Leiden University Medical Center

Eurotransplant Reference Laboratory

First experiences with the virtual crossmatch

Status after 42 days

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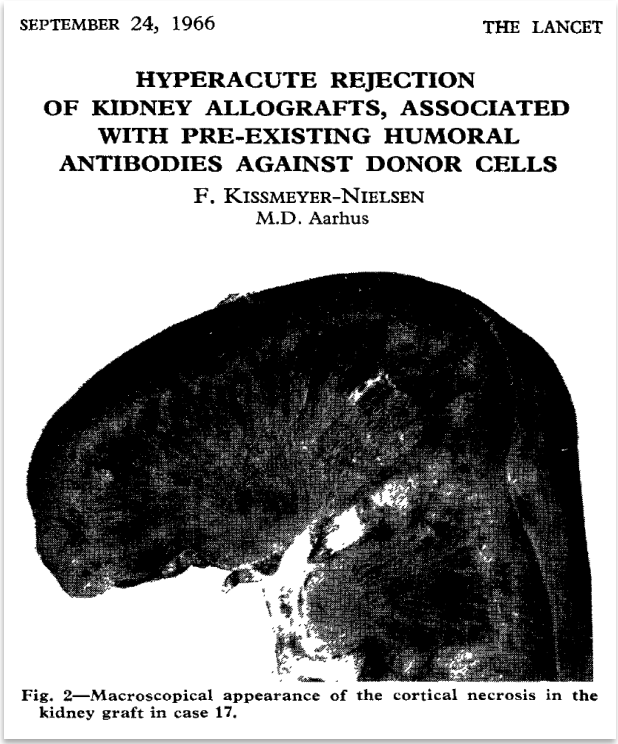
Eurotransplant Reference Laboratory

EXTRA MURAL MEETING 2023

History of the crossmatch

- The presence of pre-transplant donor-reactive antibodies can lead to hyperacute rejection

- Pre-transplant crossmatching prevents transplantation into recipients with pre-existing donor-reactive antibodies



SEPTEMBER 24, 1966 THE LANCET

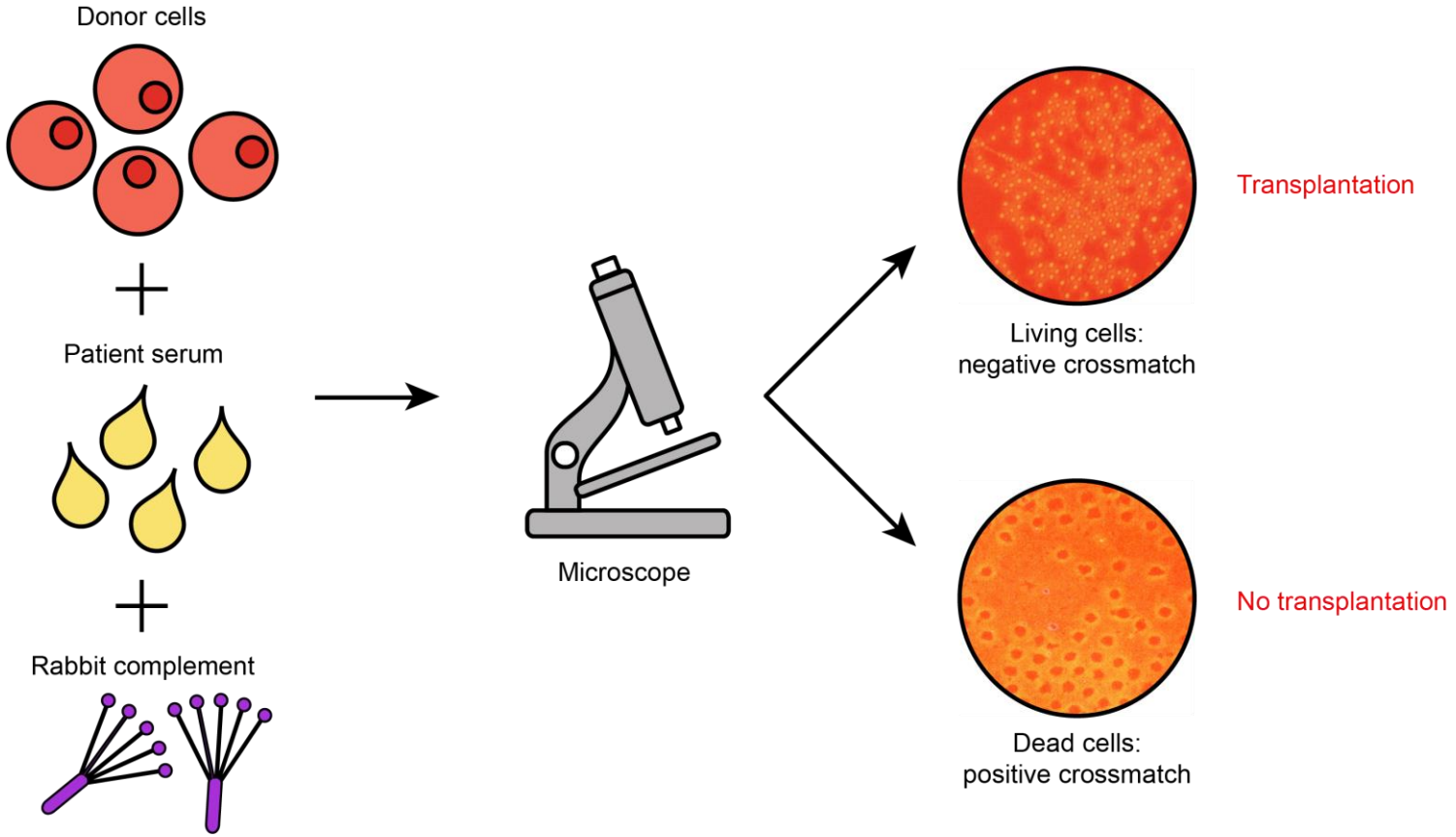
HYPERACUTE REJECTION OF KIDNEY ALLOGRAFTS, ASSOCIATED WITH PRE-EXISTING HUMORAL ANTIBODIES AGAINST DONOR CELLS

F. KISSMEYER-NIELSEN
M.D. Aarhus

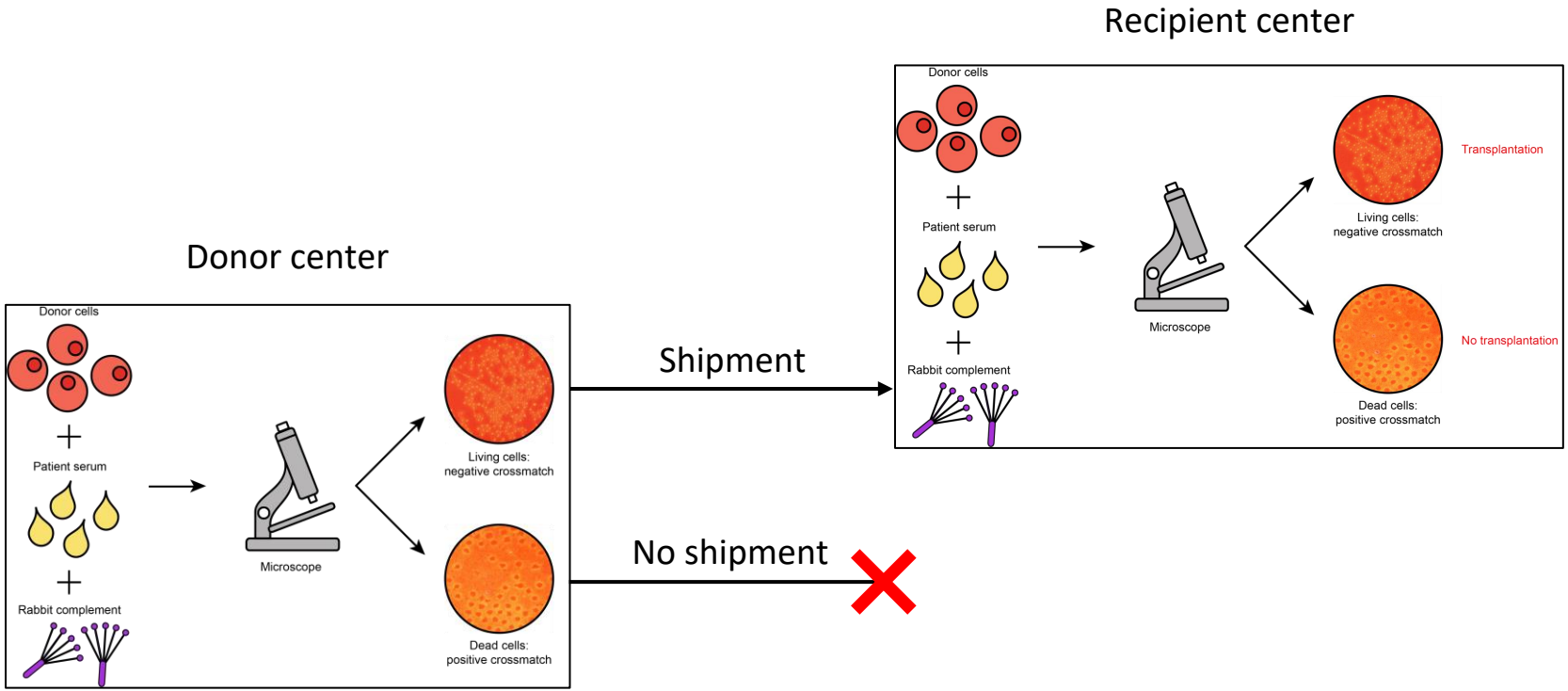
Fig. 2—Macroscopical appearance of the cortical necrosis in the kidney graft in case 17.

Kissmeyer-Nielsen *et al.*, Lancet 1966

Consequence of the outcome of the CDC crossmatch



Organ offer to immunized patient

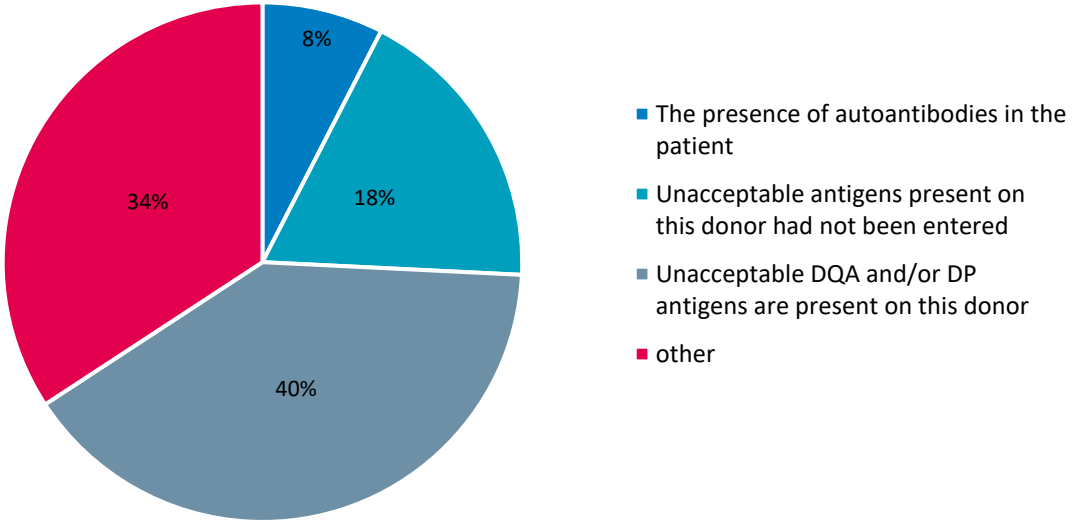


Problems of physical donor center crossmatch

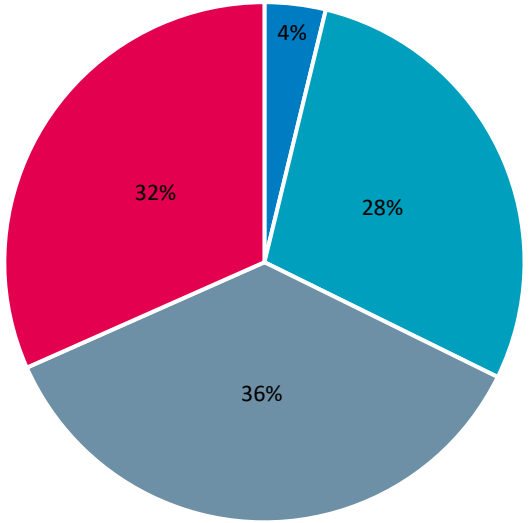
- Mandatory serum exchange program required
- No immunological history known at donor center
- False positive crossmatches due to irrelevant antibodies not directed against HLA
- Low sensitivity for HLA class II antibodies (only unseparated or T cell crossmatches are valid)
- Long cold ischemia times

Positive donor-center crossmatches

Explanations Sept 2018-Sept 2019 (n=225)



Explanations Sept 2019 -Sept 2020 (n=158)



Towards virtual crossmatch

- Organs carrying unacceptable antigens are not allocated to the specific patient
- To replace donor-center crossmatch with virtual crossmatch to prevent unnecessary organ shipment
- More detailed characterization of HLA antibodies and all relevant HLA antibody specificities can be registered

Requirements

- Alternative parameter of being sensitized: **virtual PRA** instead of % PRA in antibody screening
- Proper definition of unacceptable antigens
- More extensive donor HLA typing: HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, -DPA1

Stepwise introduction virtual crossmatch in Eurotransplant

- 01-05-2018 Start monitoring positive physical donor center crossmatches
- 01-02-2020 Introduction of the virtual PRA (vPRA)
- 23-11-2022 Unacceptable antigen upload extended

- 24-01-2023 Extension to vPRA on 11 loci and allelic level
  Introduction of virtual crossmatch with shadow phase

- **24-04-2023 Abandoning the physical donor center crossmatch**

Registration of unacceptable antigens

Two levels of registration

- Antigen level (broad and split), for example A9 or A24
- Allele level (allowing for entering allele specific antibodies), for example A*24:02

Loci

- HLA class I: HLA-A, -B, -C
- HLA class II: HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, -DPA1

Unacceptable Antigens	vPRA
*A*02:05 A3 A26 A34 B7 DRB5*01:01 DQ5 DQ6 DQA-01 DPB1*05:01*	78,56%

HLA typing requirements

Ideally, high resolution (2nd field) typing during deceased donor procedures is required but not yet possible. A practical solution of submitting ambiguous typing results has been introduced.

Types of ambiguity

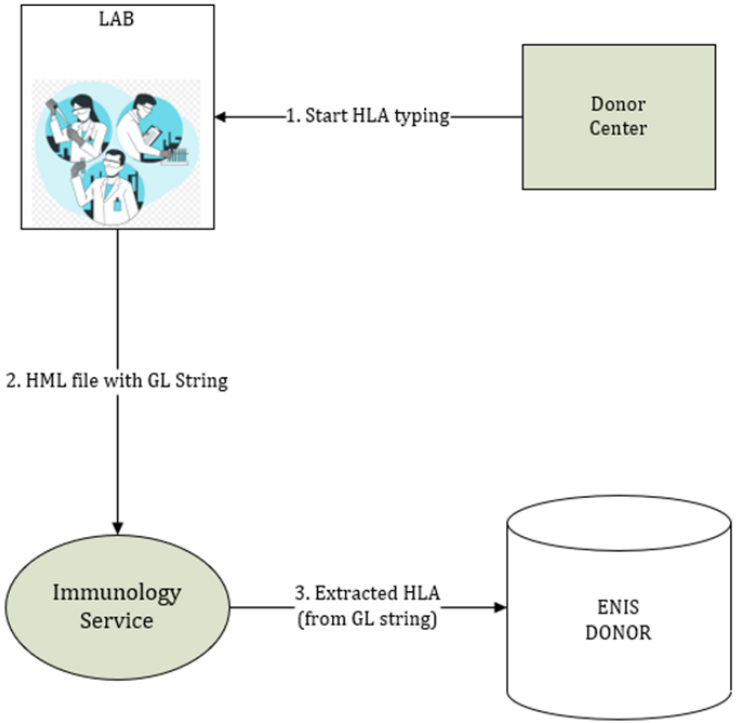
- Allele ambiguity: HLA-A*02:01/HLA-A*02:02
- Genotype ambiguity: HLA-A*02:01/HLA-A*02:02+HLA-A*03:01| HLA-A*02:07+HLA-A*03:06

Transfer of ambiguous typing results must be done electronically in a standardized format: **Histoimmunogenetics Markup Language** (HML), which contains Genotype List strings (GL strings)

Data transfer through HML files

HML files can be uploaded to the **ET Immunology Service**, after which data processing takes place:

- HML validation
  - Incorrect format
  - Not all 11 loci present
- A set of filters results in all ambiguities on second field level that are Common and Intermediate in the European population (e.g. HLA-A*02:01)
- An automatic conversion to match determinants (full phenotype) is made (e.g. HLA-A2)



HLA pre-filter v1.0

- HML files derived from techniques used on call contain many ambiguities
- Including alleles that are not **C**ommon or **I**ntermediate in the European population beyond the second field
- Pre-filter containing all **C**ommon **I**ntermediate and **W**ell-**D**ocumented alleles to filter out rare alleles on 3rd field
- Prevents the majority of incorrectly generated match determinants

Cw9 Cw9	**C*03:03:01:01**	**C*03:03:04**
Cw10	**C*03:02:16**	
	C*03:04:38	
	C*03:04:60	

Allele
C*03:02:02
C*03:03:01
C*03:03:02
C*03:03:04
C*03:03:05
C*03:03:07
C*03:03:16
C*03:04:01
C*03:04:02
C*03:04:04
C*03:04:05
C*03:04:08
C*03:04:09
C*03:04:11
C*03:04:13
C*03:04:14
C*03:04:15
C*03:04:17
C*03:04:19
C*03:04:27
C*03:04:42

HLA full phenotype

HLA full phenotype

- Based on the HML files, three scenarios of full phenotype are possible:
  - Full phenotype generated and approved
  - Full phenotype invalid and manually adjusted
  - Full phenotype disapproved and manually adjusted
- Full phenotype manually entered

HLA matching

- HLA matching remains unaltered; HLA-A and HLA-B on broad level and HLA-DR on split level

Example of processed HML file

Upload Donor HLA

Upload HML File — 2 Evaluate HLA Typing

Typing Date 04-07-2022 **HLA/IMGT Allele Database** 3.43.0

	CIWD Genotype	Generated Full Phenotype
A	A*01:01, A*01:03, A*01:25, A*02:01, A*02:04, A*02:24, A*02:30	A1, A2
B	B*07:02, B*07:07, B*07:14, B*07:15, B*50:01	B7, B50 (21)
C	C*06:02, C*06:07, C*07:02, C*07:10, C*07:25, C*07:27, C*07:46	Cw6, Cw7
DRB1	DRB1*04:04, DRB1*04:10, DRB1*04:50, DRB1*07:01, DRB1*07:05, DRB1*07:07	DR4, DR7
DRB345	DRB4*01:01, DRB4*01:02, DRB4*01:03	DR53
DQB1	DQB1*02:02, DQB1*03:02	DQ2, DQ8 (3)
DQA1	DQA1*02:01, DQA1*03:01	DQA-02, DQA-03
DPB1	DPB1*06:01, DPB1*15:01	DP-06, DP-15
DPA1	DPA1*01:03, DPA1*01:04	DPA-01
Publics		Bw6

I declare that I * ◉ **APPROVE** the generated full phenotype and agree that the uploaded donor HLA typing will be used for matching.

○ **DISAPPROVE** the generated full phenotype.

< Previous Save

Manually adjusted full phenotype

Donor HLA Typing

+ Upload Donor HLA

Entry Date	Typing Date	TT Lab	Typing Material	HLA/IMGT Allele Database	Generated Full Phenotype	Status
16-12-2022 11:26	*31-10-2022*		*Spleen*	*3.43.0*	***Invalid***	*Active*

	CIWD Genotype	Generated Full Phenotype (Invalid)	Manually adjusted Full Phenotype
A	A*02:01, A*02:05, A*02:11, A*02:12, A*02:17, A*02:27, A*02:29, A*02:30, A*11:01, A*11:02, A*11:05, A*11:29	A2, A11	A2, A11
B	B*40:02, B*40:13, B*44:02, B*44:03, B*44:04, B*44:21, B*44:27, B*44:29	B44 (12), B61 (40), B47	B44 (12), B61 (40)
C	C*02:02, C*02:07, C*02:10, C*04:01, C*04:04, C*04:07	Cw2, Cw4	Cw2, Cw4
DRB1	DRB1*07:01, DRB1*11:04, DRB1*11:06, DRB1*11:08, DRB1*11:19, DRB1*11:28, DRB1*11:29, DRB1*11:39, DRB1*11:43	DR11 (5), DR7	DR11 (5), DR7
DRB345	DRB3*02:01, DRB3*02:02, DRB3*02:06, DRB3*02:11, DRB3*02:17, DRB4*01:01, DRB4*01:02, DRB4*01:03	DR52, DR53	DR52, DR53
DQB1	DQB1*02:01, DQB1*02:02, DQB1*03:01, DQB1*03:09, DQB1*03:19	DQ2, DQ7 (3)	DQ2, DQ7 (3)
DQA1	DQA1*02:01, DQA1*05:01, DQA1*05:02, DQA1*05:03, DQA1*05:05, DQA1*05:09	DQA-02, DQA-05	DQA-02, DQA-05
DPB1	DPB1*04:02, DPB1*14:01	DP-03, DP-0402	DP-03, DP-0402
DPA1	DPA1*01:03, DPA1*01:04, DPA1*02:01, DPA1*02:07	DPA-01, DPA-02	DPA-01, DPA-02
Publics		Bw4, Bw6	Bw4, Bw6

BR-DHLA-12: The full phenotype is invalid because it has more than two match determinants of type broad for locus B.

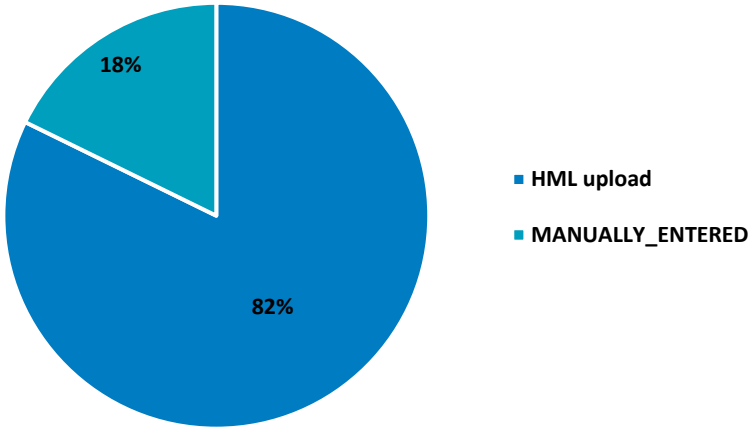
Virtual crossmatch process

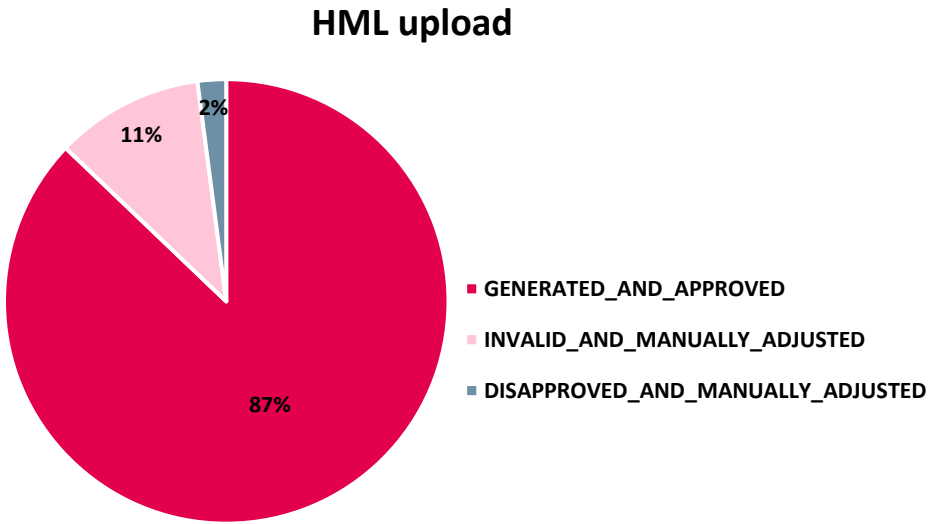
- CIWD genotype is used for virtual crossmatch on allele level

- Generated or adjusted full phenotype is used for virtual crossmatch on antigen level

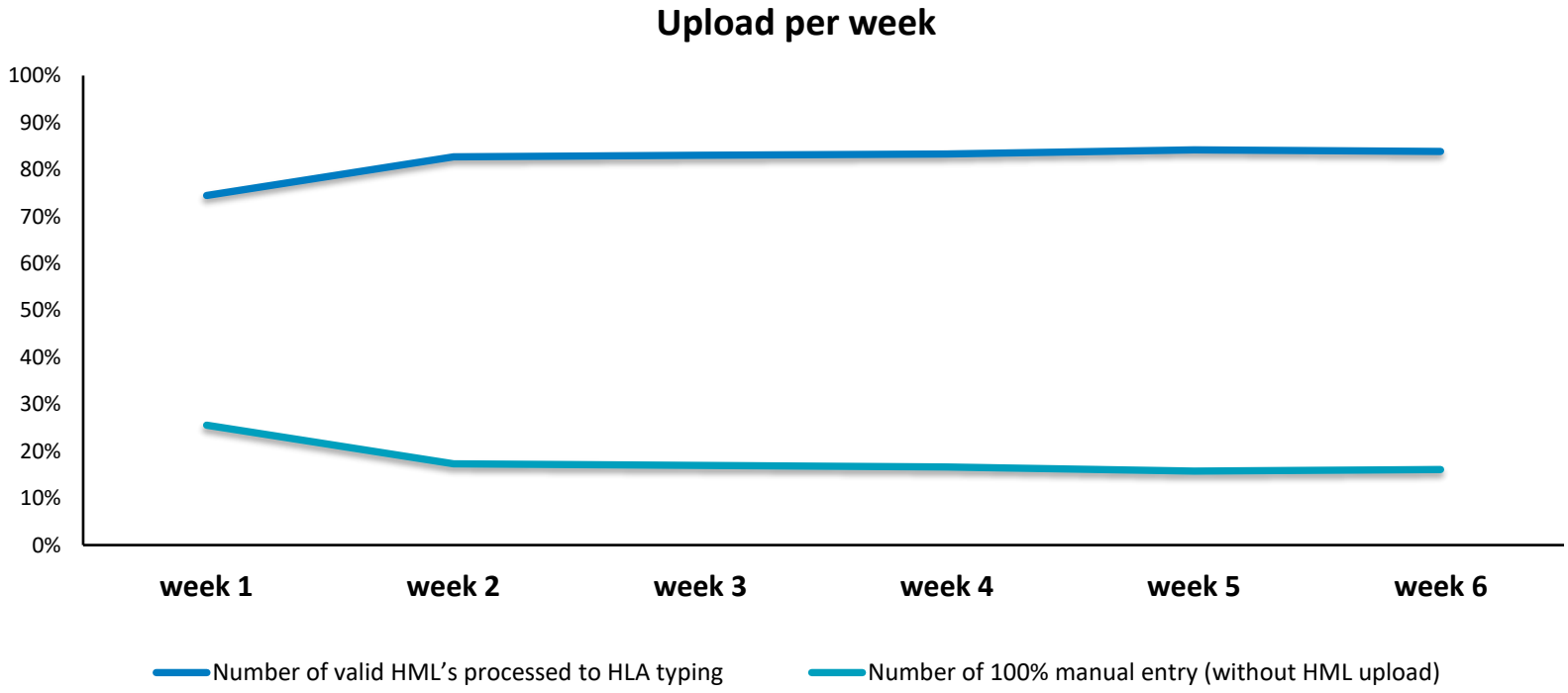
Unacceptable Antigens	CIWD genotype	Generated Full phenotype
A*02:05 A3 A26 A34	A*02:01 A*02:04 A*02:11 A*02:24 A*02:30 A*02:151 A*03:01 A*03:33	A2 A3
B7	B*07:02 B*07:09 B*07:15 B*07:47 B*27:05 B*27:09 B*27:10 B*27:51	B7 B27 Bw4 Bw6
	C*02:02 C*07:02 C*07:10 C*07:17 C*07:25 C*07:27 C*07:46	Cw2 Cw7
	DRB1*03:01 DRB1*03:06 DRB1*03:13 DRB1*03:23 DRB1*13:01	DR3 DR17 DR6 DR13
DRB5*01:01	DRB3*01:01 DRB3*01:02 DRB3*01:16	DR52
DQ5 DQ6	DQB1*02:01 DQB1*02:14 DQB1*06:03 DQB1*06:07 DQB1*06:14 DQB1*06:41 DQB1*06:44	DQ1 DQ6 DQ2
DQA-01	DQA1*01:03 DQA1*01:10 DQA1*05:01 DQA1*05:02 DQA1*05:10	DQA-01 DQA-05
DPB1*05:01	DPB1*01:01 DPB1*16:01 DPB1*127:01	DP-01 DP-10
	DPA1*01:03 DPA1*02:01 DPA1*02:03	DPA-01 DPA-02

So far, 82% of donor HLA typing data received is through HML, with 87% of HML files without need of adjustment (24/01 – 07/03)



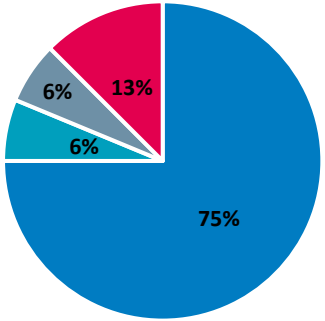


Increased HML upload over time (24/01 – 03/03)

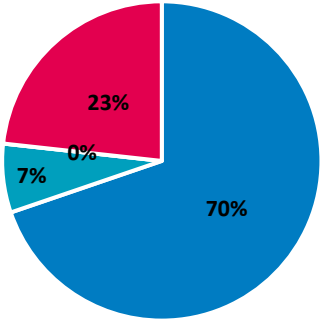


Upload per country (24/01 – 07/03)

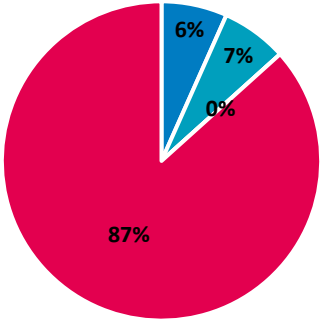
Austria



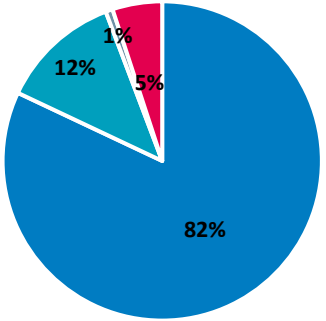
Belgium



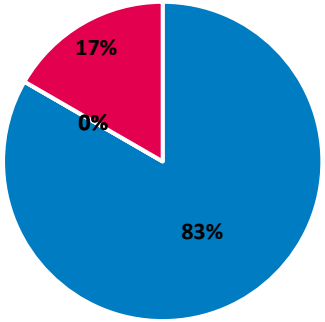
Croatia



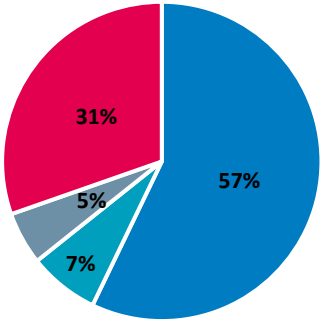
Germany



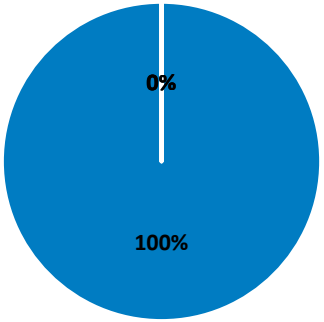
Hungary



Netherlands



Slovenia



- GENERATED_AND_APPROVED
- INVALID_AND_MANUALLY_ADJUSTED
- DISAPPROVED_AND_MANUALLY_ADJUSTED
- MANUALLY_ENTERED

Reasons for manually adjustment

- A locus with 3 match determinants due to genotype ambiguity with rare HLA allele
  - B7 B8 **B42** → HLA-B*07:02:01:01/…+HLA-B*08:01:01:01/… or HLA-B*08:156+HLA-B*42:01:01:02/HLA-B*42:01:04/…

- Incorrect match determinant due to common allele present in genotype ambiguity
  - DQ7 + **DQ2** → DQB1*03:01:01:01/…. + DQB1*03:01:01:01 | DQB1*02:01:24/DQB1*02:53Q/... + DQB1*03:52/….

- A match determinant missing due to rare allele
  - **DR52** absent in generated full phenotype due to rare DRB3*02 allele typing

Manual entry

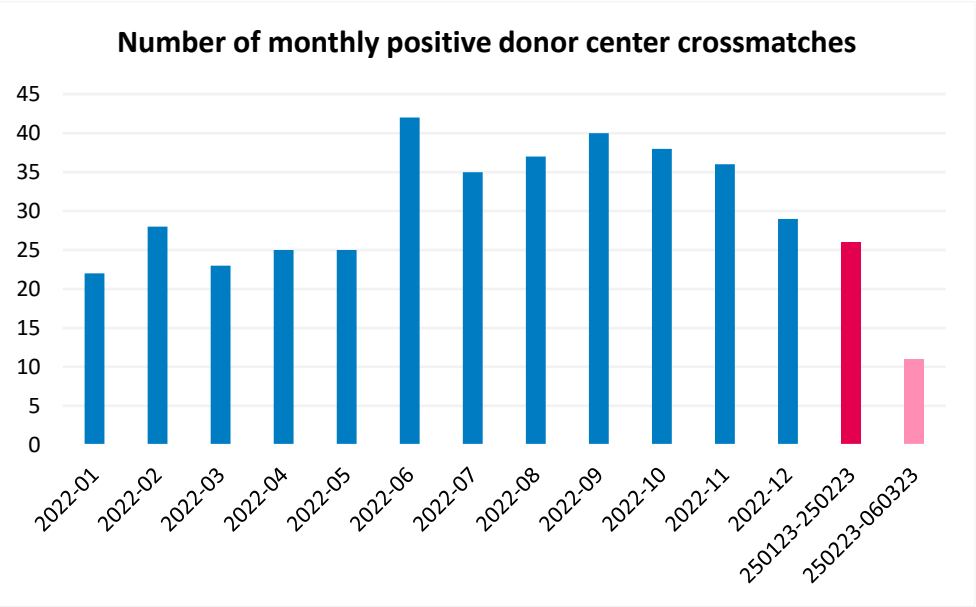
- Invalid HML files
  - Incorrect GL string in HML files
  - Loci (except for DRB3/4/5) is missing in the HML file a manual entry is required:
    - 2x due to missing DPB1 typing
    - 2x due to missing DPA1 typing

- No HML files are uploaded by laboratories

Positive donor center crossmatches

Currently, a shadow phase is running

- For every immunized patient, a physical donor center crossmatch is still performed
- In case of manual upload, the virtual crossmatch is negative in case of allele-specific antibodies



25/01 – 25/02	14/26 are crossmatches for own center 15/26 no unacceptable antigens defined
25/02 – 06/03	5/11 are crossmatches for own center 5/11 no unacceptable antigens defined

Take home message

- Conversion of alleles to match determinants is a service to the labs to enable full electronic data transfer in the vast majority of cases
- Like all HLA typing software, the ET immunology service occasionally needs human intervention
- ET and ETRL are actively working on solving recurring issues to improve accuracy
- Verification of the generated full phenotype (HLA typing) by technician of HLA laboratory is required
- Proper definition of unacceptable antigens is essential
- **Virtual crossmatch is only as good as the data submitted to the system**

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