EUROPEAN FEDERATION FOR IMMUNOGENETICS

MANUAL for INTER-LABORATORY EXCHANGES

adopted by the EFI External Proficiency Testing Committee and approved by the EFI Executive Committee on May 9th 2018.

1. Introduction

External Proficiency Testing (EPT) is a mandatory part of the EFI accreditation process for Histocompatibility and Immunogenetics (H&I) laboratories and therefore part of the EFI Standards. The laboratories must participate in EPT programme(s) to cover all the accredited laboratory applications (HLA typing, antibody screening and identification, crossmatching, etc.) and all techniques used individually or in combination as routinely employed to produce a final result.

If no established scheme exists for a specific category (e.g. HNA antibody detection and identification) the laboratories must participate in an EPT workshop or trial offered by an EPT Provider or must take part in an inter-laboratory exchange of samples. In addition, if (an) EPT scheme(s) or EPT workshop(s)/trial(s) for a specific category exist(s) but the laboratories have no access, the laboratories must at least participate in an inter-laboratory exchange of samples.

Lacking access is defined by one of the following reasons:

- Samples from outside its country will not reach the laboratory within acceptable time.
- Samples from outside its country are blocked from entering the country.

High costs for the participation in established EPT programs will only be accepted in very exceptional cases. The laboratory must discuss the use of an inter-laboratory exchange instead of accessible EPT schemes with the relevant EFI Commissioner and have her/his approval.

This manual defines procedures and provides recommendations for how H&I laboratories can organize, perform and evaluate the results of inter-laboratory exchanges of H&I diagnostic samples. In general, inter-laboratory exchanges in H&I must be organized as close as possible to the EFI EPT Standards for laboratories and the EFI EPT Standards for Providers of established EPT schemes.

2. Purpose of inter-laboratory exchanges

The purpose of inter-laboratory exchanges is the comparison and evaluation of laboratory histocompatibility testing in organ transplantation and/or hematopoietic stem cell transplantation in a certain country/region/group of countries in a professional and scientifically based manner with an educational objective. The ultimate aim of an inter-laboratory exchange should be to have an EPT program established in a country/region/group of countries, where beforehand no EPT program was available or accessible (see Chapter 1).

In addition, the following benefits of inter-laboratory exchanges can be defined:

- Help laboratories to appraise their performance and monitor improvements externally, through continuous operation, regular distributions of samples and performance evaluation
- Produce reports which are designed to be clear, informative and structured to assist interpretation
- Assess technical, analytical and interpretative performance of a laboratory.

3. General information

It is important that the organizer(s) have personnel with the necessary technical competence and expertise in any planned inter-laboratory exchange(s). This is particularly important to allow advice and education to be offered to participants in the case of any technical difficulties. The organiser may wish to create an advisory group of relevant experts to aid in providing advice and assistance for inter-laboratory exchange participants.

The organizer should consider assigning a unique code to each participant to maintain confidentiality, when required. All information supplied to the organizer should be treated as confidential, unless the participant waives confidentiality.

4. Costs

The implementation of an inter-laboratory exchange causes costs for the organizing laboratory and for the participating labs.

Costs for inter-laboratory exchanges consist of:

- Staff for preparation of shipment, organization of the exchange, assessment of results, publishing certificates
- Materials, disposables and reagents, e.g. blood, serum, sample tubes, packaging materials, cell culture medium.
- Shipment costs
- Other costs, e.g. for meetings, discussion of the results, education

Possible ways to cover costs are:

- Participants pay a fee
- Participants pay for shipment costs
- A third party (e.g. a Ministry of Health) pays (parts) of the costs. In this case there can be limitation(s) for the participants (e.g. residing in a certain region or country, being active in e.g. solid organ transplantation, etc.).

5. Participants

Prior to the implementation of any inter-laboratory exchange it should be described which laboratories can participate (from one country, a certain region, any country, involved in solid organ transplantation and/or stem cell transplantation). The number of participants will normally be less than 10, with a minimum of 2. When higher numbers of participants are reached the exchange can be changed into an official EPT program, which will follow the EFI EPT Standards for EPT Providers.

It is recommended to have the organization of the inter-laboratory exchange in the hands of one laboratory, from where also the shipment of samples is done.

6. Manual for the participants

A short manual or protocol describing the procedures of the inter-laboratory exchange is recommended. The manual should describe the conditions that participants and organizer must fulfil with respect to the following issues:

- Numbers and type (material) of samples
- Shipment dates and methods for testing
- Instructions for handling/storing the samples, including any safety requirements
- Procedures for preparing the samples before testing
- Instructions for analysing and reporting results
- Deadlines and method(s)for sending results to the organizer
- Description of result analysis and performance evaluation by the organizer and reports of the evaluation for laboratories including the certificates (if applicable)

In addition, the participants must be informed that inter-laboratory exchange samples must be tested and interpreted in an identical manner to a laboratory's routine clinical samples and that collusion between laboratories is not allowed.
Revision of the manual should be done on a regularly basis (recommended after the first completion of an exchange and afterwards at least once a year). It is important that all participants are familiar with the manual.

7. Samples

For the types and numbers of samples, preparation of samples, handling of samples it is recommended to follow the current EFI EPT Standards. The minimum numbers of samples are described in the EFI Standards version 7.0, in section D. Inter-laboratory exchanges may use fewer samples.

Possible types of samples:

- Whole Blood (for tests that require viable lymphocytes it may be beneficial to add a suitable tissue culture medium e.g. RPMI 1640 with a suitable anticoagulant e.g. tri-sodium citrate)
- Isolated lymphocyte preparations (in a suitable tissue culture medium e.g. Park/Terasaki medium)
- Isolated DNA resuspended in water or appropriate stabilizing buffer

Blood, lymphocyte and DNA samples can be obtained from regular normal healthy blood donors at a local Blood Service Blood bank. Blood samples for inter-laboratory exchange must be tested for infectious diseases as if for patient use. Serum obtained from parous women or transplanted organ recipients can be used as a source of HLA antibodies. All donors of EPT material must give formal consent.

Preparation of samples:
It is important to apply stringent procedures for sample preparation to ensure homogeneity and that high quality samples are distributed to participants. Procedures should be in place during sample preparation, labelling and packaging to prevent samples being contaminated or mixed up.

- Blood samples for serological testing (e.g. HLA Phenotyping, CDC crossmatching) should ideally be prepared and distributed to participants within 24 hours of the donor being bled to maintain adequate lymphocyte viability. It may be beneficial to add a suitable tissue culture medium (e.g. RPMI 1640) with a suitable anticoagulant (e.g. tri-sodium citrate) to blood samples for serological testing to maintain sample quality.

- For serological testing, isolated lymphocytes may be prepared for participants with extended shipping times. Lymphocyte preparations are isolated from whole blood samples by density gradient media separation, with the resultant lymphocytes suspended in a suitable tissue culture medium (e.g. Park/Terasaki medium) for transport (protocol available as an addendum of this manual).

- Blood samples for molecular testing (e.g. HLA typing using PCR) may be bled up to 5 days prior to distribution as cell viability is not an issue. These samples may be distributed without any additives.

- For sera, it is advisable to add a preservative to prevent microbial contamination (e.g. 0.01% sodium azide).

- When DNA extracts are issued their DNA concentration should be indicated. DNA can be isolated from whole blood or lymphocyte suspensions (protocol available as an addendum of this manual).

- Blood and serum samples must be tested for the following disease markers: HIV, HBsAg, HCV and syphilis. Protocols should be accessible for the participants.

As with all biological material, samples should be considered as potentially hazardous. Notify the participants to handle with caution and apply accepted standards of Good Laboratory Practice. A reference sample should be kept by the organizer in case of dispute over results.

Distribution, packaging and handling and storage upon arrival:
Samples are sent to named individuals in each participating laboratory. Packaging must conform to IATA Packing Instruction 650. All samples can be transported at ambient temperature and should be processed as soon as possible on receipt.

Samples should be tested by participants as soon as possible after delivery. If storage is necessary, they should be treated and stored as clinical samples. It is important to ensure sample uniformity by carefully mixing each exchange sample prior to testing using the procedure applied to clinical samples.

Sample testing:
Samples of inter-laboratory exchanges must be tested in the same way as patient material to ensure that the exchange and assessments are a reliable measure of the quality of laboratory patient testing. The inter-laboratory exchange is intended to be educational in nature, so if problems are identified this will allow improvements in the quality of patient testing. Participants must inform the organizer if they are unable to test samples and if the samples arrived in bad condition or with substantial delay.

8. Results, assessment and (un)acceptable performance

Deadlines:
It is recommended to have deadlines that resemble closely the time needed for a comparable patient sample. E.g. for cross matches or typing a relatively short deadline (weeks) can be used, whereas for screening for antibodies a longer deadline can be used (months).

Submission of results:
Submission of results should preferably be done in a uniform manner, using uniform nomenclature. The organizer should have standard result forms available (sent by

regular mail, preferably via e-mail or on a website). It is also important to record which methods, vendors and lot numbers are used by the participants. Examples of forms are available as addenda at the end of this document. It is easiest and most cost effective to have forms sent and returned by e-mail or via a website.

Participants are expected to return 100% of results. Where a laboratory is unable to return a set of results, an explanation must be provided in writing. Participants should make every effort to return their results on or before the deadline date indicated. If an exchange allows submissions in handwriting, they should be clear and legible. Participants must be asked to resubmit forms that are unclear or incorrectly completed.

Late results may be accepted if assessment and reports have not been completed. Results cannot be accepted for assessment or performance review once the results of the inter-laboratory exchange are published. Requests for extensions to report deadlines should be made to the organizer.

Analysis:
Analysis is preferentially based upon a reference value (from the organizing centre/ from a local H&I reference centre/from an EFI accredited reference centre). In case of problems with a reference value it is recommended to have the sample(s) analysed by one or two external reference centres. The regional EFI EPT Committee Coordinators can be of assistance (see Chapters 10, 11, and 12). A list can be found on the EFI website (http://www.efiweb.eu/efi-committees/ept-committee.html).

Results of the analysis must be published to the participants, with discrepancies clearly marked. This can be done by sending a report to all participants, preferentially by e-mail. In this report it must be clear to each participant which result belongs to the participant, either by laboratory name or by a unique code.

Successful performance:
If an inter-laboratory exchange program just has started, a certificate of participation clearly stating the number of samples tested and the number of discrepancies is sufficient for each participant. Once the inter-laboratory exchange is established, the EPT standards for Providers, section 25 (Successful performance in EPT) should be used, with reference values, as long as the number of participants does not exceed 10. Reference values must be generated by the organizer or come from an EFI accredited laboratory.

Satisfactory performance should be based on achieving a specified number of sample (patient) reports in agreement with reference values in a calendar year. These should be set according to those established by EFI (minimum performance standards) or greater. Laboratories not meeting the ‘Satisfactory Performance’ criteria must receive written notification as soon as it occurs, offering advice and assistance.

Certificates
At the end of the inter-laboratory exchange period (usually a year) a certificate should be issued in English (if nationally organized a duplicate in the language of the country may be provided).

The certificate must contain the following information:
- Name and address of the exchange organizer
- Full name and/or the code of the participant.
- Date certificate issued.
- Period covered by the inter-laboratory exchange
- Description of the exchange assessed.
- Total number of samples tested.
- Number of discrepancies related to the total number of samples and related to the other participants results
- Participant performance, when possible. Otherwise a participation certificate is sufficient.

9. Education

It is recommended to have a general meeting every year for all participants. This can be used to discuss results of the exchange, difficulties in the exchange, the use of new techniques, and other topics that are of importance to the participants. A meeting in conjunction with an annual meeting of the national H&I society will reduce travelling and accommodation costs.

10. Advice from (regional) EPTC coordinator(s)

For information and difficulties during the start of an inter-laboratory exchange program, the organizer can always seek advice from the EFI EPT Committee or a (regional) EPTC member. Names and e-mail addresses are available on the EFI website (http://www.efiweb.eu/efi-committees/ept-committee.html).

11. Registry for EFI

Like EPT programs and providers, inter-laboratory exchanges and their organizers can be registered by EFI. Contact the EPTC for more information.

12. Towards an EPT program

When the inter laboratory exchange program is established and has sufficient participants it can develop to an EPT program. The requirements for an EPT program

are described in the EFI standards for Providers (latest version). Contact the EPTC for more information.

Addendum 1: SOP for

Preparation of blood samples from buffy coats

This SOP may be used to prepare a set of blood samples with concentrated lymphocyte content to be distributed to laboratories with the aim of performing lymphocytotoxic assays or DNA isolation for proficiency testing.

The buffy coats can be obtained from a local blood bank.

- In advance: Make labels for the tubes and label the tubes (coagulation tubes).
- Work up the buffy coats one by one to prevent mistakes. Work in a flow cabinet under sterile conditions.
- Prepare the dilution buffer: Add 5 ml L-Glutamine (200mM, Gibco) to 500 ml RPMI (Gibco).
- Add 15 ml Sodium-Citrate (0.11mol/l) to a sterile 250 ml flask
- Add the buffycoat and mix gently
- Add at least dilution buffer up to a total volume of about 225 ml for up to 30 tubes of 7 ml.
- Mix well and divide all in the labelled tubes (7 ml per tube).
- Left overs can be used for isolation of DNA.

When all tubes are ready, the tubes (each from a different buffy coat) are sent to each participating laboratory. Packaging must conform to IATA Packing Instruction 650. The tubes are packed in a “transport blister” with absorbing material, and this is packed in a “safety bag”. The safety bag is packed together with an accompanying letter in a small post box. Samples can be transported at ambient temperature and should be processed as soon as possible on receipt.

Addendum 2: SOP for

Preparation of lymphocytes from whole blood

This SOP may be used to prepare a set of samples containing isolated and concentrated lymphocytes to be distributed to laboratories with the aim of performing lymphocytotoxic assays or DNA isolation for proficiency testing.

Blood bag units can be obtained from a local blood bank.

All steps should be performed in a sterile cabinet.

- Prepare sufficient quantity of sterile RPMI + additives for the process:
  Take a sterile 500ml bottle of RPMI. Add 5ml of sterile antibiotic antimycotic solution. Add 5ml 31% w/v Tri-sodium citrate solution, sterilised by filtration
  Repeat for other bottles of RPMI as required.
- Prepare a sufficient number of 10ml aliquots of sterile separation medium (density gradient media, e.g. Lymphoprep) in 30ml universal tubes for the volume of blood to be layered (approximately one 10ml aliquot of separation media per 15-20ml of blood).
- Label each tube containing the separation media with the proficiency testing sample number. Depending on the total amount of cells required, a set of tubes must be marked with the same numbers.
- Carefully layer the blood onto the surface of the separation media.
- Centrifuge the containers for 30 minutes at 1.400 x g in an appropriate centrifuge. Ensure that the centrifuge braking speed is set at a low level.
- After centrifugation remove the tubes. The blood sample should have separated into layers with the mononuclear cells forming a distinct white band at the interface between the plasma and separation medium. Some contamination with red blood cells (RBCs) may be present and requires no action.
- Using a sterile 3ml plastic pipette, carefully transfer each mononuclear cell layer into a separate sterile 30ml universal container labelled with the sample number. Try to avoid harvesting the lymphoprep as this may contain granulocytes. Checks must be made at this stage and all subsequent transfer steps to ensure that the sample identifiers match.

- Re-suspend the mononuclear cells by topping up the container with sterile RPMI plus additives. Mix the contents gently by inversion.
- Centrifuge the containers for 10 minutes at 700 x g.
- After centrifugation check to ensure that a cell pellet has formed and then pour off the supernatant into a waste container or sink leaving the mononuclear cell pellet.
- Add 2-3ml Park/Terasaki medium to re-suspend and pool all cells into a single container. Add an appropriate amount of Park/Terasaki medium for the total volume required.
- Dispense the cells in Park/Terasaki into suitable labelled containers for distribution to the participating laboratories. The container should be periodically mixed to ensure homogeneity of sample during the aliquoting procedure.
- The sample lymphocyte count and cell viability must be checked prior to distribution. Take 50ul of cell suspension, add 50ul of stain to visualise live and dead cells (e.g. ethidium bromide, Acridine Orange). View under the microscope to assess cell viability and lymphocyte count.

When all tubes are ready, the tubes (each from a different blood bag) are sent to each participating laboratory. Packaging must conform to IATA Packing Instruction 650. The tubes are packed in a “transport blister” with absorbing material, and this is packed in a “safety bag”. The safety bag is packed together with an accompanying letter in a small post box. Samples can be transported at ambient temperature and should be processed as soon as possible on receipt.

Addendum 3: SOP for

DNA isolation using spin columns

This SOP may be used for the preparation of large scale genomic DNA from whole blood using a manual extraction method (commercially available spin columns).

The commercial DNA blood kits provide a rapid method for the isolation of high quality genomic DNA for PCR amplification from whole blood, isolated lymphocytes, cultured cells and bone marrow. The method does not require the use of organic solvents or proteinase K, and consequently is less hazardous and more reliable than conventional DNA isolation techniques.

The commercially available kits contain lysis and washing solutions. The solutions must be stored according to manufacturer's guidelines. Some kits also use proteinase K solution (stored at -20°C). So called "Mini" kits should be used for smaller amounts of blood (e.g. 200µl), for washing steps small table centrifuges (microfuge) may be used (Eppendorf).

DNA must only be extracted from EDTA anti-coagulated blood. Clotted samples must not be processed. Heparin-containing samples cannot be processed as the Heparin may interfere with the PCR reaction.

- The sample tube label name, and number should be checked at all subsequent transfer stages.
- Add 4ml of whole blood or buffy coat from 5 to 10 ml of blood to a labelled universal tube.
- Add 9ml of Lysis Solution. Some kits also provide Proteinase K solution which must be added according the manufacturer's instructions. Mix by inversion, and incubate at room temperature for 10 minutes mixing occasionally.
- Centrifuge for 5 minutes at 2.000 x g (3.000 rpm based on a 200mm radius centrifuge). Decant the supernatant leaving behind a visible white cell pellet (please note if the sample requires an extra red cell lysis step please repeat from step 5).
- Gently re-suspend any residual red cell supernatant/debris with 3ml PBS without disturbing the white cell pellet. Using a disposable tip pipette, gently remove the supernatant/PBS leaving the white cell pellet in about 100-200µl supernatant/PBS.
- Vortex the tube vigorously to resuspend the white cell pellet.

- Add 3ml of Cell Lysis Solution to the tube and pipette up and down to lyse the cells. Note, it is important to resuspend the cells as far as possible in the cell lysis solution as this greatly affects the DNA yield. If cell clumps are visible after mixing, incubate at 37°C until the solution is homogenous. The sample may be stored at room temperature for up to 18 months at this stage.
- Add 1ml of Protein Precipitation Solution to the cell lysate and vortex vigorously for 20 - 30 seconds.
- Centrifuge for 5 minutes at 2.000 x g (3.000 rpm based on a 200mm radius centrifuge), and carefully pour the supernatant into another 20ml Universal. Discard the protein pellet.
- Add 3ml of isopropanol and mix by inversion several times until the DNA precipitates and forms a visible clump.
- Using a disposable tip pipette, carefully transfer the precipitated DNA (by picking up the DNA on the end of the pipette tip) to a 1.5ml Eppendorf tube containing 1ml of 70% (v/v) ethanol and mix by inversion. Dispose of waste isopropanol and ethanol down the drain, flushing with water.
- Centrifuge at 13.000 x g in microfuge for 1 minute. Drain the tube completely.
- Add 200µl of DNA hydration solution. Incubate for 1 hour at 55°C vortexing occasionally, or for 15-20 minutes at 55°C vortexing vigorously every 2 or 3 minutes. Store the sample between 2-8°C for 3 - 5 days and at -20-40°C when typing is completed.

Addendum 4: SOP for

DNA isolation by salting out method

This SOP may be used for the preparation of eukaryotic intact chromosomal DNA, pure grade, for use in PCR reactions.

Reagents:

Reagent	Vendor	Order number	Storage temp.
Absolute Ethanol	Merck	100983	RT
10 % SDS	Sigma	71725	RT
1 x Erythrocyte Lysis (EL)		n.a.	RT
1 x SE		n.a.	RT
Proteinase K	Invitrogen	23530-015	4°C
6 M NaCl		n.a.	RT
70 % Ethanol		n.a.	4°C
TE-4		n.a.	RT

Solution	Reagents	Quantity necessary for solution
EDTA 0,5M pH 8,5	EDTA	186 gr/L
10x EL - buffer pH 7,4	1.55 M NH_4CL 0.1 M $KHCO_3$ 0,01 M EDTA	165,8 g/ 2L 20 g/ 2L 40 ml/ 2L (0,5 M, pH 8,5)
20 mM $MgCl_2$	$MgCl_2$ X $6H_2O$	406,6 mg/100ml
NaCl 6M (saturated)	NaCl	350 g/L
Proteinase K (Invitrogen)	20 mg/ml	5 ml Proteinase K buffer/ 100 mg 60' 56°C
Proteinase K buffer	10 mM Tris 20mM $CaCl_2$ in 50% glycerol	2 ml (1M, pH 7.5) /200 ml 4 ml (1M) /200 ml 100 ml (100% glycerol) /200 ml
10x SE-buffer pH 8,0	0,75 M NaCl 0,25 M EDTA	43,8 g/L 93 g/L
10% SDS	Natrium Dodecyl Sulfate	100 g/L
TE $^{-4}$ pH 7,4	10 mM TRIS 10^{-4} M EDTA	1,21 g/L 0,2 ml/L (0,5 M, pH 8,5)

Apparatus and consumables:

- 50ml tubes
- 1.7ml Eppendorf tubes (sterile)
- Centrifuge
- Waterbath 37°C
- Vortex
- Refrigerator
- Heatblock or waterbath 56°C

Protocol for whole blood, per 10 ml tube:

- Bring 10ml blood in a 50ml tube and add 40ml erythrocyte lysis buffer (EL). Mix for 1 min. Incubate at least 1 hour at 4°C (refrigerator). All erythrocytes will lyse, nucleated cells remain intact.
- Collect the nucleated cells by centrifugation (5', 2.000 x g), pour off supernatant.
- Resuspend the pellet in 45ml EL, mix and spin down once more (5', 2.000 x g) pour off supernatant.
- Resuspend the pellet in 3ml 1x SE-buffer. Lyse the nucleated cells by adding 15µl proteinase K and 300 µl 10% SDS. Mix and incubate at 37°C (waterbath), overnight (DNA is released when SDS is added).
- The next day: Remove the tubes from the 37°C waterbath. Cool down to room temperature and preticipate the proteins by adding 1ml 6M NaCl (saturated solution). Mix gently at first and then vortex for 30 sec. until the solution looks milky. Spin down the proteins (5', 2.000 x g) and poor supernatant (this contains the DNA!) in a clean 50 ml tube.
- If there is any proteins still visible, mix and spin down once more, and poor the upper clear solution into a new clean tube.
- Preticipate the DNA by gently adding 2.5 x volume ethanol absolute. Mix very gentle until ethanol and supernatant are mixed completely and a DNA cloud becomes visible.
- Fish out the DNA, with a glass pipet.

- Wash the DNA shortly in 70% EtOH and dissolve in 500 µl TE-4 at 56°C. Every now and then mix gently. When necessary (high concentrations) extra TE-4 can be added. After concentration measurement, the concentration can be further adapted.

Protocol for buffy coats:

- Isolate lymphocytes by density gradient media separation.
- Wash the cells in PBS and spin down.
- Resuspend the pellet in 15ml 1x SE-buffer. Lyse the white cells by adding 75µl proteinase K and 1.500µl 10% SDS. Mix well. Incubate overnight at 37°C (waterbath).
- Cool the tubes to room temperature, preticipate proteins in the solution by adding 5ml 6M NaCl (saturated solution). Mix gently at first, followed by mixing firmly for 30 sec. on a vortex. Spin the denatured proteins down during 5 minutes at 2.000 x g.
- The clear solution on top contains the DNA, poor this into a clean 50 ml tube. If there is any proteins still visible, spin down once more, and poor the upper clear solution into a new clean tube.
- Preticipate the DNA by adding 2.5 x volume Ethanol absolute. Mix very gently until ethanol and supernatant are mixed completely and a DNA cloud becomes visible.
- Fish out the DNA, with a glass pipet.
- Wash the DNA shortly in 70% EtOH and dissolve in 2.500µl TE-4, at 56°C. Every now and then mix gently. When necessary (high concentrations) extra TE-4 can be added. After concentration measurement, the concentration can be further adapted.

RESULT FORM

CYTOTOXIC CROSSMATCHING

Lab Name/ID: _____

Sample identification: _____

Date samples received: _____ Date samples tested: _____

Cell viability: PBL ___% T cells ___% B cells ___%

Results without DTT

	PBL without DTT		T Cell without DTT		B Cell without DTT	
	Result for assessment	Reaction strength	Result for assessment	Reaction strength	Result for assessment	Reaction strength
Serum A						
Serum B						
Serum C						
Serum D						
Serum E						

Results with DTT

	PBL with DTT		T Cell with DTT		B Cell with DTT	
	Result for assessment	Reaction strength	Result for assessment	Reaction strength	Result for assessment	Reaction strength
Serum A						
Serum B						
Serum C						
Serum D						
Serum E						

COMMENTS (including reasons why sample(s) could not be tested):

RESULT FORM

FLOW CYTOMETRY CROSSMATCHING

Lab Name/ID: _____

Date samples received: _____ Date samples tested: _____

Sample identification: _____ Cell viability % _____

Units for Cytometer readings:	

Please report crossmatch results compared to your local negative control.

T Cell Results

Please indicate your results by marking with a cross one box only for each serum:

Serum Number	Positive	Negative	Equivocal	Not Tested	Cytometer readings
A	☐	☐	☐	☐	
B	☐	☐	☐	☐	
C	☐	☐	☐	☐	
D	☐	☐	☐	☐	
E	☐	☐	☐	☐	

B Cell Results

Please indicate your results by marking with a cross one box only for each serum:

Serum Number	Positive	Negative	Equivocal	Not Tested	Cytometer readings
A	☐	☐	☐	☐	
B	☐	☐	☐	☐	
C	☐	☐	☐	☐	
D	☐	☐	☐	☐	
E	☐	☐	☐	☐	

Controls

(used for participant information only)

	Cytometer readings	
	T Cell	**B Cell**
Local negative control		
Local positive control		
Positive result value *(What value above the negative control indicates a positive result, e.g., 2SD, 20 linear channel shift?)*		

COMMENTS (including reasons why the sample(s) could not be tested):

RESULT FORM

HLA PHENOTYPING / SEROLOGICAL HLA TYPING

Lab Name/ID: _____

Date samples received (DD/MM/YYYY): _____ Date samples tested (DD/MM/YYYY): _____

Sample identification: _____

Phenotype Results:

HLA-A	HLA-A	HLA-B	HLA-B	HLA-C	HLA-C	HLA-DR	HLA-DR	HLA-DQ	HLA-DQ

CELL VIABILITY: Unseparated cells % ____ T-cells % ____ B-cells % ____

COMMENTS:

RESULT FORM

DNA HLA TYPING TO 1st / 2nd FIELD RESOLUTION

Lab Name/ID: _____

Date samples received (DD/MM/YYYY): _____ Date samples tested (DD/MM/YYYY): _____

Sample identification: _____

CLASS I RESULTS:

A*	A*	B*	B*	C*	C*

CLASS II RESULTS:

DRB1*	DRB1*	DRB3*	DRB4*	DRB5*	DQA1*	DQA1*	DQB1*	DQB1*	DPA1*	DPA1*	DPB1*	DPB1*

COMMENTS:

RESULT FORM

HLA ANTIBODY DETECTION

Lab Name/ID: _____

Sample identification: _____

Date samples received (DD/MM/YYYY): _____ Date samples tested (DD/MM/YYYY): _____

IgG RESULTS

Indicate if HLA **IgG** Class I / Class II antibodies are present for each sample and mark the detection method(s)

Sample ID	HLA **IgG CI** antibody present (Positive/Negative/NT)	CDC	FCM	ELISA	LUMINEX	HLA **IgG CII** antibody present (Positive/Negative/NT)	CDC	FCM	ELISA	LUMINEX
		☐	☐	☐	☐		☐	☐	☐	☐
		☐	☐	☐	☐		☐	☐	☐	☐
		☐	☐	☐	☐		☐	☐	☐	☐
		☐	☐	☐	☐		☐	☐	☐	☐
		☐	☐	☐	☐		☐	☐	☐	☐
		☐	☐	☐	☐		☐	☐	☐	☐

Please mark the methods used to test these samples:

☐ CDC ☐ Luminex Single Antigen

☐ ELISA ☐ Luminex Mixed/Multi

☐ Flow cytometry

☐ Other (please specify):

Does this apply to all samples?: ☐ Yes ☐ No

If no, please provide further details & Sample IDs:

Please indicate if the samples have been treated:

☐ No treatment ☐ Dilution

☐ Heat Inactivation ☐ EDTA

☐ Adsorb Out/SeraClean Beads

☐ Other (please specify):

Does this apply to all samples?: ☐ Yes ☐ No

If no, please provide further details & Sample IDs:

Comments

MANUAL for INTER-LABORATORY EXCHANGES by the EFI EPTC

RESULT FORM

HLA ANTIBODY SPECIFICITY ANALYSIS

Lab Name/ID:_____

Sample _____ **CLASS I IgG RESULTS**

If antibody is present, indicate the detection method(s) by ☒ the relevant box(es).

Please mark the methods used to test this sample:

CDC ☐ Flow cytometry ☐

ELISA ☐ Luminex ☐

Other (please specify):

Please indicate if this sample has been treated:

No treatment ☐ Dilution ☐

Heat Inactivation ☐ EDTA ☐

Adsorption Beads ☐ Other (please specify):

Did this sample have 'high background' in the Luminex assay? No ☐ Yes ☐ Neg Cont Serum batch:

HLA-	CDC	FCM	ELISA	LUMINEX
A1	☐	☐	☐	☐
A2	☐	☐	☐	☐
A203	☐	☐	☐	☐
A210	☐	☐	☐	☐
A3	☐	☐	☐	☐
A23	☐	☐	☐	☐
A24	☐	☐	☐	☐
A2403	☐	☐	☐	☐
A25	☐	☐	☐	☐
A26	☐	☐	☐	☐
A34	☐	☐	☐	☐
A66	☐	☐	☐	☐
A11	☐	☐	☐	☐
A29	☐	☐	☐	☐
A30	☐	☐	☐	☐
A31	☐	☐	☐	☐
A32	☐	☐	☐	☐
A33	☐	☐	☐	☐
A74	☐	☐	☐	☐
A68	☐	☐	☐	☐
A69	☐	☐	☐	☐
A36	☐	☐	☐	☐
A43	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
A80	☐	☐	☐	☐
B51	☐	☐	☐	☐
B5102	☐	☐	☐	☐
B5103	☐	☐	☐	☐
B52	☐	☐	☐	☐
B7	☐	☐	☐	☐
B703	☐	☐	☐	☐
B8	☐	☐	☐	☐
B44	☐	☐	☐	☐
B45	☐	☐	☐	☐
B13	☐	☐	☐	☐
B64	☐	☐	☐	☐
B65	☐	☐	☐	☐
B62	☐	☐	☐	☐
B63	☐	☐	☐	☐
B75	☐	☐	☐	☐
B76	☐	☐	☐	☐
B77	☐	☐	☐	☐
B38	☐	☐	☐	☐
B39	☐	☐	☐	☐
B3901	☐	☐	☐	☐
B3902	☐	☐	☐	☐
B57	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
B58	☐	☐	☐	☐
B18	☐	☐	☐	☐
B49	☐	☐	☐	☐
B50	☐	☐	☐	☐
B4005	☐	☐	☐	☐
B54	☐	☐	☐	☐
B55	☐	☐	☐	☐
B56	☐	☐	☐	☐
B27	☐	☐	☐	☐
B2708	☐	☐	☐	☐
B35	☐	☐	☐	☐
B37	☐	☐	☐	☐
B60	☐	☐	☐	☐
B61	☐	☐	☐	☐
B41	☐	☐	☐	☐
B42	☐	☐	☐	☐
B46	☐	☐	☐	☐
B47	☐	☐	☐	☐
B48	☐	☐	☐	☐
B53	☐	☐	☐	☐
B59	☐	☐	☐	☐
B67	☐	☐	☐	☐
B71	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
B72	☐	☐	☐	☐
B73	☐	☐	☐	☐
B78	☐	☐	☐	☐
B81	☐	☐	☐	☐
B82	☐	☐	☐	☐
Cw1	☐	☐	☐	☐
Cw2	☐	☐	☐	☐
Cw9	☐	☐	☐	☐
Cw10	☐	☐	☐	☐
Cw4	☐	☐	☐	☐
Cw5	☐	☐	☐	☐
Cw6	☐	☐	☐	☐
Cw7	☐	☐	☐	☐
Cw8	☐	☐	☐	☐
Cw12	☐	☐	☐	☐
Cw14	☐	☐	☐	☐
Cw15	☐	☐	☐	☐
Cw16	☐	☐	☐	☐
Cw17	☐	☐	☐	☐
Cw18	☐	☐	☐	☐

Allele specific antibodies

If 'allele-specific' antibodies are identified, **tick the antibody specificity box <u>in addition to</u> detailing the antibodies in this box**.

*E.g. if an antibody is found to be positive with the A*68:01 product only and is negative with other A*68 allele products, tick the A68 box and indicate A*68:01 in this box.*

Official WHO specificities listed (except HLA-C), for other specificities detected use "allele specific" box.

LUMINEX KITS USED

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LM1 Class I ID	☐	_____	_____	_____
LMX Deluxe Kit	☐	_____	_____	_____

Other (please specify): _____

<u>IMMUCOR</u>

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LSA1 SA Class I	☐	_____	_____	_____
LSA I&II SA Class I&II	☐	_____	_____	_____

LUMINEX KITS USED

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LS1PRA Class I	☐	_____	_____	_____
LS12PRA Class I&II	☐	_____	_____	_____

Other (please specify): _____

<u>ONE LAMBDA</u>

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LS1A04 SA Class I	☐	_____	_____	_____
LSM12 Mixed Class I&II	☐	_____	_____	_____

RESULT FORM

HLA ANTIBODY SPECIFICITY ANALYSIS

Lab Name/ID:

Sample ___ **CLASS II IgG RESULTS**

If antibody is present, indicate the detection method(s) by ☒ the relevant box(es).

Please mark the methods used to test this sample:

CDC ☐ Flow cytometry ☐

ELISA ☐ Luminex ☐

Other (please specify):

Please indicate if this sample has been treated:

No treatment ☐ Dilution ☐

Heat Inactivation ☐ EDTA ☐

Adsorption Beads ☐ Other (please specify):

Did this sample have 'high background' in the Luminex assay? No ☐ Yes ☐ Neg Cont Serum batch:

HLA-	CDC	FCM	ELISA	LUMINEX
DR1	☐	☐	☐	☐
DR103	☐	☐	☐	☐
DR15	☐	☐	☐	☐
DR16	☐	☐	☐	☐
DR17	☐	☐	☐	☐
DR18	☐	☐	☐	☐
DR4	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
DR11	☐	☐	☐	☐
DR12	☐	☐	☐	☐
DR13	☐	☐	☐	☐
DR14	☐	☐	☐	☐
DR1403	☐	☐	☐	☐
DR1404	☐	☐	☐	☐
DR7	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
DR8	☐	☐	☐	☐
DR9	☐	☐	☐	☐
DR10	☐	☐	☐	☐
DR51	☐	☐	☐	☐
DR52	☐	☐	☐	☐
DR53	☐	☐	☐	☐

HLA-	CDC	FCM	ELISA	LUMINEX
DQ5	☐	☐	☐	☐
DQ6	☐	☐	☐	☐
DQ2	☐	☐	☐	☐
DQ7	☐	☐	☐	☐
DQ8	☐	☐	☐	☐
DQ9	☐	☐	☐	☐
DQ4	☐	☐	☐	☐

DQA AND DP ANTIBODIES – NOT ASSESSED

DQA1*				
01:01	☐	☐	☐	☐
01:02	☐	☐	☐	☐
01:03	☐	☐	☐	☐
01:04	☐	☐	☐	☐
02:01	☐	☐	☐	☐
03:01	☐	☐	☐	☐
03:02	☐	☐	☐	☐
03:03	☐	☐	☐	☐
04:01	☐	☐	☐	☐
05:01	☐	☐	☐	☐
05:03	☐	☐	☐	☐

DQA1*				
05:05	☐	☐	☐	☐
06:01	☐	☐	☐	☐
DPB1*				
01:01	☐	☐	☐	☐
02:01	☐	☐	☐	☐
03:01	☐	☐	☐	☐
04:01	☐	☐	☐	☐
04:02	☐	☐	☐	☐
05:01	☐	☐	☐	☐
06:01	☐	☐	☐	☐
09:01	☐	☐	☐	☐

DPB1*				
10:01	☐	☐	☐	☐
11:01	☐	☐	☐	☐
13:01	☐	☐	☐	☐
14:01	☐	☐	☐	☐
15:01	☐	☐	☐	☐
17:01	☐	☐	☐	☐
18:01	☐	☐	☐	☐
19:01	☐	☐	☐	☐
20:01	☐	☐	☐	☐
23:01	☐	☐	☐	☐
28:01	☐	☐	☐	☐

DPA1*				
01:03	☐	☐	☐	☐
01:04	☐	☐	☐	☐
01:05	☐	☐	☐	☐
02:01	☐	☐	☐	☐
02:02	☐	☐	☐	☐
03:01	☐	☐	☐	☐
04:01	☐	☐	☐	☐

Allele specific antibodies

If 'allele-specific' antibodies are identified, **tick the antibody specificity box in addition to detailing the antibodies in this box**.

*E.g. if an antibody is found to be positive with the DRB1*13:01 product only and is negative with other DRB1*13 allele products, tick the DR13 box and indicate DRB1*13:01 in this box.*

Please list any other DQA and DP antibodies in the allele specific antibody box

LUMINEX KITS USED

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LM2 Class II ID	☐	____	____	____
LMX Deluxe Kit	☐	____	____	____

Other (please specify): ____

IMMUCOR

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LSAII SA Class II	☐	____	____	____
LSA I&II SA Class I&II	☐	____	____	____

LUMINEX KITS USED

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LS2PRA Class II	☐	____	____	____
LS12PRA Class I&II	☐	____	____	____

Other (please specify): ____

ONE LAMBDA

		+/- cut off value	Control Bead MFI Positive	Control Bead MFI Negative
LS2A01 SA Class II	☐	____	____	____
LSM12 Mixed Class I&II	☐	____	____	____