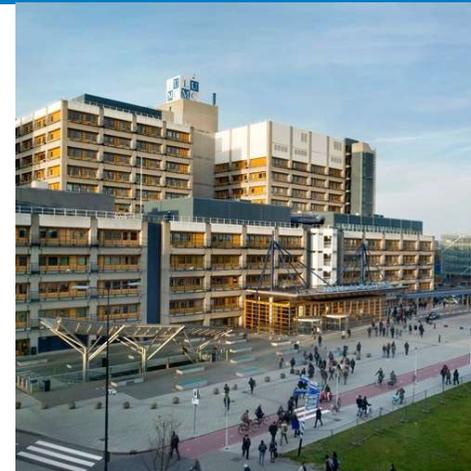


External quality control for histocompatibility testing in solid organ transplantation

EQALM 2021

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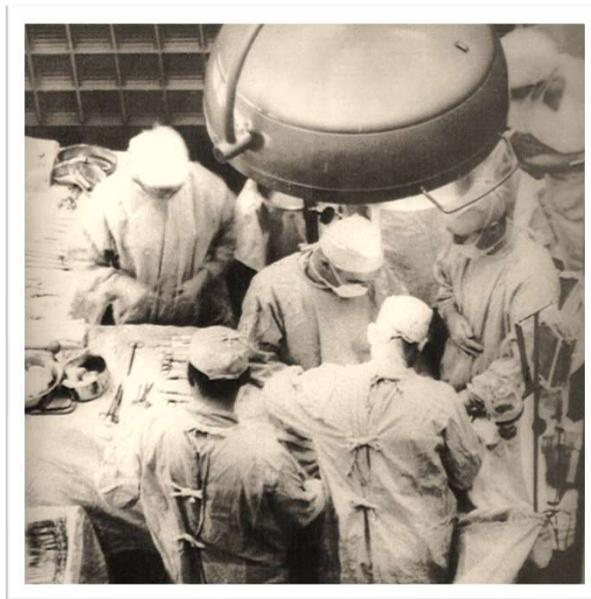


- **Human Leukocyte Antigens** and their importance in (kidney) transplantation
- Eurotransplant and the Eurotransplant Reference Laboratory
- From cell exchanges to proficiency testing
- Some practical aspects of the Eurotransplant EPT

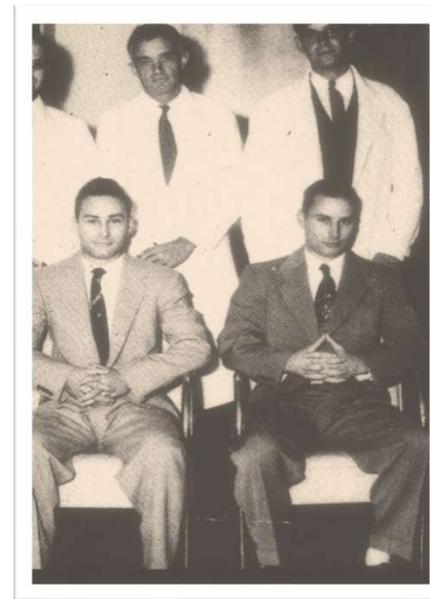


courtesy by Ilias Doxiadis

Transplantation without immunosuppressive drugs is only possible in monozygotic twin combinations

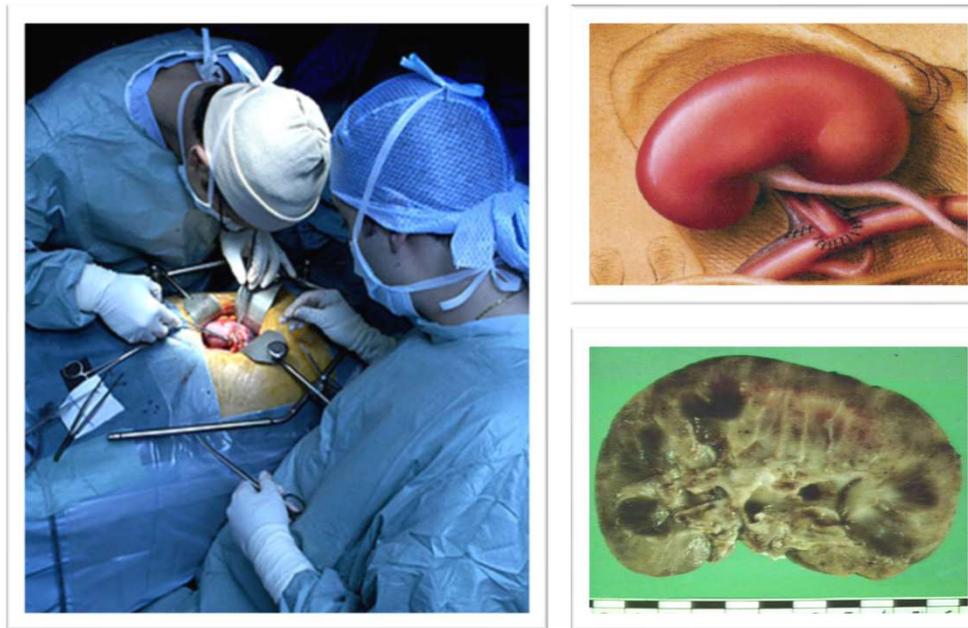


First successful kidney transplantation



By Joseph Murray, Boston - 1954

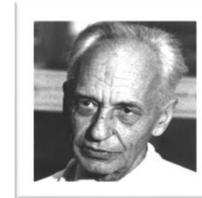
In all other situations, transplantation without further medication will lead to graft rejection



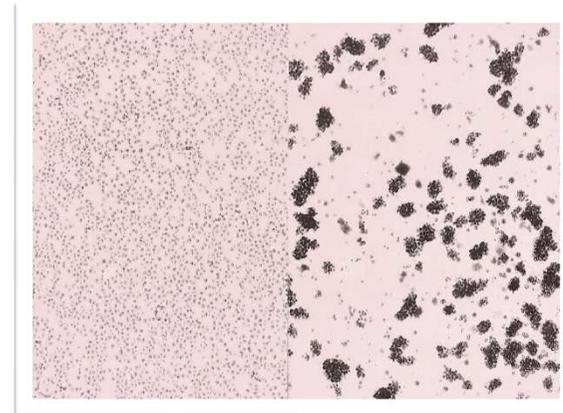
The history of HLA and HLA antibodies started around 1954 in France



- Professor Jean Dausset: recipient of Nobel Prize of Medicine in 1980



- Studies on patients who developed leukopenia after blood transfusions
- Blood transfusions can induce antibodies which cause agglutination of white blood cells



Studies in twins showed that the targets of the antibodies were genetically determined

Pregnancy can also induce HLA antibodies



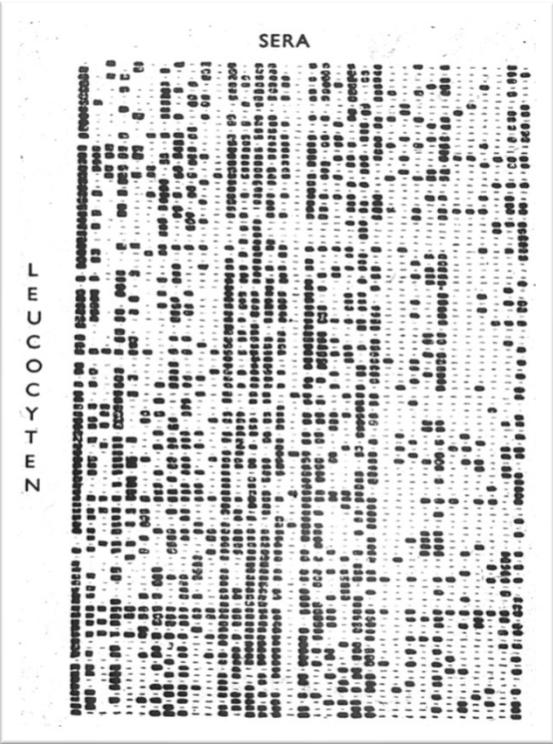
Jon van Rood

Rose Payne

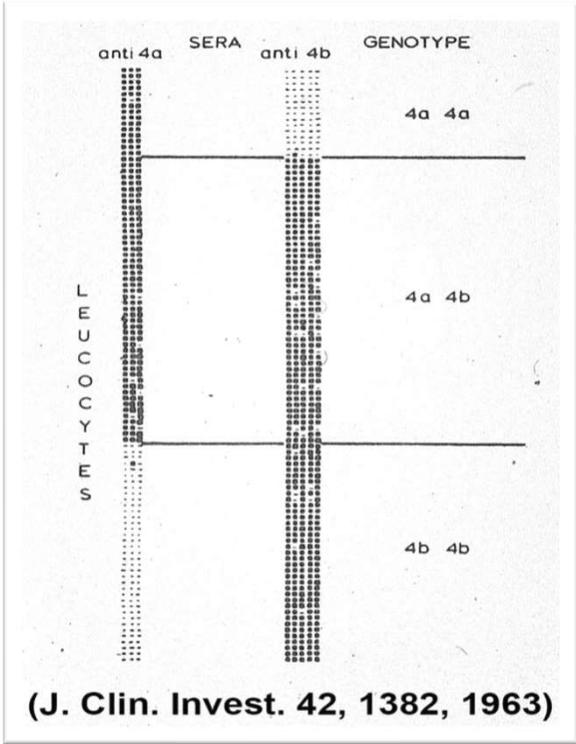
1958



Analyses of the complex serum reactions led to more knowledge of the alleles of the HLA system



manuscript. The operations with the IBM 604 have been carried out by the Rijkscentrale voor Mechanische Administratie under the supervision of M. A. Janssen, those with the IBM 101 by the Nederlandse Stichting voor Statistiek under the supervision of Dr. J. J. M. van Tulden. Financial support was provided by the Hippocrates Fund for Medical Research.



(J. Clin. Invest. 42, 1382, 1963)

Van Rood et al, J Clin Invest 42, 1382, 1963

Human Leukocyte Antigens: background



HLA is encoded on several genes:

- HLA class I: HLA-A, -B and -C
- HLA class II: HLA-DR, -DQ and -DP

HLA is differentially expressed:

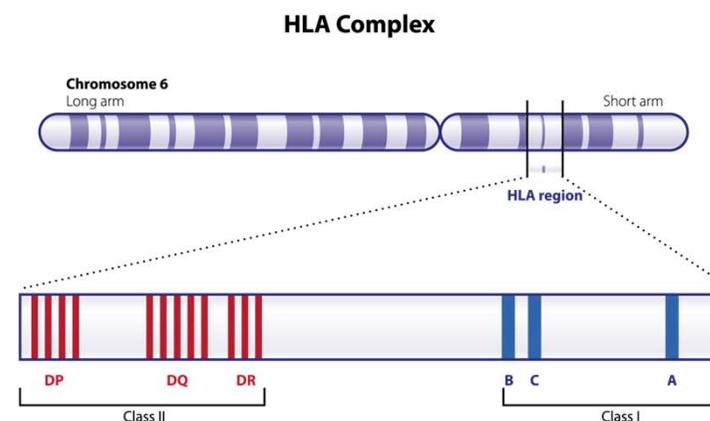
- HLA class I: expressed on all nucleated cells
- HLA class II: expressed on specialized immune cells and on activated endothelial cells

Haplotypes are inherited as a set: 1 from mother, 1 from father

HLA is extremely polymorphic:

(June 2021; <http://hla.alleles.org/nomenclature/stats.html>)

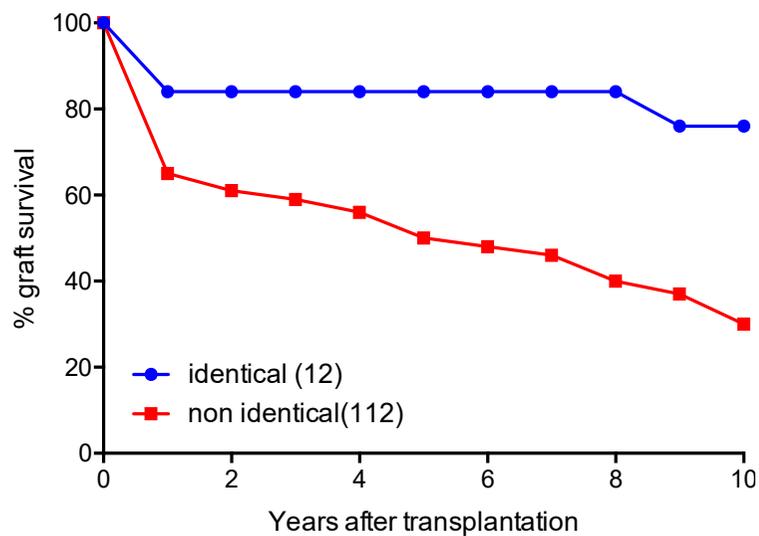
HLA-A	HLA-B	HLA-C	HLA-DR	HLA-DQ	HLA-DP
6921	8181	6779	3801	2033	1862



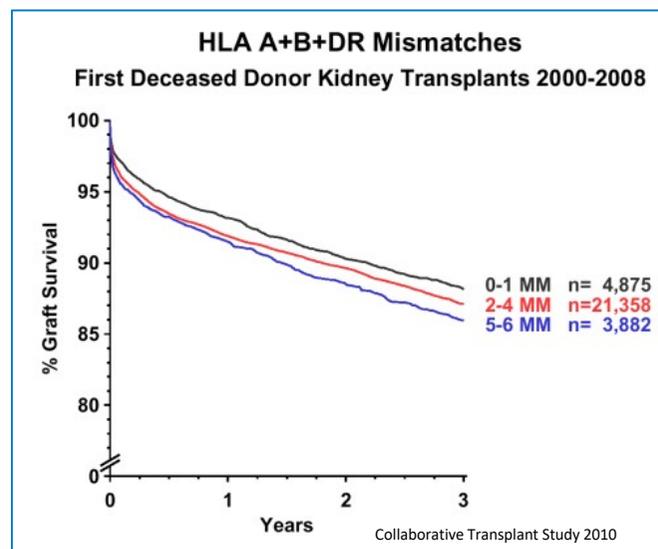
HLA matching enhances kidney graft survival



Matching on basis of 9 known serologically defined HLA antigens (1967-1972)



HLA matching is still relevant in the presence of efficient immunosuppression



Risk of pre-existing HLA antibodies in transplantation: Hyperacute rejection



- The presence of pre-transplant donor-reactive HLA-antibodies can lead to hyperacute rejection
- HLA-specific antibodies prior to transplant can be present due to pregnancy, blood transfusions or prior transplants
- Pre-transplant crossmatching prevents transplantation into recipients with pre-existing donor-reactive antibodies

SEPTEMBER 24, 1966

THE LANCET

HYPERACUTE REJECTION OF KIDNEY ALLOGRAFTS, ASSOCIATED WITH PRE-EXISTING HUMORAL ANTIBODIES AGAINST DONOR CELLS

F. KISSMEYER-NIELSEN
M.D. Aarhus

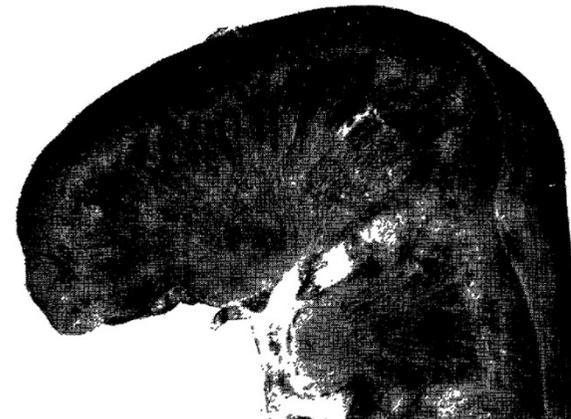
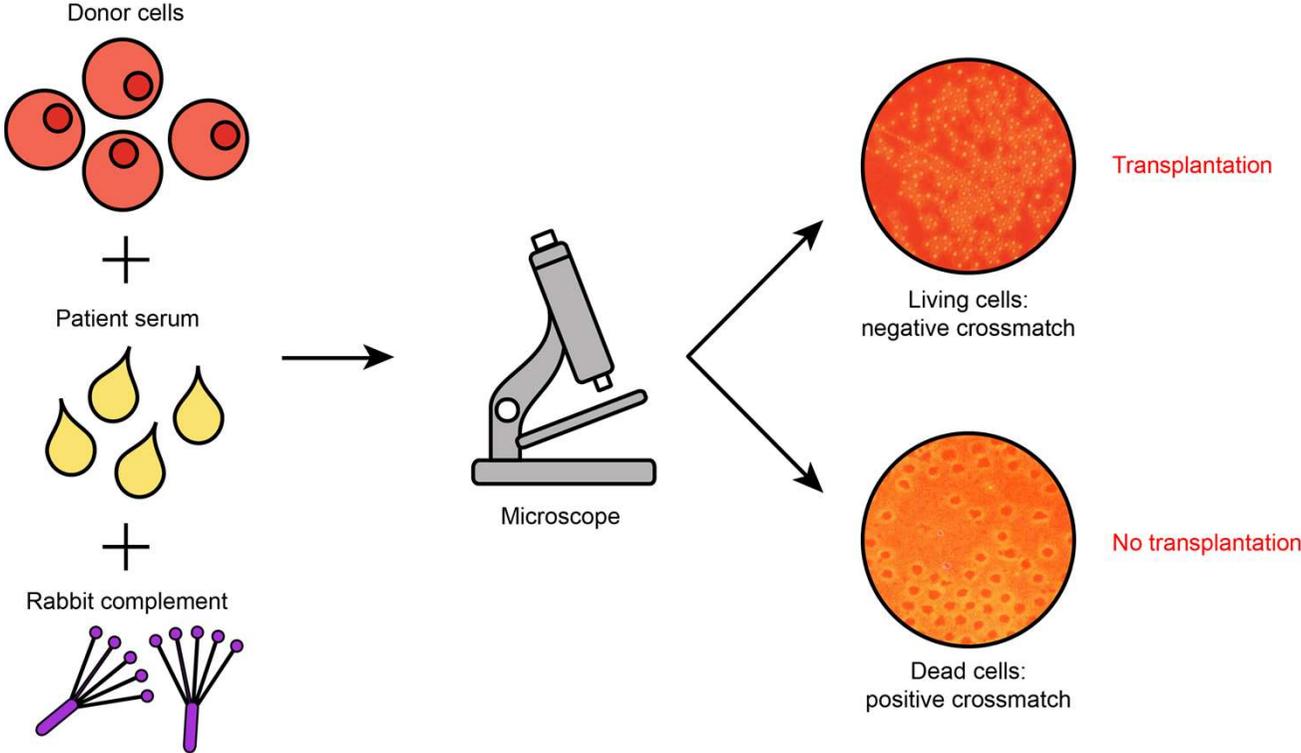


Fig. 2—Macroscopical appearance of the cortical necrosis in the kidney graft in case 17.

Kissmeyer-Nielsen *et al.*, Lancet 1966

The complement dependent cytotoxicity (CDC) test and crossmatch



Please note that the principle of this test is also used in screening for HLA antibodies and for HLA typing

Histocompatibility laboratory Results important in solid organ transplantation



HLA typing of recipient and donor

- Serology (CDC)
- DNA based techniques

HLA-specific antibody status of the recipient

- Serological CDC screening assays,
- ELISA and flow-cytometry based techniques
- HLA-coated bead based techniques (Luminex)

Crossmatch with donor cells and recipient serum

- CDC Crossmatch
- Flow cytometry

Eurotransplant



Eurotransplant was founded in 1967 by Jon van Rood (Cooperation between centers in Austria, Belgium, Luxemburg , Germany and the Netherlands)

In the past years Croatia, Slovenia and Hungary have joined as member states.

Goal:

....Eurotransplant acts as a mediator between donor hospitals and transplant centers for the benefit of patients in need of an organ transplant in all its member states....serving a total population of around 137 million people...



The ETRL was founded in the 1970's

Its initial goal was to increase the reliability of transplantation-related histocompatibility testing, including HLA typing, crossmatching, and screening for HLA specific antibodies in patient sera.

=> Provide typing serum sets

=> Retyping of all organ donors of affiliated centers



Eurotransplant EPT; Historical perspective

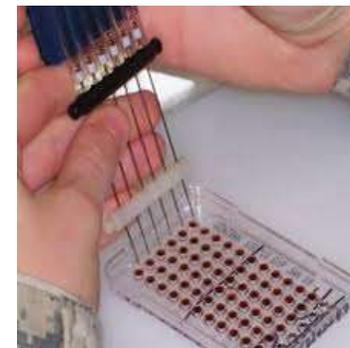


Cell exchanges started in 1978

The primary purpose of the cell exchange (1984):

- Help to improve the definition of existing HLA antigens
- Detect and define new HLA antigens
- Provide quality control of sera and methodologies

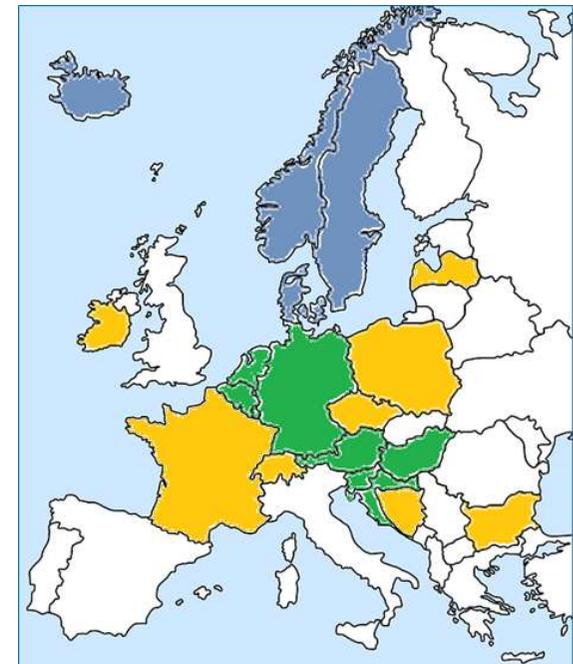
Loon et al, Tissue Antigens 1984



Eurotransplant Reference Laboratory



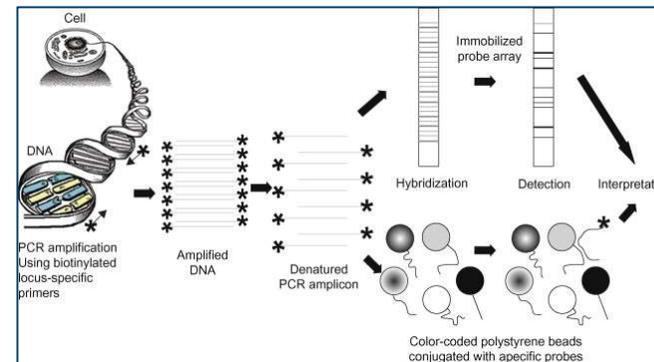
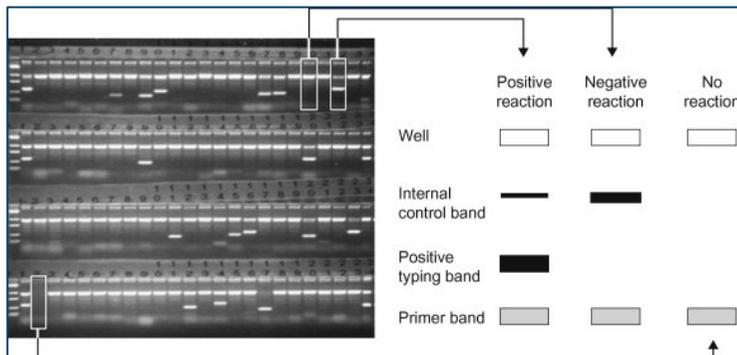
- The cell exchanges slowly evolved to the Eurotransplant EPT as we know today, with EPT schemes on HLA-typing, crossmatching and HLA-antibody testing
- These EPT schemes are mandatory for all 44 tissue typing laboratories affiliated to Eurotransplant
- The EPT is also open for other laboratories in Europe
- In total 79 participants



Eurotransplant EPT schemes (1) HLA typing



- 4 shipments of 3 blood samples each (HLA typed buffy coats from the Dutch National blood bank)
- HLA typing is done serologically and with DNA techniques
- SSP, SSO, RT-PCR, (Sanger Sequencing, NGS)



[Molecular Diagnostics](#) Techniques and Applications for the Clinical Laboratory 2010, Pages 367-379

- Results are assessed on basis of consensus or reference typing
- Since the introduction of DNA based typing mainly clerical errors are seen

Eurotransplant EPT schemes (2) Detection and Identification of HLA Antibodies



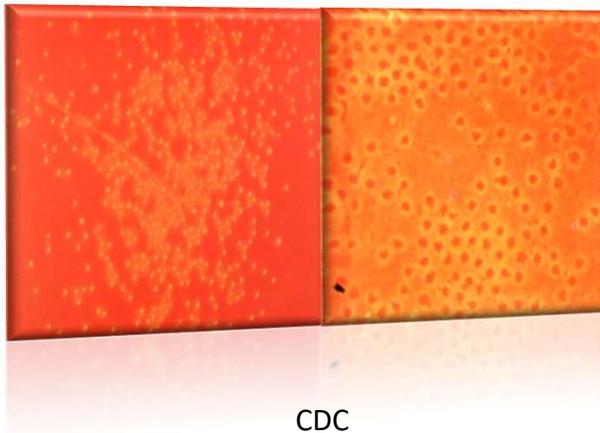
- 12 sera per year; one shipment
- Sera with HLA-specific antibodies collected from women after pregnancy are used



Eurotransplant EPT schemes (2) Detection and Identification of HLA Antibodies

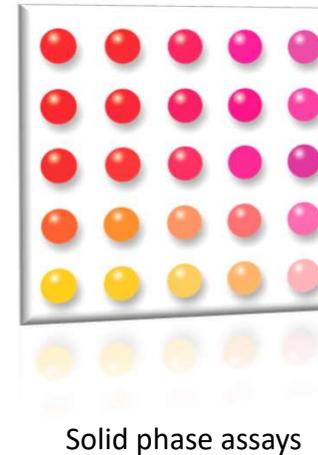


- Assays used:
CDC (mandatory for Eurotransplant affiliated centers)



CDC

- Solid Phase Assays (Single antigen Bead based)



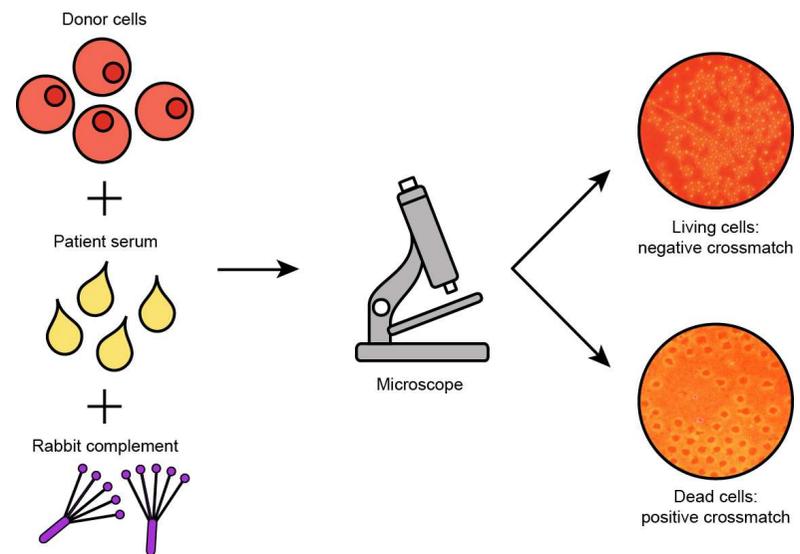
Solid phase assays

- Assessment on basis of consensus
- When 75% (CDC) or 95% (SPA) of the participants assign a certain antibody, this is in consensus. Missing specificities are considered discrepant.

Eurotransplant EPT schemes (3) Crossmatching



- 4 shipments of 3 blood samples and 3 sera each (9 crossmatches per shipment)
- The technique used within Eurotransplant is CDC, the result can be either negative or positive
- Qualitative assessment on basis of 75% consensus



Eurotransplant EPT schemes (4) Patient Based Cases



- From 2016 this scheme runs with 3 cases yearly
- Mandatory for Eurotransplant affiliated centers, all other centers are invited to participate.
- The exercises usually involve an organ offer to a recipient. The participants formulate an advice to transplant or not on immunological grounds, considering the results of the laboratory tests.
- An overview/summary of results is published on the EPT website
- Results are subject of discussion during the annual Eurotransplant extra mural meeting
- Cases can be of help to understand:
 - Difficult real-life cases
 - Local policies
 - Policy (changes) within Eurotransplant

Summary



In kidney transplantation it is of major importance for graft survival to:

- Match donor and recipient on basis of HLA type
 - Test for HLA antibodies before transplantation
 - Do a crossmatch to prevent (hyper)acute rejection of the organ
-
- Eurotransplant organizes the allocation of organs within affiliated centers
 - The ETRL helps to improve quality of tests necessary in kidney transplantation
-
- The ETRL organizes proficiency testing for HLA typing, crossmatching, antibody detection and identification and patient-based cases.
 - An annual certificate with the results is provided to each participant.



courtesy by Ilias Doxiadis

Thank You!

