Chapter 10

Histocompatibility Testing

Change record

Date	Author	Version	Change reference
16-11-2016	J. de Boer	4.1	Erratum outdated screening (§10.3)
04-08-2016	S. Heidt	4.1	Paragraph 10.4.5: 'retyping of the donor must be performed' has been changed into 'should be performed'
21-06-2016	S. Heidt	4.0	Major textual adjustments Paragraph 10.2.2 and 10.6.5 added
18-06-2015	S. Heidt	3.1	Textual adjustment paragraph 10.3.2
17-03-2015	S. Heidt	3.0	Textual adjustments
08-03-2013	J. de Boer	2.0	Textual adjustments
13-09-2012	C.M. Tieken	1.1	Text added page 2
10-03-2012	I. Doxiadis	1.0	

The Eurotransplant Manual contains the rules and regulations for the implementation and specification of national legislation and national guidelines for waiting list management, organ procurement and allocation. It has been prepared with the best of knowledge and the utmost care. In case of discrepancies between the content of this manual and national binding provisions, the following applies:

- Insofar, as provisions about the acceptance of organ recipients to the waiting list are concerned, this manual has only an informative character. Only the national provisions which are applicable for the transplant centers are relevant and legally binding.
- For the allocation of organs only the national provisions are legally binding. The display of the allocation provisions in this Manual are based on these legally binding national provisions. As far as necessary, they have been specified by Eurotransplant in this Manual. Deviations from such specifying Eurotransplant provisions cannot be considered as a breach of the national provisions as long as the latter are not violated.

Eurotransplant cannot be held liable for a potentially wrongful description in this Manual of procedures, in connection with the organ allocation, as long as the actual allocation follows national provisions.

Chapter 10 – Histocompatibility Testing Table of Contents

10.1. General

All Tissue Typing Centers (TTC) providing and/or handling histocompatibility data and participating in the frame of Eurotransplant (ET) must have a valid accreditation of the European Federation for Immunogenetics (EFI) or the American Society for Histocompatibility and Immunogenetics (ASHI) and participate in the External Proficiency Testing scheme of Eurotransplant without any sample selection.

The Standards for Histocompatibility Testing of EFI in their latest valid version apply for all described procedures unless stated otherwise in the present manual.

The recommendations released by the Board of ET regarding histocompatibility testing, screening, and crossmatching must be followed after approval by the respective national authorities.
Within ET the official WHO HLA nomenclature is used, as indicated in the latest nomenclature report. For allocation purposes “matching determinants” are generated at the ET allocation office from the report of HLA typing of recipients and donors according to 10.7 addendum HLA tables of the ET manual.

The TTC is responsible for the accuracy, reliability, and consistency of all relevant histocompatibility data of their recipients and donors reported to ET. They must follow the written and valid Standard Operation Procedures (SOP) released by the laboratory to meet the requirements of the ET Manual and the EFI Standards.

The Eurotransplant Reference Laboratory (ETRL) is an integral part of ET with the following duties and responsibilities:

- Organize and oversee all ET EPT exercises and release of an annual report and annual certificates.
- Provide expertise and practical aid in the area of histocompatibility testing to ET-affiliated TTC including a 24 hours / 7 days a week on call service for immunological questions regarding organ allocation and allocation of organs through the Acceptable Mismatch (AM) program.
- Help ET-affiliated TTC in defining acceptable mismatches for recipients awaiting a renal transplant and review every application for recipients to enter the AM Program. Decide on whether a patient is eligible to participate in the AM program based on transparent and uniform criteria.
- Visit TTC and help in solving histocompatibility related problems.
- Organize the annual ET Tissue Typers Meeting and other meetings relevant to the Tissue Typers community within ET.

10.1.1 Registration of transplant recipients

Relevant histocompatibility and immunological data of all potential organ recipients and donors are registered centrally in the Eurotransplant Network Information System (ENIS).

To avoid clerical errors, only trained personnel must report the results of HLA typing and screening to ENIS, preferably via the TTC of the recipient and preferably by electronic

communication. The local TTC is responsible for correct transmission of the results of HLA typing and antibody screening.

10.1.2 Material for histocompatibility testing

Prior to entering a recipient on the waiting list for organ transplantation, HLA typing and a screening for HLA-specific antibodies must be performed for kidney, pancreas, combined kidney/pancreas, intestine, heart, lung and should be done for liver. Every potential transplant recipient should be HLA typed on two separate occasions using two different samples.
The TTC affiliated to the transplant center should have standard policies on the requirements of samples for performing HLA typing and antibody screening and on the tests to be performed for a recipient before entering the waiting list.
In general, all required material must be sent to the TTC affiliated to the transplant center where a recipient is registered. The samples must be labeled according to the EFI Standards. The samples must be accompanied by the necessary administrative information (immunizing events: transfusions, pregnancies, previous transplants). An up-to-date listing of the TTC affiliated to ET is available at the ET office.

Typing for organ donors and crossmatching must follow the Standard Operation Procedures (SOP) released by the TTC, following the recommendations of ET and EFI. All immunologically relevant data (i.e. HLA typing, crossmatch and screening data) reported to ET must be controlled for clerical errors. Every mistake or inconsistency must be reported immediately for correction to ET.

10.2. Typing for HLA class I (A, B, C) and class II (DR, DQ)

10.2.1 Minimum requirements for HLA typing

Every recipient and every organ donor must be typed for HLA-A, -B, -C, -DR, and -DQ. Serological and DNA typing for HLA-A and -B is accepted. For HLA-DR, HLA-C and HLA-DQ DNA typing must be performed.

If the phenotype of a potential recipient shows less than ten HLA-A, -B, -C, -DR, -DQ antigens, a family typing or molecular typing must be performed to confirm homozygosity. Extended DNA typing is also accepted for the definition of homozygosity. For organ donors first field (two digits) DNA typing is accepted for the definition of homozygosity.

HLA typing data of the donor must be reported directly to ET preferably by electronic communication, or by fax using the form HLA typing of organ donor: matching data, available on the ET website (https://members.eurotransplant.org/cms/mediaobject.php?file=HLA.pdf). This form is a worksheet and not a report as defined by EFI.
The minimum requirement for HLA typing for donors and recipients to be communicated to ET is at the serological split level, as described in 10.7 addendum HLA tables (with the exception of HLA-B14). Providing ET with this level of HLA typing is required for proper vPRA calculation, as well as the virtual crossmatch.
HLA alleles, as determined by DNA typing, can also be reported to ET, as long as the alleles are present in 10.7 Addendum HLA Tables of the ET manual. In cases where there are no

serological equivalents defined, the most probable serological equivalent of a DNA typing result should be given (e.g. C*12 is translated to Cw12). In case of homozygosity, the homozygous antigen must be entered in duplicate for both donors and recipients.
Entering of Bw4/Bw6 should be done on basis of HLA-B antigens only.
The HLA typing provided is translated to "matching determinants" at the ET office for allocation purposes, whereas the full HLA typing of donors is used for the virtual crossmatch.

10.2.2 HLA retyping of donor samples

From EPT data, it is known that the error rate in HLA typing is around 3%. To prevent an organ to be transplanted on the basis of erroneous HLA data, retyping of all donors by the recipient center is recommended. In case of a discrepant HLA typing, the recipient center must report to ET, upon which the donor center and recipient center are notified and have to come to a consensus HLA type. If no consensus between the two labs is reached, the ETRL is issued to perform the reference typing.

10.3. HLA antibody Screening

Sera from all potential organ recipients should be screened for HLA-specific antibodies at regular time intervals. For potential kidney recipients on the waiting list a screening must be performed every three months. The screening of sera from potential kidney recipients on the waiting list must be carried out in time to prevent outdated screening (i.e. >180 days in between two antibody screenings), leading to removal of the recipient from the allocation list.
For other organs than kidneys, according the national legislations, recipients should be screened for HLA specific antibodies prior to entering the waiting list. In addition, further screenings are requested after every immunizing event.

A screening for HLA-specific antibodies for all potential organ recipients should be performed at 2 and 4 weeks after every immunizing event, e.g. blood transfusion, transplantation, pregnancy and graft removal. The screening policies must follow the recommendations of the National Bodies, ET, EFI, and the transplant center.

The TTC must check the waiting list with respect to histocompatibility related aspects. The screening is performed by the TTC affiliated to the transplant center where the recipient is registered. The information must be recorded in the recipient file and reported to ENIS.

The TTC must use solid phase screening methods for the definition of antibodies against HLA class I and HLA class II . A complement dependent cytotoxicity (CDC) screening for HLA specific antibodies must be done at least once a year. The TTC must report a %-PRA value for every positive screening performed. For HLA antibody positive sera, the HLA specificities must be reported, and unacceptable HLA antigens must be defined.

10.3.1 Screening for HLA specific antibodies (%-PRA value)

The %-PRA value represents the percentage of donors in a panel to which antibodies in the recipient's serum react in CDC. For the time being, the definition of the %-PRA value must be done using a panel of HLA typed cells or a panel of HLA antigens in case of solid phase assays.

Unacceptable antigens must be defined and a virtual PRA (vPRA) will be calculated automatically based on the unacceptable antigens, and included in the ENIS data file. Starting 2016, the vPRA will be accepted as the only indication that a patient is sensitized, and a %-PRA without indication of the specificity of the antibodies will not be accepted anymore.

If the %-PRA value is >5%, an auto-crossmatch must be performed to exclude autoantibodies. The TTC must report this information to ET. The serum of recipients with alloantibodies, >5% PRA must be included in the crossmatch serum exchange, unless these are found only in solid phase assays. The TTC must tick the appropriate box in the respective window of ENIS.
The recipient's TTC must update the antibody status of the recipients on the waiting list after every screening and check whether recipients have an outdated screening. Furthermore, the TTC must define the autoantibody status of the recipients, and must distribute the sera with a CDC %-PRA value >5%, unless otherwise stated.

10.3.2 Autoantibodies

The identification of autoantibodies or transplantation irrelevant antibodies must be performed by the TTC. Such antibodies may lead to high panel reactive antibody values (%-PRA) and can often lead to false positive crossmatches. Therefore, the screening and the auto-crossmatch using the recipient's own cells must be done with and without dithiothreitol (DTT) at least once. In case the serum of the patient is sent around for crossmatching, the TTC must report to ET whether the recipient has autoantibodies or not.
The %-PRA value must be based on alloantibody reactivity only and consist of full numbers only. The serum of recipients with autoantibodies only will not be included in the crossmatch serum exchange. The serum of recipients with a mixture of auto- and alloantibodies with a %-PRA value <6% are not included in the crossmatch serum exchange.

10.3.3 Unacceptable HLA antigens

Unacceptable HLA antigens are HLA antigens that are forbidden as donor HLA mismatches. HLA antigens, towards which the recipient has formed alloantibodies defined with CDC in the current serum must be reported as unacceptable HLA antigens and entered into ENIS. Both HLA class I and HLA class II specificities can be entered as unacceptable antigens. Depending on the policy of the transplant center, additional specificities can be entered into the unacceptable HLA antigen field. These can be based on historical antibodies against mismatched HLA antigens of the previous organ donor, or paternal HLA antigens in case the recipient has been pregnant.

Not all HLA antibody specificities detected by solid phase assays only (CDC negative) are necessarily unacceptable antigens. All plausible antibody specificities detected with solid phase techniques only should be considered a risk factor and can be entered as unacceptable antigens according the policy of the transplant center (center or patient dependent). A direct link from defined HLA antibody specificities to unacceptable antigens is neither desirable nor possible. The responsible TTC must confirm every unacceptable HLA antigen separately. From the list of unacceptable antigens, the vPRA value will be automatically calculated within ENIS. This value appears in the immunological report of the recipient. The definition of unacceptable HLA antigens must be discussed by the transplant center and the TTC.

For kidneys allocation through ETKAS, no offer will be made if an organ donor expresses these

unacceptable HLA antigens (a synonym of virtual crossmatch positive). In case the donor HLA type is not known at time of the ESP and the heart/lung match it may be possible that an organ offer is made which harbors antigens that are listed as unacceptable for this patient. For lung(s) and pancreas this policy is not yet implemented. This policy is not applicable to liver allocation.

Based on the unacceptable antigens and blood group of a patient, the chance to receive an offer of a compatible renal transplant can be calculated. This Donor Frequency Calculator ETKAS has been developed by the ETRL (http://etrl.org/FREQ_DONORS_ETKAS/Information.aspx) and will be implemented into ENIS in 2016. The chance of receiving a compatible renal transplant within ETKAS will be used to determine eligibility for the AM program in the near future (see 10.5.1).

10.4. Crossmatch

The crossmatch is an intergral step in the decision making process for organ allocation and transplantation. The prerequisite is the definition of HLA-specificities that are found in the patients serum and found to increase the immunological risk of graft rejection. These HLA-specificities are defined as unacceptable HLA antigens and are to avoid in the donors HLA-type. This calculation is named “virtual crossmatch”.
The crossmatch using the recipient serum and lymphocytes of the prospective donor is usual done with the CSDc technique.

10.4.1 The “allocation” crossmatch

No allocation will be done to a recipient, whose immunological profile shows unacceptable HLA antigens that are present in the HLA phenotype of the donor (positive virtual crossmatch). For patients with cytotoxic antibodies in the current serum, a CDC crossmatch must be performed in the donor center to avoid organ shipment to recipients having preformed antibodies against donor HLA antigens, which are not included in the recipient specific profile as unacceptable HLA antigens. For the allocation crossmatch procedure, the TTC must use CDC on unseparated cells or T cells (B cells optionally) for the allocation crossmatch with or without DTT as requested by the recipient center via the ET crossmatch list or the local transplant center. The TTC can use additional techniques and include historical sera in the decisive crossmatch for local patients.

10.4.2 The “transplantation” or “decisive” crossmatch”

The “transplantation” or “decisive” crossmatch is performed in the TTC where the recipient is registered, or the TTC cooperating with the recipient’s transplant center. Here, other than the above-mentioned targets, B cells may be used. The evaluation of this decisive crossmatch prior to transplantation follows the SOP established by the TTC and follows the recommendations of the National Bodies, the transplant center, ET and EFI. It is the responsibility of the TTC to adhere to these recommendations.

For kidney and combined kidney/pancreas transplantation a crossmatch must be performed before transplantation using current sera as specified by the recipient transplant center and TTC unless otherwise decided by the National Bodies. In addition, historical (peak) sera should be included. In case a prospective crossmatch is not performed, the reason, final decision, and

outcome of the possible transplantation must be documented at the TTC, following the EFI standards. In case no prospective crossmatch is performed, the crossmatch with the pre-transplant serum must be performed and documented retrospectively.

For organs other than kidney, at least a retrospective crossmatch should be done for recipients who either harbor HLA-specific alloantibodies, or have had an alloimmunizing event such as pregnancy, blood transfusion, or previous transplantation. Unless otherwise decided by the transplant center, for recipients waiting for heart, lung, pancreas, and small bowel or a combination of those organs and being allosensitized, a crossmatch must be performed. Recipients with cytotoxic antibodies may require a prospective crossmatch.

10.4.3 Shipment of cell material for crossmatching

Anti-coagulated (citrate or heparin) peripheral blood, a piece of spleen and / or lymph nodes in phosphate buffered saline or equivalent must be included in the designated container. A sufficient number (if available) of isolated lymphocytes can also be sent. Labeling of every vial and all information included must include the Eurotransplant donor number and must follow the EFI Standards. In case of reshipment of the organ to a second recipient center, all material for histocompatibility testing must be placed back in the organ box.

10.4.4 Donor TTC

The donor TTC must perform all transplantation relevant immunological assays for post-mortal organ donors and recipients including HLA typing, screening for HLA-specific antibodies and crossmatching. In Germany, the donor TTC is named regional TTC.
The allocation crossmatches must be performed for local recipients irrespective of their immunization status and for sensitized (>5% PRA) non-local recipients selected by the ET allocation office.
For autoantibody positive recipients a crossmatch with and without DTT must be performed by the donor TTC and the results must be reported to the ET allocation office when indicated on the allocation crossmatch list.

The donor TTC must apply policies allowing quick and reliable results avoiding any unnecessary prolongation of the cold ischemia period.

10.4.5 Recipient TTC

Besides typing and screening for HLA specific antibodies, the recipient TTC (in Germany the regional TTC) must perform the decisive crossmatch for transplantation of the selected recipient and potential back-up of local/regional recipients selected by the ET allocation office. In addition, retyping of the donor should be performed. In special cases, the decisive crossmatch can be performed retrospectively (see 10.4.2).

For allosensitized recipients, a decisive crossmatch with and without DTT must be performed. The recipient's transplant center decides upon acceptance or denial of the offer. Transplantation can only be performed in case of a negative crossmatch, unless otherwise decided by the local transplant center. The reasons must be reported to ET before transplantation.

The recipient TTC and transplant center are responsible for the decision on the histocompatibility of the transplant.

10.4.6 Crossmatch serum exchange program

Dialysis centers collect sera of their potential kidney transplant recipients four times a year and send these to their affiliated TTC. These sera are screened for HLA specific antibodies, from which unacceptable HLA antigens are defined.
ET provides the TTC with a mailing list of all TTC performing crossmatches. An additional list of all potential recipients of the local TTC is included. Labels for each potential kidney recipient are printed locally.

For patients awaiting kidney transplantation only, sera with an allo-PRA value of >5% as determined by CDC are included in the crossmatch serum exchange program. Sera from recipients with antibodies found in solid phase assay only, and recipients with transplantation irrelevant antibodies only, are not included in the exchange program. For the latter group of recipients this information must be provided in the respective screens in ENIS.

The TTC sends the serum samples together with a list of the recipients, of whom serum is included, following the national postal regulations. The receiving TTC must check if all sera have been included as stated on the accompanying information. In case a serum or sera are missing, the receiving TTC must immediately inform the sending TTC.

10.4.7 Procedure

Use Beckman tubes type PAT22 or identical clones from other companies. The tubes must be labeled with the locally printed labels or with labels provided by ET if applicable.

The following procedure is recommended:
- Label the tubes and fill with 50-250 microliter recipient serum
- Per recipient sufficient tubes must be prepared corresponding to the latest list of TTC participating in the crossmatch serum exchange
- The ET-number and the first 4 digits of the name of the recipient serum should be marked on the TTC list, which is sent to the TTC participating in the crossmatch serum exchange The sera must be listed in numerical order
- For administrative purposes, a copy of the list should remain locally

In the receiving TTC the following steps are required:
- The accompanying list must be checked. Any inconsistency must be reported to the sending TTC
- New crossmatch sera must be put in the crossmatch serum storage system immediately after arrival, allowing a quick retrieval of the most current serum
- For administrative purposes, the lists of the different TTC must be kept until the next exchange

10.4.8 Sera from non-kidney transplant candidates

Screening of sera from potential recipients of organs and tissues other than kidney is identical to the procedure described above. In case of allo-immunized patients, sera should be sent to

the TTC performing donor typing and crossmatching. Sera older than one calendar year should be discarded.
Germany only: The sera of all potential recipients of a pancreas transplant must be sent to all German TTC.

10.5. Acceptable Mismatch (AM) Program

The AM program has been established to increase the chance of highly sensitized kidney transplant candidates to receive a crossmatch negative offer. The program is open for all potential kidney transplant recipients of ET affiliated countries. The organ offer is mandatory. No crossmatch will be performed at the donor center if the patient is offered an organ through the AM program.

10.5.1 Eligibility of a recipient for the AM Program

The potential recipient should be on the ETKAS waiting list for at least two years, as defined by the date of first dialysis. Panel reactive cytotoxic antibodies resulting in a PRA value of ≥85% must be detectable in the serum of two different bleeding dates of the recipient or ≥85% v-PRA calculated from the unacceptable HLA antigens reported by the transplant center of the recipient, provided they are predominantly detectable in CDC. This policy will change in the near future: renal transplant candidates will only be eligible for entering the AM program when the chance of receiving a compatible donor organ within the ET area through ETKAS allocation is <2%, as calculated within ENIS. This calculation is based on the unacceptable antigens defined by the TTC affiliated to the recipient center in combination with the blood group of the potential recipient.

Recipients are not eligible for the AM program if:
- No unacceptable antigens are reported
- The recipient possesses mainly solid phase defined HLA antibodies not detectable in CDC

In case a recipient center removes unacceptable HLA antigens, the ETRL will re-evaluate whether the recipient still fulfills the criteria for the AM waiting list.

For recipients with cytotoxic HLA antibodies, the ETRL will accept the contribution of additional solid phase defined antibodies to the AM status, when these specificities can be explained by earlier transplantation, e.g. HLA mismatches of the previous donor(s), or a specific sensitization of the recipient, e.g. HLA antigens of the partner or children in women.
For every patient on the AM waiting list, the ETRL calculates the chance that a suitable donor becomes available in the ET donor population based on the patient's blood group, own HLA type and acceptable HLA antigens. The recipients are then divided into two categories:

Low chance of receiving a donor kidney: donor frequency ≤ 0.1%
High chance of receiving a donor kidney: donor frequency > 0.1%

A donor frequency of 0.1% represents a chance of 1- 2 organ donors per year (based on immunological grounds only).

10.5.2 Exceptions

Patients listed for both a liver and kidney transplant can be entered into the AM program but will not receive priority in case of a combined liver/kidney transplant, since in this situation ranking will be done on basis of the MELD score. The AM priority in this setting will only apply to patients (fulfilling the above mentioned criteria) who receive a kidney after liver transplant.

10.5.3 Selection of recipients upon availability of a donor organ

The AM program runs for every organ donor, and recipients are selected on the basis of blood group compatibility and HLA compatibility of the donor with the recipient's own HLA type in combination with the acceptable antigens.

The HLA-A, -B, -C, and -DR, -DQ typing of the organ donor is entered into ENIS. Potential recipients will be selected on the basis of their own HLA-A, -B and -DR antigens in combination with the HLA-A, -B and -DR acceptable antigens. These acceptable antigens are regarded as recipient's own HLA antigens. Full compatibility between donor and recipient including the acceptable antigens is a prerequisite for allocation of kidneys via the AM program. Matching is based on "split" HLA class I antigens and "split" HLA-DR antigens as already done for all recipients on the renal waitlist. Preferentially, acceptable HLA-C and HLA-DQ antigens should also be defined.

For recipients with a high chance of receiving a donor kidney as defined above, the following minimal match criteria apply: one HLA-B and one HLA-DR, or two HLA-DR antigens shared with the patient's own HLA antigens. For recipients with a low chance of receiving a donor kidney as defined above, or urgency status HU no minimal match criteria apply.

The ETRL immunologist on duty is informed about every potential offer for a recipient included in the AM program. In case of a potential offer through the AM program, the ETRL immunologist on duty checks the HLA typing of the organ donor, the HLA typing of the recipient, the acceptable and unacceptable antigens, and the reported HLA specific antibodies. After approval by the ETRL immunologist on duty, the respective transplant center is informed, and if accepted by the transplant center, the kidney must immediately be dispatched. The crossmatch must be performed in the recipient TTC. In case of a negative crossmatch, the transplantation can be performed. Repeat HLA mismatches for broad and split HLA-A, -B, -DR antigens are regarded as a contraindication for transplantation, unless otherwise reported. HLA-C and HLA-DQ specificities reported as unacceptable antigens are taken into consideration The ETRL immunologist on duty will deny an offer if unacceptable antigens are reported in the donor HLA typing, or if the minimal match criteria are not met (when applicable).

The order in which the kidneys will be offered in case of multiple potential AM recipients is based on the calculated chance to receive an organ within the AM program as provided by the ETRL (Donor Frequency calculator Acceptable Mismatch Program). Recipients with the lowest chance get the highest priority.

10.6. ET proficiency testing (EPT)

ET being an organ exchange organization relies on the work of the affiliated TTC. An essential

step in maintaining the high standards of histocompatibility related matters within ET is the fulfillment of the ETRL External Proficiency Testing exercises. This is the only EPT scheme where a center to center comparison within ET is possible. Therefore, all ET affiliated laboratories entering data into ENIS must participate in all EPT exercises without any sample selection, and must fulfill the requirements of EFI. The ETRL has established the EPT scheme in order to assess, maintain, and improve the quality of HLA typing, screening for HLA specific antibodies and crossmatching of TTC affiliated to ET. The participants are informed at the end of each calendar year how the EPT scheme of the following year will be organized, and what data are required for the analysis and certificates. The results of the EPT form the basis for future decisions of bodies such as the Tissue Typing Advisory Committee or the Kidney Advisory Committee of ET. The participants must use the local SOP for the EPT. The Standards released by the External Proficiency Testing Committee, and approved by the Executive Committee of EFI, form an essential basis for the Histocompatibility quality control and assurance within ET. Modification of any of those Standards is done if deemed necessary.

All results must be communicated to the ETRL through the EPT website (https://www.etrl.org/), with the exception of the patient-based cases. These results can be returned by email to etrl@eurotransplant.org. In general, the participants can download the analysis of the results from the EPT website within four weeks after the deadline. When analyzed, results are published on the website and participants will be notified by e-mail. Every participant receives these results in an open way (i.e. disclosing the identity of the laboratory) with the center code as provided by ET. The participants receive the analysis of the results via the EPT website. Every participant receives by December 31 of every calendar year latest a certificate of performance, which states whether the TTC fulfills the criteria for the particular EPT exercise. In case of irregularities, changes in the certificate can be made within two months of issuing. When a TTC is not fulfilling the requirements, it will be supported by the ETRL with respect to corrective actions. A summary of the EPT results is included in the Annual Report of ET.

The actual schemes include EPT exercises for: HLA typing, crossmatching, screening detection and identification and patient-based cases. In addition, a serum crossmatching EPT is designed in case of postal or customs problems. At the beginning of each new cycle, the TTC receives information from the ETRL regarding the EPT schemes. This information is published on the EPT website.

From 01-01-2016, the EPT scheme is divided into 2 arms. The first arm is to fulfill the requirements as stated by EFI. To this aim, raw data from the EPT exercises without any interpretation need to be reported. The second arm is designed to fulfill the requirements for ET, which are aimed at harmonization between ET affiliated laboratories. Interpretation of crossmatch results (*i.e.* final result), as well as classifying sera based on %-PRA into non-immunized (<6% PRA), immunized (≥6% and <85% PRA), or highly immunized (≥85% PRA)

10.6.1 EPT on HLA typing

This EPT is performed 4 times per year and consists of a shipment of peripheral blood from healthy blood donors for HLA typing. The TTC are divided into two groups for logistical reasons: 1) TTC performing postmortal organ donor typing, in addition to recipient typing and screening for HLA specific antibodies (donor TTC) and 2) TTC performing recipient typing and screening for HLA specific antibodies (recipient TTC) and TTC not affiliated to ET.
The results must be reported according to the minimum requirement for HLA typing as communicated to ET, which is at the serological split level, as described in 10.7 addendum HLA

tables (with the exception of HLA-B14). All TTC submitting transplantation relevant HLA typing results to ET must participate without any selection of samples. When the typing is not in consensus, the typing result of the organizer is regarded the correct typing. In case a participant disapproves with the results, the secretary of the TTAC must be informed by e-mail. The issue will then be discussed in the following TTAC meeting.

10.6.2 EPT on crossmatching

This EPT is performed 4 times per year using the peripheral blood samples distributed for the EPT on typing and selected sera from the EPT for screening for HLA specific antibodies. All TTC performing crossmatches for postmortal organ donors must participate. The TTC must perform all crossmatches with and without DTT. The TTC must use unseparated lymphocytes, and/or separated T cells, and may use B cells for the crossmatch according to the local SOP. Both raw data and interpreted results will be analyzed by the ETRL for EFI and ET requirements, respectively.

10.6.3 EPT on screening

This EPT is performed once per year and consists of a shipment of 12 sera of transplant recipients or multiparous women. All TTC reporting screening data to ENIS must participate in the EPT on screening. The TTC must report the PRA value with and without DTT, the existence of HLA class I and/or HLA class II antibodies, and the specificity (-ies). Methods reported in the local SOP must be used. All ET affiliated TTC must report screening identification results tested with CDC. The use of additional methods is allowed. The analysis of this EPT will be performed as stated in the respective information published on the ETRL website and reported to the participants.

10.6.4 EPT on serum crossmatch

This EPT is designed for TTC not having received the samples for crossmatch EPT in time, because of postal or customs problems. This EPT is only available for selected TTC and for a short period of time. A set of defined sera is sent to the TTC where selected HLA typed suspensions must be used. The results must be reported immediately to the ETRL. The standards of the External Proficiency Testing Committee of EFI apply.

10.6.5 EPT on patient based cases

The ETRL will publish 3 patient based cases each EPT year. The deadline is two weeks after publication. Various data about a patient will be provided, so that is possible for the participant to make a decision whether the transplant can proceed on an immunological basis. Participation is mandatory for ET affiliated TTC.

10.7. Addendum HLA Tables

10.7.1 HLA-A

ANTIGEN	SPLIT	BROAD	PUBLIC
A1			
A*01:01		A1	
A*01:02		A1	
A*01:XX		A1	
A2			
A*02:01		A2	
A*02:02		A2	
A*02:03		A2	
A*02:05		A2	
A*02:06		A2	
A*02:07		A2	
A*02:10		A2	
A*02:11		A2	
A*02:17		A2	
A*02:XX		A2	
A3			
A*03:01		A3	
A*03:02		A3	
A*03:XX		A3	
A9			
A23		A9	
A*23:01	A23	A9	
A*23:XX	A23	A9	
A24		A9	
A*24:02	A24	A9	
A*24:03	A24	A9	
A*24:07	A24	A9	
A*24:08	A24	A9	
A*24:XX	A24	A9	

ANTIGEN	SPLIT	BROAD	PUBLIC
A10			
A25		A10	
A*25:01	A25	A10	
A*25:XX	A25	A10	
A26		A10	
A*26:01	A26	A10	
A*26:02	A26	A10	
A*26:03	A26	A10	
A*26:08	A26	A10	
A*26:XX	A26	A10	
A34		A10	
A*34:01	A34	A10	
A*34:02	A34	A10	
A*34:XX	A34	A10	
A66		A10	
A*66:01	A66	A10	
A*66:02	A66	A10	
A*66:XX	A66	A10	
A11			
A*11:01		A11	
A*11:02		A11	
A*11:XX		A11	

ANTIGEN	SPLIT	BROAD	PUBLIC
A19			
A29		A19	
A*29:01	A29	A19	
A*29:02	A29	A19	
A*29:XX	A29	A19	
A30		A19	
A*30:01	A30	A19	
A*30:02	A30	A19	
A*30:04	A30	A19	
A*30:XX	A30	A19	
A31		A19	
A*31:01	A31	A19	
A*31:XX	A31	A19	
A32		A19	
A*32:01	A32	A19	
A*32:XX	A32	A19	
A33		A19	
A*33:01	A33	A19	
A*33:03	A33	A19	
A*33:XX	A33	A19	
A74		A19	
A*74:01	A74	A19	
A*74:XX	A74	A19	
A28			
A68		A28	
A*68:01	A68	A28	
A*68:02	A68	A28	
A*68:03	A68	A28	
A*68:XX	A68	A28	
A69		A28	
A*69:01	A69	A28	
A*69:XX	A69	A28	
A36			
A*36:01		A36	
A*36:XX		A36	
A43			
A*43:01		A43	
A*43:XX		A43	
A80			
A*80:01		A80	
A*80:XX		A80	

B = BROAD
S = SPLIT
P = PUBLIC
A = ALLELE

10.7.2 HLA-B

ANTIGEN	SPLIT	BROAD	PUBLIC
B5			BW4
B51		B5	
B*51:01	B51	B5	
B*51:02	B51	B5	
B*51:07	B51	B5	
B*51:08	B51	B5	
B*51:XX	B51	B5	
B52		B5	
B*52:01	B52	B5	
B*52:XX	B52	B5	
B7			BW6
B*07:02		B7	
B*07:03		B7	
B*07:04		B7	
B*07:05		B7	
B*07:09		B7	
B*07:XX		B7	
B8			BW6
B*08:01		B8	
B*08:XX		B8	
B12			
B44		B12	BW4
B*44:02	B44	B12	
B*44:03	B44	B12	
B*44:04	B44	B12	
B*44:05	B44	B12	
B*44:10	B44	B12	
B*44:XX	B44	B12	
B45		B12	BW6
B*45:01	B45	B12	
B*45:XX	B45	B12	
B*50:02	B45	B12	
B13			BW4
B*13:01		B13	
B*13:02		B13	
B*13:XX		B13	
B14			BW6
B*14:XX		B14	
B64		B14	
B*14:01	B64	B14	
B65		B14	
B*14:02	B65	B14	
B15			
B*15:XX		B15	
B62		B15	
B*15:01	B62	B15	BW6
B*15:05	B62	B15	BW6
B*15:07	B62	B15	BW6
B*15:24	B62	B15	BW4
B*15:25	B62	B15	BW6
B*15:27	B62	B15	BW6
B*15:30	B62	B15	BW6
B63		B15	BW4
B*15:16	B63	B15	
B*15:17	B63	B15	
B75		B15	BW6
B*15:02	B75	B15	
B76		B15	BW6
B*15:12	B76	B15	
B77		B15	BW4
B*15:13	B77	B15	

ANTIGEN	SPLIT	BROAD	PUBLIC
B70			BW6
B71		B70	
B*15:10	B71	B70	
B*15:18	B71	B70	
B72		B70	
B*15:03	B72	B70	
B16			
B38		B16	BW4
B*38:01	B38	B16	
B*38:02	B38	B16	
B*38:XX	B38	B16	
B39		B16	BW6
B*39:01	B39	B16	
B*39:02	B39	B16	
B*39:05	B39	B16	
B*39:06	B39	B16	
B*39:10	B39	B16	
B*39:XX	B39	B16	
B17			BW4
B57		B17	
B*57:01	B57	B17	
B*57:02	B57	B17	
B*57:03	B57	B17	
B*57:XX	B57	B17	
B58		B17	
B*58:01	B58	B17	
B*58:02	B58	B17	
B*58:XX	B58	B17	
B18			BW6
B*18:01		B18	
B*18:XX		B18	
B21			
B49		B21	BW4
B*49:01	B49	B21	
B*49:XX	B49	B21	
B50		B21	BW6
B*40:05	B50	B21	
B*50:01	B50	B21	
B*50:XX	B50	B21	
B22			BW6
B54		B22	
B*54:01	B54	B22	
B*54:XX	B54	B22	
B55		B22	
B*55:01	B55	B22	
B*55:02	B55	B22	
B*55:XX	B55	B22	
B56		B22	
B*56:01	B56	B22	
B*56:XX	B56	B22	
B27			
B*27:02		B27	BW4
B*27:03		B27	BW4
B*27:05		B27	BW4
B*27:06		B27	BW4
B*27:07		B27	BW4
B*27:08		B27	BW6
B*27:XX		B27	BW4

ANTIGEN	SPLIT	BROAD	PUBLIC
B35			BW6
B*35:01		B35	
B*35:02		B35	
B*35:03		B35	
B*35:05		B35	
B*35:08		B35	
B*35:12		B35	
B*35:17		B35	
B*35:43		B35	
B*35:XX		B35	
B37			BW4
B*37:01		B37	
B*37:XX		B37	
B40			BW6
B*40:XX		B40	
B60		B40	
B*40:01	B60	B40	
B61		B40	
B*40:02	B61	B40	
B*40:06	B61	B40	
B41			BW6
B*41:01		B41	
B*41:02		B41	
B*41:XX		B41	
B42			BW6
B*42:01		B42	
B*42:XX		B42	
B46			BW6
B*46:01		B46	
B*46:XX		B46	
B47			BW4
B*47:01		B47	
B*47:XX		B47	
B48			BW6
B*48:01		B48	
B*48:XX		B48	
B53			BW4
B*53:01		B53	
B*53:XX		B53	
B59			BW4
B*59:01		B59	
B*59:XX		B59	
B67			BW6
B*67:01		B67	
B*67:XX		B67	
B73			BW6
B*73:01		B73	
B*73:XX		B73	
B78			BW6
B*78:01		B78	
B*78:XX		B78	
B81			BW6
B*81:01		B81	
B*81:XX		B81	
B82			BW6
B*82:01		B82	
B*82:XX		B82	
B83			BW6
B*83:01		B83	
B*83:XX		B83	
BW4			
BW6			

10.7.3 HLA-C

ANTIGEN	SPLIT	BROAD	PUBLIC
Cw1			
C*01:XX		Cw1	
Cw2			
C*02:XX		Cw2	
Cw3			
C*03:XX		Cw3	
Cw10		Cw3	
C*03:02	Cw10	Cw3	
C*03:04	Cw10	Cw3	
Cw9		Cw3	
C*03:03	Cw9	Cw3	
Cw4			
C*04:XX		Cw4	
Cw5			
C*05:XX		Cw5	
Cw6			
C*06:XX		Cw6	
Cw7			
C*07:XX		Cw7	
Cw8			
C*08:XX		Cw8	
Cw12			
C*12:XX		Cw12	
Cw13			
C*13:XX		Cw13	
Cw14			
C*14:XX		Cw14	
Cw15			
C*15:XX		Cw15	
Cw16			
C*16:XX		Cw16	
Cw17			
C*17:XX		Cw17	
Cw18			
C*18:XX		Cw18	

10.7.4 HLA-DR

ANTIGEN	SPLIT	BROAD	PUBLIC
DR1			
DRB1*01:01		DR1	
DRB1*01:02		DR1	
DRB1*01:03		DR1	
DRB1*01:XX		DR1	
DR2			DR51
DR15		DR2	
DRB1*15:01	DR15	DR2	
DRB1*15:02	DR15	DR2	
DRB1*15:03	DR15	DR2	
DRB1*15:XX	DR15	DR2	
DR16		DR2	
DRB1*16:01	DR16	DR2	
DRB1*16:02	DR16	DR2	
DRB1*16:XX	DR16	DR2	
DR3			DR52
DR17		DR3	
DRB1*03:01	DR17	DR3	
DRB1*03:04	DR17	DR3	
DR18		DR3	
DRB1*03:02	DR18	DR3	
DRB1*03:03	DR18	DR3	
DRB1*03:XX		DR3	
DR4			DR53
DRB1*04:01		DR4	
DRB1*04:02		DR4	
DRB1*04:03		DR4	
DRB1*04:04		DR4	
DRB1*04:05		DR4	
DRB1*04:06		DR4	
DRB1*04:07		DR4	
DRB1*04:08		DR4	
DRB1*04:09		DR4	
DRB1*04:10		DR4	
DRB1*04:11		DR4	
DRB1*04:12		DR4	
DRB1*04:XX		DR4	

ANTIGEN	SPLIT	BROAD	PUBLIC
DR5			DR52
DR11		DR5	
DRB1*11:01	DR11	DR5	
DRB1*11:02	DR11	DR5	
DRB1*11:03	DR11	DR5	
DRB1*11:04	DR11	DR5	
DRB1*11:05	DR11	DR5	
DRB1*11:XX	DR11	DR5	
DR12		DR5	
DRB1*12:01	DR12	DR5	
DRB1*12:02	DR12	DR5	
DRB1*12:XX	DR12	DR5	
DR6			DR52
DR13		DR6	
DRB1*13:01	DR13	DR6	
DRB1*13:02	DR13	DR6	
DRB1*13:03	DR13	DR6	
DRB1*13:04	DR13	DR6	
DRB1*13:05	DR13	DR6	
DRB1*13:06	DR13	DR6	
DRB1*13:XX	DR13	DR6	
DR14		DR6	
DRB1*14:01/54	DR14	DR6	
DRB1*14:02	DR14	DR6	
DRB1*14:03	DR14	DR6	
DRB1*14:04	DR14	DR6	
DRB1*14:05	DR14	DR6	
DRB1*14:06	DR14	DR6	
DRB1*14:07	DR14	DR6	
DRB1*14:08	DR14	DR6	
DRB1*14:09	DR14	DR6	
DRB1*14:10	DR14	DR6	
DRB1*14:XX	DR14	DR6	

ANTIGEN	SPLIT	BROAD	PUBLIC
DR7			DR53
DRB1*07:0		DR7	
DRB1*07:2		DR7	
DR8			
DRB1*08:0		DR8	
DRB1*08:0		DR8	
DRB1*08:0		DR8	
DRB1*08:0		DR8	
DRB1*08:0		DR8	
DRB1*08:2		DR8	
DR9			DR53
DRB1*09:0		DR9	
DRB1*09:2		DR9	
DR10			
DRB1*10:0		DR10	
DRB1*10:2		DR10	
DR51			
DRB5*01:01			DR51
DRB5*01:02			DR51
DRB5*02:01			DR51
DRB5*02:02			DR51
DRB5*XX			DR51
DR52			
DRB3*01:01			DR52
DRB3*02:			DR52
DRB3*03:01			DR52
DRB3*XX			DR52
DR53			
DRB4*01			DR53
DRB4*XX			DR53

10.7.5 HLA-DQ

ANTIGEN	SPLIT	BROAD	PUBLIC
DQ1			
DQ5		DQ1	
DQB1*05:01	DQ5	DQ1	
DQB1*05:02	DQ5	DQ1	
DQB1*05:03	DQ5	DQ1	
DQB1*05:04	DQ5	DQ1	
DQB1*05:XX	DQ5	DQ1	
DQ6		DQ1	
DQB1*06:01	DQ6	DQ1	
DQB1*06:02	DQ6	DQ1	
DQB1*06:03	DQ6	DQ1	
DQB1*06:04	DQ6	DQ1	
DQB1*06:05	DQ6	DQ1	
DQB1*06:06	DQ6	DQ1	
DQB1*06:07	DQ6	DQ1	
DQB1*06:08	DQ6	DQ1	
DQB1*06:09	DQ6	DQ1	
DQB1*06:XX	DQ6	DQ1	
DQ2			
DQB1*02:01		DQ2	
DQB1*02:02		DQ2	
DQB1*02:XX		DQ2	
DQ3			
DQ7		DQ3	
DQB1*03:01	DQ7	DQ3	
DQB1*03:04	DQ7	DQ3	
DQ8		DQ3	
DQB1*03:02	DQ8	DQ3	
DQ9		DQ3	
DQB1*03:03	DQ9	DQ3	
DQB1*03:XX		DQ3	
DQ4			
DQB1*04:01		DQ4	
DQB1*04:02		DQ4	
DQB1*04:XX		DQ4	