Chapter 10

Histocompatibility Testing

Change record

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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.

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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 (EFI-Standards; http://www.efiweb.eu) 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.
(http://www.eurotransplant.org/cms/index.php?page=etrl1)

In ET the official WHO HLA nomenclature is used, as indicated in the latest nomenclature report. For allocation and subsequent documentation purposes "matching determinants" are used for the report of HLA typing of recipients and donors according to the listing of the ETRL (http://etrl.eurotransplant.org).

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 ETRL is an integral part of Eurotransplant with the following duties and responsibilities:

1. Organize and oversee all EPT exercises and release an annual report and annual certificates.
2. Provide expertise and practical aid in the areas of histocompatibility testing to ET-associated TTC including a 24 h / 7 days a week on call service for

immunological questions in organ allocation and allocation of organs via the Acceptable Mismatch (AM) Program.

3. Help affiliated TTC in defining acceptable mismatches for recipients awaiting a renal transplant and control every application for recipients entering the AM Program, taking the decision to accept or deny the application.
4. Visit TTC and help in solving histocompatibility related problems and
5. Organize the annual ET Tissue Typers Meeting and other Meetings relevant to the Tissue Typers Community within Eurotransplant.

10.1.1 Registration of transplant recipients

Relevant histocompatibility and immunological data of all potential organ recipients and donors are registered centrally in ET.
Every potential transplant recipient should be HLA typed on two different occasions using two different samples. Every recipient and every organ donor **must** be typed for HLA-A, -B, -DR and –should be typed for HLA-C, and -DQ,.

If the phenotype of a potential recipient shows less than six (ten) HLA-A, -B, (-C), -DR, (-DQ) antigens, a family typing or molecular typing should be done for the definition of possible 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.

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.

10.1.2 Material for histocompatibility testing

Prior to enter a recipient on the waiting list for organ transplantation HLA typing and a screening for HLA specific antibodies **must** be done. The treating physician should contact the TTC affiliated to the transplant center and request information for the material before applying.

In general, all material **must** be sent to the TTC, affiliated to the transplantation center (TC), where a recipient is registered. The samples **must** be labeled according to the EFI-Standards, (http://www.efiweb.eu/index.php?id=102). The samples **must** be accompanied by the needed administrative information, provided by the TTC. An up-to-date listing of the TTC affiliated to ET is available at the central office of ET.

Typing for organ donors and crossmatching **must** follow the Standard Operation Procedures (SOP) released by the TTC, following the recommendations of ET and EFI.

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

Serological and molecular typing for HLA-A and B is accepted. For HLA-DR, HLA-C and HLA-DQ a molecular typing **must** be performed. HLA typing data translated to "matching determinants" are reported directly to ET (http://etrl.eurotransplant.org).

NB: All immunologically relevant data (i.e. HLA typing and screening data) reported to ET **must** be controlled for clerical errors. Every mistake or inconsistency **must** be reported immediately for correction to ET.

* provided the acceptance of the Eurotransplant affiliated countries.

10.3 Screening

Sera from all potential organ recipients should be screened for HLA specific antibodies at regular time intervals: e.g. for kidney recipients a screening every three months **must** be performed unless otherwise specified by the TC or the National Bodies.

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. In all cases, the screening **must** be carried out in time to prevent outdated screening leading to removal of the recipient from the allocation list. The screening should follow the recommendations of the National Bodies, ET, EFI, and the TC.

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

The identification of autoantibodies or transplantation irrelevant antibodies **must** be done 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 autocrossmatch using the recipient's own cells **must** be done with and without dithiothreitol (DTT). The TTC **must** report to ET if the recipients have autoantibodies or not. The TTC **must** use solid phase screening methods for the definition of antibodies against HLA Class I and HLA Class II (generic assay). 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 screening performed or in case of changes of the unacceptable HLA mismatches list a virtual PRA value (see below).

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

The %-PRA value represents the percentage of donors in a panel reacting positively with the recipient's serum. The definition of the %-PRA value **must** be done using a

panel of HLA typed cells or by entering the specificities found as unacceptable antigens followed by a calculation of the virtual PRA value. Solid phase assays where a %-PRA value can be defined or calculated are also accepted. In case of complement dependent cytotoxicity (CDC +/- DTT) the panel **must** allow the definition of the %-PRA value and the definition of the HLA specificities recognized by the recipient serum:

- The number of HLA typed cell suspensions used **must** be ≥50.
- If the %-PRA value is >5%, an autocrossmatch **must** be done to exclude autoantibodies. The TTC **must** report this information to ET.
- The %-PRA value **must** be based on alloantibody reactivity only and consists 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.
- 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 control if recipients have an outdated screening. Furthermore, the TTC **must** define the autoantibody status of the recipients, and **must** distribute the sera with a %-PRA value >5%, unless otherwise stated.

For other organs than kidneys, recipients should be screened for HLA specific antibodies prior to enter the waiting list. In addition, further screenings are requested after every immunizing event.

10.3.2 Unacceptable HLA mismatches (vPRA)

Unacceptable HLA mismatches are HLA antigens against which a recipient has formed alloantibodies (current and / or historical depending on the policy of the TC). These antigens are used for the calculation of the vPRA value. Following this policy, mismatched HLA antigens of the previous organ donor or the HLA antigens of the

partner of the recipient, can be reported as unacceptable mismatches in ENIS. No offer will be made if an organ donor expresses these unacceptable HLA mismatches (a synonym of virtual crossmatch positive). .HLA antigens, towards which the recipient has formed alloantibodies defined in the current serum, **must** be reported as unacceptable mismatches and entered in ENIS after informing the TC. A direct link from defined specificities and unacceptable mismatches is not possible. The responsible TTC **must** confirm every unacceptable HLA specificity separately. From the list of these unacceptable mismatches the virtual PRA value will be automatically calculated. This value appears in the immunological report.

10.4 Crossmatch

The crossmatch using the recipient serum and lymphocytes of the prospective donor is an integral step in the decision making process in transplantation. For kidney and combined kidney/pancreas transplantation a crossmatch **must** be done before transplantation using current sera as specified by the recipient TC and TTC unless otherwise decided by the National Bodies. In addition, historical (peak) sera should be included. In case a crossmatch is not prospectively performed, the reasons, final decision, and outcome of the possible transplantation **must** be documented in the TTC, following the EFI standards (http://www.efiweb.eu/index.php?id=102).
For organs other than kidney a crossmatch should be done for recipients who either have HLA specific alloantibodies, or had an alloimmunizing event such as pregnancy, blood transfusion, and previous transplantation. Unless otherwise decided by the TC recipients waiting for heart, lung, pancreas, and small bowel or a combination of those organs and being allosensitized, a crossmatch **must** be performed.

The TTC **must** use CDC for the crossmatch with or without DTT as requested by the recipient center via the ET allocation office/ENIS (allocation crossmatch) or the local TC and can use additional techniques if the screening for HLA specific antibodies have been performed with the same methods and at the same degree of sensitivity (transplantation or decisive crossmatch).

10.4.1 The "allocation" crossmatch

The "allocation" crossmatch is performed in the donor center. It aims to avoid organ dispatch to recipients having preformed antibodies against the donor, which are not included in the recipient specific profile as unacceptable HLA antigens. In this profile in addition to the class I also the class II specificities can be entered. For the crossmatch procedure unseparated cells or T cells **must** be used as targets with addition or not of DTT according to the request forwarded by the allocation office. The use of any other target is not applicable. Crossmatches with B cells can be done only in case of local recipients or recipients from co-operating centers, where a formal request from the TC is present.

10.4.2 The "transplantation" or "decisive" crossmatch"

The "transplantation" or "decisive" crossmatch is the one done in the TTC where the

recipient is registered or the Center co-operating with the recipients TC. Here, other than the above mentioned targets, unseparated cells and T cells, can be used, e.g. B cells or endothelial cells. 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 TC, ET and EFI. It is the responsibility of the TTC to adhere to these recommendations.

10.4.3. Shipping 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 respective container together with a sufficient number (if available) of isolated lymphocytes. Labeling of the vials and all information included **must** include the ET donor number **and must** follow the EFI-Standards.

10.4.4 Donor TTC

The donor TTC **must** perform all transplantation relevant immunological assays for postmortal organ donors and recipients as HLA typing, screening for HLA specific antibodies and crossmatching. In Germany the donor TTC is named regional TTC. The crossmatches **must** be done 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 and the results must be reported to the ET allocation office when indicated by the ET allocation office.

The TTC must apply policies allowing quick and reliable results avoiding any 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.

For allosensitized recipients a crossmatch with and without DTT **must** be performed when indicated by the ET allocation office and the result **must** be reported in this way to the ET allocation office. The recipients TC 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 TC. The reasons **must** be reported to ET before transplantation.

The recipient TTC and TC are responsible for the histocompatibility of the transplant, including crossmatching.

10.4.6 Crossmatch serum exchange program

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 recipient are printed locally. Dialysis centers collect sera of their potential kidney recipients four times a year and send them to their affiliated TTC.

The sera are screened for HLA specific antibodies and their %-PRA (v-PRA) value.

For recipients awaiting kidney transplantation only, sera with an allo-PRA value of >5% as depicted in the CDC are included in the crossmatch serum exchange program. Sera from recipients with antibodies found in sold 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 given in the respective screens in ENIS.

The TTC ships the serum samples together with a list indicating the recipients, of whom serum is included, following the national postal regulations. The receiving TTC **must** control if all sera have been included. In case 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.
- Fill the tubes with 50-250 microliter recipient serum.
- Avoid any air bubble formation in the serum.
- Per recipient a number of tubes corresponding to the latest list of TTC participating in the crossmatch serum exchange **must** be prepared and shipped.
- The ET-Nr. and name of the recipient serum should be marked on the TTC list, which is sent to the TTC participating in the crossmatch serum exchange.
- For control reasons a copy of the list should remain locally.

In the receiving TTC the following steps are recommended:

- The accompanying list **must** be controlled. 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 control purposes, the lists of the different TTC **must** be kept until the next exchange.

10.4.8 Sera from non-kidney recipients

Screening of sera from potential recipients of organs and tissues other than kidney is identical to the one described above. In case of immunized recipients, 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 Program (AM)

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

10.5.1 Eligibility of a recipient for the AM Program

Panel reactive cytotoxic antibodies resulting in a PRA value of ≥85% must be found in the serum of two different bleedings of the recipient or ≥85% v-PRA calculated from the unacceptable HLA antigens reported by the transplantation center of the recipient, provided they mainly activate complement.

Recipients are not eligible for the AM if:

no unacceptable antigens are reported
the recipient possesses solid phase defined HLA antibodies only

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

In recipients with cytotoxic HLA antibodies, the ETRL will accept the contribution of solid phase defined antibodies to the AM status, when these specificities are explained by earlier transplantation, e.g. HLA mismatches of the previous donor (s), or sensitization of the recipient, e.g. HLA antigens of the partner or children in women.

The recipient should wait at least two years, as defined by the date of first dialysis, before inclusion in the AM program.

For every AM recipient the ETRL calculates the chance that a suitable donor becomes available in the ET donor population. The recipients are then divided into two categories:

Low chance for a donor, which implies a frequency of 0.1% or less

High chance for a donor which represents a frequency of more than 0.1%

A frequency of 0.1% represents a chance of 1- 2 organ donors per year.

10.5.2 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.

The HLA-A, B, C, and DR, DQ typing of the organ donor is entered in the Eurotransplant Network Information System (ENIS). Potential recipients will be selected on the basis of their own HLA-A, B, C and DR, DQ antigens in combination with the AM. The AM are regarded, as recipients own HLA antigens. Full compatibility between donor and recipients including the AM is a prerequisite for allocation of kidneys via the AM program. Matching is based on the "split" HLA class I antigens and the "split" HLA-DR antigens as already done for all recipients on the renal waitlist.

For recipients with a low chance for a suitable donor no minimal criteria apply.

For recipients with a high chance for an organ the following minimal **sharing** criteria apply:

- 1HLA-B and 1 HLA-DR

or

- 2 HLA-DR antigens

The ETRL immunologist on duty is informed about every potential offer for a recipient included in the AM program:

A. In case the offer results from the AM program the immunologist on duty controls 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 immunologist on duty of the ELTR the respective TC is informed, and if accepted by the TC, 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. Repeated 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 only when the organ donor is typed for. In case an organ donor is not typed for HLA-C and DQ then the association table is used for the decision making of the immunologist on duty. An organ will not be offered if the recipient has specific antibodies to the associated antigens. The Immunologist on duty will **deny** an offer if unacceptable antigens are reported or the minimal criteria are not met.

The order by which the kidneys will be offered in case of multiple recipients is according to the calculated chance to receive an organ as provided by the ETRL (Donor Frequency Calculator). Recipients with the lowest chance get the highest priority.

B. If the offer results from the ET-Kidney Allocation System the Immunologist on duty controls the HLA typing of the organ donor, the HLA typing of the recipient, the unacceptable HLA antigens and the antibody specificities reported. The Immunologist on duty advises to offer or not the organ. The final decision is in the hand of the transplantation center of the recipient. Here an allocation crossmatch **must** be done.

10.6 ET proficiency testing (EPT)

ET being an organ exchange organization **must** rely on the work of the affiliated TTC. One of the essential steps in maintaining the high standards of histocompatibility related matters within ET is the External Proficiency Testing Exercises. This is the only EPT where a center to center comparison in Eurotransplant is possible. Therefore, all ET affiliated laboratories entering data in ENIS **must** participate in all EPT without any sample selection and **must** fulfill the requirements of EFI. The ETRL has established these schemes 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 beginning of the calendar year how the EPT scheme will be organized and what data are required for the analysis and the certificate. The results of the EPT form the basis for future decisions of bodies 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 in ET.

Modification of any of those Standards is done if deemed important. Every participant receives the results in an open way and with the center code given by ET. The participants receive the analysis of the results either separately by e-mail or as a link and the results are finally published on the web. Every participant receives by January 31 of every calendar year latest a certificate of performance, where the fulfillment or not of the requirements can be mentioned. In case of any inconsistency, changes in the certificate can be done until March 1, of the calendar year latest. A summary of the results is presented in the Annual Report of ET. The actual schemes include external proficiency testing exercises (EPT) for: HLA typing, crossmatching, and screening. In addition, a serum crossmatching EPT is designed in case of e.g. postal or custom problems.
At the beginning of each new period the TTC receives information from the ETRL regarding the EPT schemes. This information is publicized on the ETRL website.

10.6.1 EPT on HLA typing

This EPT performed 4 times per year, 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 and 2) TTC performing recipient typing and screening for HLA specific antibodies. The results **must** be reported back as matching determinants. All TTC submitting transplantation relevant HLA typing results to ET **must** participate without any selection of samples. The typing result of the organizer represents the correct typing. In case a participant disapproves with the results, the secretary of the TTAC must be informed via e-mail. The point will then be discussed in the following TTAC meeting. The participants receive the analysis of the results within four weeks after deadline.

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. In case participants are using methods resulting in low viability of B cells, jeopardizing the incubation with DTT, the participants must use DTT with unseparated cells as targets. The TTC are free to use unseparated lymphocytes, and/or separated T and/or B cells for the crossmatch following the local SOP's. The results **must** be reported back to the organizer.

10.6.3 EPT on screening

This EPT is performed once per year and consists of a shipment of 12 sera of recipients or multiparous women with HLA specific antibodies. 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 MHC class I and/or MHC class II antibodies, and the specificity (-ies). . Methods reported in the local SOP **must** be used. The use of additional methods is possible. The analysis of this EPT will be performed as stated in the respective information publicized on the ETRL site and reported to the participants.

10.6.4 EPT on serum crossmatch

This EPT is designed for TTC having problems in receiving in due time the samples for the crossmatch EPT, because of postal or custom problems. The EPT is only for selected TTC and 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 back to the ETRL. The standards of the External Proficiency Testing Committee of EFI apply.

10.7 Forms

All forms are on the ETRL part of the Eurotransplant site

http://www.eurotransplant.org/cms/index.php?page=etrl1