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Transplantation cross match kit (XMStep)

Reference	Test
FCXM	100

Reactivity: Human
Source: Supernatant proceeding from an *in vitro* cell culture of a cell hybridoma.
Purification: Affinity chromatography
Composition: Mouse anti-human monoclonals antibodies conjugated with a fluorochromes in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN_3).

Reference	Reagent provided	Clone	*Isotype*	Flurochrome
FCXM	Anti-IgG 5 µl/test (0,5 ml)	iMHP6017	Mouse IgG2a	FITC

RECOMMENDED USAGE

Immunostep's Transplantation cross match kit intended use for determine presence of anti-HLA antibodies preformed in a patient's serum against the lymphocytes of a possible donor by flow cytometry.

CLINICAL RELEVANCE

Human leukocyte antigens (HLAs) are antigens compound of molecules found on the surface of almost every cell in an individual's tissues, and also on white blood cells. When two people share the same HLA, they are said to be a "match", that is, their tissues are immunologically compatible with each other.

The presence of recipient antibodies against antigens expressed on donor white cells is a major risk factor for early rejection or graft loss after kidney transplant.[1] In this sense, is indicated the HLA crossmatch study between donor cells and recipient serum prior to transplantation.

Analysis of specific antibodies against B and T lymphocytes by flow cytometry allows clinicians to perform transplant cross-matching tests by detecting antibodies specific for HLA class I and class II as well as other antigens also expressed in these cells.[2-4] Thereby, a negative crossmatch justified proceeding with the transplant, whereas a positive crossmatch was considered a contraindication for kidney transplantation.

PRINCIPLES OF THE TEST

The anti-IgG monoclonal antibody recognize anti-donor antibodies against T-lymphocytes (anti-HLA class I antibodies) and B-lymphocytes (anti-HLA class I and/or HLA class II antibodies) on the surface of donor cells.

To identify these antibodies, the cells are incubated with the serum patient and the anti-IgG. Sample is analysed by flow cytometry.

APPROPRIATE STORAGE AND HANDLING CONDITIONS.

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the date indicated.

Once the vial is open, the product is stable for 90 days.

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS ⚠

a) The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR RESEARCH USE ONLY.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (Heparin, EDTA, citrate, acid citrate dextrose (ACD) and citrate phosphate dextrose (CPD) may be used) [1,2].
For optimum results, the sample should be processed during the six hours following the extraction. Samples which cannot be processed within the 72 hours following the extraction should be discarded.

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ISOLATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS

Isolate the donor cells by density gradient centrifugation with FICOLL ™ or any other media or method that allows the separation of mononuclear cells [3-5].

MATERIALS REQUIRED BUT NOT PROVIDED

- Negative control serum.
- Positive control serum.
- Recipient serum sample.

SAMPLE PREPARATION.

Plan the following samples for IgG detection:

– Negative control serum.
– Positive control serum.
– Recipient serum sample.

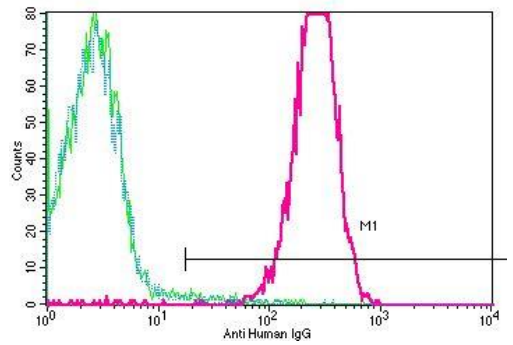
1. Resuspend and adjust previously isolated cells to 3x10e6 cells/ml in PBS.
2. Divide as follows the isolated cells between the number of 12x75-mm cytometer tube for testing:
   - Tube 1: 200 ul cellular suspension + 40 ul negative control serum.
   - Tube 2: 200 ul cellular suspension + 40 ul positive control serum.
   - Tube 3: 200 ul cellular suspension + 40 ul undiluted recipient serum.
3. Incubate in the dark for 30 minutes at room temperature (20-25°C).
4. Wash 2 times by adding 2 ml of PBS, mix in the vortex and centrifuge at 540g for seven minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet.
5. Add the suggested volume of the vial to each tube.
6. Incubate in the dark for 30 minutes at room temperature (20-25°C).
7. Wash 1 time by adding 2 ml of PBS, mix in the vortex and centrifuge at 540g for seven minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet.
8. Resuspend the pellet in 500µl of PBS

Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out

FLOW CYTOMETRY ANALYSIS

General protocol data acquisition cytometer will be used. The specific settings and gates that should be used for flow cytometry is determined by the instrument and must be decide by end user.
Below is Representative flow cytometric histograms are shown of lymphocytes labelling applying the protocol described in point 7:

(a)



(b)

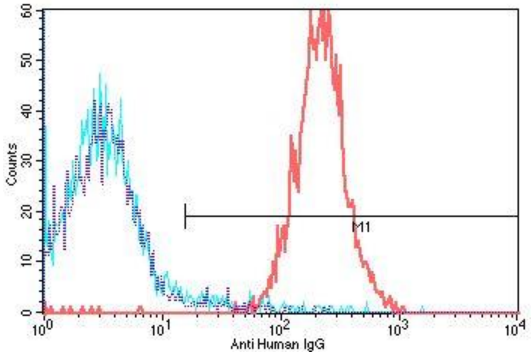


Fig. 1: Representative flow cytometry overlays histogram of IgG in lymphocytes. (a) Human peripheral blood B lymphocytes with positive control serum (pink), negative control serum (green) and negative recipient serum (blue). (b) Human peripheral blood T lymphocytes with positive control serum (red), negative control serum (blue) and negative recipient serum (violet).

LIMITATIONS OF THE PROCEDURE

Due to the risk of negative results in sera with higher levels of antibodies, it is recommended to process an additional tube with a serum diluted or introduce in the protocol an additional washing step after the incubation of the sera, to prevent the free immunoglobulins of serum can reduce the specific staining.

Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

REFERENCE VALUES

A known and well characterized negative control serum must be used to set FL1 baseline-values. A positive control serum should provide a reference for positive staining compared to negative control.

Each laboratory establishes its own reference values. For example: The test is positive when the mean serum fluorescence value of the receptor exceeds 3 times the value of the negative control.

WARRANTY

Immunostep products are guaranteed as to the quantity and contents stated on the product label at the time of delivery to the customer. Immunostep disclaims any other warranty. Responsibility for

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Immunostep is limited to replacement of products or refund the purchase price.

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