

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 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 framework 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 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 by the different ET matching algorithms from the reported HLA typing data 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 in general and allocation of organs through the Acceptable Mismatch (AM) program in particular.
- 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 are authorized to 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 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-C, HLA-DR, 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. In case of proven homozygosity, the homozygous antigen must be entered in duplicate. For organ donors first field (two digits) DNA typing is accepted for the definition of homozygosity. However, when for a donor typing a homozygous antigen is found and not proven as described above, this antigen should be reported only once to ET to comply to EFI standards.

HLA typing data of the donor must be reported directly to ET preferably by electronic communication, or by e-mail 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 a non-expressed allele is found, the accompanying antigen must not be entered to prevent unnecessary denial of organ offers. Entering of Bw4/Bw6 should be done on basis of HLA-B antigens only. The HLA typing provided is translated to “matching determinants” by the different ET matching algorithms for allocation purposes, whereas the full HLA typing of donors is used for the virtual crossmatch.

In case of stem cell transplantation before organ transplantation, a new HLA typing and ABO blood group typing should be performed.

10.2.2 Patients with uncommon HLA type

Patients with an uncommon HLA type might have allele specific HLA antibodies to the common allele. Most organ offers matched for this (common) allele will result in a positive cross match. The uncommon HLA antigen of the patients can be deleted in ENIS to prevent such organ offers. The change must be submitted for acceptance to the ETRL.

10.2.3 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, accompanied by a critical review of the unacceptable antigens listed. 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 exclusion of the recipient from the allocation list.

For other organs than kidneys, according to the national legislations, recipients should be screened for HLA specific antibodies prior to entering the waiting list, as well as on an annual basis. In addition, further screenings are required 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. For HLA antibody positive sera, the HLA specificities should be reported, and in case these are deemed unacceptable, these unacceptable HLA antigens must be reported.

10.3.1 Screening for HLA specific antibodies

The CDC screening for determining the presence of complement fixing antibodies must be done using a panel of HLA typed cells. Solid phase screening methods must be used for the definition of antibodies against HLA class I and HLA class II. Unacceptable antigens must be defined, ideally on basis of both CDC screening and solid phase screening data. The number of HLA typed cell suspensions used should be ≥ 50 . Upon registering the unacceptable antigens in ENIS the virtual PRA (vPRA) will be calculated automatically based on these unacceptable antigens, and included in the ENIS data file. Starting January 28, 2020, the vPRA will be accepted as the only parameter to indicate that a patient is sensitized.

In case the patient has unacceptable antigens and has known CDC reactive antibodies at time of registration, or develops these in a subsequent serum sample, an auto-crossmatch must be performed to exclude that CDC reactivity is based on autoantibodies. The TTC must report this information to ET. The serum of recipients with $>0\%$ vPRA and known CDC reactive antibodies must be included in the crossmatch serum exchange.

The recipient's TTC must update the HLA antibody and unacceptable antigen 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 vPRA value $>0\%$ and known CDC reactivity due to allo-antibodies, unless otherwise stated.

10.3.2 Autoantibodies

The identification of autoantibodies or transplantation irrelevant antibodies must be performed by the TTC. Such antibodies can 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 serum of recipients with autoantibodies only will not be 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, repeat mismatches, or spousal 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 to 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. Unacceptable antigens should be entered at the split antigen level. In case all split antigens from a broad antigen are unacceptable, the broad antigen should be entered as unacceptable. 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 allocation through ETKAS, ESP or the heart/lung match, no offer will be made if an organ donor expresses unacceptable HLA antigens of a given patient (a synonym of virtual crossmatch positive)¹. 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 ABO blood group of a patient, the chance to receive an offer of an ABO identical or -compatible renal transplant can be calculated. There are three calculators based on the blood group; ABO identical, ABO compatible and ABO ET compatible. These Donor Frequency Calculators have been developed by the ETRL (<https://etrl.org/>) and are also implemented into ENIS

10.4. Crossmatch

The crossmatch is an integral step in the decision-making process for organ allocation and transplantation. Both virtual and physical crossmatches can be performed. The prerequisite for the virtual crossmatch is the definition of HLA antibody specificities that are deemed to be unacceptable HLA antigens and are to be avoided in the donor's HLA-type.

The physical crossmatch using the recipient serum and lymphocytes of the prospective donor is usually done with the CDC technique. Currently, two types of physical crossmatches exist in ET, the allocation crossmatch and the decisive crossmatch. An overview of the shipment and crossmatch rules is depicted in table 10.4. All crossmatch results must be communicated to the ET allocation office.

¹ In case the donor HLA type is not known at time of the ESP or 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.

HLA antibody status	Unacceptable antigens defined	vPRA	Serum shipment	Allocation crossmatch	Decisive crossmatch
No HLA antibodies or only non-complement fixing HLA antibodies	No	0%	No	No	Yes (may be retrospective)
	Yes	>0%	No	No	Yes
Complement fixing HLA antibodies	Yes	>0%	Yes	Yes	Yes

Table 10.4. Overview of serum shipment and crossmatch rules

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 is optional, but these results are not allowed to be taken into consideration for final results of the crossmatch) 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 allo-immunizing 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 allo-immunized, 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 donor HLA typing 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 (>0% vPRA and known CDC reactivity) 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 recipient HLA 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 allo-sensitized 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 vPRA value of >0% from patients with known CDC reactive antibodies 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. Preferentially, barcoded labels should be used.

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. For the procedure of application AM status: see the ETRL website.

10.5.1 Eligibility of a recipient for the AM Program

The potential AM recipient must be on the ETKAS waiting list for at least two years in total, as defined by the date of first dialysis (both adult and pediatric). Patients must have status T(ransplantable) and must be typed for HLA-A, -B, -C, -DR and -DQ at the split antigen level (except for HLA-B14) to be eligible for acceptance in the AM program. Panel reactive cytotoxic HLA antibodies resulting in a vPRA value of $\geq 85\%$ must be detectable in the serum of two different bleeding dates of the recipient. In addition to CDC reactivity, those antibodies solely detected by solid phase assays will only contribute to the AM status when the 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. In case of a female with suspected immunization due to pregnancy, HLA typing of child(ren) and/or father of children is required for eligibility check.

Preferentially, acceptable HLA-C and HLA-DQ antigens should also be defined. Current and historical sensitization are regarded equally important. Unacceptable antigens need to be confirmed by screening of a peak serum by the ETRL.

Recipients are not eligible for the AM program if:

- No unacceptable antigens are reported.
- The recipient possesses solely solid phase defined HLA antibodies not detectable in CDC which cannot be explained by a sensitization event.

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.

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 criteria mentioned above) 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 the ET modified blood group compatibility and HLA compatibility of the donor with the recipient's own HLA type in combination with the acceptable antigens. The AM allocation overrides the center profile with regards to local minimal match criteria. One must bear in mind that acceptable

mismatches are truly acceptable and should not be a reason to decline an organ offer.

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. In principle, minimal match criteria of sharing of one HLA-B antigen and one HLA-DR antigen, or two HLA-DR antigens on the split antigen level with the patient's own HLA antigens are adhered to.

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 and the minimal match criteria. Upon calculation of the chance that a suitable donor becomes available, the recipients are divided into two categories:

Low chance of receiving a donor kidney: donor frequency $\leq 0.1\%$
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). For recipients with a low chance of receiving a donor kidney as defined above, or urgency status HU, the minimal match criteria are reduced to one HLA-DR match at the broad antigen level.

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, the chance of receiving a kidney through the AM program, 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. In case of identical chance, the patient with the longest waiting time within the AM program gets prioritized.

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.etril.org/>), with the exception of the patient-based cases. These results can be returned by email to etril@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 negative or not-immunized (0% PRA) and positive or immunized (>0%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 deceased donor HLA 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). Homozygosity rules as described in section 10.2.1 apply. 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 each time the 3 peripheral blood samples distributed for the EPT on typing and 3 selected sera only used for crossmatching. All TTC performing crossmatches for deceased 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. The final results must be based on unseparated lymphocytes and/or separated T cells only. 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, different from those sera sent for crossmatching. All TTC reporting screening data to ENIS must participate in the EPT on screening. The TTC must report the presence or absence of CDC reactive antibodies 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. Next to this at least one solid phase test must be used to define HLA class I and class II antibodies. 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	ANTIGEN	SPLIT	BROAD	PUBLIC	ANTIGEN	SPLIT	BROAD	PUBLIC
A1				A10				A19			
A*01:01		A1		A25		A10		A29		A19	
A*01:02		A1		A*25:01	A25	A10		A*29:01	A29	A19	
A*01:XX		A1		A*25:XX	A25	A10		A*29:02	A29	A19	
A2				A26		A10		A*29:XX	A29	A19	
A*02:01		A2		A*26:01	A26	A10		A30		A19	
A*02:02		A2		A*26:02	A26	A10		A*30:01	A30	A19	
A*02:03		A2		A*26:03	A26	A10		A*30:02	A30	A19	
A*02:05		A2		A*26:08	A26	A10		A*30:04	A30	A19	
A*02:06		A2		A*26:XX	A26	A10		A*30:XX	A30	A19	
A*02:07		A2		A34		A10		A31		A19	
A*02:10		A2		A*34:01	A34	A10		A*31:01	A31	A19	
A*02:11		A2		A*34:02	A34	A10		A*31:XX	A31	A19	
A*02:17		A2		A*34:XX	A34	A10		A32		A19	
A*02:XX		A2		A66		A10		A*32:01	A32	A19	
A3				A*66:01	A66	A10		A*32:XX	A32	A19	
A*03:01		A3		A*66:02	A66	A10		A33		A19	
A*03:02		A3		A*66:XX	A66	A10		A*33:01	A33	A19	
A*03:XX		A3		A11				A*33:03	A33	A19	
A9				A*11:01		A11		A*33:XX	A33	A19	
A23		A9		A*11:02		A11		A74		A19	
A*23:01	A23	A9		A*11:XX		A11		A*74:01	A74	A19	
A*23:XX	A23	A9						A*74:XX	A74	A19	
A24		A9						A28			
A*24:02	A24	A9						A68		A28	
A*24:03	A24	A9						A*68:01	A68	A28	
A*24:07	A24	A9						A*68:02	A68	A28	
A*24:08	A24	A9						A*68:03	A68	A28	
A*24:XX	A24	A9						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	ANTIGEN	SPLIT	BROAD	PUBLIC	ANTIGEN	SPLIT	BROAD	PUBLIC
B5			BW4	B70			BW6	B35			BW6
B51		B5		B71		B70		B*35:01		B35	
B*51:01	B51	B5		B*15:10	B71	B70		B*35:02		B35	
B*51:02	B51	B5		B*15:18	B71	B70		B*35:03		B35	
B*51:07	B51	B5		B72		B70		B*35:05		B35	
B*51:08	B51	B5		B*15:03	B72	B70		B*35:08		B35	
B*51:XX	B51	B5		B16				B*35:12		B35	
B52		B5		B38		B16	BW4	B*35:17		B35	
B*52:01	B52	B5		B*38:01	B38	B16		B*35:43		B35	
B*52:XX	B52	B5		B*38:02	B38	B16		B*35:XX		B35	
B7			BW6	B*38:XX	B38	B16		B37			BW4
B*07:02		B7		B39		B16	BW6	B*37:01		B37	
B*07:03		B7		B*39:01	B39	B16		B*37:XX		B37	
B*07:04		B7		B*39:02	B39	B16		B40			BW6
B*07:05		B7		B*39:05	B39	B16		B*40:XX		B40	
B*07:09		B7		B*39:06	B39	B16		B60		B40	
B*07:XX		B7		B*39:10	B39	B16		B*40:01	B60	B40	
B8			BW6	B*39:XX	B39	B16		B61		B40	
B*08:01		B8		B17			BW4	B*40:02	B61	B40	
B*08:XX		B8		B57		B17		B*40:06	B61	B40	
B12				B*57:01	B57	B17		B41			BW6
B44		B12	BW4	B*57:02	B57	B17		B*41:01		B41	
B*44:02	B44	B12		B*57:03	B57	B17		B*41:02		B41	
B*44:03	B44	B12		B*57:XX	B57	B17		B*41:XX		B41	
B*44:04	B44	B12		B58		B17		B42			BW6
B*44:05	B44	B12		B*58:01	B58	B17		B*42:01		B42	
B*44:10	B44	B12		B*58:02	B58	B17		B*42:XX		B42	
B*44:XX	B44	B12		B*58:XX	B58	B17		B46			BW6
B45		B12	BW6	B18			BW6	B*46:01		B46	
B*45:01	B45	B12		B*18:01		B18		B*46:XX		B46	
B*45:XX	B45	B12		B*18:XX		B18		B47			BW4
B*50:02	B45	B12		B21				B*47:01		B47	
B13			BW4	B49		B21	BW4	B*47:XX		B47	
B*13:01		B13		B*49:01	B49	B21		B48			BW6
B*13:02		B13		B*49:XX	B49	B21		B*48:01		B48	
B*13:XX		B13		B50		B21	BW6	B*48:XX		B48	
B14			BW6	B*40:05	B50	B21		B53			BW4
B*14:XX		B14		B*50:01	B50	B21		B*53:01		B53	
B64		B14		B*50:XX	B50	B21		B*53:XX		B53	
B*14:01	B64	B14		B22			BW6	B59			BW4
B65		B14		B54		B22		B*59:01		B59	
B*14:02	B65	B14		B*54:01	B54	B22		B*59:XX		B59	
B15				B*54:XX	B54	B22		B67			BW6
B*15:XX		B15		B55		B22		B*67:01		B67	
B62		B15		B*55:01	B55	B22		B*67:XX		B67	
B*15:01	B62	B15	BW6	B*55:02	B55	B22		B73			BW6
B*15:05	B62	B15	BW6	B*55:XX	B55	B22		B*73:01		B73	
B*15:07	B62	B15	BW6	B56		B22		B*73:XX		B73	
B*15:24	B62	B15	BW4	B*56:01	B56	B22		B78			BW6
B*15:25	B62	B15	BW6	B*56:XX	B56	B22		B*78:01		B78	
B*15:27	B62	B15	BW6	B27				B*78:XX		B78	
B*15:30	B62	B15	BW6	B*27:02		B27	BW4	B81			BW6
B63		B15	BW4	B*27:03		B27	BW4	B*81:01		B81	
B*15:16	B63	B15		B*27:05		B27	BW4	B*81:XX		B81	
B*15:17	B63	B15		B*27:06		B27	BW4	B82			BW6
B75		B15	BW6	B*27:07		B27	BW4	B*82:01		B82	
B*15:02	B75	B15		B*27:08		B27	BW6	B*82:XX		B82	
B76		B15	BW6	B*27:XX		B27	BW4	B83			BW6
B*15:12	B76	B15						B*83:01		B83	
B77		B15	BW4					B*83:XX		B83	
B*15:13	B77	B15						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	DR51/52/53	ANTIGEN	SPLIT	BROAD	DR51/52/53	ANTIGEN	SPLIT	BROAD	DR51/52/53
DR1				DR5			DR52	DR7			DR53
DRB1*01:01		DR1		DR11		DR5		DRB1*07:01		DR7	
DRB1*01:02		DR1		DRB1*11:01	DR11	DR5		DRB1*07:XX		DR7	
DRB1*01:03		DR1		DRB1*11:02	DR11	DR5		DR8			
DRB1*01:XX		DR1		DRB1*11:03	DR11	DR5		DRB1*08:01		DR8	
DR2			DR51	DRB1*11:04	DR11	DR5		DRB1*08:02		DR8	
DR15		DR2		DRB1*11:05	DR11	DR5		DRB1*08:03		DR8	
DRB1*15:01	DR15	DR2		DRB1*11:XX	DR11	DR5		DRB1*08:04		DR8	
DRB1*15:02	DR15	DR2		DR12		DR5		DRB1*08:05		DR8	
DRB1*15:03	DR15	DR2		DRB1*12:01	DR12	DR5		DRB1*08:XX		DR8	
DRB1*15:XX	DR15	DR2		DRB1*12:02	DR12	DR5		DR9			DR53
DR16		DR2		DRB1*12:XX	DR12	DR5		DRB1*09:01		DR9	
DRB1*16:01	DR16	DR2		DR6			DR52	DRB1*09:XX		DR9	
DRB1*16:02	DR16	DR2		DR13		DR6		DR10			
DRB1*16:XX	DR16	DR2		DRB1*13:01	DR13	DR6		DRB1*10:01		DR10	
DR3			DR52	DRB1*13:02	DR13	DR6		DRB1*10:XX		DR10	
DR17		DR3		DRB1*13:03	DR13	DR6		DR51			
DRB1*03:01	DR17	DR3		DRB1*13:04	DR13	DR6		DRB5*01:01			DR51
DRB1*03:04	DR17	DR3		DRB1*13:05	DR13	DR6		DRB5*01:02			DR51
DR18		DR3		DRB1*13:06	DR13	DR6		DRB5*02:01			DR51
DRB1*03:02	DR18	DR3		DRB1*13:XX	DR13	DR6		DRB5*02:02			DR51
DRB1*03:03	DR18	DR3		DR14		DR6		DRB5*XX			DR51
DRB1*03:XX		DR3		DRB1*14:01/54	DR14	DR6		DR52			
DR4			DR53	DRB1*14:02	DR14	DR6		DRB3*01:01			DR52
DRB1*04:01		DR4		DRB1*14:03	DR14	DR6		DRB3*02:			DR52
DRB1*04:02		DR4		DRB1*14:04	DR14	DR6		DRB3*03:01			DR52
DRB1*04:03		DR4		DRB1*14:05	DR14	DR6		DRB3*XX			DR52
DRB1*04:04		DR4		DRB1*14:06	DR14	DR6		DR53			
DRB1*04:05		DR4		DRB1*14:07	DR14	DR6		DRB4*01			DR53
DRB1*04:06		DR4		DRB1*14:08	DR14	DR6		DRB4*XX			DR53
DRB1*04:07		DR4		DRB1*14:09	DR14	DR6					
DRB1*04:08		DR4		DRB1*14:10	DR14	DR6					
DRB1*04:09		DR4		DRB1*14:XX	DR14	DR6					
DRB1*04:10		DR4									
DRB1*04:11		DR4									
DRB1*04:12		DR4									
DRB1*04:XX		DR4									

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	