Rules for the Eurotransplant External Proficiency Testing on HLA typing, crossmatching, and screening for HLA specific antibodies (2019)

The external proficiency testing (EPT) exercises follow the daily work of histocompatibility and immunogenetics laboratories in the field of solid organ transplantation. It reflects the decision making process for accepting or denying an organ offer for a given patient on immunological basis. Therefore, the sera used for screening will be used for the crossmatch procedure in the respective EPT. Since the complement dependent cytotoxicity (CDC) test is the method used within Eurotransplant (ET) for final decision whether a transplant can go ahead on immunological basis, all participants must report screening results using the standard CDC technique as minimum requirement. In addition, the participants must perform dithiothreitol (DTT) incubation to detect possible transplantation irrelevant antibodies of the IgM type.

Number of samples

HLA typing: 4 times 3 tubes of peripheral blood for typing are shipped. In total 12 samples have to be typed.
Cross matching: 4 times 3 tubes of peripheral blood are shipped, to be cross matched with 3 designated different sera from the screening EPT. The cells are the same cells as the typing samples. In total 36 cross matches have to be performed.
Screening: For screening and screening identification 12 sera are shipped (in one shipment). These sera are also used for cross matching.
For Eurotransplant affiliated centers no selection of samples is allowed (Eurotransplant Manual, chapter 10.1 and 10.6).

Techniques accepted for the EPT organized by the ETRL

HLA typing

For typing HLA-A and HLA-B, serology and/or DNA techniques may be used, whereas typing for HLA-C, HLA-DR and HLA-DQ must be performed by using DNA techniques. The HLA typing must be reported as serological equivalents on split level, as described in the ET manual, table 10.7.1-5.

Crossmatching

Crossmatching must be performed with CDC, using either unseparated cells or T cells. Additionally, B cell crossmatching is allowed. For interpretation and reporting of final results, only unseparated cell and/or T cell crossmatches must be used.
Crossmatches must be performed with and without DTT, to exclude possible autoantibodies.

Screening Antibody Detection

1. CDC +/- DTT, resulting in definition of % PRA. Results can be entered into three categories: PRA < 6% (not immunized), PRA ≥6% and <85% (immunized), PRA ≥85% (highly immunized).
2. Flow cytometry (FCM), resulting in definition of % PRA for HLA class I and class II (results can be entered into three categories similar to CDC).
3. ELISA, leading to detection of antibodies directed at HLA class I and HLA class. Results can be entered in two categories: POS (positive) and NEG (negative).
4. Luminex for detection of antibodies directed at HLA class I and HLA class II. Results can be entered into two categories: POS (positive) and NEG (negative).
5. Any other method for detection of antibodies directed at HLA class I and HLA class II. Results can be entered into two categories: POS (positive) and NEG (negative).

Screening Antibody Identification

1. CDC with and without the use of DTT. This method is mandatory for all ET affiliated laboratories. In addition to the regular CDC assay, the participants must perform dithiothreitol (DTT) incubation to detect possible transplantation irrelevant antibodies of the IgM type.
2. Solid phase assays from cell lysates. This method is abbreviated as **SPA**:
   a) ELISA for specification of antibodies directed at HLA class I and class II.

b) Luminex based methods specification of antibodies directed at HLA class I and class II. Any other method for specification of antibodies directed at HLA class I and class II.

3. Solid phase assays using single HLA antigens. This method is abbreviated as **SPA SA**:
   a) ELISA for specification of antibodies directed at HLA class I and class II.
   b) Luminex based methods for specification of antibodies directed at HLA class I and class II.
   c) Any other method for specification of antibodies directed at HLA class I and class II.
4. Solid phase assays using single HLA antigens as targets and allow the detection of complement fixing antibodies. This method is abbreviated as **SPA SA plus**, and includes C1q and C3d assays:
   a) Luminex based methods for specification of complement fixing antibodies directed at HLA class I and class II.
   b) Any other method for specification of complement fixing antibodies directed at HLA class I and class II.

Patient-based EPT

During each EPT cycle, a patient based case will be sent to all participating centers at three separate occasions. Participation is mandatory for ET affiliated centers and optional for other centers. For each case all necessary patient and donor data will be provided. Participants have to decide on basis of these data whether the transplant can go ahead on an immunological grounds. Participants can submit their answers by returning the filled in patient based case forms.

Submission and analysis of EPT results

All results , except for the patient based cases, must be submitted using the ETRL EPT website (https://www.etrl.org/). Only after authorization by a representative from the laboratory the results can be evaluated by the ETRL. Analysis of the reported results will be performed by the ETRL for all categories. For all EPT schemes, the analysis is performed based on consensus rules as described below.

Assessment of results

As basis for result assessment the most recent EFI standards for EPT providers are adhered to.

HLA-Typing

For HLA typing a 75% consensus rule is applied. When an HLA-typing is not in consensus, a reference typing using NGS will be performed. This will be done at the NLBTT laboratory according to the SLA (Service Level Agreement) between the ETRL and NLBTT.

Crossmatch consensus

For crossmatching a 75% consensus rule is applied. This means that when 75% or more of the participants give the same result for a crossmatch, this result is considered in consensus. Discrepant results will be marked and scored for all categories (unseparated cells, T cells, B cells and final results).

Antibody detection consensus

For antibody detection a 75% consensus rule is applied. This means that when 75% or more of the participants give the same result, this result is considered in consensus. Discrepant results will be marked and scored for all categories (CDC, FCM, ELISA, Luminex and final results).

Antibody specificity consensus

For the CDC assay a 75% consensus rule is used. A specificity is regarded as detectable when 75% of the participants report this specificity. If 95% of the participants report that a serum does not include a certain specificity, then this specificity is regarded as absent in the serum.
For all SPA varieties, a 95% consensus rule will be used for all antigens reported. A specificity is regarded as detectable when 95% of the participants report this specificity. If 95% of the

participants report that a serum does not include a certain specificity, then this specificity is regarded as absent in the serum.

Patient-based EPT
Answers and motivations are collected and analyzed. During the annual extramural meeting the patient cases will be discussed.

Analysis of results by the ETRL

Certificates are issued by the ETRL based on the performance in the EPT. The following results on the certificate are possible: fulfilled / not fulfilled / participated. Certificates will be issued for all techniques as a participation certificate. The certificate for (not) fulfilling the requirements can only be awarded if a minimum number of 10 participants report results in the respective category. For certificates of successful performance (fulfilled), the participant needs to meet the following criteria as described in the latest version of the Eurotransplant Manual and the latest version of the EFI Standards (7.0) and the latest version of the EFI standards for providers (7.1) as described below.

HLA typing
All samples must be typed (serological and/or DNA-based typing). The typing EPT exercise is successfully performed in case of 90% correct complete typing results.
When two samples are swapped and both HLA types are correct this will count as one discrepancy. Every discrepant typing will be mentioned on the certificate. When the maximum discrepancy rate is exceeded, the participant will get the chance to correct this by performing additional typing.

Crossmatch
All crossmatches must be performed. The crossmatch EPT exercise is successfully performed in case the participant reports 85% correct results of the total number of results reaching consensus. Discrepancy rates are counted as percentage within a certain type of crossmatch (e.g. B cells with DTT). When a center exceeds the maximum discrepancy rate, it will have the chance to perform additional crossmatches in order to correct this.

Screening Antibody detection:
All samples must be tested for the presence or absence of HLA antibodies. The antibody detection EPT exercise is successfully performed in case the participant reports 80% correct results of the total number of results reaching consensus.
Discrepancy rates are counted as a percentage of the different categories (CDC +/- DTT, FCM HLA class I/II, ELISA HLA class I/II, luminex HLA class I/II and the final results HLA class I/II). When a center exceeds the maximum discrepancy rate, it will get the opportunity to test additional sera in order to correct this.

Screening Antibody identification:
All samples must be tested for the identification of HLA-specific antibodies. The antibody identification EPT exercise is successfully performed in case the participant reports 75% or more of the consensus specificities in all samples.
Discrepancy rates are counted as a percentage of total number missing specificities divided by the total number of consensus specificities. When a center exceeds the maximum discrepancy rate, it will have the chance to test additional sera in order to correct this.

Patient based cases
Three patient based cases are sent throughout the EPT year. For ET affiliated centers it is mandatory to send an answer to each case, before the deadline. Other centers are encouraged to join. A certificate of participation will be send at the end of each EPT year. Answers are analysed and categorised by the ETRL and will be discussed during the extra mural meeting in subsequent the year.