3.11.1, 3.11.2
3a.2p	• knowledge of the standards of practice expected from these techniques	3.1.1, 3.1.3, 3.1.4, 3.1.5, 3.2.2, 3.8.7, 3.8.8
2b.4p	• experience of performing these techniques	3.1.5, 3.8.1, 3.8.2, 3.8.4, 3.8.5, 3.8.8, 3.9.1-3.9.3, 3.10.2, 3.10.4, 3.10.6, 3.11.1, 3.11.2, 5.3, 5.4, 5.5, 6.3
2b.4p	• the ability to solve problems that might arise during the routine application of these techniques (troubleshooting)	3.8.1-3.8.3, 3.9.3, 3.10.7, 5.1, 5.3, 5.4, 5.9, 6.3
2c.2g	• understanding of the principles of quality control and quality assurance	3.2.1, 3.2.2, 3.8.8, 3.9.3, 5.5, 5.9, 6.2
2c.1p	• experience of the use of quality control and quality assurance techniques including restorative action when performance deteriorates	3.2.3, 3.9.3, 5.9, 6.4, 6.8, 6.9

4-RESEARCH AND DEVELOPMENT		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
2b.1p	• ability to read and critically appraise the literature	1.2.1, 2.1, 2.2, 2.4, 4.2, 4.3, 5.6, 5.7
2b.1p	• ability to develop the aims and objectives associated with a project	1.2.1, 2.2, 4.3, 5.6, 5.7, 5.8
2b.1p	• ability to develop an experimental protocol to meet the aims and objectives in a way that provides reliable and robust data (i.e. free of bias)	1.2.1, 5.6, 5.7, 5.8
2b.1p	• ability to perform the required experimental work ability to produce and present the results (including statistical analysis)	1.2.1, 4.2, 4.3, 5.6, 5.7, 5.8
2b.1p	• ability to critically appraise results in the light of existing knowledge and the hypothesis developed and to formulate further research questions	1.2.1, 2.1, 2.2, 4.2, 4.3, 5.6, 5.7, 5.8
2b.1p	• ability to present data and provide a critical appraisal to an audience of peers – both spoken and written	1.2.1, 2.1, 2.2, 2.4, 2.6, 2.7, 4.2, 5.6, 5.8, 5.9

5-COMMUNICATION		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
-	• ability to assess a situation and act accordingly when representing the specialty	5.1, 5.2, 5.3, 5.4, 5.6, 5.8, 6.1-6.11
1b.2p	• ability to respond to enquiries regarding the service provided when dealing with clinical colleagues	55.1, 5.2, 5.3, 5.4, 5.8, 6.1-6.11
1b.4g	• ability to communicate with patients, carers and relatives, the public and other healthcare professionals as appropriate	55.1, 5.2, 5.3, 5.4, 5.8, 6.1-6.11
1b.5p	• ability to communicate the outcome of problem solving and research and development activities	2.4, 5.1, 5.2, 5.3, 5.4, 5.6, 5.7, 5.8, 6.1, 6.3
2b.1p 1b.5p	• evidence of presentation of scientific material at meetings and in the literature	2.4, 5.6, 5.7, 5.8

6-PROBLEM SOLVING		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
2a.2g 2c.1g	• to assess a situation	5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.9, 6.1-6.11
2b.1g	• determine the nature and severity of the problem	5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.9, 6.8
2b.1g	• call upon the required knowledge and experience to deal with the problem	5.1, 5.2, 5.3, 5.4, 5.6, 5.9, 6.8
2b.1g	• initiate resolution of the problem	5.1, 5.2, 5.3, 5.4, 6.1-6.11
-	• demonstrate personal initiative	5.1, 5.2, 5.3, 5.4, 5.8, 6.1-6.11

7-MANAGEMENT		
HPC Standards of Proficiency Codes for Clinical Scientist	**AREA OF COMPETENCE**	**INDICATE SECTION(S) IN PORTFOLIO WHERE COMPETENCE IS DEMONSTRATED**
1a1.g	• Understanding of the legal and ethical boundaries of the modality, and the ethical aspects of scientific research.	2.2, 3.1.1, 3.1.2, 3.2, 4.3
1b.1g, 1a.5g	• Ability to recognise the limits of personal practice and when to seek advice.	1.3, 5.1, 5.4
1a.6g	• Ability to manage personal workload and prioritize tasks appropriately.	5.1, 5.2, 5.3, 5.4, 5.6, 6.1, 6.2, 6.3
2c.2g 1a.3g	• Understanding of the principles of clinical governance including clinical audit, accreditation requirements relevant to the modality. The importance of confidentiality, informed consent and data security	3.1.3, 3.2.1, 3.2.3, 3.7.2
1b.3g	• Ability to contribute effectively to work undertaken as part of a multi-disciplinary team	5.1, 5.2, 5.3, 5.4, 5.5, 5.7, 6.1, 6.3, 6.5, 6.11
	• Ability to supervise others as appropriate to area of practice. Understanding of the role of appraisal in staff management and development.	5.11, 6.1, 6.2, 6.6, 6.7
1a.7g 1a.8g	• Understanding of the need for career-long self-directed learning and the importance of continuing professional development.	2.8, 5.6, 5.10
3a.3g	• Understanding of the need for, and ability to establish and maintain, a safe practice environment.	3.1.1-3.1.5, 3.2.1-3.2.3, 6.9
	• Understanding of the structure and organization of the department and how it fits into the local clinical setting, General understanding of the way the modality is structured and practised in other locations within the UK. Basic understanding of the importance of financial accountability, budgetary control and resource management.	2.3, 3.6.1-3.6.4, 5.1, 5.2, 5.3, 5.4, 5.7, 6.1

Note:

The above are the generic competences that must be met by all Clinical Scientists. These competences have also been mapped onto specific subjects. Copies of these can be obtained from the ACS Administrative Office and the website.