This issue of the EFI Newsletter contains a draft proposal of the EFI Standards prepared by the Standards and QA Committee in Granada on 27 March 2001, and accepted by the EFI Committee on 31 May 2001.

These new Standards, referred to as Version 5, are based mainly on the last revised ASHI Standards (submitted for public comment in October 2000), and on suggestions of Dr. Paul Reekers summarising comments from several EFI Commissioners and Inspectors.

The following areas have been revised:

- **Section B**: the discrimination between technologist and technician has been suppressed.
- **Section C** has been modified to incorporate former sections C (General Comments) and O (Records and Test Reports). It now also contains new items on continuing education, on the evaluation of test management systems, and on client service evaluation.
- **Sections E and F** (Serological HLA Class I and Class II typing) have been simplified and pooled in a single section.
- **Section G** (Antibody screening) now includes items on the use of flow cytometry and of microparticles targets.
- **Section J** (Marrow and stem cell transplantation) has been implemented to include Class I and II DNA typing at a level appropriate to the transplant protocol and optimal donor selection. This allows a full compliance with the most recent NMDP requirements.
- **Section N** (Nucleic acid analysis) contains a few minor modifications, e.g. the use of dedicated equipment for pre-PCR is a must, specificity of SSO probes must be demonstrated (as was the case for SSP), and SSP-typing now contains a short section on electrophoresis.
- **Section O** (Flow cytometry): in all instances the word “shall” has been replaced by “must”.

Please review these Standards and send your comments to me at fax no: 41-22-372.93.90 or by e-mail (Jean-Marie.Tiercy@medecine.unige.ch) BEFORE 15th DECEMBER 2001. All comments must include the full name, address and fax number of the sender. It is also recommended that proposals are accompanied by detailed arguments.

On behalf of the Standards and QA Committee I would like to thank you for your collaboration that is essential to the success of the Accreditation programme.

PD Dr. Jean-Marie Tiercy
Chair,
EFI Standards and QA Committee
... from the editor’s desk

Dear EFI Member,

As you will notice from the front-page announcement, the bulk of this delayed issue is taken by the draft of the 5th version of the EFI Standards. The production of the Standards has been one of the major undertakings and successes of EFI together with the Accreditation Programme and the QA Programmes. Please take time to read the latest version and send your comments to Jean Marie Tiercy.

This is the last issue of the EFI Newsletter of which I am the Editor. I have been editing EFI Newsletters since the first issue back in August 1991. So after ten years it is time to give up and let someone else bring new ideas, format and design to the Newsletter. I have enjoyed editing and preparing all of the past 34 issues. I would like to thank the EFI Committee and all its past and present Members for their help and advice. All of you who have contributed articles, notices and news over the years to the Newsletters, my great thank you. And many thanks to the Advertisers whose generous support of the EFI Newsletter and of EFI over the years kept the EFI Membership subscription fee at a very reasonable price.

I wish the new Editor of the EFI Newsletter every success and I look forward to reading future issues. I hope to keep in touch with many of you by reading your future contribution. EFI is a great Society and deserves full-hearted support from all its Members.

Dr Peter T. Klouda
EFI Newsletter Editor

Important Announcement to EFI Members

For security reasons we need to change the username password for the EFI website on a regular basis. Changes to the password will be announced in the EFI Newsletter and will apply from the publication date of the Newsletter in which the change is announced.

The new username and password from March 2001 are:

Username: efimember
Password: europe
URL: http://www.efiweb.org/members/

James Robinson, EFI Webmaster

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Dear Colleagues

We are pleased to be able to give you preliminary information about the EFI Conference, which will take place in Strasbourg. The preliminary scientific programme consists of plenary sessions and teaching sessions and has been arranged by the scientific committee (F. Claas, F. Garrido, G.B. Ferrara, J.F. Eliaou, R. Wassmuth, S. Marsh) and by E. van den Berg Loonen and G. Fischer.

Preliminary programme

Tuesday March 19th
14.30-14.45 Opening ceremony
14.45-15.30 Ruggero Ceppellini Lecture
16.00-18.00 First plenary session: Allorecognition and transplantation tolerance
- Mechanisms of allorecognition
- Towards transplantation tolerance
- How to monitor transplantation tolerance
- Mixed chimaerism and transplantation tolerance

Wednesday March 20th
09.00-11.00 Second plenary session: Human genome
- Genomics of the MHC
- DNA micro arrays
- Use of microsatellites and SNPs for gene localisation
- The application of spectrotyping and TRECs for measuring T cell diversity and maturation
11.30-13.00 Teaching session 1: Basic DNA typing; low and medium resolution techniques
Abstract session 1
14.30-16.00 Best abstracts
16.30-18.00 Teaching session 2: Screening and crossmatching for HLA class I antibodies
Abstract session 2

Thursday March 21st
9.00-11.00 Third plenary session: Innate and acquired immunity
- TREM-1 and innate immunity
- Toll-like receptors and innate immunity
- Specific immune response to bacterial infections
- Co-evolution of MHC and pathogens
11.30-13.00 Teaching session 3: Advanced DNA typing; high resolution techniques
Abstract session 3
14.00-15.30 Poster viewing
Abstract session 4
16.00-17.30 Teaching session 4: Screening and crossmatching for HLA class II antibodies
Abstract session 5
17.30 General Assembly

Friday March 22nd
8.30-10.00 Teaching session 5: Biostatistics
Abstract session 6
10.30-12.30 Fourth plenary session: Immunogenetics
- Relevance of cytokine gene polymorphisms
- Role of NK receptors
- MIC gene polymorphisms
- MHC, odour and partner choice
11.30-13.00 Abstract session 4
14.00-15.30 Poster viewing
Abstract session 5
16.00-17.30 Teaching session 6: Screening and crossmatching for HLA class II antibodies
Abstract session 6
17.30 General Assembly

GENERAL INFORMATION

Abstract sessions. Six abstract sessions will be organised. The topics will be defined after selection of the abstracts to be presented.

Call and selection of abstracts. Only original abstract forms can be used. The scientific committee will select abstracts for oral and poster presentation. The presenting author will be informed by January 22nd, 2002. Deadline for receipt of abstracts is November 26th, 2001. The selected abstracts will be published in the European Journal of Immunogenetics.

A registration form, 2 abstract forms and the preliminary programme has been sent to EFI Members sent to you in October 2001.

Transport: preferential fares can be obtained from Air France, the official carrier, and from the SNCF (train) as mentioned on the registration form.

Commercial exhibition: Commercial companies involved in the HLA field will show their products and answer all your enquiries.

Bursaries. A number of bursaries will be awarded to EFI members who have been selected to present an abstract at the Conference. Only one bursary per laboratory will be awarded and priority will be given to individuals who have not received a bursary previously.

Applications for bursaries should be received before 15th January 2002 and should include a short CV, a letter from the laboratory director and a copy of the abstract submitted to the Conference. The application may be sent by letter, fax or e-mail. Members of the EFI Executive Committee will evaluate the applications and their decisions will be communicated to the applicants before 22 January 2002. Please indicate your fax number and/or an e-mail address on the application so that you can be notified rapidly. The successful applicants will each receive 460 Euros paid by cheque or cash, at the first day of the Congress.

Reduced registration fee. Copy of notification of bursary from the EFI must be attached.

All bursaries will be awarded on the strict condition that the recipient submits a report (2-3 type written pages, on a floppy disk or by e-mail) on any scientific session of the Conference. Selected reports will be published in the EFI Newsletter (after editing to avoid redundancy). Recipients of bursaries, who do not submit a report will not be eligible for bursaries in the future, nor will anybody else from their laboratory!

Requests for bursaries should be sent to the EFI treasurer:
Mogens Thomsen, Inserm U 466, Institute L. Bugnard CHU Rangueil, 31403 Toulouse cedex 4, France
Fax + 33 56 13 22 084, e-mail: thomsen@rangueil.inserm.fr

For additional abstract forms or further information, please contact:
M.M. Tongio, Etablissement Français du Sang-Alsace, 10 Rue Spieermann,
BP 36, 67005 Strasbourg cedex, France
tel + 33 3 88 2125 07, fax + 33 3 88 2125 44, e-mail: hla@etss.u-strasbg.fr

Welcome to Strasbourg.
Julia Bodmer Young Scientist Award

As a tribute to Julia Bodmer, the late EFI President, who through her enthusiasm and knowledge encouraged very many researchers in the field of immunogenetics, an award will be established to recognise the work of a young scientist.

The award will be associated with an oral presentation of the award winner’s work in a plenary session at the EFI histocompatibility conference. The recipient will receive free registration to the Conference, and a grant of 1,000 Euro to cover travel and lodging expenses. Candidates should be EFI members under the age of 35. Applicants for the award should submit a CV, including a list of their publications and a letter of support from the head of their department.

Competitors for the 2002 Award should send their applications before 15 January 2002 to the Chairman of the Scientific Committee of the next EFI conference:

Prof. Dr. Frans Claas,
Dept of Immunohematology and Blood Transfusion,
LUMC, E3-Q, PO Box 9600, 2300 RC Leiden, The Netherlands
phone: (31) 71 5263800; fax: (31) 71 5216751;
e-mail: fhjclaas@lumc.nl

Rare Alleles: A Correction and an Update

There are so far few data on the occurrence of rare HLA variants in the routine histocompatibility typing practice. In the paper entitled “How rare are rare HLA alleles” published in the EFI Newsletter of February 2001, we reported a list of HLA class I and II alleles that have been identified in our two laboratories by high resolution typing performed for unrelated bone marrow searches over a period of about 2 years. Rare alleles that were typed for other clinical purposes or for external QC exercises or were not been included in this survey.

Unfortunately DR3 subtypes had been omitted in Table 1. During our screening we indeed encountered only 2 alleles, DRB1*03011 and DRB1*0302, out of the 20 DRB1*03 alleles assigned so far by the Nomenclature Committee.

Furthermore, by extending our investigation over an additional year (until September 2001) the following alleles have been encountered once:

HLA class I: A*0305, B*1511, B*1522, B*2703, B*4421, B*5108, Cw*1505
HLA class II: DRB1* 1605, DRB1* 1607, DRB1*1127, DRB1* 1128.

In future it will be interesting to investigate whether the occurrence of such rare variants can be correlated to different population groups. In this respect the experience of other laboratories involved in unrelated bone marrow donor searches would be a valuable contribution to this issue.

J.-M. Tiercy,
University Hospital, Geneva,

V. Dubois and L. Gebuhrer,
Laboratoire d’Histocompatibilité, Lyon
An advanced course on “Immunogenetics and Immunology of solid organ and bone marrow transplantation” was held at the CEFPAS (Centre for the permanent formation and education of National Health Service personnel) in Caltanissetta (Italy) from the 21st to 25th of May 2001. The course was the third of a series organised in Southern Italy in the last two years, covering different biological and clinical aspects of solid organ and bone marrow transplantation. The aim of the course was to illustrate the molecular and cellular bases of transplant immunogenetics and immunology as well as the latest advances in the field, both at clinical and laboratory level.

The course was organised by Dr. Carlo Carcassi (Department of Internal Medicine, University of Cagliari), Italy) and Dr. Raimondo Marceno (Transfusion Medicine Unit, Cervello Hospital, Palermo, Italy) under the scientific patronage of AIBT (Italian Association for Immunogenetics and Transplantation Biology), GITMO (Italian Group for Bone Marrow Transplantation), SIE (Italian Society of Haematology), SIES (Italian Society of Experimental Haematology) and SIMTI (Italian Society of Transfusion Medicine).

Fifty researchers (medical doctors and biologists) and eight technicians, from Italian HLA laboratories or clinical Departments involved in solid organ transplantation and bone-marrow programmes attended the Course.

Sixty-one teachers and tutors contributed to the Course sessions. These covered the following topics: Genetics of HLA system including non-classical class I and Class II molecules and minor histocompatibility antigens; molecular methods for the analysis of histocompatibility systems; biology and physiology of T and NK cells; cytokine and cytokine receptor gene polymorphism analysis; effector mechanisms in tumour and transplant immunology (anti-tumour immunological responses and immunological escape, acute and chronic rejection of solid organ allografts, GVHD, graft versus tumour reactions, tolerance induction); mechanisms of action of immunosuppressive drugs; application of HLA matching in solid organ and bone-marrow transplantation and clinical results.

The final session was devoted to the discussion of future applications in transplantation and tumour immunology, with particular emphasis on some of the new approaches such as use of non myeloablative conditioning regimens or haploidentical donors in allogeneic bone-marrow transplantation, stem cell transplantation, corneal limbus implants, antitumor adoptive immunotherapy and antitumour vaccines.

At the end of the Course the participants were asked to fill in anonymously a questionnaire, from which it was possible to derive a very positive evaluation of all the different aspects of the Course. Lectures were published in a volume that was distributed to the attendants. Moreover, some of the slides of the lectures have been collated in a single CD.

Arcangelo Nocera,
Genoa, Italy

7th International Workshop on MHC Evolution and VIII Complement Genetics Workshop and Conference
October 2002, Perth, Western Australia

The MHC Evolution meeting continues the traditions established at earlier meetings organised by Ronald Bontrop, Jan Klein, Jonathon Howard, Ward Wakefield, Ulf Gyllensten and Masanori Kasahara. We expect to be able to compare MHC sequence maps of many vertebrates and to identify at least some of the corresponding clusters in invertebrates. We anticipate that there will be considerable interest in innate immunity, NK, viral receptors and self/nonself recognition systems including the complement system. Other areas of expected interest include Primate and human evolution and migration, MHC diseases and functions, peptide presentation, olfactory receptors, retroviral insertions, xenotransplantation, duplications and polymorphism.

Recently, we agreed to a request to host the Complement Genetics Workshop and Conference in association with the MHC Evolution meeting. The last meeting was organised by Peter Schneider and Christian Rittner in Mainz and dramatically improved understanding of the structure and polymorphism of the MHC Complement genes.

We are confident each group will benefit through the presence of the other. However we are conscious of the need to limit numbers so as to retain the excellent interaction achieved at previous meetings. To this end, we also propose to encourage pre and post meeting satellites. Some of the topics suggested include Retroviral infection in evolution, MHC microsatellites, Forensic applications, Fish immunity and disease and the marsupial MHC and Immunity. A technical session on phylogenetic analysis has also been proposed.

At this stage, we are seeking expressions of interest and your help in extending and correcting our mailing list. Please respond to the above email address (cmi@cyllene.uwa.edu.au) and we will then invite you to contribute further through our web page.

Roger Dawkins, Silvana Gaudieri, Yurek Kulski
EFI ELECTIONS 2002

Nominations are requested for candidates for:

**Deputy Secretary and Councillors (two vacancies)**

Nominations should be seconded by ten paid-up EFI Members from at least two countries and signed by the candidate. No person can nominate or second more than two candidates.

Completed nomination forms, accompanied by the candidate’s Curriculum Vitae of not more than 200 words, should be sent to Ieke Schreuder, EFI Secretary, by January 2, 2002.

Dr G. M. Th. Schreuder,
Dept. of Immunohematology and Blood Transfusion,
E3-Q, Leiden University Medical Centre,
PO Box 9600, 2300 RC Leiden, The Netherlands Fax: +31-71-5248203/5216751

NOMINATION FORM (please print or type)

Name of Candidate:........................................................................................................................................................................

Nomination for Councillor  ☐ Deputy Secretary  ☐

Nominated by:

Name:                                                               Signature:                                      Country:

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Signature of Candidate                                                Address of Candidate

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Date.................................................................................................................................
Reminiscences from the Central East European Workshop on HLA

Andrzej Lange, Bartosz Grywacz, Anna Laba
L. Hirszfeld Institute of Immunology & Experimental Therapy & National Polish Bone Marrow Donor Registry, Wroclaw, Poland

In 1994, Prof. Gyöző Petranyi initiated a meeting in Hungary of representatives from several Central-East European countries to discuss mutual interests in the development of techniques for standardisation of HLA typing. At that time an informal organisation, the Central European Transplant group (CET) was formed. One of the practical aspects of bringing together people working in the field of immunogenetics in East and Central Europe was the CET Quality Control Workshop on DNA Based HLA Typing organised by Dr Gottfried Fisher from Vienna.

The following conferences in Bratislava, Wroclaw and Liblice proved that mutual exchange of information is interesting and may have practical aspects. All the conferences confirmed the feeling of the fruitfulness of co-operation between East and Central European Laboratories. Fortunately, at the same time the support from the European Commission for establishing multinational co-operation in the field of recipient – donor selection for transplantation became available. The dedicated work of Dr Sandor Vari and several other colleagues from East and Central European Countries was awarded with a grant. This was a project RETRANSPALNT within the 4th Framework Programme of the European Commission. Both Retransplant and at present StemNet runs in the frame of the 5th Framework Programme of the European Commission.

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Improvement in HLA technology and organisation of marrow donors registry was generously supported, but most of all the grant provided the platform for meetings and discussions. All these positive feelings of the benefits of mutual co-operation between Central-East European countries prompted us in Wroclaw to organise the proficiency testing for HLA class I antigens typing.

So far five trials have been successfully completed. This activity was in line with the EFI policy to organise proficiency testing trials in serology in small groups of countries providing easy postal handling of samples. In the meantime, after successful completion of the Retransplant project, a new initiative of the former Retransplant group of people was awarded with another grant. StemNet project runs in the frame of the 5th Framework Programme of the European Commission. Both Retransplant and at present StemNet, supporting mutual international co-operation, should bring a real improvement in the activity in the field of recipient – donor selection for stem cell transplantation.

The time was, therefore, right to organise a meeting of all workers practically participating in the Proficiency Testing trials. The Central East European Workshop on “HLA Typing: Methodological and Quality Control Aspects” was organised by the Commission of Transplantation of the Polish Academy of Sciences and the L. Hirszfeld Institute of Immunology in Wroclaw on the 7-8th June 2001.

The introductory lecture on general requirements and clinical significance of HLA donor – recipient matching in solid organ and haematopoietic stem cell transplantation by Prof. Andrzej Lange constituted a basis for open discussion.

The lecture by Dr Iwona Podobinska from the Institute of Transplantology, Warsaw Medical University addressed the actual status and standardisation approach in recipient selection for solid organ transplantation in Poland. The differences in the algorithm and logistics of donor-recipient matching in solid organ and marrow transplantation was discussed and mutual understanding of problems and personal dedication of people involved built a solid platform to work out a common approach and reach internationally recognised standards.

The presentation on organisation and mutual co-operation between HLA laboratories and clinical BMT units by Dr Tomasz Pacuszek from BMT unit in Wroclaw gave a clinician’s view and stemmed from the experience reached in the Retransplant project. It was based on discussions between technologists free from administrative or personal attitudes.

The cornerstone of the workshop was the presentation of the results of the Proficiency Testing of HLA class I typing for Central/East Europe. All participants agreed to continue with the Testing programme and expressed their feeling of its usefulness. The participants agreed that in the future two separate batches of control samples will be distributed, one batch for serology and the other for DNA typing of class I specificities. The organisers of the proficiency testing took up this suggestion and from October this year samples for both techniques - serology and DNA - will be dispatched to participating laboratories.

In her talk, Lidia Karabon from Wroclaw, shared some experiences from the EFI accreditation process with emphasis on practical laboratory aspects indicated by the EFI guidelines.

Two important practical workshops were also organised.

The Laboratory of the National Polish Bone Marrow Donors Registry hosted Dr. Roswitha Keller from GenoVision, who presented a novel DNA isolation technique and PCR-SSP sets for HLA locus A, B and DR typing. In the afternoon the results of HLA typing were evaluated with a help of Score software. It was interesting to see we all have similar doubts when analysing bands on a gel.

Dr. Ivan Balazs from The LifeCodes Corporation US gave a presentation on QuickType, a laboratory procedure for high throughput molecular typing of HLA class I and II loci, and Life Match - a microsphere based methodology for HLA DNA-based typing and antibody screening. This was a live workshop with a discussion that was conducted not only in the venue of the workshop but also during an informal get-together party, organised in a Restaurant Szwejk. The name Szwejk has a special connotation of a “clever fellow”. We do hope, that the typically Slavic Szwejk’s sense of humour and his attitude to life - which helped him to survive in a changing environment - will be with us in the future and that our efforts to meet the internationally recognised standards in the field of immunogenetics and recipient – donor matching in transplantation will be successful.

Prof. Andrzej Gorski, the Polish National Consultant in immunology, run the last part of the conference, summing up the discussion supporting the need of international co-operation in recipient-donor matching in Europe. Among practical conclusions it appeared evident that the range of possibilities created by modern technologies is overwhelming, but still it must be adjusted to the laboratories needs. Issues such as the level of resolution, the time and the scale required will be different in different laboratories, suited to the needs of the environment in which we are working. In this context the need for standardisation in the field of immunogenetics appears crucial in order to be able to keep a common language and the spirit of mutual co-operation.
These Standards have been approved and adopted by the EFI Executive Committee. They are based, with minor changes, on Standards originally prepared by the American Society for Histocompatibility and Immunogenetics (ASHI).

These Standards have been established for the purpose of ensuring accurate and dependable histocompatibility testing consistent with the current state of technological procedures and the availability of reagents.

These Standards establish minimal criteria, which all histocompatibility laboratories must meet if their services are to be considered acceptable. Many laboratories, because of extensive experience and long-established programmes of reagent procurement and preparation and/or testing for organ transplantation when relevant, will exceed the minimal requirements of these Standards.

Certain Standards are obligatory. In these instances, the Standards use the word “must”. Some Standards are highly recommended but not absolutely mandatory. In these instances the Standards use words like “should” or “recommended”.

Procedures to be used in histocompatibility testing often have multiple acceptable variations. The accuracy and dependability of each procedure must be documented in each laboratory or by published data from other laboratories.

Some procedures have sufficient documentation of effectiveness to warrant their use in clinical service even though they are not available in or obligatory for all laboratories.

The use of the name of the European Federation for Immunogenetics as certification of compliance to these Standards may only be made by laboratories, which have been accredited through the EFI accreditation process.

The laboratory must employ one or more individuals who meet the qualifications and fulfils the responsibilities of the Director, Technical Supervisor, and General Supervisor.

A Director/Technical Supervisor must hold an earned doctoral degree in a biological science, or be a physician, or have an equivalent qualification approved by the EFI Executive Committee and 1) must have had four years experience in immunology or cell biology, two of which were devoted to training in human histocompatibility testing, or 2) five years of working experience in human histocompatibility testing. The Director must have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on a sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing and reflected by external measures such as participation in national or international workshops or publications in peer-reviewed journals. He/she is available on site commensurate with workload at the laboratory, provides adequate supervision of technical personnel, utilises his/her special scientific skills in developing new procedures and is held by the laboratory.
C1.000 Facilities.
C1.100 In accordance with national regulations, laboratory space must be sufficient so that all procedures can be carried out without crowding to the extent that errors may result. The following facilities must be adequate and immediately available to the laboratory: refrigerators, freezer storage of reagents and specimens, storage of records.

C1.200 Lighting and ventilation must be adequate.
C1.300 Refrigerators and freezers must be maintained at temperatures optimal for storage of each type of sample or reagent. They must be monitored every working day. Recording thermometers are recommended for mechanical refrigerators or freezers. These should be coupled to alarm systems with an audible alarm where it can be heard 24 hours a day. In laboratories where liquid nitrogen is utilised for storage of frozen cells, the level of liquid nitrogen in the cell freezers must be monitored at intervals which will ensure an adequate supply at all times. Ambient temperature and/or the temperature of incubators in which test procedures are carried out must be monitored every working day to ensure that these procedures are carried out within temperature ranges specified in the laboratory's procedure manual.

C1.400 Laboratories performing procedures which require cell culture must have a laminar flow hood or other appropriately aseptic work area. Incubators must be monitored every working day in relation to temperature (37°C) and CO₂ concentration (5%±1%) and should be appropriately humidified.

C1.500 Laboratories using radioactive materials must store radioactive materials and conduct procedures using radioactive materials in a designated section of the laboratory. Radioactive materials must be disposed of at locations designated by local institutions. (=P2.1110)

C1.600 Laboratories performing amplification of nucleic acids must use physical and/or biochemical barriers to prevent DNA contamination. Pre-amplification procedures must be performed in an area, which excludes amplified DNA that has the potential to serve as a template for amplification in any of the genetic systems tested in the laboratory.

C1.700 Equipment maintenance and function checks.

C1.710 The laboratory must establish and employ policies and procedures for the proper maintenance of equipment, instruments and test systems by 1) defining its preventive maintenance programme for each instrument and piece of equipment, and by 2) performing and documenting function checks on equipment with at least the frequency specified by the manufacturer.

C1.800 The laboratory must document compliance with all applicable national and local laws which relate to laboratory employee health and safety; fire safety; and the storage, handling and disposal of chemical, biological and radioactive materials.

C1.900 Computer assisted analyses.
C1.910 Computer assisted analyses must be reviewed, verified and signed by the Supervisor and/or Laboratory Director before issue. (=P2.6550)

C1.920 The computer software programme used for analyses must be identified and validated.

C2.000 Specimen submission and requisition.
C2.100 The laboratory must have available and follow written policies and procedures regarding specimen collection.

C2.110 The laboratory must perform tests only at the written or electronic request of an authorised person. The laboratory must assure that the requisition includes: 1) the patient's or donor's name or other method of specimen identification to assure accurate reporting of results; 2) the name and address of the authorised person who ordered the test; 3) date of specimen collection; 4) time of specimen collection, when pertinent to testing; 5) source of specimen (e.g. bone marrow, spleen cells).

C2.120 Blood or tissue samples must be individually labelled with the name, and/or other unique identification marker of the individual and the date of collection. When multiple blood containers are collected, each container must be individually labelled.

C2.130 The laboratory must maintain a system to ensure reliable specimen identification, and must document each step in the processing and testing of patient specimens to assure that accurate test results are recorded.

C2.140 The laboratory must have criteria for specimen rejection and a mechanism to assure that specimens are not tested when they do not meet the laboratory's criteria for acceptability.

C2.200 Blood samples must be obtained using a location, which does not compromise
C3.100 All procedures in use in the laboratory must be detailed in a procedure manual, which is immediately available where the procedures are carried out. The use of product inserts provided by manufacturers is not acceptable in place of the procedure manual. The procedure manual must be reviewed at least annually by the Director and written evidence of this review must be in the manual. Any changes in procedures must be initialled and dated by the Director at the time they are initiated.

C4.000 Quality assurance.
C4.100 Proficiency testing and competency evaluation.
C4.110 The laboratory must participate in external proficiency testing programme(s) to cover all techniques in use for accredited activities.
C4.120 For proficiency testing, the laboratory must be in compliance with valid regulations formulated by the EFI proficiency Testing Committee and approved by the EFI Board.
C4.130 If a laboratory's performance in external proficiency testing programme(s) is unsatisfactory in any category for which EFI accreditation is sought, the laboratory must participate in an enhanced proficiency testing programme in that category until performance is deemed satisfactory.
C4.140 Proficiency test samples must be tested in a manner comparable to that for testing patient samples.
C4.150 The laboratory must, at least four times a year, give each individual performing tests a characterised specimen as an unknown to verify his or her ability to reproduce test results. The laboratory must maintain records of these results for each individual.
C4.160 The Director/Technical Supervisor or designee must evaluate the competence of each technologist annually.
C4.170 The laboratory Director and technical staff must participate in continuing education relative to each category for which EFI accreditation is sought.
C4.200 Systems evaluation.
C4.210 The laboratory must establish and employ policies and procedures, and document actions taken when 1) test systems do not meet the laboratory's established criteria including quality control results that are outside of acceptable limits; and when 2) errors are detected in the reported patient results. In the latter instance, the laboratory must promptly a) notify the authorised person ordering or individual utilising the test results of reporting errors; b) issue corrected reports, and c) maintain copies of the original report as well as the corrected report for at least two years.

C4.220 The laboratory must have an ongoing mechanism for monitoring and evaluating test management systems.
C4.230 If a laboratory performs the same test using different methodologies or instruments, or performs the same test at multiple sites, the laboratory must compare the test results obtained by different methods or instruments or at different sites.
C4.240 The laboratory must have a mechanism to identify and evaluate test results that appear inconsistent with relevant criteria such as patient information.
C4.300 Client service evaluation.
C4.310 The laboratory must document problems that result from breakdowns in communication between the laboratory and the authorised individual who orders tests or receive results.
C4.320 All complaints and problems reported to the laboratory must be documented. Complaints must be investigated and corrective action taken when necessary.
C4.330 The laboratory must, upon request, make available to clients a list of tests employed by the laboratory.
C4.400 Quality assurance evaluation.
C4.410 The laboratory must document and assess problems identified during quality assurance reviews, discuss them with the staff, and take corrective actions necessary to prevent recurrences.
C4.420 The laboratory must have an ongoing mechanism to evaluate corrective action taken. Ineffective policies and procedures must be revised based on the outcome of the evaluation.
C4.430 The laboratory must maintain documentation of all quality assurance activities including problems identified and corrective actions.
C4.440 The laboratory must maintain permanent files of all internal and external quality control tests.

C5.000 Records and test reports.
C5.100 The laboratory must maintain records of subjects tested for two years or longer depending on local regulations.
C5.110 These records must include log books, worksheets, and at least a summary of results obtained.
C5.120 Work sheets must clearly identify the sample tested, the reagents and methods that were used, the test performed, the date of the test and the person performing the test.
C5.130 For each cell-serum combination, the results must be recorded in a manner which indicates the approximate percent of cells killed. The numerical scores used in the International workshop procedure (0,1,2,4,6,8) should be used. Other numerical codes can also be used.
C5.140 Reports or records, as appropriate, should include a brief description of the specimen (blood, lymph node, spleen, bone marrow, etc.) used for testing.
C5.150 Molecular typing. A permanent record must be kept which is appropriate to the technique used, such as a photographic record of a gel, a membrane, an autoradiograph, an electronic file, or the raw data out from a sequencer.
C5.160 Records may be only saved in computer files, provided that back-up files are maintained to ensure against loss of data.
C5.200 For marrow transplantation, donor records should be maintained so that donors can be rapidly retrieved according to HLA type.
C5.300 The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner.
C5.400 The report must contain:
   a. The name of the individual tested or unique identifier of each individual tested and relationship to the patient if applicable.
   b. The date(s) of collection of sample when pertinent.
   c. The date of the report.
   d. The test results.
   e. The techniques used.
   f. Appropriate interpretations and the signature of the Laboratory Director, or, in his/her absence, by a designee who meets the requirements of General Supervisor.
C5.410 The laboratory must indicate on the test report information regarding the condition and disposition of specimen that did not meet the laboratory’s criteria for acceptability.
C5.500 Laboratories must have a procedure in place for resolving any tissue typing discrepancies that may occur between laboratories.
C6.000 Testing referred to other laboratories.
C6.100 An accredited laboratory may engage another laboratory to perform testing not done by the primary laboratory. In that event, the subcontracting laboratory must be accredited by the European Federation for Immunogenetics or by ASHI, if the testing is covered by EFI Standards. If genetic systems not covered by EFI Standards (ABO, RBC enzymes, virology etc.) are subcontracted, the subcontracting laboratory should document expertise and/or accreditation in those systems. The identity of the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the reports.

D - HLA ALLELES AND ANTIGENS

D1.000 Terminology of HLA alleles and antigens must conform to the latest report of the WHO Committee on Nomenclature.
D1.100 Potential new alleles or antigens not yet approved by the WHO Committee must have a local designation which cannot be confused with WHO terminology.
D1.200 Phenotypes and genotypes must be expressed as recommended by the WHO Committee, as in the following examples:
D1.210 Single alleles: HLA-B*07. Single antigens: HLA-B7 (or B7 if HLA is obvious from context).
D1.220 HLA type. Serological assignment: HLA-A, 30; B7, 44; CW5, DR1, 4; DQ5, 7. DNA assignment: HLA-A*02, *30; B*07, *44; CW*05, *16; DRB1*01, *04; DQB1*05, *0301.
D1.230 Genotype. Serological assignment: HLA-A2, B44, CW5, DR1, DQ5 / A30, B7; CW-, DR4, DQ7. DNA assignment: HLA-A*02, B*44, CW*05, DRB1*01, DQB1*05 / A*30, B*07, CW*16, DRB1*04, DQB1*0301.
D1.240 The locus designation must always be included.
D1.250 Broad antigens and the epitopes Bw4 and Bw6 may be reported in brackets.
D1.300 If only a single antigen or allele is found at a locus by serological typing or DNA typing, the phenotype may include it twice only if homozygosity is proven by family studies. Conversely, a “blank antigen or allele” can only be assigned if proven by family studies.

D2.000 Determination of haplotypes and genotypes can only be done by family studies.
D2.100 All available members of the immediate family should be typed.
D2.110 Typing for HLA-A, B and DR locus alleles or antigens is mandatory.
D2.112 Typing for HLA-C, and/or DQ and/or DP is mandatory until the ambiguities have been resolved.
D2.113 Reports of HLA family studies must include haplotype assignments and an explanation of recombination when this occurs.

D2.200 Unrelated individuals.
D2.210 The probability of possible haplotypes, given the phenotype, may be determined from known haplotype frequencies in the relevant population.
The haplotype frequencies used should be from the most complete and reliable studies available.

The haplotype frequencies used should be those most appropriate for the ethnic group of the subject.

Reports of probable haplotypes based on population frequencies should clearly indicate that they were so derived.

E - SEROLOGICAL HLA CLASS I AND CLASS II TYPING

E1.000 HLA-A, -B and -C locus antigens.
E1.100 The laboratory must be able to type for the HLA-A and -B specificities, which are officially recognised by the WHO and for those deemed relevant by EFI.
E1.200 Typing for HLA-C locus antigens is not mandatory.
E2.000 HLA-DR, -DQ, and -DP antigens.
E2.100 The laboratory must be able to type for the HLA-DR specificities, officially recognised by the WHO and for those deemed relevant by EFI.
E2.200 Typing for HLA-DQ and -DP antigens is not mandatory.
E3.000 HLA Class I and II typing techniques.
E3.100 Techniques used must be those, which have been established to define HLA Class I and II specificities optimally.
E3.200 Control reagents.
E3.210 Each typing tray must contain at least one positive control antibody, previously shown to react with cells expressing class I and class II antigens.
E3.220 Typing results may be invalid if the positive control fails to react as expected.
E3.230 Each typing must include at least one negative control serum. The negative control should be one previously shown to lack leukocyte reactive antibody.
E3.240 Cell viability in the negative control well at the end of incubation must be sufficient to permit accurate interpretation of results. For most techniques, viability should exceed 80%.
E3.250 In assays in which cell viability is not required, results on positive and negative controls must be sufficiently discriminatory to permit accurate interpretation of results.
E3.260 Procedures to deal with control serum failures in typing or crossmatch trays must be described in the laboratory manual.
E3.300 Target cells.
E3.310 Separation of B lymphocytes is not required if a technique is used which distinguishes between T and B lymphocytes or in assays in which antibodies with well-defined specificity are used which only define HLA Class II molecules.
E3.400 Antigen assignments.
E3.410 Each HLA-A, B, C antigen should be defined by at least two sera, if both are operationally monospecific. If multispecific sera must be used, at least three partially non-overlapping sera should be used to define each HLA-A, B, C antigen.
E3.420 Each monoclonal antibody used for alloantigen assignment must be used at a dilution and with a technique in which it demonstrates specificity comparable to antigen assignment by alloantisera on a well-defined cell panel.
E3.430 Each HLA Class II antigen should be defined by at least three sera, if all are operationally monospecific. If multispecific sera must be used, at least five partially non-overlapping sera must be used to define each HLA Class II antigen.
E3.440 Criteria for antigen assignment must be described in the laboratory manual.
E3.500 Control of antibody specificity.
E3.510 Cell panels of known HLA type must be used to prove the specificity of new antibodies. The panel cells should include at least one example of each HLA antigen the laboratory should be able to define.
E3.600 Typing sera.
E3.610 It is recommended that the specificity of typing sera obtained locally be confirmed in at least one other HLA laboratory.
E3.620 A reagent grade typing serum is validated only after confirmation of specificity. Specificity determinations must include supporting statistical analysis.
E3.630 Specificity of individual sera received from other laboratories or commercial sources must be confirmed to ensure that they reveal the same specificities in the receiving laboratory.
E3.640 Each lot of new typing trays must be evaluated by testing either with at least five different cells of known phenotype representing major specificities or in parallel with previously evaluated trays.
E3.700 Complement.
E3.710 Each batch of complement must be tested to determine that it mediates cytotoxicity in the presence of specific antibody but is not cytotoxic in the absence of specific antibody. The complement must be kept at an appropriate temperature.
E3.720 The test should employ multiple dilutions of complement to ensure that it is maximally active at least one dilution beyond that intended for use.
E3.730 The test should be carried out with at least two antibodies, which should react with at least two different test cells and at least one cell, which should not react. A strong and a weak antibody should be selected for the test, or serial dilutions of a single serum may be used.
E3.740 Complement should be tested separately for use with each type of target cell, since a different dilution or preparation may be required for optimal performance.

F - MIXED LEUKOCYTE CULTURE TESTS

F1.000 Mixed leukocyte culture (MLC) test
F1.100 At the start of the culture, cell viability should exceed 80%.
F1.200 Serum used in the culture medium must be
screened for support of cellular proliferation and the absence of cytotoxic antibodies and
must be sterile.

F1.300 MLC cultures must be incubated for length of time sufficient for detection of a maximal
proliferative response.

F1.400 The negative control for each responder cell
must consist of responder cells stimulated with autologous cells. MLC compatibility
cannot be determined if a responder cell’s autologous control counts are
inappropriately high.

F1.500 The positive control for each responder cell
must consist of either of the following: a) responder cells separately stimulated with
cells from 3 or more unrelated individuals; or b) responder cells separately stimulated
with cells from 2 unrelated individuals and a pool of cells from at least 3 other
individuals. MLC compatibility cannot be
determined if cells being tested as responder
cells do not respond adequately to unrelated
cells.

F1.600 In each MLC test, stimulator cells must be
shown to be capable of stimulating unrelated cells.

G - ANTIBODY SCREENING

G 1.000 Techniques.

G1.100 A complement-dependent cytotoxic technique must be used for the detection of
antibody to HLA antigens unless the laboratory has performed and documented
testing to validate that another technique identifies alloantibody to HLA antigens at a
level of sensitivity equivalent or superior to that of its cytotoxic technique.

G1.110 To detect antibodies to HLA class II antigens, a technique must be used that distinguishes
them from antibodies to HLA class I antigens.

G1.120 Reports of results of antibody screening must include identification of the technique
used.

G1.200 Sera.

G1.210 Sera must be tested at a concentration
determined to be optimal for detection of
antibody to HLA antigens. The dilution(s)
must be documented.

G1.220 Negative control sera must include a serum
from non-alloimmunised human donor(s). Each assay must include negative control(s).

G1.230 Positive control sera should be from highly
alloimmunised individuals and documented
to react with HLA antigens. The antibodies
must be of the appropriate isotype for each
assay. Each assay must include positive
control(s).

G1.300 Panel cells.

G1.310 Target cells may be mononuclear cells from
peripheral blood, lymph nodes, spleen or
cell lines.

G1.320 To detect HLA class II specific antibodies, B
lymphocytes, chronic lymphocyte
leukaemia cells or B cell lymphoblastoid
lines may be used.

G1.330 The panel of HLA antigens must include
sufficient panel cell donors to ensure that
they are appropriate for the population
served and the use of the data.

G1.340 For assays intended to provide information
on antibody specificity, documentation of
the HLA class I and class II phenotypes of
the donors of the panel cells must be
provided.

G1.350 To identify the specificity of an antibody
with certainty, the laboratory should test the
serum with additional cells expressing and
lacking the candidate antigen and cross-
reacting antigens.

G 2.000 Antibody screening by complement-dependent
cytotoxicity.

G2.100 An HLA specific positive control for the
activity of the complement and a negative
control for the viability of the test cells must
be included on each tray.

G2.200 If sera are screened after treatment with
dithiothreitol, then IgG and IgM positive
controls must be included.

G 3.000 Antibody screening by flow cytometry.

G3.100 Laboratories performing assays using flow
cytometry must conform to the standards in
section O1.1000: Instrument Standardisation/
Calibration and section O2.0000 Flow
Cytometric Crossmatch Technique.

G3.200 Negative control sera must include a serum
from non-alloimmunised human donor(s). Each assay must include negative control(s).

G3.300 Positive control sera should be from highly
alloimmunised individuals and documented
to react with HLA antigens. The antibodies
must be of the appropriate isotype for the
assay. Each assay must include positive
control(s).

G3.400 There must be methods to control for non-
specific binding of antibody to target
material.

G3.500 Each laboratory must document its own
threshold for significant levels of antibody
binding. Any change in technique, protocol
or instrumentation requires that the
characterisation of the positive threshold be
repeated.

G3.600 Antibody screening using cell targets.

G3.610 If cells pooled from multiple individuals are
used for a present/not present detection of
antibody, the cells used must cover the
major antigen specificities. The laboratory
must document the criteria for defining
major specificities and the number of
individuals included.

G3.620 For assignment of antibody specificity, the
composition of the cell panel must conform
to the standards in section G1.300 Panel Cells.

G3.700 Antibody screening using microparticle targets.

G3.710 If microparticles coated with HLA class I or
class II antigens are used for present/not present
detection of anti-HLA antibodies in
serum, the antigens coating the
microparticles must be obtained from a
sufficient number of individuals to cover the
major antigen specificities. The number of
individuals and antigen specificities must be
Antibody screening by ELISA

For assignment of antibody specificity using panels of HLA-antigen coated microparticles, the manufacturer must provide documentation specifying the HLA antigens on each microparticle reagent and an analysis of the distribution of HLA antigens on the panel in use.

Antibody screening by ELISA

Laboratories using ELISA techniques for antibody screening must conform to standards in Section P.

Negative control sera must include a serum from non-alloimmunised human donor(s).

Positive control sera should be pooled from highly alloimmunised individuals and documented to react with HLA antigens. The antibodies must be of the appropriate isotype for each assay.

A control reaction lacking only HLA antigen must be included in the test system.

Sera must be tested at a concentration determined to be optimal for detection of antibody to HLA antigens. The dilution must be documented.

The panel for HLA antigens must conform to the standards in Section G1.300 Panel Cells.

Antigens obtained from pooled cells may be used for a present/not present detection of antibody. Cells from a sufficient number of individuals must be used to cover the major antigen specificities. The number of individuals must be documented.

For assays intended to provide information on antibody specificity, the manufacturer must provide documentation of the HLA class I and class II phenotypes of the donors of the panel cells.

H - RENAL and/or PANCREAS TRANSPLANTATION

If cadaver donor transplants are done, personnel for the required histocompatibility testing, interpretation of results and provision of advice for the clinical transplant team must be available 24 hours a day, seven days a week.

Antibody screening.

Laboratories must have a documented policy in place to evaluate the sensitisation of each patient at the time of their initial evaluation.

Laboratories must have a programme to periodically screen serum samples from each patient for antibody to HLA antigens. Samples must be collected 3 monthly or as stipulated by the national and/or international organ exchange organisations. The laboratory must have a policy establishing the frequency of screening serum samples and must have data to support this policy.

Laboratories should maintain a record of potentially sensitising events for each patient. Serum samples should be collected and stored after each of these events for possible subsequent screening for antibodies to HLA and/or use in crossmatch tests.

The specificity of detected HLA antibodies should be defined and recorded.

Studies should be performed to distinguish antibodies to HLA antigens from antibodies with other specificities.

Crossmatching.

Crossmatching must be performed prospectively.

Crossmatching for the detection of HLA specific antibodies must use techniques equivalent in sensitivity to the screening test. Techniques documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test, such as prolonged incubation, washing or augmentation with antiglobulin or flow cytometry may also be used.

Crossmatches must be performed with potential donor unseparated lymphocytes or with T enriched lymphocytes. B-cell crossmatches must be performed if required by the relevant transplantation programmes.

For lymphocytotoxic crossmatching, an HLA specific positive control for the activity of complement and a negative control for the viability of the donor lymphocytes must be included for each tray.

If crossmatches are performed after treatment of the patient sera with dithiothreitol, then IgG and IgM positive and negative controls must be included.

Sera samples.

Sera must be tested at a dilution that is optimal for each assay. For lymphocytotoxicity crossmatches, sera must be tested undiluted and should be tested at one or more dilutions.

Sera obtained 14 days after a potentially sensitising event should be included in a final crossmatch.

Final crossmatches performed prior to transplantation should utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient has HLA class I lymphocytotoxic antibodies or has had a recent sensitising event. Otherwise, a serum collected within 3 months should be used.

Serum samples used for crossmatching should be retained in the frozen state for future use.

HLA typing.

Prospective typing of donor and recipient for HLA-A, B and DR antigens is mandatory.

Typing donor and recipient for HLA-C, DQ and DP antigens is optional.

Family donors.

All available members of the immediate family should be typed for accurate haplotype assignment.

Final crossmatches performed prior to transplantation should utilise a recipient
serum sample collected within the previous 48 hours before transplant if the recipient has HLA class I lymphocytotoxic antibodies or has had a recent sensitising event. Otherwise, a serum collected within three months should be used.

**H 6.000** Cadaver donors.

**H 6.100** Donors may be typed using lymphocytes from lymph nodes, spleen or peripheral blood.

**I - OTHER ORGAN TRANSPLANTATION**

**I1.000** In cases where patients are at high risk for allograft rejection (e.g. patients with histories of allograft rejection, patients with high levels of preformed Class I HLA antibodies), donors and recipients should be typed for HLA-A, B and DR antigens whenever possible.

**I2.000** Patients at high risk for allograft rejection should be screened whenever possible for the presence of HLA-A, B and DR alloantibodies.

**I3.000** Cross-matching.

**I3.100** Sera from patients at high risk for allograft rejection should be prospectively cross-matched whenever possible. Cross-matching must use techniques equivalent in sensitivity for the detection of HLA specific antibodies to the screening test. Techniques documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test, such as prolonged incubation, washing or augmentation with antiglobulin or flow cytometry may also be used. Cross-match results should be available prior to transplantation of a presensitised patient.

**I3.200** Final crossmatches performed prior to transplantation should utilise a serum sample collected within the previous 48 hours before transplant if the recipient has HLA class I lymphocytotoxic antibodies or has had a recent sensitising event. Otherwise, a serum collected within three months should be used.

**I3.300** If the patient receives a blood transfusion, has an allograft that is rejected or removed, or experiences any other potentially sensitising event, a serum sample obtained at least 14 days post-sensitisation should be used in the final cross-match.

**I3.400** Whenever possible, non-renal organs and tissues for recipients at high risk for allograft rejection should come from cross-match negative donors (i.e., cross-match with unseparated lymphocytes or enriched T-cells is less than 20% above background).

**J - MARROW AND STEM CELL TRANSPLANTATION**

**J1.000** Histocompatibility testing for related donors.

**J1.100** HLA-A, B, DR typing of all available members of the immediate family must be done to establish inheritance of haplotypes.

**J1.110** HLA typing for HLA identical siblings must include adequate testing to definitively establish HLA identity by descent. Extended Class I and Class II typing by DNA methods or augmented testing (e.g. MLC, T cell precursor frequency) should be performed as appropriate for the transplant protocol and optimal donor selection.

**J1.120** HLA typing for intrafamilial potential donors who are not HLA identical siblings must include Class I and Class II typing by DNA methods at a level that is appropriate for the transplant protocol and optimal donor selection. Augmented testing (e.g. bidirectional MLC, T cell precursor frequency) should be performed as appropriate for the transplant protocol and optimal donor selection.

**J1.130** For final selection of a related donor, HLA typing of both donor and recipient must be repeated using a new typing sample from each such that each individual’s typing is confirmed.

**J1.140** Laboratories not able to perform extended Class I and Class II typing by DNA methods must arrange for an EFI or ASHI accredited laboratory performing these tests to undertake confirmatory testing as described in J1.120. The latter laboratory must be able to perform DNA based HLA Class I and Class II typing to a level of resolution with 4 digits (e.g. A*0201, DRB1*1107). Where the ambiguities cannot be resolved, all the alternatives must be reported.

**J2.000** Histocompatibility testing for unrelated donors.

**J2.100** Volunteer bone marrow donors registries.

**J2.110** The donor must give his/her informed consent according to the national legislation before blood is taken for typing and before the donor is placed on a list of donors available to be called.

**J2.120** Typing of the donors must be performed by serology or DNA methods at a level of resolution with at least 2 digits (e.g. A2 or A*02, DR11 or DRB1*11).

**J2.200** Unrelated donors selected for bone marrow transplantation.

**J2.210** HLA typing for unrelated donors must include Class I and Class II typing by DNA methods at a level that is appropriate for the transplant protocol and optimal donor selection. Augmented testing (e.g. bidirectional MLC, T cell precursor frequency) should be performed as appropriate for the transplant protocol and optimal donor selection.

**J2.220** Laboratories typing unrelated donors selected for bone marrow donation must be able to type the donor and the recipient for HLA Class I and Class II by DNA methods, to a level of resolution with 4 digits (e.g. A*0201, DRB1*1107). Where the ambiguities cannot be resolved, all the alternatives must be reported.

**J2.230** (now included in C5.500.)

For final selection of an unrelated donor, HLA typing of both donor and recipient
must be repeated using a new typing sample from each such that each individual's typing is confirmed. HLA typing must be performed using a DNA method as described in J2.240.

K - PLATELET AND GRANULOCYTE TRANSFUSION

K1.000 HLA typing.
K1.100 The patient should be typed for HLA-A and HLA-B.
K1.200 The donors should be selected according to national regulation/legislation.
K2.000 Cross-matching.
K2.100 Lymphocyte cross-matches are optional.
K2.200 Cross-matching by techniques which utilise donor platelets or granulocytes as the target cells are preferred.

L - DISEASE ASSOCIATION

L1.000 Complete HLA typing is an appropriate option.
L1.100 Typing may also be limited to all products of a single or limited number of HLA loci.
L2.000 Typing for a single antigen (e.g., HLA-B27).
L2.100 Cell controls must be tested on each batch.
L2.110 The control cells must include at least two cells known to express the specified antigen.
L2.120 The control cells must also include two cells for each crossreacting antigen, which might be confused with the specific antigen.
L2.130 The control cells must also include at least two cells lacking the specific and crossreacting antigens.
L2.200 Serum controls must be tested at the time of typing.
L2.210 Serum controls must include a positive and negative control.
L2.220 Serum controls should also include two sera for each antigen which crossreacts with the specified antigen (if available).
L2.300 Sera to define each antigen must meet requirements of Section E as appropriate.

M - PATERNITY TESTING

M1.000 Paternity testing must be restricted to laboratories whose Director fulfils the general Director qualifications (B2.000) and in addition is qualified by advanced training and/or experience in paternity testing.
M1.100 The competency of the technical staff in relation to paternity testing must be the responsibility of the Director.
M1.200 The laboratory Director and technical staff performing paternity testing must participate in continuing education relative to the field of paternity testing.
M1.300 A qualified individual must be available for legal testimony in the case, as needed.
M2.000 Laboratories utilising genetic systems in addition to HLA must be able to document expertise and/or accreditation in those systems.
M2.100 An accredited laboratory may engage another laboratory to perform genetic testing for systems not used by the primary laboratory. In that event, the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the report (see M7.000).

M3.000 Subject identification.
M3.100 Evidence for verifiable means of identification for subjects must be recorded at the time the blood sample is taken.
M3.200 Recommended evidence includes photographs, fingerprints and the number(s) of identification cards displaying the subject’s picture (e.g., drivers license).
M3.300 Specimens received from an outside collecting facility must also have a mean for positive identification unless this requirement has been waived by mutual consent of the individuals involved.
M3.400 A record must be kept at the testing facility of all identifying information including, but not limited to, name, relationship, ethnic group, place and date of collection of sample. Information about each individual must be verified by the signature of that person or the guardian.
M3.500 The date of birth of the child and recent transfusion history (past three months) of each individual to be tested must be recorded.
M4.000 Sample identifications.
M4.100 Each tube must be labelled immediately prior to collection of the sample to avoid mix-up of samples.
M4.200 The label must include the full name of the subject, the date and the initials of the blood drawer.
M4.300 The phlebotomist’s name must be part of the permanent record.
M4.400 A record of the “chain of custody” of the sample must be maintained.
M5.000 HLA testing requirements for paternity testing.
M5.100 Each test sample must be plated on two separate trays or tray sets each containing a minimum of one monospecific or two multispecific sera defining each HLA-A and B locus antigen tested. The sera defining a particular specificity should be from different donors. The trays must be read independently.

M6.000 Calculations.
M6.100 Computer assisted analyses must be reviewed, verified and signed by the Supervisor and/or Laboratory Director before issue.
M6.200 The computer programme, which is utilised for analyses must be documented.
M6.300 If only manual calculations are done, they must be done in duplicate.
M6.400 Gene and haplotype frequencies should have been obtained from examination of populations of adequate size.

M7.000 Reports.
M7.100 Each report must be released only to authorised individuals and must contain:
M7.110 The name of each individual tested and the relationship to the child.
M7.120 The ethnic origin(s) assigned by the laboratory to the mother and alleged father(s) for the purpose of calculation.

M7.130 The phenotypes established for each individual in each genetic system examined.

M7.140 A statement as to whether or not the alleged father can be excluded. When there is no exclusion the report must contain:

M7.141 The individual Paternity Index for each genetic system reported.

M7.142 The cumulative Paternity Index.

M7.143 The probability of paternity expressed as a percentage. The prior probability(ies) used to calculate the probability of paternity must be stated.

M7.144 Other mathematical or verbal expressions are optional. If they are included in the report, such expressions should be defined and explained.

M7.150 If the results are inconclusive, an explanation as to the nature of the problem.

M7.160 The signature of the laboratory Director.

N - NUCLEIC ACID ANALYSIS

The nucleic acid analysis standards apply to histocompatibility testing.

N1.0000 DNA extraction.

N1.1000 DNA must be purified by a standard method that has been reported in the scientific literature and validated in the laboratory.

N1.2000 If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material.

N2.0000 Amplification-based assays.

N2.1000 Amplification.

N2.1100 Use of physical and/or biochemical barriers to prevent DNA contamination (carryover) is required. Pre-amplification procedures must be performed in a dedicated work area that excludes amplified DNA that has the potential to serve as a template for amplification in the HLA typing assays (e.g., PCR product, plasmids containing HLA genes). Physical separation and restricted traffic flow is recommended. Use of a static air hood or a Class II biological safety cabinet is recommended. Biochemical procedures can be used to inactivate amplified products.

N2.1200 Other pre-amplification physical containment must include use of dedicated lab coats, gloves and disposable supplies. Frequent cleaning with dilute acid or bleach and/or UV treatment of work surfaces must be performed.

N2.1300 Equipment and reagents.

N2.1310 Use of dedicated equipment for pre-amplification procedures is mandatory.

N2.1311 Use of dedicated pipettors is required. Positive displacement pipettes or filter-plugged tips are recommended.

N2.1312 Thermal cycling instruments must precisely and reproducibly maintain the appropriate temperature of samples. Accuracy of temperature control for samples must be verified on a regular basis.

N2.1320 Reagents.

N2.1321 All reagents (solutions containing one or multiple components) utilised in the amplification assay must be dispensed in aliquots for single use or reagents can be dispensed in aliquots for multiple use if documented to be free of contamination at each use. When reagents are combined to create a master mix, it is recommended that one critical component (e.g. Mg++) be left out of the aliquot.

N2.1322 Reagents (e.g., chemicals, enzymes) must be stored and utilised under conditions recommended by the manufacturer (i.e., storage temperature, test temperature, buffer, concentration). Reagents used for amplification must not be exposed to post-amplification work areas.

N2.1323 For commercial kits, the source, lot number, expiration date, and storage conditions must be documented. Each laboratory is responsible for the accuracy of typing. Reagents from different lots of kits must not be mixed.

N2.1324 Primers must be stored under conditions that maintain specificity and sensitivity.

N2.1325 Methods that utilise two consecutive steps of logarithmic amplification are especially susceptible to errors related to PCR carryover (contamination) and special attention must be paid to containment of amplified products (e.g. physical separation, work flow and enhanced contamination monitoring). Standard N2.1100 applies to all components of the second amplification except template. Addition of the template for the second amplification must be physically separated from the pre-amplification work area and the post-amplification work area. Use of pipettors dedicated to each work area (i.e. first amplification and analysis) is required.

N2.1400 Amplification templates.

N2.1410 Templates must not be exposed to post-amplification work areas.

N2.1420 DNA or cDNA (from RNA templates) is satisfactory. DNA from any nucleated cells or RNA from any cells expressing the HLA product may be used. If RNA is used, appropriate positive controls for reverse transcription must be included.

N2.1430 Nucleic acids must be prepared and stored in a manner, which does not result in artefacts or inhibition of the amplification reaction. The acceptable range for the amount of target must be specified and validated.

N2.1500 Primers.

N2.1510 The specificity and sequence of primers must be determined. The HLA locus and allele(s) must be defined.

N2.1520 Conditions, which influence specificity or quantity of amplified product must be demonstrated to be satisfactory for each set of primers.
N2.1530 Reference material should be used to test and periodically reconfirm the specificity and product quantity of each lot of primers.

N2.1600 Contamination.

N2.1610 Monitoring for nucleic acid contamination must be performed. Controls must be tested using the method that is routinely used to detect HLA types.

N2.1611 Negative controls (no nucleic acid) must be included in each amplification assay. Another negative control might include open tubes in the work area.

N2.1612 In order to minimise the detection of minor contaminants and the occurrence of stochastic fluctuation the number of cycles should be set at a level sufficient to detect the target nucleic acid but insufficient to detect small amounts (e.g. <10 molecules) of contaminating template.

N2.1613 Routine wipe-tests of pre-amplification work areas must be performed. If amplified product is detected, the area must be cleaned to eliminate the contamination and measures must be taken to prevent future contamination.

N2.1700 Controls.

N2.1710 The quantity of specific amplification products must be monitored (e.g., gel electrophoresis hybridisation).

N2.1720 Criteria for accepting or rejecting an amplification assay must be specified.

N2.1730 If presence of an amplified product is used as the end result, controls must be included to detect amplification failure in every amplification mixture. Amplification specificity must be monitored on a periodic basis.

N2.2000 Amplified product (Nucleic acid targets).

N2.2100 Variation in the amount of amplified product must be monitored (e.g., hybridisation with a consensus probe, gel electrophoresis). The acceptable range for the amount of available target must be specified.

N2.3000 Oligonucleotide probes and hybridisation with amplified DNA.

N2.3100 The specificity and target sequence of probes must be defined.

N2.3200 Probes must be stored under conditions, which maintain specificity and sensitivity.

N2.3300 Probes must be utilised under empirically determined conditions that achieve the defined specificity. The specificity must be demonstrated and maintained for each lot of probe. Each lot of probes must be tested for specificity and product quantity using reference material under optimised conditions and reconfirmed periodically.

N2.3400 Hybridisation must be carried out under empirically determined conditions that achieve the defined specificity.

N2.3500 The specificity of hybridisation should be confirmed using positive and negative controls for hybridisation with each probe. The controls should be capable of detecting cross-hybridisation with closely related sequences.

N2.3600 Reuse of nucleic acids (probes or targets) bound to solid supports must only be undertaken after demonstrating that previous signals are no longer detectable.

N2.3700 Reuse of nucleic acids in solution (probes or targets) must only be undertaken with controls included to ensure that the sensitivity and specificity of the assay are unaltered.

N2.3800 Incubators and water baths must be monitored for precise and accurate temperature maintenance every time the assay is performed.

N2.4000 Labelling of nucleic acids and detection.

N2.4100 The specificity and sensitivity of the labelling and detection method should be established and reproducible.

N2.4200 The specificity and sensitivity must be maintained for each lot of reagents (e.g., antibodies, probes, indicator molecules).

N2.4300 Enzymes must be stored and utilised under conditions recommended by the manufacturer (i.e., storage temperature, test temperature, buffer, concentration) to ensure correct enzymatic activity. The enzymatic activity of each lot should be confirmed before use.

N2.5000 Analysis.

N2.5100 Acceptable limits of signal intensity must be specified for positive and negative results. If these are not achieved, corrective action is required.

N2.5200 The method of assignment of alleles must be specified.

N2.5300 Two independent interpretations of alleles must be specified.

N2.5400 Analysis.

N2.5400 Now included in C5.400.

N2.6000 Nucleotide sequencing.

N2.6100 Sequencing templates.

N2.6110 Standards in N2.1420 and N2.1430 must be followed for preparation of templates.

N2.6110 Templates must have sufficient purity specificity (e.g. locus or allele-specificity), quantity and quality to provide interpretable primary sequencing data. The method for preparing sequencing templates must reliably generate appropriate length sequencing templates that are free of inhibitors of subsequent reactions (e.g. residual primer extension) and free of contaminants that cause sequencing artefacts. Methods must ensure that preparation of sequencing templates does not alter the accuracy of the final sequence (e.g. mutations created during cloning, preferential amplification).

N2.6120 Reagents used in preparation of sequencing templates (e.g. enzymes, biochemicals) must be stored and utilised under conditions recommended by the manufacturer. The appropriate performance of each lot must be documented before results of tests using these reagents are reported.

N2.6200 Methods utilising primer extension.

N2.6210 The specificity and polymorphism of the target sequence must be documented.

N2.6220 Primers must be used under empirically
determined conditions that achieve the defined specificity of amplification. The amplification conditions must be demonstrated by the laboratory to achieve defined specificity and must yield adequate quantity of specific product. Each lot of primer should be tested for specificity and product quantity using reference material (e.g. DNA) under routine conditions and reconfirmed periodically.

N.2.6230 Conditions for primer extension (e.g. polymerase type, polymerase concentration, primer concentration, concentration of nucleotide triphosphates, concentration of terminators) must be appropriate for the template (e.g. length of sequence, GC content).

N.2.6240 The specificity and sensitivity of the labelling and detection methods must be documented (e.g. demonstrating correct signal strength for a control sequence) in the laboratory before results are reported.

N.2.6250 Satisfactory performance of each lot of reagent (e.g. nucleotides, enzymes) must be documented before results using these reagents are reported. Reagents must be stored under conditions that maintain optimal performance.

N.2.6300 Electrophoresis.

N.2.6310 The laboratory must establish scientifically and technically sound criteria for accepting each gel and each lane of a gel.

N.2.6320 Satisfactory performance of each lot of reagents that influence the quality and accuracy of sequencing data of the gel (e.g. acrylamide, buffer and salt concentration) should be documented before results using these reagents are reported. Acceptable electrophoretic conditions (e.g. temperature, voltage, duration) must be established. Conditions should be recorded for each run. Reagents must be stored under conditions that maintain acceptable performance.

N.2.6400 Nucleotide and allele assignments.

N.2.6410 Criteria for acceptance of primary data must be established (e.g. correct assignments for nonpolymorphic positions, definition of sequencing region, criteria for peak intensity, baseline fluctuation, signal-to-noise ratio and peak shapes). Validation might include sequencing of samples representative of all polymorphic motifs that are frequently encountered in the routine sample population to detect sequence-specific artefacts. Sequencing of both strands of at least one representative of each polymorphic motif is recommended during validation. Established sequence-specific characteristics should be documented and utilised in routine interpretation of data.

N.2.6420 Routine sequence assignments must be based on analysis of sequence data from complementary strands of DNA unless it is documented that the sequencing method consistently yields accurate sequence assignments using data from only one strand of DNA. If assignments are routinely based upon data from one strand of DNA, periodic confirmation of complementary strands is recommended. If base assignments are frequently difficult to interpret, routine sequencing of both strands is recommended. If a sequence suggests a novel allele or a rare combination of alleles, the sequences of both strands must be determined.

N.2.6430 A scientifically and technically sound method must be established for interpretation, acceptance, and/or rejection of sequences from regions which are difficult to resolve (e.g. compression, ends).

N.2.6440 Two independent interpretations of the primary data are recommended in order to resolve doubtful nucleotide positions.

N.2.6450 Automated systems and computer programmes for nucleotide assignments must be validated prior to use.

N.2.6500 Allele assignments.

N.2.6510 HLA locus and alleles must be defined for every template/ primer combination. Each unknown sequence must be compared with the sequences of all alleles that are recognised by the WHO provided that the nucleotide sequences are readily available (i.e. in a locus specific alignment in conjunction with the WHO nomenclature Committee for Factors of the HLA System which appears periodically in the public domain). Databases of sequences must be accurate and conform to the most recent compilation of sequences published in conjunction with the WHO.

N.2.6520 Ambiguous combinations of alleles must be defined for each template/primer combination.

N.2.6530 Methods must ensure that sequences contributed by amplification primers are not considered in the assignment of alleles.

N.2.6540 Two independent assignments of alleles are recommended when alleles are assigned manually.

N.2.6550 The laboratory must maintain records that define the sequence data base utilised to interpret the primary data. This database must be updated periodically. If a determined sequence is ambiguous (i.e., more than one possible interpretation of available data), the report must indicate all possible allelic combinations.

N.2.7000 Typing using sequence-specific amplification.

N.2.7100 The specificity of each primer combination must be defined.

N.2.7200 Each amplification reaction must include procedures to detect technical failures (e.g. an internal control such as additional primers or templates that produce a product that can be distinguished from the typing product).

N.2.7300 In each amplification assay (i.e. set up of amplification mixtures for one or more samples) controls should be used to detect contamination with previously amplified products (e.g., a special primer pair internal to all amplification products or a
Primers must be utilised under empirically determined conditions that achieve the defined specificity for templates used in routine testing. Each set of primers must be tested for amplification specificity and product quantity using reference material under optimised conditions. The frequency of testing each primer set must ensure that all primer pairs have appropriate sensitivity and specificity of amplification. The specificity and sensitivity must be maintained in heterozygous samples.

N2.7510 Negative and positive DNA amplification controls must be included in the electrophoretic run.

N2.7520 Size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each gel.

N2.7530 The amount of DNA per lane must not alter the rate of sample migration with respect to the migration of controls.

N2.7540 Optimal electrophoretic conditions must be determined for each genetic locus, acceptable ranges must be established and their use documented.

N2.7550 the laboratory must establish criteria for accepting each gel.

N2.7600 Detection of nucleic acid fragments.

P2.7640 The specificity and sensitivity of the detection method must be established and reproducible.

N2.7700 Analysis.

N2.7710 Acceptable quantitative limits of signal intensity must be specified.

N2.7720 The method of assignment of alleles must be designated.

N2.7730 Two independent interpretations of primary data are recommended.

N2.8000 Restriction fragment length polymorphism of amplified products.

N2.8100 Restriction nucleases.

N2.8110 HLA locus and Target allele(s) or variants must be defined for each RFLP type.

N2.8120 Enzymes must be stored and utilised under conditions recommended by the manufacturer (i.e., storage temperature, test temperature, buffer, concentration) to ensure correct enzymatic activity. The appropriate performance of each lot of enzyme must be documented before results using these reagents are reported.

When amplified DNA is digested, controls of amplified DNA, which will produce fragments of known sizes, must also be digested in parallel to monitor complete digestion.

Electrophoresis.

All relevant standards from section N2.7500 must be applied.

Detection of nucleic acid fragments.

All relevant standards from section N2.7600 must be applied.

Analysis.

All relevant standards from section N2.7700 must be applied.

Other methods.

If alternative methods (e.g., SSCP, heteroduplex, DGGE) are used for HLA typing, established procedures must be validated and must include sufficient controls to ensure accurate assignment of types for every sample. All relevant standards from the above sections must be applied.

Automated systems and computer programmes must be validated prior to use and tested routinely for accuracy and reproducibility of manipulations.

O - FLOW CYTOMETRY

The Flow cytometry standards apply to MHC testing including B27 typing.

An optical standard consisting of latex beads or other uniform particles must be run to ensure proper focusing and alignment of all lenses in the path for both the exciting light source and signal (light scatter, fluorescence, etc.) detectors.

The optical standard must be run at the start of each daily or shift operation as any time maintenance or adjustment of the instrument during operation is likely to have altered optical alignment.

The results of optical focusing/alignment must be recorded in daily quality control log.

A threshold value for acceptable optical standardisation must be established for all relevant signals for each instrument and the focusing procedure repeated until these values are achieved or surpassed.

In the event a particular threshold value cannot be attained, a written protocol for instituting corrective action must be available. This protocol should include appropriate corrective actions including clear guidelines describing when a service is warranted.

The flow cytometer should be cleaned regularly in accordance with the manufacturer’s instructions.

The flow cytometer must be serviced regularly exactly as recommended by the manufacturer. The dates of the service visits, the faults detected and the comments of the service engineer must be recorded.
A fluorescent standard for each fluorochrome to be used must be run to ensure adequate amplification of the fluorescent signal(s) on a day-to-day basis. This standard may be incorporated in the beads or other particles used for optical standardisation or may be a separate bead or fixed cell preparation.

The fluorescent standard must be run at the start of each daily or shift operation and any time maintenance or adjustment of the instrument during operation, which has altered the gain or high voltage settings.

The results of fluorescent standardisation must be recorded in a daily quality control log.

In the event that acceptable fluorescence separation cannot be attained, a written protocol for instituting corrective action must be available. This protocol should include appropriate corrective action including clear guidelines describing when a service call is warranted.

If performing analyses using two or more fluorochromes simultaneously, an appropriate compensation procedure must be used to eliminate “spill over” into the other fluorescence detectors.

Compensation must be carried out using peripheral blood lymphocytes stained with suitable fluorochromes or with other dual stained particles, which have been shown to be suitable by the laboratory.

For laser based instruments, the current input (amps) and laser light output (milliwatts), at the normal operating wavelength measured after the laser is peaked and normal operating power set, should be recorded as part of a daily quality control record.

Flow cytometric crossmatch technique

A multi-colour technique is recommended. However, if a single colour technique is used, the purity of the isolated cell population must be documented and should be of sufficient purity to define the population for analysis.

The binding of human immunoglobulin must be assessed with a fluorochrome labelled F(ab') anti-human IgG specific for the Fc region of the heavy chain.

In order to assess binding of human immunoglobulin to cell population(s), the sub population(s) should be identified by two or three colour staining with differently labelled monoclonal antibodies to the appropriate CD marker(s) (e.g. phycoerythrin conjugated CD3 monoclonal antibody to identify T cells).

Two or three colour staining of other immunoglobulin classes may also be justified.

Control sera must be tested at the same time and under the same conditions as the sera under test. Tests must be done in duplicate as a minimum requirement.

The normal human serum (negative) control should be from non-immunised and otherwise healthy individuals and may be a pool of several donors. It must be screened by flow cytometry to ensure lack of reactivity against human leukocytes.

The positive control should be human serum containing antibodies of the appropriate isotype, specific for the HLA antigens or any other alloantigens deemed to be important for detection in the crossmatch. Positive controls should react with lymphocytes of all humans.

The anti-human IgG reagent should be titered to determine the dilution with optimal activity (signal to noise ratio). If a multicolour technique is employed, the reagent must not demonstrate crossreactivity with the other immunoglobulin reagents used to mark the cells.

Reagents.

The specificity of monoclonal antibodies shall be verified by published and/or manufacturer’s documentation and/or local documented quality control testing.

Reagents must be stored according to manufacturer’s instructions or according to conditions verified to maintain stability by documented local tests.

Monoclonal antibodies which have been reconstituted from lyophilised powder form for storage at 4°C should be centrifuged according to the manufacturer’s instructions or locally documented procedures to remove micro aggregates prior to use in preparation of working stains.

Interpretation.

Each laboratory must establish its own crossmatch protocol, standardising and optimising all reagents used, incubation times and temperatures.

Whether reporting mean, mode or median channel shifts, relative mean fluorescence, or number of molecules of fluorescent marker, each laboratory must establish its own threshold for positive crossmatches.

Any significant change in protocol or instrumentation requires that characterisation of the positive threshold be repeated.

HLA typing by flow cytometry (e.g. HLA B27). Terminology used must be defined and/or conform to nomenclature recommended/approved by the most recent WHO nomenclature committee meeting.

Cell preparation.

The method used for cell preparation should be documented to yield appropriate preparations of viable cells.

The viability of cell preparations should be recorded and should exceed the laboratory’s established minimum
O3.2300 Labelling of specimens.
O3.2310 A negative reagent control(s) shall be run for each test cell preparation. This control should consist of monoclonal antibody(ies) of the same species and subclass and should be prepared/purified in the same way as the monoclonal(s) used for phenotyping.
O3.2311 For indirect labelling, an irrelevant primary antibody, if available, and in all cases, the same secondary antibody(ies) conjugated with the same fluorochrome(s) used in all relevant test combinations.
O3.2312 For direct labelling, an irrelevant antibody conjugated with the same fluorochrome and at the same fluorochrome: protein ratio used in all relevant test combinations.
O3.2320 Whether analysed directly or fixed prior to analysis, labelled cells must be analysed within a time period demonstrated by the laboratory to avoid significant changes in test results. Control samples must be analysed within the same period after staining as the test samples.
O3.3000 Reagents.
O3.3100 The specificity of monoclonal antibodies must be verified through tests with appropriate control cells prepared and tested by the same method employed in the laboratory's test sample analysis.
O3.3200 Cell controls must be tested for each batch of monoclonal antibodies received.
O3.3210 The control cells must include at least five cells known to express the specified antigen.
O3.3220 The control cells must also include two cells for each cross-reacting antigen, which might be confused with the specific antigen.
O3.3230 The control cells must also include at least two cells lacking the specific and cross-reacting antigens.
O3.3300 The quantities of reagents used for each test sample must be determined by the manufacturers or from published data and whenever possible should be verified locally by appropriate titration procedures.
O3.3400 Reagents must be stored according to manufacturer's instructions or according to conditions verified to maintain stability by documented local tests.
O3.3500 Monoclonal antibodies, which have been reconstituted from lyophilised powder form for storage at 4°C should be centrifuged according to the manufacturer's instructions or locally documented procedures to remove microaggregates prior to use in preparation of working stains.
O3.3600 A single monoclonal antibody must be used to define an antigen provided its specificity has been sufficiently verified by local testing.
O3.3700 Minimum reactivity for assignment of a positive reaction must be established by the laboratory.
O3.3710 Each batch of tests must include a cell sample known to express the antigen under test as a positive control.
O3.3800 If the monoclonal antibody(ies) is (are) known or found to react with antigens other than the one specified, a written protocol must explain how its presence or absence is finally determined.

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P - ENZYME-LINKED IMMUNO SORBENT ASSAY (ELISA)

P1.000 Instrument standardisation/calibration.
P1.100 The ELISA reader.
P1.110 The light source must produce the intensity and wavelength of light required for the test system.
P1.120 Precise movement of the plate must be verified and recorded.
P1.130 Periodic calibration must be performed according to the instrument manufacturer's instructions and must be documented.
P1.200 Assays must be performed with calibrated dispensing instruments (pipettes). Calibration must be performed routinely and must be documented.
P1.300 Microplate washer performance must be checked monthly and acceptable performance documented.
P2.000 ELISA technique.
P2.100 If commercial kits are used, the manufacturer's instructions must be followed unless the laboratory has performed and documented testing to support a deviation in technique or analysis.
P2.200 Reagents must be stored at the temperature and for no longer than the duration specified by the manufacturer.
P2.300 Each assay must contain a positive control, a negative control and reagent controls. The dilution of reagents and test specimens must be documented.
P2.400 Sample identity and proper plate orientation must be maintained throughout the procedure.
P2.500 The lot numbers and optical density values of the reference reagents and the controls must be recorded for each assay. These values must fall within acceptable limits for the assay to be valid.
P2.600 The volume and number of washes must be recorded for each assay.
P2.700 New lots of reagents must be validated by side-by-side testing with a lot known to give acceptable performance or by testing with