European Federation for Immunogenetics

STANDARDS FOR HISTOCOMPATIBILITY & IMMUNOGENETICS TESTING

Version 9.0

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Standards

SECTION A – GENERAL POLICIES

A1	These Standards have been approved and adopted by the EFI Executive Committee
A2	They are based on Standards originally prepared by the American Society for Histocompatibility and Immunogenetics (ASHI)
A3	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
A4	These Standards establish minimal criteria, which all histocompatibility laboratories must meet if their services are to be considered acceptable
A5	Many laboratories, because of extensive experience, will exceed the minimal requirements of these Standards
A6	Certain Standards are obligatory. In these instances, the Standards use the word "must"
A7	Some Standards are highly recommended but not absolutely mandatory. In these instances the Standards use words like "should" or "recommended"
A8	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
A9	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
A10	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
A11	All relevant data necessary for compliance evaluation must be available for inspections
A12	**Testing Referred To Other Laboratories**
A12.1	An accredited laboratory may engage another laboratory to perform testing not done by the primary laboratory
A12.2	The subcontracting laboratory:
A12.2.1	Must be accredited by EFI or by ASHI, if the testing is covered by EFI standards
A12.2.2	Should have documented expertise and/or accreditation in genetic systems not covered by EFI Standards
A13	**Partial Testing In Other Laboratories**
A13.1	An accredited laboratory may engage another laboratory to perform part of the testing i.e. core laboratory
A13.2	When core facilities are used:

A13.2.1	They must fulfil relevant standards
A13.2.2	Facilities must be available for inspections
A14	Transplant Protocol
A14.1	A transplant protocol must:
A14.1.1	be a written document
A14.1.2	describe the roles and responsibilities of the H&I laboratory and the clinical team
A14.1.3	meet the requirements of the transplant programme
A14.1.4	be relevant to the particular transplant programme. If the H&I Laboratory supports multiple transplant programmes a transplant protocol must be in place with each
A14.1.5	be agreed, and signed, by the Laboratory Director and the Clinical Director
A14.2	The transplant protocol must meet the minimal requirements in sections E and F

SECTION B – PERSONNEL QUALIFICATIONS

B1	For the purposes of this document, EFI defines the Director as the person who is responsible for the H&I laboratory activities for which accreditation is applied for
B2	The laboratory must employ one or more individuals who meet the qualifications and fulfil the responsibilities of:
B2.1	**The Director and/or Co-Director**
B2.1.1	A Director, that must:
B2.1.1.1	Hold a qualification approved by EFI, such as an ESHI or national diploma, earned doctoral degree in a biological science, or be a physician, and
B2.1.1.2	Have minimum qualifying experience equivalent to either of the following:
B2.1.1.3	Four years' relevant experience two of which were devoted to full time training in human H&I testing, or
B2.1.1.4	Four years of working experience at full time in human H&I testing
B2.1.1.5	Additional qualifications required according to national legislation also apply
B2.1.2	If these tests are performed in a laboratory seeking accreditation where H&I testing is not performed; for chimaerism, KIR, HPA, HNA two years of working experience at full time
B2.1.3	Have documentation of professional competence in the appropriate activities in which the laboratory is engaged. This should be based on sound knowledge of the fundamentals of immunology, genetics and histocompatibility testing as appropriate to the areas in which accreditation is sought
B2.1.4	If a Co-Director is appointed, this person must also fulfil Standards B3.1.1 - B3.1.3

B2.1.5	The Director and/or Co-Director must:
B2.1.5.1	Be available on site to supervise the laboratory for at least 80% of the week
B2.1.5.1.1	when applicable remote accessibility for supervision and direction may be permitted as a supplement to being on site
B2.1.5.2	Provide adequate supervision of technical personnel
B2.1.5.3	Utilise his/her special scientific skills in developing new procedures
B2.1.5.4	Be held responsible for the proper performance, interpretation and reporting of all laboratory procedures
B2.1.5.5	Ensure the laboratory's successful participation in proficiency testing
B2.1.5.6	Be informed of the relevant national legislation and professional standards
B2.1.5.7	Comply with the relevant national legislation and professional standards
B2.1.5.8	Demonstrate active participation in clinically relevant professional development, such as national or international conferences or workshops
B2.1.6	The Director or Co-Director should:
B2.1.6.1	Have publications in peer-reviewed journals
B2.2	**Technical Staff**
B2.2.1	A Technical Supervisor, that must:
B2.2.1.1	Have minimum qualifying experience equivalent to either of the following:
B2.2.1.1.1	Hold a bachelor's degree, ETHIQ, or equivalent and have three years' relevant experience in the areas for which accreditation is sought under the supervision of a qualified Director or Co-Director
B2.2.1.1.2	Five years of supervised experience if a bachelor's degree has not been earned
B2.2.1.2	If these tests are performed in a laboratory not performing H&I, one year of supervised experience for chimaerism, KIR, HPA, HNA is required
B2.3	A Quality Manager, who must establish and maintain a comprehensive quality management programme covering all aspects for the accredited facility addressed by these standards
B2.4	The resources of the laboratory must be sufficient to accommodate the workload
B3	**Competency Evaluation and Continuous Education**
B3.1	The Director/Co-Director or designee must:
B3.1.1	Evaluate the competence of each technologist to accurately perform tests. This must be done at least yearly for each technique the technologist performs and must be based on a defined process

B3.1.2	Maintain records of these evaluations for each individual
B3.2	The Laboratory Director and the technical staff must participate in continuing education relating to each category for which EFI accreditation is sought

SECTION C – QUALITY ASSURANCE

C1	**MANAGEMENT**
C1.1	**Laboratory Procedure Manual**
C1.1.1	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
C1.1.2	For each procedure:
C1.1.2.1	A documented review by the Director/Co-Director or a delegated individual with appropriate qualifications must be performed at least biennially
C1.1.2.2	Any changes in procedures must be approved and documented by the Director/Co-Director/ delegated individual at the time they are initiated
C1.1.2.3	Each deviation from the standard operation procedure must be documented
C1.2	**Quality Assurance Evaluation**
C1.2.1	The laboratory must:
C1.2.1.1	Hold quality assurance reviews
C1.2.1.2	Document, assess problems identified in these reviews and discuss them with the staff
C1.2.1.3	Take corrective actions necessary to prevent recurrences
C1.2.1.4	Have an ongoing mechanism to evaluate corrective action taken. Ineffective policies and procedures must be revised based on the outcome of the evaluation
C1.2.2	The laboratory must maintain documentation of all quality assurance activities including problems identified and corrective actions taken, for a minimum of two years or longer, depending on local, or national regulations
C1.2.3	The laboratory must maintain permanent files of all internal and external quality control tests according to any regulation to which the laboratory is obliged to abide, but for a minimum of four years
C1.3	**Systems for Continuous Test Evaluation and Monitoring**
C1.3.1	The laboratory must establish and employ policies and procedures, and document actions taken when:
C1.3.1.1	Test systems do not meet the laboratory's established criteria
C1.3.1.2	Quality control results are outside of acceptable limits

C1.3.1.3	Errors are detected in the reported patient results. In this instance, the laboratory must:
C1.3.1.3.1	Promptly notify the authorised person ordering or individual utilising the test results of reporting errors
C1.3.1.3.2	Issue corrected reports
C1.3.1.3.3	Maintain copies of the original report as well as the corrected report for at least two years
C1.3.1.3.4	Laboratories must have a procedure in place for resolving any discrepancies that may occur between laboratories
C1.3.2	The laboratory must have mechanisms in place for continuous monitoring of all test systems and equipment used, including:
C1.3.2.1	Validation/verification, before introduction into routine use, of all new tests, by systematic comparative evaluation of results obtained in parallel with the new and the standard system
C1.3.2.2	For commercial kits, any deviation from the manufacturer's specifications must be validated and documented
C1.3.2.3	Regular evaluation of results obtained in external and internal QC testing
C1.3.2.4	Regular monitoring of test validity in routine testing, by recording observations diverging from the expected results (e.g. cross-reactivity of probes or primer mixes, day-to-day variations)
C1.3.2.5	Comparing test results and documenting inconsistencies, if the same test is performed using different techniques
C1.3.2.6	Identifying and evaluating inconsistencies between test results and clinical data or diagnostic parameters provided
C1.3.2.7	Written evidence of the ongoing monitoring process must be available in the laboratory for each method performed
C1.3.2.8	Controls and procedures to identify sample mix- up
C1.3.3	Each sample processed must be traceable through the whole process including data analysis and reporting
C1.3.4	Each step in the processing and testing of samples must be documented to assure that accurate test results are recorded
C1.3.5	QC parameters for optimal performance must be specified, documented and in range for every assay
C1.4	The laboratory must document compliance with all applicable national and local laws which relate to:
C1.4.1	Employee health and safety
C1.4.2	Fire safety
C1.4.3	Storage, handling and disposal of:
C1.4.3.1	Chemical material
C1.4.3.2	Biological material
C1.5	**Client Service Evaluation**
C1.5.1	All complaints and problems reported to the laboratory must be:

C1.5.1.1	Documented
C1.5.1.2	Investigated
C1.5.1.3	Followed by corrective action when necessary
C1.5.2	The laboratory must, upon request, make available to clients a list of tests employed by the laboratory
C2	**TECHNICAL**
C2.1	**Facilities**
C2.1.1	The following facilities must be adequate and immediately available to the laboratory:
C2.1.1.1	Sufficient space so that all procedures can be carried out without crowding to the extent that errors may result, in accordance with national regulations
C2.1.1.2	Lighting
C2.1.1.3	Ventilation
C2.1.1.4	Refrigerators
C2.1.1.5	Freezers
C2.1.1.6	Storage for:
C2.1.1.6.1	Reagents
C2.1.1.6.2	Specimens
C2.1.1.6.3	Records
C2.1.1.7	Uninterruptible or emergency power supplies must be used for essential equipment
C2.1.2	To ensure that procedures are carried out within temperature ranges specified in the laboratory's procedure manual, the relevant temperatures, including ambient, must be monitored every working day:
C2.1.3	Laboratories using radioactive materials must have a designated section of the laboratory for:
C2.1.3.1	The storage of materials
C2.1.3.2	Conducting procedures
C2.1.3.3	Radioactive materials must be disposed of at locations designated by local institutions
C2.1.4	Laboratories performing amplification of nucleic acids must use:
C2.1.4.1	A dedicated work area with restricted traffic flow
C2.1.4.2	Physical and/or biochemical barriers to prevent DNA contamination, including the use of dedicated
C2.1.4.2.1	Equipment

C2.1.4.2.2	Laboratory coats
C2.1.4.2.3	Disposable supplies
C2.1.5	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
C2.1.6	All activities occurring from and including thermal cycling must take place in the post-amplification area
C2.1.7	For methods that use two consecutive steps of logarithmic amplification the addition of the template for subsequent amplifications:
C2.1.7.1	Must occur in a post PCR area in a dedicated work space
C2.1.7.2	Must use dedicated equipment and consumables
C2.2	**Equipment**
C2.2.1	The laboratory must establish and employ policies and procedures for the proper maintenance of equipment, instruments and test systems by:
C2.2.1.1	Defining its preventive maintenance programme for each instrument and piece of equipment at least once a year
C2.2.1.2	Performing and documenting function including calibration checks on equipment with at least the frequency specified by the manufacturer
C2.2.1.3	The result of the performance test must be:
C2.2.1.3.1	Acceptable
C2.2.1.3.2	Documented
C2.2.2	Cleaning
C2.2.2.1	There must be a procedure for regular cleaning of the instrument
C2.2.2.2	The frequency and protocol for cleaning must conform to manufacturer’s instructions, if available
C2.2.2.3	Cleaning must be documented
C2.2.3	**Dispensing Equipment**
C2.2.3.1	The laboratory must use calibrated dispensing instruments (e.g. pipettes, etc.) to perform assays
C2.2.3.1.2	Calibration must be performed according to the manufacturer’s specifications
C2.2.3.1.3	Calibration of dispensing instruments must be performed at least once a year
C2.2.3.1.4	Calibration must be documented

C2.2.4	**Temperature Controlled Equipment**
C2.2.4.1	Acceptable ranges for each temperature-controlled equipment must be documented
C2.2.4.2	Must be monitored to detect unacceptable temperatures
C2.2.4.3	Refrigerators and freezers should be coupled to recording thermometers
C2.2.4.4	Refrigerators and freezers should be coupled to alarm systems with an audible alarm where it can be heard 24 hours a day
C2.2.4.5	Corrective actions for when the temperature is outside the documented acceptable range must be defined and documented
C2.2.4.6	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
C2.2.5	**Cell Culture**
C2.2.5.1	Laboratories performing procedures which require cell culture must have the following:
C2.2.5.2	Laminar Flow Hoods or other appropriately aseptic work area
C2.2.5.3	Incubators, which must be:
C2.2.5.3.1	Appropriately humidified and
C2.2.5.3.2	Monitored daily in relation to:
C2.2.5.3.2.1	Temperature
C2.2.5.3.2.2	CO2 concentration (5% ± 1%)
C2.2.6	**Image Capture Device**
C2.2.6.1	Where a scanner is used for acquisition of the raw data, a second visual reading must be performed to confirm data
C2.2.7	**Bead Analyser**
C2.2.7.1	General Instrument standardisation and maintenance
C2.2.7.1.1	An automated integrated multi-parameter standardisation must be performed, for which
C2.2.7.1.1.1	The reagents specified by the manufacturer to perform this test must be used
C2.2.7.1.1.2	The instrument must only be used if the test has passed
C2.2.7.1.1.3	The frequency of standardisation must conform to manufacturer's instructions and must be performed at any time that the temperature delta check is not correct

C2.2.8	**Sequencer**
C2.2.8.1	Sequencing platform and the version of associated software, reagents and accessories must be specified
C2.3	**Reagents**
C2.3.1	**Storage and labelling**
C2.3.1.1	All reagents must be properly stored according to manufacturers' instructions or locally-specified conditions to maintain reactivity and specificity
C2.3.1.2	Reagents, solutions, culture media, controls, calibrators and other materials must be labelled to indicate:
C2.3.1.2.1	Identity and when significant, titre, strength or concentration
C2.3.1.2.2	Storage requirements
C2.3.1.2.3	Expiration date and other pertinent information
C2.3.1.2.4	Preparation date for in house reagents
C2.3.1.2.5	For storage of larger numbers of identical samples, it might be acceptable to use short-cut labelling of individual samples if the short-cut notation is explained on the outside of the storage container
C2.3.2	**Shipment and Lot Control**
C2.3.2.1	The appropriate performance of individual products must be documented before results using these reagents are reported for:
C2.3.2.1.1	Each shipment, and
C2.3.2.1.2	Each lot
C2.3.2.2	For commercial kits, the following information must be documented:
C2.3.2.2.1	Source
C2.3.2.2.2	Lot number
C2.3.2.2.3	Expiry date
C2.3.2.2.4	Storage conditions
C2.3.2.3	Reagents from different lots of commercial kits must not be mixed, unless either:
C2.3.2.3.1	Specified by the manufacturer, or
C2.3.2.3.2	Validated and documented with appropriate quality control in the laboratory
C2.3.2.4	There must be policies and procedures to address at least:

C2.3.2.4.1	Reagent standardisation and optimisation
C2.3.2.4.2	Reagent validation
C2.3.2.4.3	Incubation times
C2.3.2.4.4	Incubation temperatures
C2.3.2.5	Controls
C2.3.2.5.1	Each lot and shipment of antibody testing kits must be tested with a negative and a positive control serum
C2.3.2.5.2	Dilution of reagents and controls must be defined and documented
C2.3.2.5.3	Negative control serum must be:
C2.3.2.5.3.1	A serum from non-alloimmunised human donor(s), and
C2.3.2.5.3.2	Screened and found to be negative by bead array screening methods.
C2.3.2.5.4	Positive control serum must be:
C2.3.2.5.4.1	a human serum,
C2.3.2.5.4.2	Specific for HLA antigens
C2.3.2.5.4.3	Of the appropriate isotype
C2.3.2.5.5	Appropriate controls for background and reagents must be included in each test (i.e. negative control bead, positive control bead)
C2.3.2.6	Commercial kits must be used according to the manufacturer's instructions, or
C2.3.2.7	The laboratory must perform and document testing to support a deviation in the technique or analysis
C2.3.2.8	Each lot of reagents must be validated and shown to have comparable reactivity to a previously validated lot
C3	**PREANALYTICAL**
C3.1	**Specimen Submission and Requisition**
C3.1.1	The laboratory must have available and follow written policies and procedures regarding specimen collection
C3.1.2	The laboratory must perform tests only at the written or electronic request of an authorised person
C3.1.3	The laboratory must assure that the requisition includes:
C3.1.3.1	The patient's or donor's name or other method of specimen identification to assure accurate reporting of results

C3.1.3.2	The name and address of the authorised person or of the service who ordered the test
C3.1.3.3	Date of specimen collection
C3.1.3.4	Time of specimen collection, when pertinent to testing
C3.1.3.5	Source of specimen (e.g. bone marrow, spleen cells) if pertinent
C3.1.4	Blood or tissue samples must be individually labelled with:
C3.1.4.1	The name, and/or other unique identification marker of the individual
C3.1.4.2	Date of collection
C3.1.5	When multiple blood containers are collected, each container must be individually labelled
C3.1.6	All biological samples must be handled and transported in accordance with the understanding that they could transmit infectious agents
C3.1.7	The laboratory must provide all service users with information about the requirement for
C3.1.7.1	Sample labelling
C3.1.7.2	Anticoagulant / preservation media
C3.1.7.3	Sample packaging
C3.1.7.4	Regulations relating to postal transport
C3.1.7.5	The laboratory should warn users that failure to meet these requirements may result in sample rejection.
C3.2	**Sample Acceptance**
C3.2.1	The laboratory must:
C3.2.1.1	Maintain a system to ensure reliable specimen identification
C3.2.1.2	Document each step in the processing and testing of patient specimens to assure that accurate test results are recorded
C3.2.1.3	Have criteria for specimen rejection
C3.2.1.4	Have mechanism to assure that specimens are not tested when they do not meet the laboratory's criteria for acceptability
C3.3	**Phlebotomy Service**
C3.3.1	If the laboratory provides a phlebotomy service:
C3.3.1.1	Blood samples must be obtained using a location, which does not compromise aseptic techniques
C3.3.1.2	The donor's skin must be prepared by a technique, which ensures minimal possibility of:
C3.3.1.2.1	Infection of the donor
C3.3.1.2.2	Contamination of the sample
C3.3.1.3	All needles and syringes must be for single use and disposable

C4	**DATA STANDARDS**
C4.1	The laboratory must have available a policy considering the data integrity of all information management systems used including:
C4.1.1	Storage and back-up of data (input, raw data, intermediate and final data) must be defined in accordance with the national laws
C4.1.2	Limiting access to data based on user role
C4.1.3	The prevention of unauthorised access to data
C4.1.4	The storage of data, both internal and external to information management systems, and how data is transferred between internal and external storage
C4.2	**Allele database**
C4.2.1	Must be documented
C4.2.2	Must be updated at least once a year with the most current version of the IPD-IMGT/HLA database
C4.3	**Databases of HLA sequences used to interpret the primary data**
C4.3.1	Must be documented
C4.3.2	Must be accurate
C4.3.3	Must be archived or a record retained according to any regulation the laboratory is obliged to abide, but for a minimum of four years
C4.4	**Bioinformatics**
C4.4.1	Algorithms for modification of raw sequence reads must be described in detail and validated (i.e. sequence trimming, quality filtering)
C4.4.2	Detailed documentation and validation of the bioinformatics process supporting the analysis, interpretation and reporting results must be established
C4.4.3	Revalidation of bioinformatics processes must be performed after upgrading or changes of any affected components
C4.4.4	Storage and back-up of data (input, raw data, intermediate and final data) must be defined in accordance with the national laws
C4.4.5	The version of the bioinformatics process must be traceable for each sample analysed
C4.4.6	Each sample processed must be traceable through the whole process including data analysis and reporting
C4.5	**Computer Assisted Analysis**
C4.5.1	The Laboratory Director, the Supervisor or a designated individual must
C4.5.1.1	Review
C4.5.1.2	Verify

C4.5.1.3	Sign computer assisted analyses before issue
C4.5.2	The computer software programme used for analyses must be:
C4.5.2.1	Identified
C4.5.2.2	Validated/Verified before use
C4.6	**Records**
C4.6.1	The laboratory must maintain the following records:
C4.6.1.1	Records of subjects tested for two years or longer, depending on local regulations. These records must include:
C4.6.1.1.1	Log books
C4.6.1.1.2	Worksheets, that must clearly identify:
C4.6.1.1.2.1	The sample tested
C4.6.1.1.2.2	The reagents used
C4.6.1.1.2.3	The methods used
C4.6.1.1.2.4	The test performed
C4.6.1.1.2.5	The date of the test
C4.6.1.1.2.6	The person performing the test
C4.6.1.1.3	A brief description of the specimen (blood, lymph node, spleen, bone marrow, etc.) used for testing
C4.6.1.1.4	results obtained
C4.6.1.1.5	the report issued
C4.6.2	Records may be only saved in computer files, provided that back-up files are maintained to ensure against loss of data
C4.6.3	For molecular typing, a 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 read out from a sequencer
C4.6.3.1	The record must be kept according to any regulation to which the laboratory is obliged to abide, but for a minimum of two years

SECTION D – EXTERNAL PROFICIENCY TESTING

D1	**PROCEDURE OF EPT**
D1.1	Registration for EPT schemes
D1.1.1	The laboratory must participate in EPT programme(s) to cover

D1.1.1.1	All the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.)
D1.1.1.2	All techniques used individually or in combination as routinely employed to produce a final result
D1.1.2	If no established scheme exists for a specific category (e.g. HNA antibody detection and identification) laboratory must participate in an EPT workshop or trial offered by an EPT Provider or must take part in an inter-laboratory exchange of samples
D1.1.3	If (an) EPT scheme(s) or EPT workshop(s)/trial(s) for a specific category exist(s) but the laboratory has no access, the laboratory must at least participate in an inter-laboratory exchange of samples.
D1.2	The laboratory must prospectively define core and supplemental techniques according to the Accreditation Application.
D1.2.1	Core techniques are used individually or in combination to produce a final result
D1.2.2	Supplemental techniques are used occasionally for rare cases in combination with core techniques to refine final results
D1.2.3	For HLA disease association or pharmacogenetic testing: If a different technique is used than that applied for routine HLA genotyping (e.g. a positive/negative kit for the HLA allele(s)/antigen(s) of interest), it is considered a core technique and must be tested through a relevant HLA disease association/pharmacogenetic EPT scheme.
D1.3	The laboratory must
D1.3.1	Prospectively document the relevant EPT schemes or workshops on an annual basis
D1.3.2	Have a predetermined policy for testing EPT samples and must document this prior to the annual commencement of the EPT cycle
D1.3.3	Have a predetermined policy if they select individual shipments or samples for EPT
D1.3.4	Have a predetermined policy for the selection of samples or shipments for supplemental techniques
D1.4	EPT samples must be
D1.4.1	Tested by the same techniques as routinely employed for clinical samples, either individually or in combination
D1.4.2	Interpreted in a manner comparable to routine clinical samples
D1.4.3	Incorporated into the laboratory's routine workload
D1.4.4	If the same sample is tested for more than one accreditation category, e.g. both antibody testing and antibody identification, the results must be analysed independently
D1.4.5	If the same sample is tested by more than one technique in the same accreditation category, e.g. low resolution typing, the laboratory should make only one report to the provider, but must keep results obtained by different techniques available for inspection
D1.5	The minimum number of samples applies to all core techniques used to produce a final result:

D1.5.1	HLA typing:
D1.5.1.1	Serological typing: 10 samples
D1.5.1.2	Each low resolution DNA-based typing technique: 10 samples
D1.5.1.3	Each high resolution DNA-based typing technique: 10 samples
D1.5.1.4	Each allelic resolution DNA-based typing technique: 10 samples
D1.5.1.5	HLA disease association or pharmacogenetic testing: 10 samples if a different technique is used than that for HLA genotyping
D1.5.1.6	HPA/HNA/KIR/MICA typing: 10 samples
D1.5.1.7	HLA antibody detection: 10 samples for HLA class I and 10 samples for HLA class II. The same samples can be used for the detection of both classes.
D1.5.1.8	HLA antibody identification by CDC: 10 samples
D1.5.1.9	HLA antibody identification by solid phase assays: All HLA class I and II antibody positive samples as defined in D1.5.1.7. If the HLA antibody identification is a separate scheme the minimum number is 10 samples. A laboratory may test only for class I or class II antibodies according to their clinical requirements and D1.1
D1.5.1.10	HNA/HPA/MICA antibody detection and identification: 5 samples
D1.5.1.11	Crossmatching: 20 tests of different donor/recipient combinations of each accredited cell subtype (B-/T-/unseparated cells) which must include a minimum of two cell samples and 10 different sera.
D1.5.1.12	Haematopoietic chimaerism and engraftment monitoring: 10 tests of different donor/recipient mixtures in the range 0% - 100% excluding the reference donor and recipient samples
D.1.5.2	For supplemental techniques the number of EPT samples tested must be in accordance to the predetermined policies described in D1.2 and D1.3.
D2	**REPORTING OF EPT RESULTS**
D2.1	For phenotyping/genotyping schemes participants must report:
D2.1.1	The antigen specificities and alleles identified, unless a specific HLA disease association or pharmacogenetic scheme is used. In such cases, either the HLA type or a positive/negative result for the HLA allele(s)/antigen(s) of interest can be reported, in line with the EPT provider scheme instructions in accordance with Section F.
D2.1.2	The method(s) used
D2.2	For the detection of HLA class I and/or class II antibodies, participants must report:
D2.2.1	The presence or absence of HLA class I and/or class II antibodies
D2.2.2	The antibody specificities identified
D2.2.3	The methods used

D2.3	For crossmatching, participants must report:
D2.3.1	The test results
D2.3.2	The method(s) used
D2.4	For haematopoietic chimaerism and engraftment monitoring, participants must report:
D2.4.1	The test results as a percentage of donor and/or recipient chimaerism
D2.4.2	The method(s) used
D2.4.3	Details of the kit(s) and the manufacturer(s) used
D3	**LABORATORY PERFORMANCE**
D3.1	A laboratory must declare all results of EPT schemes according to D1.1 to the EFI Accreditation Program.
D3.2	If a laboratory's performance in EPT programme(s) is unsatisfactory in any category for which EFI accreditation is sought, the laboratory must:
D3.2.1	Continue to participate in an EPT program for that category
D3.2.2	Document the Director’s review and ensure that any corrective action is implemented and reviewed for efficacy
D3.2.3	Where possible test additional EPT samples if provided by the same Provider or participate in an additional EPT programme in that category
D3.3	Laboratories must not engage in inter-laboratory communication pertaining to EPT results until after the reporting deadline has passed
D3.4	Laboratories must not send their own EPT samples or results for analysis to another laboratory until after the reporting deadline has passed
D3.5	Participating laboratories must ensure that all the following EPT related documents are maintained and are made available to EFI inspectors for assessment:
D3.5.1	All data and analyses produced for all techniques
D3.5.2	Results submitted to the EPT Provider
D3.5.3	EPT summary/scheme reports
D3.5.4	Certificates generated by the EPT Provider
D3.5.5	Outcomes of investigations of any unsatisfactory results
D3.5.6	Corrective or preventive actions

SECTION E – ANALYSIS PROCESSES

E1	**METHODS**
E1.1	**CDC**
E1.1.1	**Reagents For CDC**
E1.1.1.1	Complement
E1.1.1.1.1	Each lot and shipment of complement must be evaluated by either:
E1.1.1.1.1.1	Testing with at least 3 previously evaluated trays for every application for which it is intended, or
E1.1.1.1.1.2	Testing a combination of at least 3 sera and 2 cells selected to include negative, weak positive and strong positive reactions
E1.1.1.1.2	The test must employ multiple dilutions of complement to ensure that it is maximally active at least one dilution beyond that intended for use
E1.1.1.1.3	Complement must be tested separately for use with each type of target cell
E1.1.1.1.4	Each new lot and shipment of complement must be tested and evaluated to determine that:
E1.1.1.1.4.1	It mediates cytotoxicity in the presence of specific HLA antibody
E1.1.1.1.4.2	It is not cytotoxic in the absence of HLA specific antibody
E1.1.1.2	Controls
E1.1.1.2.1	Each tray must include:
E1.1.1.2.1.1	At least one positive control antibody, which must either be a validated monoclonal antibody or sera from highly alloimmunised individuals and documented to react with HLA antigens or a control specified by a manufacturer.
E1.1.1.2.1.2	At least one negative control serum that lacks leukocyte reactive antibodies
E1.1.1.2.2	Procedures that deal with control serum failures in typing, antibody or crossmatch trays must be described in the laboratory manual
E1.1.1.2.3	If the positive control fails to react as expected, there must be a procedure in place as whether to accept or reject the test
E1.1.1.2.4	The minimum viability of the cells and the reactivity of control sera required for the validation of a serological typing must be described in the laboratory manual
E1.1.1.3	**Reagents For Serological Typing**
E1.1.1.3.1	In House Reagents
E1.1.1.3.1.1	Cell panel of known HLA type:

E1.1.1.3.1.1.1	Must be used to prove the specificity of new antibodies
E1.1.1.3.1.1.2	Should include at least one example of each HLA antigen the laboratory is required to define
E1.1.1.3.1.2	Each monoclonal antibody used for alloantigen assignment must be used with an established technique at a dilution which demonstrates specificity comparable to antigen assignment by alloantisera on a well-defined cell panel
E1.1.1.3.1.3	For reagent grade typing serum:
E1.1.1.3.1.3.1	Confirmation of specificity must be performed
E1.1.1.3.1.3.2	Supporting statistical analysis must be recorded
E1.1.1.3.1.4	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
E1.1.1.3.2	Commercial Typing Trays: Lots And Shipments
E1.1.1.3.2.1	Each lot of typing trays must be evaluated by testing, either:
E1.1.1.3.2.1.1	At least five different cells of known phenotype representing major specificities
E1.1.1.3.2.1.2	In parallel with previously evaluated trays with at least five cells of known phenotype
E1.1.1.3.2.2	Each new shipment of previously evaluated typing trays must be verified with at least one cell of known phenotype
E1.1.2	**Technical Requirements**
E1.1.2.1	**Recording Test Results**
E1.1.2.1.1	For testing by Complement Dependent Cytotoxicity, each serum-cell combination must be recorded in a manner which indicates the percentage of cells killed. Numerical scores used should be:
E1.1.2.1.1.1	Scores used by the International Workshop (0,1,2,4,6,8), or
E1.1.2.1.1.2	Other numerical codes
E1.1.2.2	**HLA typing**
E1.1.2.2.1	For each of the following loci, the laboratory must be able to type for HLA specificities which are officially recognised by the WHO:
E1.1.2.2.1.1	HLA-A and -B when applying for accreditation in the category of class I by serology
E1.1.2.2.1.2	HLA-DR when applying for accreditation in the category of class II by serology
E1.1.2.2.2	Techniques used must be those, which have been established to define HLA Class I and II specificities optimally
E1.1.2.2.3	Antigen Assignments
E1.1.2.2.3.1	Each HLA-A and -B antigen must be defined by:

E1.1.2.2.3.1.1	At least two sera when available, if both are operationally monospecific, or
E1.1.2.2.3.1.2	If multispecific, at least three partially non-overlapping sera
E1.1.2.2.3.2	Each HLA Class II antigen should be defined by:
E1.1.2.2.3.2.1	At least three sera, if all are operationally monospecific
E1.1.2.2.3.2.2	At least five partially non-overlapping sera if multispecific
E1.1.2.2.3.3	Criteria for antigen assignment must be described in the laboratory manual
E1.1.2.2.3.4	Ambiguity in antigen definition by serological typing must be referred for confirmation by DNA based methods
E1.1.2.2.4	Typing for a single antigen by CDC
E1.1.2.2.4.1	Cell Controls must:
E1.1.2.2.4.1.1	Be tested on each batch
E1.1.2.2.4.1.2	Include at least two cells known to express the specified antigen
E1.1.2.2.4.1.3	Include at least two cells for each cross reacting antigen, which might be confused with the specific antigen
E1.1.2.2.4.1.4	Include at least two cells lacking the specific and cross reacting antigens
E1.1.2.2.4.2	Serum Controls must
E1.1.2.2.4.2.1	Be tested at the time of typing
E1.1.2.2.4.2.2	include a positive and negative control
E1.1.2.2.4.2.3	Serum controls should also include two sera for each antigen which cross reacts with the specified antigen (if available)
E1.1.2.3	**Antibody Testing**
E1.1.2.3.1	If sera are screened after treatment with dithiothreitol, IgG and IgM positive controls must be used
E1.1.2.3.2	Panels
E1.1.2.3.2.1	The panel must include sufficient HLA antigens to ensure that they are appropriate for the population served and the use of the test results. Must be documented in the lab
E1.1.2.3.2.2	For assays intended to provide information on antibody presence or antibody identification, documentation of the HLA class I and/or class II specificities of the panel must be provided

E1.2	**Enzyme-Linked Immuno Sorbent Assay (ELISA)**
E1.2.1	**Equipment for ELISA**
E1.2.1.1	**Microplate Washer**
E1.2.1.1.1	The performance of the microplate washer must be checked at least monthly.
E1.2.1.2	**ELISA Reader**
E1.2.1.2.1	The light source must produce the intensity and wavelength of light required for the test system
E.1.2.1.2.2	Periodic calibration must be performed according to the manufacturer's instructions
E1.2.2	**Technical Requirements for ELISA**
E1.2.2.1	Sera must be tested at a concentration determined to be optimal for the detection of antibody to HLA antigens with the test system used
E1.2.2.2	Quality Management and Controls
E1.2.2.2.1	Sample identity and proper plate orientation must be maintained throughout the procedure
E1.2.2.2.2	The lot numbers and optical density values for the reference reagents, controls and test samples must be recorded for each assay
E1.2.2.2.3	The test results must meet defined criteria for reference reagents and controls in order for the test to be valid
E1.2.2.2.4	Negative Control
E1.2.2.2.4.1	A negative control must be included in each assay, and
E1.2.2.2.4.2	Must include a serum from a non-alloimmunised human donor(s)
E1.2.2.2.5	Positive Control
E1.2.2.2.5.1	A positive control must be included in each assay and
E1.2.2.2.5.2	Must be a human serum specific for HLA antigens and of the appropriate isotype
E1.2.2.2.6	Reagent Controls
E1.2.2.2.6.1	A control lacking only HLA antigen must also be included in each assay
E1.3	**Flow Cytometry**
E1.3.1	**Reagents for Flow Cytometry**
E1.3.1.1	Specificity of labelling reagents for identification of cell subsets:
E1.3.1.1.1	The specificity of labelling reagents must be verified using a published method and/or the manufacturer's documentation and/or by local documented quality control testing

E1.3.1.1.1.2	If locally defined, the specificity of labelling reagents must be verified using appropriate control cells, prepared and tested by the same method employed in the laboratory's test sample analysis
E1.3.1.2	Secondary labelling reagents:
E1.3.1.2.1	Must be titrated to determine the dilution with optimal activity (signal to noise ratio)
E1.3.1.2.2	If a multicolour technique is employed, the reagent must not cross-react with the other immunoglobulin reagents used to label the cells or appropriate compensation must be performed.
E1.3.2	**Equipment for Flow Cytometry**
E1.3.2.1	Flowcytometer
E1.3.2.1.1	Optical Standardisation
E1.3.2.1.1.1	The optical standard must be run:
E1.3.2.1.1.1.1	Every day of instrument use unless otherwise specified by the manufacturer
E1.3.2.1.1.1.2	Any time maintenance or adjustment of the instrument during operation is likely to have altered optical alignment
E1.3.2.1.1.2	The optical standard must consist of latex beads or other uniform particles
E1.3.2.1.1.3	A threshold value for acceptable optical standardisation must be established for all relevant signals
E1.3.2.1.1.4	The results of optical focusing / alignment must be recorded and fall within the defined acceptable range
E1.3.2.1.2	Fluorescence standardisation
E1.3.2.1.2.1	The fluorescence standard:
E1.3.2.1.2.1.1	Must be run every day of instrument use unless otherwise specified by the manufacturer
E1.3.2.1.2.1.2	Must be run any time maintenance or adjustment of the instrument during operation is likely to have altered settings
E1.3.2.1.2.1.3	Must be used for each fluorochrome employed in analytical procedures
E1.3.2.1.2.1.4	The results of fluorescence standardisation must fall within the defined acceptable range
E1.3.2.1.2.1.5	The results of fluorescence standardisation must be recorded
E1.3.2.1.3	Compensation
E1.3.2.1.3.1	If performing analyses that require the simultaneous use of two or more fluorochromes, an appropriate procedure to compensate for overlap in their emission spectra must be used
E1.3.2.1.3.2	Compensation settings must be determined every day of use, and
E1.3.2.1.3.3	At any time maintenance or adjustment of the instrument during operation is likely to have altered them
E1.3.2.1.3.4	Compensation must be carried out for all fluorochromes used

E1.3.2.1.3.5	Compensation values
E1.3.2.1.3.5.1	The Compensation method and it's application must be well defined. The methodology must be reviewed to ensure it is acceptable for the test.
E1.3.2.1.3.5.2	The values used must be recorded
E1.3.3	**Technical Requirements for Flow Cytometry**
E1.3.3.1	**Antibody Testing and Crossmatching**
E1.3.3.1.1	Cell based testing
E1.3.3.1.1.1	Labelling of target cells
E1.3.3.1.1.1.1	An individual fluorochrome must be used for the identification of each population subset (multicolour technique)
E1.3.3.1.1.1.2	If a single colour technique is used, the purity of the isolated cell population
E1.3.3.1.1.1.2.1	Must be sufficient to define the population for analysis
E1.3.3.1.1.1.2.2	Must be documented
E1.3.3.1.1.2	The target sub-populations analysed
E1.3.3.1.1.2.1	Must be defined
E1.3.3.1.1.2.2	Must include a sufficient number of acquired events per sub-population, relevant to the test performed
E1.3.3.1.1.2.3	Must be identified by appropriate labelling antibodies
E1.3.3.1.1.3	The binding of human immunoglobulin on target cells must be assessed with a fluorochrome labelled F(ab') anti-human IgG specific for the Fc region of the heavy chain
E1.3.3.1.1.4	Interpretation instructions must include details of:
E1.3.3.1.1.4.1	The threshold for significant levels of antibody binding (positivity)
E1.3.3.1.1.4.2	The mechanism for reporting positive results (mean, mode or median channel shifts, relative mean fluorescence, or number of molecules of fluorescent marker)
E1.3.3.1.1.5	Acceptable reactivity required for negative, positive and secondary control reagents, in order for the test to be valid must be
E1.3.3.1.1.5.1	Defined
E1.3.3.1.1.5.2	Documented
E1.3.3.2	**Cell-based HLA typing by flow cytometry (e.g. HLA B27)**
E1.3.3.2.1	Labelling reagents for the identification of HLA specificities

E1.3.3.2.1.1	The specificity of each lot and shipment must be determined by testing:
E1.3.3.2.1.2	At least five cells known to express the target antigen
E1.3.3.2.1.3	At least two cells for each cross-reacting antigen
E1.3.3.2.1.4	At least two cells which lack the specific and cross-reacting antigens
E1.3.3.2.1.5	Acceptable criteria for validation must be defined and results must be recorded
E1.3.3.2.1.6	Each lot and shipment of labelling reagents must be shown to have comparable reactivity to the previously validated lot and shipment
E1.3.3.2.2	Controls
E1.3.3.2.2.1	Controls for HLA typing by flow cytometry must be run for each test cell preparation
E1.3.3.2.2.2	Negative Controls
E1.3.3.2.2.2.1	For direct labelling, a negative control must be conjugated with the same fluorochrome as the test
E1.3.3.2.2.2.2	For indirect labelling, a negative control should be used in conjunction with the same secondary antibody conjugated with the same fluorochrome as used for the specific antibody under test
E1.3.3.2.2.3	Positive Controls
E1.3.3.2.2.3.1	For indirect labelling, a pan-reacting anti-HLA monoclonal antibody must be included, which
E1.3.3.2.2.3.1.1	Must be tested against each cell
E1.3.3.2.2.3.1.2	Must be tested under the same conditions as the antibodies under test
E1.3.3.2.2.3.2	A control cell known to express the HLA specificity under test must be included in each run
E1.3.3.2.3	Interpretation instructions must define:
E1.3.3.2.3.1	The required reaction criteria in the negative and positive control samples for the test results to be valid
E1.3.3.2.3.2	Criteria for positivity of the HLA antigen under test
E1.3.3.2.3.3	A documented procedure must be followed for monoclonal antibodies which react with antigens other than those expected
E1.4	**Bead Array**
E1.4.1	Bead based antibody testing and crossmatching
E1.4.1.1	Interpretation instructions must include details of:
E1.4.1.1.1	The threshold for significant levels of antibody binding (positivity)

E1.4.1.2	Acceptable reactivity required for negative, positive and secondary control reagents, in order for the test to be valid must be
E1.4.1.2.1	Defined
E1.4.1.2.2	Documented
E1.5	**Nucleic Acid Analysis- General**
E1.5.1	**Reagents for Nucleic Acid Analysis**
E1.5.1.1	All reagents (solutions containing one or multiple components) must either be:
E1.5.1.1.1	Dispensed in aliquots for single use, or
E1.5.1.1.2	Dispensed in aliquots for multiple use if documented to be free of contamination at each use
E1.5.1.2	When reagents are combined to create a master mix, one critical component (e.g. DNA polymerase) should be left out of the mixture
E1.5.1.3	Each lot and shipment of commercial kits must be tested against at least one DNA sample of known type
E1.5.1.4	In house primers
E1.5.1.4.1	The specificity of primer combinations and the annealing positions must be defined
E1.5.1.5	Laboratories must:
E1.5.1.5.1	Have a policy for quality control of each lot or shipment of primers
E1.5.1.5.2	Confirm the specificity and quantity of the amplified product using reference material
E1.5.1.5.3	Use primers under empirically determined conditions that achieve the defined specificity for templates used in routine testing
E1.5.1.5.4	Test each lot of local primers for amplification specificity and quantity using reference material whenever available
E1.5.1.5.5	Test each lot of local primers with reference DNA for appropriate sensitivity and specificity
E1.5.1.6	Oligonucleotide probes
E1.5.1.6.1	The specificity of each probe and target sequence must be defined
E1.5.1.6.2	For in house kits each lot must be tested with reference DNA so that each probe is tested for specificity and signal intensity at least once
E1.5.1.6.3	The specificity and signal intensity for each probe must be defined and monitored
E1.5.1.6.4	Nucleic acid probes must be utilised under empirically determined conditions that achieve the defined specificity

E1.5.2	**Equipment for Nucleic Acid Analysis**
E1.5.2.1	**Automated Hybridization Device**
E1.5.2.1.1	The calibration of the pumps and of the heating elements must be performed according to the manufacturer's specification at least once a year
E1.5.2.2	**Real Time PCR systems**
E1.5.2.2.1	Accuracy of the instruments:
E1.5.2.2.1.1	Must be verified by annual thermal verification of the block using a calibrated device designed specifically for this purpose
E1.5.2.2.1.2	Calibration of the fluorescence detection must be performed at least annually where indicated
E1.5.2.3	**Thermal Cyclers**
E1.5.2.3.1	Accuracy of thermal cycling instruments:
E1.5.2.3.1.1	Must be verified by annual thermal verification of the block using a calibrated device or by PCR method designed specifically for this purpose
E1.5.3	**Technical Requirements for Nucleic Acid Analysis**
E1.5.3.1	If a manual allele call or interpretation of positive/negative reactions is performed two independent interpretations of primary data must be performed, except under justified special emergency situations
E1.5.3.2	Nucleic acid extraction
E1.5.3.2.1	The method used for nucleic acid extraction:
E1.5.3.2.1.1	Must be published and documented
E1.5.3.2.1.2	Must be validated in the laboratory
E1.5.3.2.2	Purity and concentration of Nucleic Acids:
E1.5.3.2.2.1	Must be sufficient to ensure reliable test results
E1.5.3.2.2.2	Should be determined for each sample, or
E1.5.3.2.2.3	If not determined for each sample, the laboratory must have tested and validated this policy
E1.5.3.2.3	If the DNA is not used immediately after purification, suitable methods of storage must be available that will protect the integrity of the material
E1.5.3.3	Electrophoresis
E1.5.3.3.1	Optimal electrophoretic conditions must be documented
E1.5.3.3.2	The laboratory must establish criteria for accepting each slab or capillary gel migration, and each lane of a gel or capillary injection

E1.5.3.3.3	When the size of an amplicon is a critical factor in the analysis of data, size markers that produce discrete electrophoretic bands spanning and flanking the entire range of expected fragment sizes must be included in each run
E1.5.4	Analysis
E1.5.4.1	Signal intensity
E1.5.4.1.1	Acceptable limits of signal intensity must be specified for positive and negative results
E1.5.4.1.2	If these are not achieved, acceptance of the results must be justified and documented
E1.5.4.2	The method of allele assignment must be designated
E1.6	**Contamination control ("wipe-test")**
E1.6.1	Contamination must be monitored for amplification products produced in the laboratory
E1.6.2	Routine wipe-tests must:
E1.6.2.1	Include pre-amplification work areas
E1.6.2.2	Include pre-amplification equipment
E1.6.2.3	Be performed at least every two months
E1.6.2.4	Be performed using a method that is at least as sensitive as routine test methods
E1.6.2.5	Include positive controls to assure proper performance of monitoring
E1.6.2.6	Include inhibition controls to assure proper performance of monitoring
E1.6.2.6.1	Actions need to be taken if inhibition control is weaker than positive control
E1.6.2.7	Include other areas of the laboratory as relevant
E1.6.3	If amplified product is detected, there must be:
E1.6.3.1	Written description of how to eliminate the contamination
E1.6.3.2	Measures taken to prevent future contamination
E1.6.3.3	Evidence of elimination of the contamination
E1.7	**Sequence-Specific Primers (SSP)**
E1.7.1	**Technical Requirements For SSP**
E1.7.1.1	Each amplification reaction must include controls 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)

E1.7.1.2	When a typing exhibits reactions with no specific amplicon or internal control amplification, the laboratory must have a policy in place on how to accept or reject the whole typing
E1.7.1.3	The laboratory must use the following data in the interpretation phase of the typing:
E1.7.1.3.1	Information derived from the validation process
E1.7.1.3.2	Information derived from previous typings with the same lot of primers
E1.7.1.4	The following must be defined and documented:
E1.7.1.4.1	Non-specific and weak amplifications
E1.7.1.4.2	Any tendency to form primer-dimer
E1.8 Assays	**Sequence-Specific Oligonucleotide Probe (SSOP) Hybridization**
E1.8.1	**Technical Requirements for SSOP**
E1.8.1.1	The amplification should be monitored by electrophoresis before the hybridization is performed
E1.8.1.2	Each assay must include:
E1.8.1.2.1	A probe internal to a conserved region of the amplified fragment
E1.8.1.2.2	Appropriate controls to validate the hybridization and the detection steps
E1.8.1.2.3	A negative (no DNA) control:
E1.8.1.2.3.1	That must be included in the hybridization and revelation steps of the assay in forward SSOP assays
E1.8.1.2.3.2	That must either be included in the hybridization and detection step of the assay or monitored by electrophoresis in reverse SSOP assays
E1.8.1.3	Interpretation
E1.8.1.3.1	Acceptable limits of signal intensity must be specified for positive and negative results
E1.8.1.3.2	If a test is accepted with probe signals outside the set limits, this must be documented and justified
E1.8.1.3.3	The laboratory must use the data derived from the validation process and from previous typings with the same lot of primers and probes in the interpretation phase of the typing
E1.8.1.3.4	Nonspecific and weak hybridization signals must be defined and documented
E1.9	**Sanger Sequencing**
E1.9.1	**Technical Requirements for Sanger Sequencing**
E1.9.1.1	Sequencing Templates:

E1.9.1.1.1	Must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data.
E1.9.1.1.2	Should be purified after amplification to eliminate the presence of dNTPs, Taq polymerase and amplification primers.
E1.9.1.1.3	Must not contain any inhibitors or contaminants affecting the sequencing reaction.
E1.9.1.1.4	Validation of the methods for template preparation must ensure that the accuracy of the final typing is not altered (e.g. mutations during cloning, preferential amplification).
E1.9.1.1.5	If cloning is used as template preparation, the sequence of at least three different clones for each allele must be determined for accurate results
E1.9.1.2	Sequencing Reaction:
E1.9.1.2.1	The specificity of the template in combination with the sequencing primer (HLA locus and alleles) must be defined.
E1.9.1.2.2	Quantity and quality of templates, sequencing primers and sequencing reagents must be sufficient to provide interpretable primary sequencing data.
E1.9.1.2.3	The conditions for the sequencing reaction must be documented and appropriate for obtaining reliable primary sequencing data.
E1.9.1.3	Nucleotide Assignment:
E1.9.1.3.1	The following criteria for acceptance of primary data must be established (peak intensity, baseline fluctuation, peak shape, correct assignment for non-polymorphic positions)
E1.9.1.3.2	The signal to noise ratio must be sufficient to ensure reliable nucleotide assignments
E.1.9.1.3.3	A scientific 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).
E.1.9.1.4	Established sequence-specific characteristics should be documented and utilized in routine interpretation of data.
E.1.9.2	Allele assignment
E.1.9.2.1	Methods for allele assignement must ensure that sequences contributed by amplification primers are not considered
E.1.9.2.2	If allele assignments are difficult to obtain by sequencing only one strand, routine sequencing of both strands should be performed
E.1.9.2.3	If a sequence suggests a novel allele or a rare combination of alleles, the sequences of both strands must be determined
E1.10	**Next Generation Sequencing**
E1.10.1	**Technical Requirements for Next Generation Sequencing**
E1.10.1.1	Targeted regions
E1.10.1.1.1	Must be defined, documented and validated according to the intended application (e.g. whole or part of an HLA gene)
E1.10.1.2	Sequencing Templates:

E1.10.1.2.1	Must have sufficient purity, specificity, quantity and quality to provide interpretable sequencing data
E1.10.1.2.2	Should be purified after amplification to eliminate the presence of dNTPs, Taq polymerase and amplification primers
E1.10.1.3	The following must be documented:
E1.10.1.3.1	Sample tagging
E1.10.1.3.2	Purification
E1.10.1.3.3	Normalization
E1.10.1.3.4	Pooling methods
E1.10.1.3.5	Length of read
E1.10.1.4	Steps must be taken to prevent creation of PCR artefacts,
E1.10.1.4.1	PCR artefacts must be documented
E1.10.1.4.2	The information must be used in the routine interpretation of data following established policies. (i.e. PCR cross-over and/or artefact)
E1.10.1.5	Controls and procedures must be established to ensure sample tracking during pooling and barcoding
E1.10.1.6	A strategy to prevent contamination must be defined:
E1.10.1.6.1	By periodic barcode rotation
E1.10.1.6.2	If barcode rotation is not possible, another approach must be validated to exclude barcode contamination
E1.10.1.7	If shotgun sequencing is used
E1.10.1.7.1	Method of fragmentation must be specified
E1.10.1.7.2	For each run the size of fragments must be documented and the selection must be specified
E1.10.1.7.3	Methods for enrichment strategies of multi gene panels must be defined.
E1.10.1.8	If nanopore sequencing is used:
E1.10.1.8.1	The number of times a flow cell is to be used must be determined and documented
E1.10.1.9	Nucleotide Assignment
E1.10.1.9.1	The signal to noise ratio must be sufficient to ensure reliable nucleotide assignments
E1.10.1.9.2	A scientific and technically sound method must be established for interpretation, acceptance and/or rejection of sequences from regions which are difficult to resolve

E1.10.1.9.3	Established sequence-specific characteristics should be documented and utilized in routine interpretation of data
E1.10.1.9.4	Percentage of low quality reads and passed filter reads must be specified and in defined range
E1.10.1.9.5	Inability to correctly analyze sequences must be documented, tracked and taken into consideration in result interpretation e.g. homopolymer regions
E1.10.1.10	Allele assignment
E1.10.1.10.1	Methods for allele assignment must ensure that sequences contributed by amplification primers are not considered
E1.10.1.10.2	Criteria for allele assignment must be established
E1.10.1.10.3	Adequate depth of coverage threshold necessary to make accurate allele calls must be established and documented empirically during the validation phase
E1.10.1.10.4	Overlap of sequences must be sufficient to determine the phase of alleles for the methods where phasing is possible
E2	**TECHNICAL APPLICATIONS**
E2.1	**Typing**
E2.1.1	Typing for a single allele-group by molecular techniques
E2.1.1.1	A positive control DNA known to encode the allele-group of interest must be included in each test
E2.1.1.2	A negative control DNA known not to encode an allele belonging to the allele-group of interest must be included in each test
E2.2	**Antibody Testing and Crossmatching**
E2.2.1	Techniques
E2.2.1.1	For the detection of antibody to HLA antigens, the laboratory must either use:
E2.2.1.1.1	A complement-dependent cytotoxic technique, or
E2.2.1.1.2	Another technique performed by the laboratory with documented validation testing, demonstrating that this technique identifies alloantibody to HLA antigens at a level of sensitivity equivalent or superior to that of its cytotoxic technique
E2.2.1.1.3	To detect antibodies to HLA class II antigens, a technique must be used that distinguishes them from antibodies to HLA class I antigens
E2.2.2	Other techniques:
E2.2.2.1	Laboratories performing assays using flow cytometry and/or bead array must also conform to the respective standards in Section E1.3 and E1.4
E2.2.3	Sera

E2.2.3.1	Serum samples stored must be retained in the frozen state
E2.2.3.2	Sera must be tested under conditions optimal for the detection of antibodies to HLA antigens including consideration of the prozone effect. This may involve pretreatment or dilution, which must be documented
E2.2.3.3	Negative control:
E2.2.3.3.1	Each assay must include a negative control
E2.2.3.3.2	The negative control must be a human serum documented to be negative in the assay of intended use
E2.2.3.4	Positive control:
E2.2.3.4.1	Each assay must include a positive control
E2.2.3.4.2	The positive control must be either a validated monoclonal antibody, or sera from highly alloimmunised individuals and documented to react with HLA antigens, or a control specified by manufacturer
E2.2.3.4.3	The antibodies used must be of the appropriate isotype for each assay
E2.2.3.5	Control sera must be tested at the same time and under the same conditions as the sera under test
E2.2.4	**Crossmatching**
E2.2.4.1	Crossmatching for the detection of HLA specific antibodies:
E2.2.4.1.1	Must use techniques at least as sensitive as the basic lymphocytotoxicity test
E2.2.4.1.2	Should use at least one technique documented to have increased sensitivity in comparison with the basic microlymphocytotoxicity test, such as prolonged incubation, antiglobulin test, ELISA, B-cell crossmatch or flow cytometry
E2.2.4.2	The antibody testing result used must be at least as sensitive as the routine crossmatch technique
E2.2.4.3	Each serum must be tested:
E2.2.4.3.1	Undiluted
E2.2.4.3.2	In duplicate
E2.2.4.4	Crossmatches must be performed:
E2.2.4.4.1	With unseparated lymphocytes or with T lymphocytes
E2.2.4.4.2	With B lymphocytes if required by the relevant transplantation programmes
E2.2.4.5	If sera are tested after treatment with dithiothreitol, IgG and IgM positive controls must be used

E2.3	Haemopoietic Chimaerism and Engraftment (HCE) Monitoring
E2.3.1	The polymorphic gene system(s) used for HCE monitoring must be identified and documented with regards to allelic variability
E2.3.2	The sensitivity of the HCE assay must be validated using DNA mixtures from two individuals at defined ratios/concentrations, before implementation into clinical use
E2.3.3	Donor and patient specific allele profiles must be:
E2.3.3.1	Determined using appropriate reference material
E2.3.3.2	Documented
E2.3.4	Optimal ranges of DNA quantity and purity must be:
E2.3.4.1	Defined
E2.3.4.2	Documented
E2.3.4.3	If a sample falls outside these optimal ranges, a statement must be included in the report
E2.3.5	Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined
E2.3.5.1	In case of multiple donors specificity and sensitivity must be defined, limitations should be addressed in the data interpretation and reported
E2.3.6	When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias
E2.3.7	When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population:
E2.3.7.1	Must be documented and
E2.3.7.2	Taken into account in the analysis of the results
E2.3.7.3	If this is not possible it must be clearly stated in the report
E2.3.8	For quantitative HCE monitoring by quantitative PCR (Q-PCR), the following must be defined
E2.3.8.1	Chemistry used
E2.3.8.2	Internal control gene
E2.3.8.3	Thresholds for positive and negative results of each reaction
E2.3.9	All steps of locally developed Q-PCR assays must be validated
E2.3.10	In addition to the requirements from standard F3.2, the report must contain
E2.3.10.1	A description of the method for the isolation/purification of cellular subsets

E2.3.10.2	The date of transplant
E2.3.10.3	Other information if deemed relevant for HCE interpretation (i.e. limited informative markers or clinical condition of the patient)
E2.4	**HPA**
E2.4.1	Clinically significant HPA specificities must be defined and documented
E2.4.2	Investigation of HPA antibodies
E2.4.2.1	Laboratories must make all reasonable efforts to include HPA antigens in their antibody testing protocol which will aid the identification of clinically significant HPA alloantibodies
E2.4.3	The antibody testing technique must
E2.4.3.1	Be validated before use
E2.4.3.1.1	Include positive and negative controls in each assay
E2.4.3.1.1.1	In glycoprotein specific assays, a positive control for each glycoprotein used should be included
E2.4.3.1.1.1.1	The specificity of detected HPA alloantibodies must be defined and recorded
E2.5	**HNA**
E2.5.1	The clinically significant HNA specificities must be defined and documented
E2.5.2	Investigation of HNA Antibodies
E2.5.2.1	Laboratories must make all reasonable efforts to include HNA antigens in their antibody testing protocol which will aid the identification of clinically significant HNA alloantibodies
E2.5.2.1.1	The antibody testing technique must
E2.5.2.1.1.1	Be validated before use
E2.5.2.1.1.1.1	Include positive and negative controls in each assay
E2.5.2.1.1.1.2	In glycoprotein specific assays, laboratories must make all reasonable efforts to include a positive control for each glycoprotein used
E2.5.2.1.1.1.2.1	The specificity of detected HNA alloantibodies must be defined and recorded.
E2.6	**KIR Typing**
E2.6.1	KIR typing must be performed using a validated KIR typing technique.
E2.6.2	Group A and B haplotypes must be determined if required by the transplant protocol
E2.6.2.1	KIR-B content must be calculated for the prospective donors if it is requested by the transplant protocol

E2.6.2.1.1	Mismatches between KIR-Ligands of recipient and donors must be searched and reported if it is requested by the transplant protocol
E2.6.2.1.1.1	KIR allelic resolution must be performed if it is requested by the transplant protocol
E2.7	**MICA**
E2.7.1	MICA typing must be performed using a validated MICA typing technique.
E2.7.2	MICA antibodies
E2.7.2.1	The antibody testing technique must
E2.7.2.1.1	Be validated before use
E2.7.2.1.1.1	Include positive and negative controls in each assay
E2.7.2.1.1.1.1	The specificity of detected MICA alloantibodies must be defined and recorded
E3	**CLINICAL APPLICATIONS**
E3.1	**Renal and/or Pancreas Transplantation**
E3.1.1	If deceased donor transplants are done:
E3.1.1.1	The following personnel must be available 24 hours a day, seven days a week:
E3.1.1.1.1	Personnel for the required histocompatibility testing
E3.1.1.1.2	Personnel for interpretation of results
E3.1.1.1.3	Personnel for advice for the clinical transplant team
E3.1.1.2	Laboratories not able to perform tests 24h/day, 7d/week must arrange with an EFI or ASHI accredited laboratory to perform tests
E3.1.2	Antibody Testing
E3.1.2.1	Laboratories must:
E3.1.2.1.1	Have a documented policy in place to evaluate the sensitisation of each patient at the time of their initial evaluation
E3.1.2.1.2	Have a programme to periodically screen serum samples from each patient for antibodies to HLA antigens by:
E3.1.2.1.2.1	Determining and recording the specificity of detected HLA antibodies
E3.1.2.1.2.2	Performing testing to distinguish HLA specific antibodies from non HLA antibodies and autoantibodies
E3.1.2.1.3	Have a policy establishing the frequency of testing serum samples and must have data to support this policy. Samples must be collected and tested, either:
E3.1.2.1.3.1	Every three months, or

E3.1.2.1.3.2	As stipulated by the national and/or international organ exchange organisations
E3.1.3	Sensitising Events
E3.1.3.1	Laboratories should maintain a record of potentially sensitising events for each patient
E3.1.3.2	Serum samples should be collected and stored after each of these events for possible subsequent testing for antibodies to HLA and/or use in crossmatch tests.
E3.1.4	Crossmatching
E3.1.4.1	Crossmatching must be performed according to national legislation applying to the laboratory and/or regulations from the national / international exchange organisation
E3.1.4.2	Crossmatching must be performed prospectively, or may be omitted if virtual crossmatching is performed
E3.1.4.3	Prospective crossmatching must be performed for all living donor transplants
E3.1.4.4	If the prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM
E3.1.5	Virtual Crossmatching
E3.1.5.1	A transplant protocol for Virtual Crossmatching must be agreed with the clinical transplant teams and documented
E3.1.5.2	There must be evidence that the eligibility of each patient has been evaluated when a virtual crossmatch has been performed
E3.1.5.3	The transplant protocol must include evidence that the clinical teams are aware of the possibility of errors in donor offer typing
E3.1.5.4	The Virtual Crossmatch result must be reported by the laboratory to the transplant clinician before the transplant proceeds
E3.1.5.5	Evidence that the Virtual Crossmatch was reported must be documented
E3.1.5.6	Patients are only eligible for Virtual Crossmatching if
E3.1.5.6.1	There have been no potential sensitising events since the last serum sample screened
E3.1.5.6.2	Sera must have been collected as defined in standard E3.1.2.1.3
E3.1.5.6.3	At least two different samples must have been tested
E3.1.5.6.4	At least one serum testing result obtained within the previous 3 months must be included unless otherwise agreed with the clinical lead in the transplant protocol
E3.1.5.6.5	Sera must be tested for antibody specificity identification by a technique of at least equivalent sensitivity to that used for crossmatching
E3.1.6	Virtual Crossmatching for Unsensitised Patients

E3.1.6.1	A prospective crossmatch may be omitted for patients:
E3.1.6.1.1	Who test consistently negative for the presence of HLA-specific antibodies, as relevant for the transplant protocol
E3.1.6.1.2	For whom there must be documented evidence that the laboratory maintains a record of potentially sensitising events
E3.1.7	Virtual Crossmatching for Sensitised Patients
E3.1.7.1	The prospective crossmatch may be omitted in carefully selected HLA immunised recipients in whom acceptable and/or unacceptable mismatches have been clearly defined and documented
E3.1.7.2	If a prospective crossmatch is omitted from an alloimmunised recipient, the method of antibody identification must rely on single antigen technology
E3.1.7.3	The Virtual Crossmatch must include data from single antigen testing performed on a sample collected within the last 3 months unless otherwise agreed in the transplant protocol
E3.1.7.4	Patients are ineligible for Virtual Crossmatching if they have antibodies against specificities for which the donor has not been typed
E3.1.8	Retrospective Crossmatching
E3.1.8.1	If retrospective crossmatches are performed according to standard E3.1.4.4
E3.1.8.1.1	They must be shown to be in concordance with the predicted negative result and this must be documented
E3.1.8.1.2	The physician in charge must be notified immediately of an unpredicted positive result
E3.1.8.1.3	There must be a re-evaluation of this policy at least annually
E3.1.8.1.4	Any additional regulations either national, or of the exchange organisation must also be applied
E3.1.9	Sera
E3.1.9.1	The laboratory must have a policy regarding the selection of relevant sera to be used in the final crossmatch procedure
E3.1.10	Final crossmatches performed prior to transplantation
E3.1.10.1	Must use a recipient serum sample collected within the previous 48 hours before transplant if the recipient has had a recent sensitising event
E3.1.10.2	Must use the most recent available serum collected as defined in standard E3.1.2.1.3
E3.1.10.3	Should use sera obtained 14 days after a potentially sensitising event
E3.1.11	HLA Typing

E3.1.11.1	Prospective typing of donor and recipient:
E3.1.11.1.1	Must include typing for HLA-A, B and DR antigens
E3.1.11.1.2	Must meet the required specification of the relevant national or international organ sharing scheme and/or regulations
E3.1.11.2	Every effort must be made to perform verification typing for recipients prior to transplantation
E3.1.11.3	Verification typing must be performed on living donors prior to transplantation
E3.2	**Other Organ Transplantation**
E3.2.1	In cases where patients are at high risk for allograft rejection (e.g. patients with histories of allograft rejection, patients with preformed HLA antibodies), donors and recipients must be typed for HLA-A, B and DR antigens
E3.2.2	Cardiothoracic patients must be screened for the presence of HLA alloantibodies, and
E3.2.2.1	Unacceptable specificities must be defined, or
E3.2.2.2	A prospective crossmatch must be performed
E3.2.2.3	Standards E3.1.2 (Antibody Testing) also applies
E3.2.3	Crossmatching
E3.2.3.1	Crossmatching must be performed according to standards in E1.1.2.4 (Crossmatching)
E3.2.3.1.1	Sera from patients at high risk for allograft rejection should be prospectively crossmatched
E3.2.3.1.2	Crossmatch results should be available prior to transplantation of a pre-sensitised patient
E3.2.3.2	Final crossmatches performed prior to transplantation should either:
E3.2.3.2.1	Utilise a recipient serum sample collected within the previous 48 hours before transplant if the recipient has had a recent sensitising event. Or,
E3.2.3.2.2	Utilise a serum collected within three months
E3.2.3.3	Sera obtained 14 days after a potential sensitising event should be used in the final cross-match
E3.2.3.4	Whenever possible, non-renal organs for recipients at high risk for allograft rejection should come from cross-match negative donors as defined by the laboratory and the transplant programme
E3.3	**Haematopoietic Stem Cell Transplantation**
E3.3.1	Typing and Antibody testing
E3.3.1.1	There must be a documented transplant protocol with each transplant program the laboratory serves, which must detail the service provided including:

E3.3.1.1.1	Patient
E3.3.1.1.2	Donor
E3.3.1.1.3	Loci typed (e.g. HLA, KIR etc.)
E3.3.1.1.4	Level of resolution
E3.3.1.1.5	Which party takes responsibility of the histocompatibility component of the transplant
E3.3.1.1.6	The transplant protocol must be signed by all parties
E3.3.2	Histocompatibility testing for related transplants
E3.3.2.1	HLA-A, -B and -DRB1 typing must be carried out on available members of the immediate family
E3.3.2.2	Must include adequate testing:
E3.3.2.2.1	To definitively establish HLA genotype identity (F1.3.2 applies), or
E3.3.2.2.2	To type at high resolution for the relevant loci defined in the transplant protocol, if only phenotype identity has been established, or
E3.3.2.2.3	To include high resolution typing for recipient and potential intra-familial donors who are not HLA identical siblings
E3.3.2.2.4	HLA typing for haploidentical donors
E3.3.2.2.4.1	Identity of the shared haplotype must be defined by descent
E3.3.2.2.4.2	If the identity of the shared haplotype can not be proven a high resolution typing must be performed
E3.3.2.3	HLA-A, B and DR typing as a minimum requirement must be repeated on both the recipient and the potential donor prior to transplantation using a new typing sample from each, so that each individual's typing is confirmed
E3.3.3	HLA typing for Donors (related cord blood unit)
E3.3.3.1	The cord blood unit must be typed using DNA methods for HLA-A, -B and -DRB1 at a minimum of low resolution (e.g. A*02, B*44, DRB1*11)
E3.3.3.2	Extended typing must be included if required by the transplant protocol (standards E3.3.2 also apply)
E3.3.3.3	Prior to transplantation, a verification typing:
E3.3.3.3.1	Must be performed for HLA-A, B and DRB1 at a minimum of low resolution
E3.3.3.3.2	Must be performed on a segment of the tubing integrally attached to the unit, if available, or otherwise, on a satellite vial

E3.3.3.4	If verification typing was not performed on a segment of the tubing integrally attached, the laboratory must recommend that an additional typing is performed on the content of the thawed unit
E3.3.4	Histocompatibility Testing for Unrelated Transplants
E3.3.4.1	Volunteer Bone Marrow Donor Registries
E3.3.4.1.1	Typing of the donors must be performed
E3.3.4.1.1.1	By serology or
E3.3.4.1.1.2	By DNA methods at a minimum of low resolution
E3.3.4.2	Typing of Units for Cord Blood Banks
E3.3.4.2.1	Typing must be performed using DNA methods for HLA-A, B and DRB1, at a minimum of low resolution
E3.3.4.2.2	Typing of additional loci or high resolution typing must be included if required by the policy of the registry, or if requested
E3.3.4.2.3	The identity of the Cord Blood Unit must be verified by HLA typing on a separate sample to demonstrate concordance of results
E3.3.4.2.4	Additional typing may be performed using any stored DNA sample, provided that the identity of the unit has previously been verified
E3.3.4.2.5	The verification of identity and the source of the sample tested must be reported back to the registry
E3.3.4.3	Histocompatibility Testing for Transplants from Unrelated Donors
E3.3.4.3.1	HLA typing for recipient and unrelated donors must:
E3.3.4.3.1.1	Be performed by DNA based methods
E3.3.4.3.1.2	Include as a minimum requirement:
E3.3.4.3.1.2.1	HLA-A/B/C and DRB1 typing at high resolution
E3.3.4.3.1.3	Include additional loci if required by the transplant protocol
E3.3.4.3.1.4	Include higher resolution levels if required by the transplant protocol
E3.3.4.3.2	Prior to transplantation using an unrelated donor, HLA typing of the recipient and donor must be repeated for verification:
E3.3.4.3.2.1	Using a different typing sample
E3.3.4.3.2.2	For HLA-A, -B, and –DRB1, as a minimal requirement
E3.3.4.3.3	For unrelated donors HLA-A,-B,-DRB1 concordant results are required on two separate samples. Registry typing is acceptable as one of the two required results
E3.3.4.4	Unrelated Cord Blood Unit Typing for Donor Selection

E3.3.4.4.1	Verification typing must be performed prior to the conditioning regimen of the recipient on a segment integregrally associated with the cord unit
E3.3.4.4.1.1	Including as a minimum requirement
E3.3.4.4.1.1.1	HLA-A and -B at low resolution, and
E3.3.4.4.1.1.2	HLA-DRB1 at high resolution
E3.3.4.4.1.1.3	Extended typing if required by the transplant protocol
E3.3.4.5	If E3.3.4.4.1 have not been met then additional verification typing must be performed prior to conditioning of the recipient and upon reception of the shipped unit
E3.3.4.5.1	Prior to the conditioning regimen of the recipient, a verification typing must be performed at a minimum level of low resolution for HLA-A, -B, and -DRB1
E3.3.4.5.1.1	On a segment of the tubing integrally attached to the unit, if available; otherwise a satellite vial shipped with the unit may be used
E3.3.4.5.1.2	If no segment is available, this step can be performed after transplantation and must be initiated as soon as possible after thawing the unit
E3.3.5	Antibody testing
E3.3.5.1	Antibody testing must be performed
E3.3.5.1.1	in case of mismatched transplant as specified by the transplant protocol
E3.3.6	Crossmatching
E3.3.6.1	Crossmatching must be performed
E3.3.6.1.1	Prior to related and unrelated transplantation if required by the local transplant protocol
E3.3.6.1.2	According to standards E1.1.2.4 (Crossmatching)
E3.3.7	MICA allelic resolution must be performed if it is requested by the transplant protocol
E3.3.8	Investigation of MICA antibodies
E3.3.8.1	For bead array techniques, standards from section E1.4 (Bead Array) also apply
E3.4	**Transfusion**
E3.4.1	HLA and Transfusion
E3.4.1.1	Platelet refractoriness
E3.4.1.1.1	Platelet refractory patients who require HLA matched platelets
E3.4.1.1.1.1	Must be typed for HLA-A and HLA-B

E3.4.1.1.1.2	If alloimmune refractoriness is suspected the patient must be tested for HLA class I antibodies
E3.4.1.1.2	To provide compatible platelets, either:
E3.4.1.1.2.1	The specificity of detected HLA antibodies against HLA-A and HLA-B must be defined and recorded, or
E3.4.1.1.2.2	Crossmatching must be performed, or
E3.4.1.1.2.3	Platelets from donors with acceptable mismatches for HLA-A and -B must be provided.
E3.4.1.1.3	All selected plateletpheresis donors used for the provision of HLA matched platelets must be typed for HLA-A and HLA-B
E3.4.1.1.4	For crossmatching using lymphocytes standards E1.1.2.4 must be followed
E3.4.2	**Transfusion Related Acute Lung Injury (TRALI)**
E3.4.2.1	For the laboratory investigation of TRALI, the sera from implicated donors must be tested for both HLA class I and class II antibodies
E3.4.2.2	The specificity of detected HLA antibodies must be defined and recorded
E3.4.2.3	If HLA specific antibodies are identified, HLA typing must be performed at least for all relevant loci on
E3.4.2.3.1	The patient
E3.4.2.3.2	The donor
E3.4.2.4	For the laboratory investigation of TRALI, the sera from implicated donors should be tested for HNA antibodies
E3.4.2.4.1	If HNA specific antibodies are identified, HNA typing must be performed at least for all relevant antigens/alleles on
E3.4.2.4.1.1	The patient
E3.4.2.4.1.2	The donor
E3.4.3	Febrile Non Haemolytic Transfusion Reactions (FNHTR) after transfusion of platelet concentrates
E3.4.3.1	The patient’s serum must be tested for the presence of HLA class I antibodies
E3.4.4	**HPA and Transfusion**
E3.4.4.1	Neonatal Alloimmune Thrombocytopenia (NAIT)
E3.4.4.1.1	The maternal serum must be investigated for the presence of HPA antibodies
E3.4.4.1.2	HPA typing of the mother, father and neonate should be performed
E3.4.4.2	Post Transfusion Purpura (PTP)
E3.4.4.2.1	The patient must be HPA typed

E3.4.4.2.2	The patient's serum must be investigated for HPA antibodies
E3.4.5	**HNA and Transfusion**
E3.4.5.1	Neonatal Alloimmune Neutropenia (NAIN)
E3.4.5.1.1	The maternal serum sample must be investigated for the presence of HNA antibodies
E3.4.5.1.2	HNA typing of the mother, father and neonate should be performed
E3.5	**Disease Association**
E3.5.1	Typing may be limited to
E3.5.1.1	all products of selected loci, or
E3.5.1.2	a limited number of HLA antigens or alleles
E3.5.1.3	The clinically relevant HLA loci and alleles must be documented for each disease association service provided
SECTION F – POST-ANALYSIS PROCESSES	
F1	**TERMINOLOGY AND NOMENCLATURE**
F1.1	**HLA**
F1.1.1	**Alleles and Antigens**
F1.1.1.1	Terminology of HLA alleles and antigens must conform to the latest report of the WHO Nomenclature Committee for Factors of the HLA System
F1.1.1.2	Potential new alleles or antigens not yet approved by the WHO Committee must have a local designation which cannot be confused with WHO terminology
F1.1.1.3	NMDP codes must only be used for recording donors or cord blood unit typings into databases or for communication of the donor, cord blood unit or recipient typing with the registries
F1.1.2	**Resolution**
F1.1.2.1	High resolution typing is defined as the identification of HLA alleles that encode the same protein sequence within the antigen binding site
F1.1.2.1.1	HLA alleles must be identified at the level of resolution which defines the first and second fields according to WHO nomenclature by at least resolving all ambiguities:
F1.1.2.1.2	Resulting from polymorphisms located within exons 2 and 3 for HLA class I loci, and exon 2 for HLA class II loci
F1.1.2.1.3	That encompass a null allele, wherever the polymorphism is located, unless it can be demonstrated that an expressed antigen is present on the cells

F1.1.2.2	Allelic resolution typing is defined as a DNA based typing result consistent with a single allele
F1.1.2.2.1	HLA alleles must be identified at the level of resolution which defines all of the fields according to current WHO nomenclature
F1.1.3	Phenotypes and Genotypes
F1.1.3.1	Phenotypes and genotypes must be expressed as recommended by the WHO Committee, as in the following examples:
F1.1.3.1.1	Single alleles: HLA-B*07. Single antigens: HLA-B7 (or B7 if HLA is obvious from context)
F1.1.3.1.2	Serological assignment: HLA-A2, 30; B7, 44; Cw7; DR1, 4; DQ5, 7
F1.1.3.1.3	DNA assignment: HLA-A*02,*30; B*07,*44; C*07,*16; DRB1*01,*04; DQB1*05, *03:01 or HLA-A*02, 30; B*07, 44: C*07, 16; DRB1*01, 04; DQB1*05, 03:01
F1.1.3.2	If an HLA typing is performed using DNA methods, it is acceptable to report an HLA serological assignment if required for the purposes of organ allocation
F1.1.3.3	The translation of alleles to serological equivalence must be performed according to a documented protocol
F1.1.4	Haplotypes
F1.1.4.1	Serological assignment: HLA-A2, B44, Cw-, DR1, DQ5 / A30, B7, Cw7, DR4, DQ7
F1.1.4.2	DNA assignment: HLA-A*02, B*44, C*16, DRB1*01, DQB1*05 / A*30, B*07, C*07, DRB1*04, DQB1*03:01
F1.1.5	Reporting Homozygosity and Heterozygosity
F1.1.5.1	If no more than one 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 or if DNA typing unequivocally demonstrates the presence of heterozygosity for two different alleles from the same specificity (e.g. DRB1*13:01/13:59, DRB1*13:03/13:33)
F1.1.5.2	A “blank antigen or allele” can only be assigned if proven by family studies
F1.1.5.3	If homozygosity has not been proven, the HLA type may be reported using a hyphen. For example:
F1.1.5.3.1	HLA-A1,3; B7,44; Cw7,- to indicate a phenotypic blank, or
F1.1.5.3.2	HLA-A*01,*03; B*07,*44; C*07,- or HLA-A*01,03; B*07,44; C*07,- for DNA based typing
F1.1.6	Reporting High Resolution Typing
F1.1.6.1	When reporting high resolution typing, where ambiguous allele combinations cannot be resolved, all the alternatives must be documented
F1.1.6.2	If all ambiguities are not included on the report, a comment must be added stating that: Other ambiguous HLA (define loci) results have not been excluded and this information is available upon request

F1.1.7	**Haplotype Assignment**
F1.1.7.1	Determination of haplotypes must be done by typing immediate family members including parents, siblings and/or children of the patient
F1.1.7.2	Genotypic identity can only be proven if both parents are available or if the segregation of the four haplotypes is clearly defined
F1.1.7.3	When appropriate, ambiguities in haplotype assignment must be resolved by:
F1.1.7.3.1	Typing for HLA-C, and/or DQ and/or DP
F1.1.7.3.2	High resolution typing
F1.1.7.4	If recombination occurs, this must be reported with the HLA haplotype assignment
F1.1.7.5	For unrelated individuals, when probable haplotypes based on population frequencies are used:
F1.1.7.6	Reports must clearly indicate that they were so derived
F1.1.7.7	The relevant references or sources must be available
F2	**Other immunogenetic markers**
F2.1	Current HPA Nomenclature (Platelet Nomenclature Committee; Vox Sanguinis 2003 85, 240-245) must be used for recording and reporting HPA antigens and HPA alloantibody specificities
F2.2	Current HNA Nomenclature (https://onlinelibrary.wiley.com/doi/full/10.1111/voxs.12121) must be used for recording and reporting HNA antigens and HNA alloantibody specificities
F2.3	Terminology of KIR genes and alleles must conform the nomenclature of WHO Nomenclature Committee for Factors of the HLA System (http://www.ebi.ac.uk/ipd/kir)
F2.3.1	KIR Haplotypes must be determined as agreed with WHO Nomenclature Committee for Factors of the HLA System (http://www.ebi.ac.uk/ipd/kir)
F2.4	Terminology of MICA genes must conform the nomenclature of WHO Nomenclature Committee for Factors of the HLA System (http://www.ebi.ac.uk/ipd/imgt/hla/align.html)
F3	**Test Reports**
F3.1	The laboratory must have adequate systems in place to report results in a timely, accurate and reliable manner
F3.2	The report must contain:
F3.2.1	The name of the individual tested or unique identifier of each individual tested and relationship to the patient if applicable
F3.2.2	The date(s) of collection of sample when pertinent
F3.2.3	The date of the report
F3.2.4	The test results
F3.2.5	The techniques used

F3.2.6	Appropriate interpretations and the signature of the Laboratory Director/Co-Director, or a designated individual
F3.2.7	Information regarding the condition and disposition of specimen that did not meet the laboratory's criteria for acceptability
F3.2.8	The identity of the subcontracting laboratory and that portion of the testing for which it bears responsibility must be noted in the reports issued.
F3.3	Reporting Antibody results
F3.3.1	Terminology of HLA antibody specificities reported must conform to the latest report of the WHO Nomenclature Committee for Factors of the HLA System
F3.3.1.1	If solid phase antibody testing methods are performed then it is acceptable to report assigned HLA serological equivalents
F3.3.1.2	If no serological equivalent has been assigned then the DNA nomenclature must be employed
F3.3.1.3	the presence of HLA antibodies should be expressed as the % PRA or calculated %PRA value
F3.4	Reporting Crossmatching results
F3.4.1	Report must include:
F3.4.1.1	Cell type used
F3.4.1.2	Serum treatment (e.g. by DTT)
F3.4.1.3	Antibodies directed against the donor (virtual crossmatch) if required by the transplant protocol
F3.4.1.4	Result of each individual technique used

Definitions

Allele	alternate forms or varieties of a gene
Alloimmunised	is a response to foreign antigens (alloantigens) from genetically dissimilar members of same species
Ambiguity	possibility of interpretation in more than one way
Antigens	a foreign substance in the body that stimulates an immune response especially the production of antibodies
Average coverage threshold	typically established for all genomic region sequenced to achieve reliable base calling
Average depth of coverage	average number of overlapping reads within the total sequenced area
barcode	an oligonucleotide that is used for labelling genomic sample for multiplexed sequencing analysis
Chromosome	thread-like, gene carrying bodies in the nucleus of a cell and are composed of DNA, which itself is composed of 4 nucleotides: A (adenine), T (thymine), C (cytosine) and G (guanine)
Cloning	to make multiple identical copies of a DNA sequence
Common	HLA alleles which have been observed in multiple populations, although not necessarily in every population or in every region of the world. Their frequencies are known, and they have been observed at frequencies of <0.001 in reference populations of at least 1500 individuals. (*Tissue Antigens* 2013 **81**:194-203)
Conditioning regimen	chemotherapy or irradiation given immediately prior to a transplant the purpose of which is to help eradicate the patient's disease prior to the transplant of HSC and to suppress the immune system
Discrepancy (typing)	a conflict or variation
DNA	a large genetic molecule that stores the genetic code for the synthesis of proteins. DNA is made out of two strands that are held together by hydrogen bonds, each strand being made up of a sugar, a phosphate group and one of four bases (adenine, guanine, cytosine or thymine)
Exon	

	is any nucleotide sequence encoded by a gene that remains present within the final RNA product of that gene after introns have been removed by RNA splicing
Extended typing	HLA typing performed to add additional information to an existing HLA assignment
Genes	units of inheritance usually occurring at specific locations, or loci, on a chromosome OR the fundamental physical and functional unit of heredity, which carries information from one generation to the next
Genotype	the genetic makeup of an individual. Genotype can refer to an organism's entire genetic makeup or the alleles at a particular locus
Haplotype	a set of closely linked alleles (genes or DNA polymorphisms) inherited as a unit
Heteroduplex	a DNA double helix formed by annealing single strands from different sources
Heterozygous	a genotype consisting of two different alleles of a gene
Homozygous	having the same allele at the same locus on both members of a pair of homologous chromosomes
High resolution typing	is defined as the identification of HLA alleles that encode the same protein sequence within the antigen recognition site OR As a set of alleles that specify and encode the same protein sequence for the antigen binding domain of an HLA molecule and that excludes alleles that are not expressed as cell- surface proteins
Histocompatibility	compatibility between the tissues of two different individuals so that one accepts a graft from the other without having an immune reaction
HLA system	the body's genetically inherited system for recognising and rejecting foreign tissues, such as transplanted organs
Immunogenetics	is a branch of medical science that explores the relationship between the immune system and genetics
Isotype (antibody)	defines the role of antibodies in the body. There are five different antibody isotypes seen in humans: IgG, IgA, IgM, IgE and IgD.
Locus	the position of a particular gene on a chromosome OR a specific location on a chromosome.
Low resolution typing	the DNA-based typing result is at the level of the digits comprising the first field in the DNA-based nomenclature

Luminex	bead array solid phase based assay
Markers (genetic)	alleles of genes, or DNA polymorphisms, used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene
Molecular techniques	the study of the molecular constitution of genes and chromosomes
Mutation	an alteration of genetic material such that a new variation is produced OR a disruption in the normal sequence of a DNA strand resulting in a different trait produced. A point mutation is caused by a change in a single base pair
Next generation sequencing	sequencing by direct detection of base insertion
Null allele (HLA)	allele in which no expressed antigen is present on the cells
Phasing	Process in sequencing whereby haplotypes are determined using overlapping sequences
Phenotype	the detectable expression of a genotype or the observable or detectable characteristics of an individual organism or the visible characteristics of an individual
Polymerase chain reaction (PCR)	a method of DNA analysis that amplifies a specific DNA region allowing rapid DNA analysis
Polymorphisms	different forms of a single gene
Primer	an oligonucleotide that binds to a specific target sequence of a gene or template by complementarities under defined conditions and is used to initiate DNA amplification
Probe	an oligonucleotide that binds to and identifies the presence of target sequences of a gene by complementarities under defined conditions. Probes may be free in liquid phase or bound to solid substrates
Primer-dimer	is a potential by-product in PCR. As its name implies a primer-dimer consists of primer molecules that have attached (hybridised) to each other
Prospectively	looking towards the future
Read coverage	number of reads

Read length	number of bases sequenced
Reagent	to include: Reagents, solutions, culture media, controls, calibrators and other materials
Recombination	the interchange of genetic sections between pairing chromosomes during meiosis that produces variations in genetic characteristics by rearranging genes. Also referred to as a “crossover”
Refractoriness	not responding to treatment/therapy e.g. Transfusion of randomly selected platelets
Retrospectively	looking backwards to the past
Sample tagging	adding barcode sequence
Sensitisation	to render an individual sensitive to foreign antigens. May follow a sensitising event such as transplantation, transfusion, pregnancy etc.
Sequencer	instrument used for the analysis and interpretation of the DNA sequence
Shotgun	a method where genomic DNA or PCR products are fragmented randomly
Temperature Controlled Equipment	to include all refrigerators, freezer, incubators, waterbaths
Uniformity of coverage	is the distribution of coverage within specific targeted regions in which variant calling will occur. Uniformity of coverage should be measured by assessing coverage across the regions that are sequenced
Verification typing	HLA typing performed on an independent sample with the purpose of verifying concordance of that typing assignment with the initial HLA typing assignment
Virtual crossmatching	Determining the presence of pre-transplant donor specific HLA – Antibodies (DSA) by comparing recipient’s HLA antibody specificities with the donor’s HLA antigens, and replacing the pre-transplant laboratory crossmatching test with an interpretive assessment of compatibility. For clarity an allocation virtual crossmatch does not constitute a transplant laboratory virtual crossmatch.
Well-Documented	Alleles which have been observed in at least five times in unrelated individuals through the use of a Sequence Based Typing method or at least three times if the allele has been observed in a specific haplotype in unrelated individuals (*Tissue Antigens* 2013 **81**:194-203)

Abbreviations

ASHI	American Society for Histocompatibility and Immunogenetics
CDC	Complement Dependent Cytotoxicity
CWD	Common and Well-Documented
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribo Nucleic Acid
dNTP	DeoxyriboNucleotide TriPhosphates
EFI	European Federation for Immunogenetics
EPT	External Proficiency Testing
ELISA	Enzyme Linked Immunosorbent Assay
ESHI	The European Specialisation in Histocompatibility & Immunogenetics (H&I)
ETHIQ	European Technical Histocompatibility and Immunogenetics Qualification
FNHTR	Febrile Non Haemolytic Transfusion Reaction
HCE	Haematopoietic Chimaerism and Engraftment (monitoring)
HNA	Human Neutrophil Antigen
HPA	Human Platelet Antigen
HSCT	Haematopoietic Stem Cell Transplantation
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMGT	ImMunoGeneTics (project)
KIR	Killer-cell immunoglobulin-like receptor
MHC	Major Histocompatibility Complex
MICA	MHC class I polypeptide-related sequence A
MICB	MHC class I polypeptide-related sequence B
NAIN	Neonatal Allo Immune Neutropenia
NAIT	Neonatal Allo Immune Thrombocytopenia
NGS	Next Generation Sequencing
NMDP	National Marrow Donor Program
PCR	Polymerase Chain Reaction
PTP	Post Transfusion Purpura
QC	Quality Control
Q–PCR	Quantitative PCR
SBT	Sequence Based Typing

SSCP	Single Strand Conformation Polymorphism
SSOP	Sequence Specific Oligonucleotide Probe
SSP	Sequence Specific Primer
TAGVHD	Transfusion Associated Graft Versus Host Disease
Taq	*Thermus Aquaticus*
TRALI	Transfusion Related Acute Lung Injury
WHO	World Health Organisation

