Leiden University Medical Center

Eurotransplant Reference Laboratory

Virtual Crossmatch in Eurotransplant: One year experience

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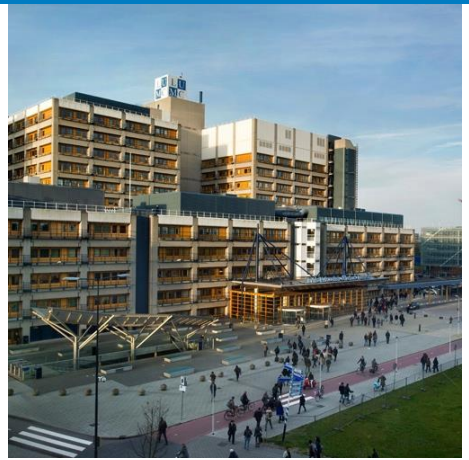
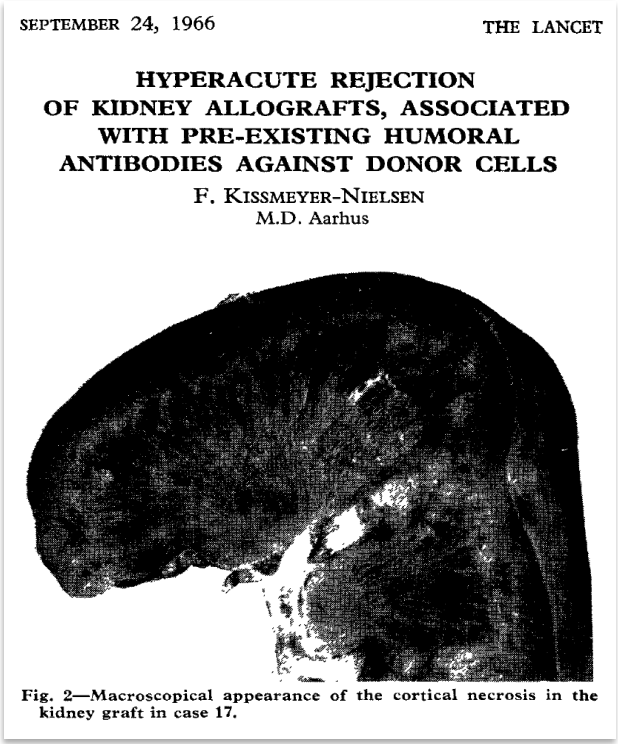


Eurotransplant

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

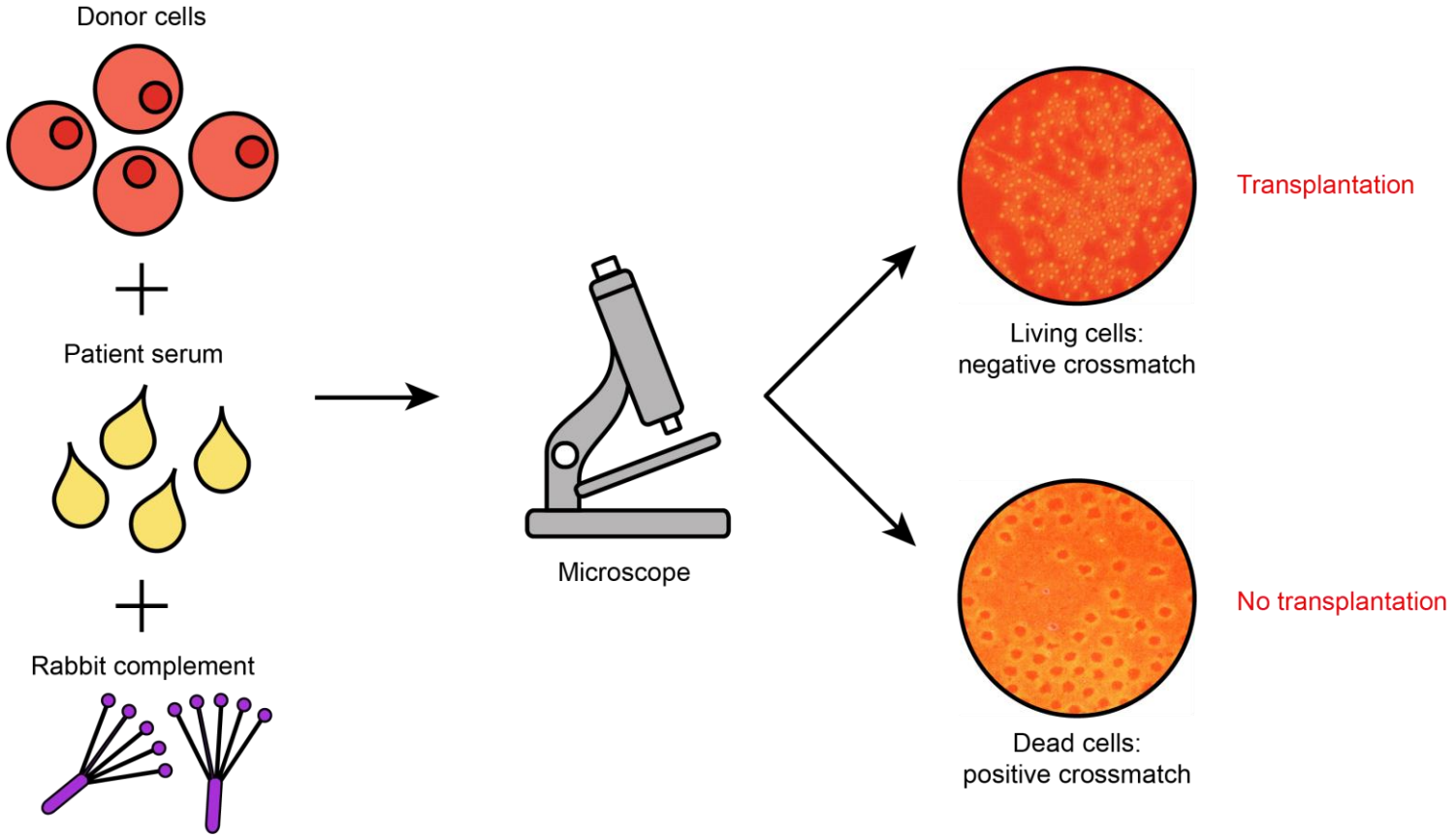
HYPERACUTE REJECTION OF KIDNEY ALLOGRAFTS, ASSOCIATED WITH PRE-EXISTING HUMORAL ANTIBODIES AGAINST DONOR CELLS

F. KISSMEYER-NIELSEN
M.D. Aarhus

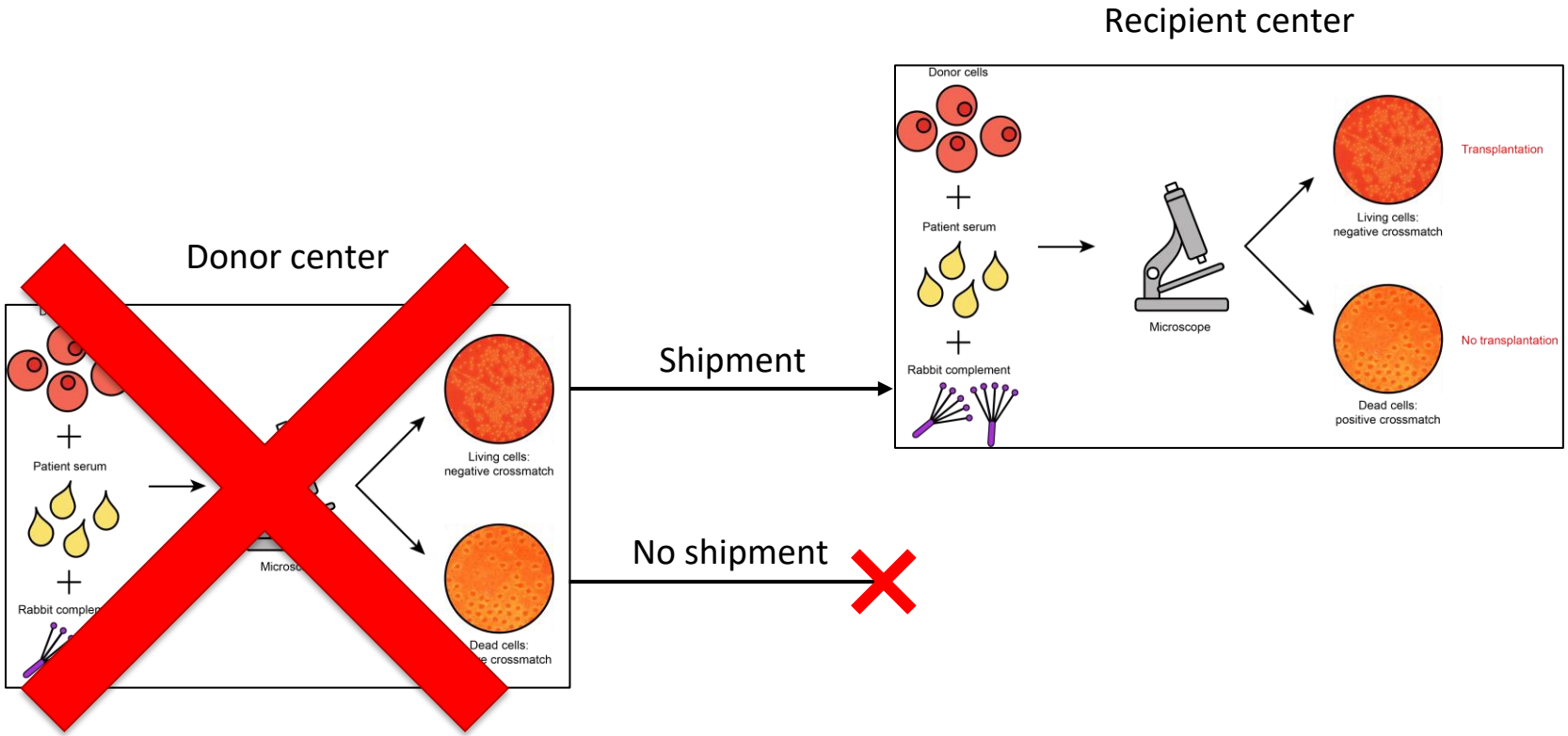
Fig. 2—Macroscopical appearance of the cortical necrosis in the kidney graft in case 17.

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

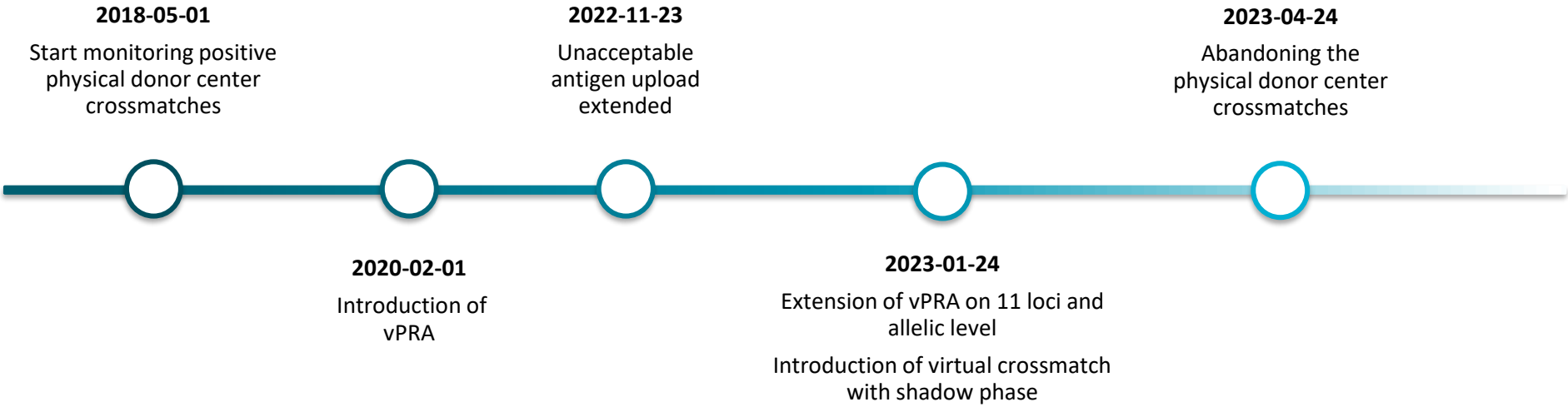
Towards virtual crossmatch in Eurotransplant

- 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: **vPRA** 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

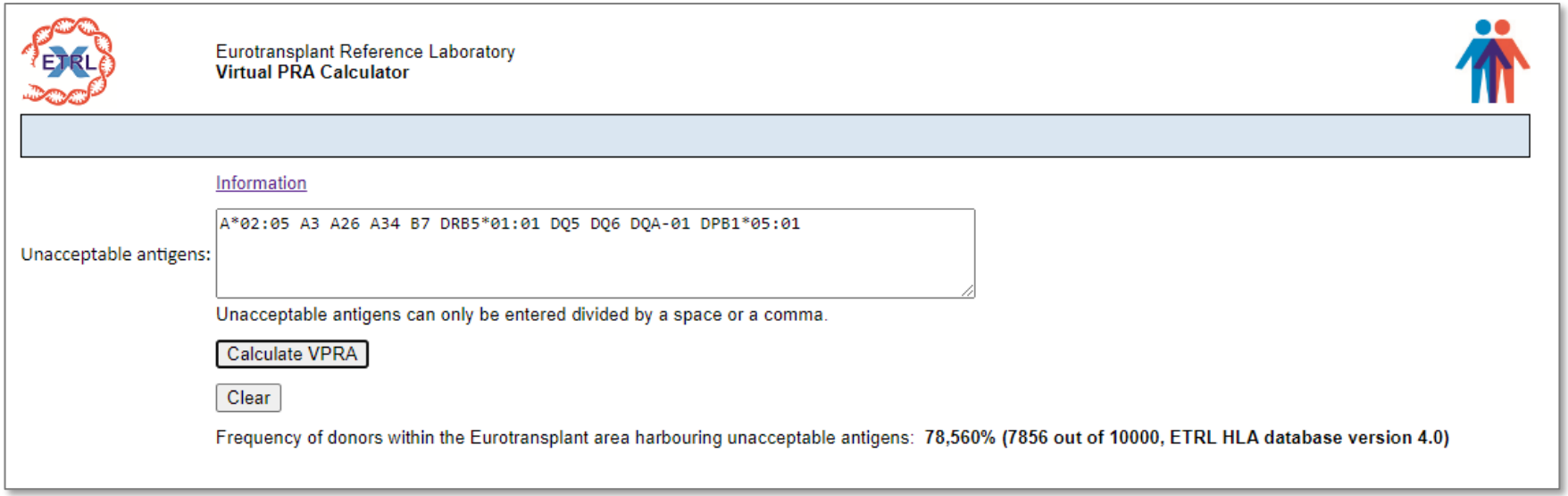


Extension of unacceptable antigens and vPRA to include all 11 loci at the allelic, split, and broad antigen level

- For the virtual crossmatch it is pivotal that for each patient the **unacceptable antigens** are properly defined
- vPRA calculator is based on **ETRL reference database v4.0**, which contains HLA data of 10.000 individuals from within the ET area
- vPRA is the **frequency of donors** within the ET area harboring unacceptable antigens

Unacceptable antigens	vPRA %
DR7	22,61
DR7 DQA-04 DQA-05 DQA-06	67,52
DR7 DQA-04 DQA-05 DQA-06 DRB3*02:01 DRB3*02:02	74,24
DR7 DQA-04 DQA-05 DQA-06 DRB3*02:01 DRB3*02:02 DPA-01	99,7

vPRA calculator on ETRL website



Eurotransplant Reference Laboratory
Virtual PRA Calculator

Information

Unacceptable antigens: A*02:05 A3 A26 A34 B7 DRB5*01:01 DQ5 DQ6 DQA-01 DPB1*05:01

Unacceptable antigens can only be entered divided by a space or a comma.

Calculate VPRA

Clear

Frequency of donors within the Eurotransplant area harbouring unacceptable antigens: **78,560% (7856 out of 10000, ETRL HLA database version 4.0)**

https://etrl.org/vPRA.aspx

For virtual crossmatch high resolution (2nd field) donor HLA typing is required

- Most ET centers use RT-PCR for HLA typing during deceased donor procedure

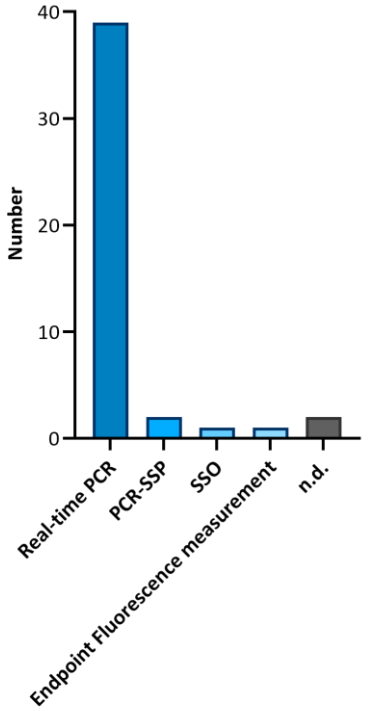
- Donor HLA typing with ambiguities
  - **Allele ambiguity**

    HLA-A*02:01/HLA-A*02:02 + HLA-A*03:01

  - **Genotype ambiguity**

    HLA-A*02:01/HLA-A*02:02 + HLA-A*03:01

    | HLA-A*02:07 + HLA-A*03:06



ETRL Survey 2024
29 centers responded
13 centers use more than one technique
n.d. centers that did not respond

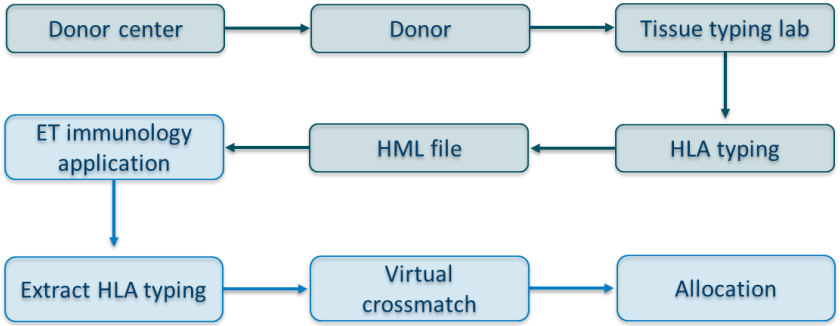
ET immunology application to electronically submit donor HLA typing

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

- HML **validation**
  - Incorrect format
  - Required 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 filters and HLA table

- Pre-filter containing all **C**ommon **I**ntermediate and **W**ell-**D**ocumented alleles to filter out rare alleles on **3rd field**
  - To filter out alleles that are not Common or Intermediate in the European population beyond the second field

- **Eurotransplant Match Determinants list** (HLA table) to filter alleles on **2nd field**
  - All alleles classified as Common and Intermediate in the EURO population of the CIWD3.0 catalogue
  - HLA-DQA1 alleles classified as Common and Well-documented in EFI v1.0 catalogue
  - HLA-DPA1 alleles frequently occurring in ET region (ETRL reference data)
  - Alleles of all loci frequently occurring in ET region or present in luminex single antigen bead kits (ETRL reference data)

- For all alleles present in the list **match determinants** were determined based on:
  - 2008 HLA dictionary
  - Serotypes as published by Osoegawa *et al*. HLA 2022, which have been approved by WHO HLA nomenclature as official nomenclature

https://etrl.eurotransplant.org/resources/hla-tables/

HLA table on ETRL website

Eurotransplant Reference Laboratory

About us | Resources | Dissemination | Meetings | Contact

- ETRL calculators
- ETRL HLA tables
- HLA-A
- HLA-B
- HLA-C
- HLA-DRB1
- HLA-DRB3/4/5
- HLA-DQB1
- HLA-DQA1
- HLA-DPB1
- HLA-DPA1

HLA-A

This HLA table is to be used for HLA typing and unacceptable antigen listing in Eurotransplant for HLA-A.

The alleles with XX in the antigen list are only to be used for patient HLA typing registration.

Assays used or typing donors and/or recipients in Eurotransplant affiliated laboratories must be capable of determining the presence (with or without ambiguities) of the alleles listed in red.

Allele	ET Match Determinant Split	ET Match Determinant Broad
A*01:01		A1
A*01:02		A1
A*01:03		A1
A*01:06		A1
A*01:25		A1
A*01:XX		A1
A*02:01		A2
A*02:02		A2
A*02:03		A2
A*02:04		A2
A*02:05		A2

https://etrl.eurotransplant.org/resources/hla-tables/

Generation of CIWD genotype and full phenotype

GL string extraction	Extract complete donor HLA typing from HML file	C*03:03:01:01/C*03:03:04/C*03:02:16/ C*03:04:38 + C*07:01:01:01/…/C*07:13:01:01/
HLA pre-filter	To filter out rare alleles on 3rd field	C*03:03:01:01/C*03:03:04/C*03:02:16/ C*03:04:38 + C*07:01:01:01/…/C*07:13:01:01/
HLA filter second field	Alleles trimmed to 2nd field & rare alleles on 2nd field are filtered out	C*03:03:01:01/C*03:03:04/C*03:02:16/ C*03:04:38 + C*07:01:01:01/…/C*07:13:01:01/
CIWD genotype	CIWD3.0 alleles on second field	C*03:03 C*07:01
Full phenotype	Match determinants based on ETRL HLA table	Cw9 Cw3 Cw7

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

Donor HLA typing

Upload Donor HLA

Upload HML File — 2 Evaluate HLA Typing

HML File test.hml **Typing Date** 29-08-2023 **HLA/IMGT Allele Database** 3.45.1

	CIWD Genotype	Generated Full Phenotype
A	A*01:01, A*01:02, A*01:06, A*01:25 - A*26:01, A*26:02, A*26:08, A*26:15	A1, A26 (10)
B	B*57:01 - B*40:01	B57 (17), B60 (40)
C	C*06:02, C*06:07 - C*07:01, C*07:02, C*07:04, C*07:06, C*07:07, C*07:10, C*07:12, C*07:17, C*07:18, C*07:19, C*07:22, C*07:25, C*07:27, C*07:40, C*07:46, C*07:60	Cw6, Cw7
DRB1	DRB1*03:01, DRB1*03:04, DRB1*03:06, DRB1*03:23 - DRB1*04:01, DRB1*04:02, DRB1*04:03, DRB1*04:04, DRB1*04:05, DRB1*04:06, DRB1*04:07, DRB1*04:08, DRB1*04:09, DRB1*04:10, DRB1*04:11, DRB1*04:13, DRB1*04:14, DRB1*04:38, DRB1*04:50	DR17 (3), DR4
DRB345	DRB3*01:01, DRB3*01:02 - DRB4*01:02, DRB4*01:03	DR52, DR53
DQB1	DQB1*02:01, DQB1*02:02 - DQB1*03:01, DQB1*03:09, DQB1*03:19	DQ2, DQ7 (3)
DQA1	DQA1*03:01, DQA1*03:02, DQA1*03:03 - DQA1*05:01, DQA1*05:02, DQA1*05:03, DQA1*05:05, DQA1*05:09	DQA-03, DQA-05
DPB1	DPB1*01:01 - DPB1*04:01	DP-01, DP-0401
DPA1	DPA1*01:03, DPA1*01:04 - DPA1*02:01, DPA1*02:07	DPA-01, DPA-02
Publics		Bw4, 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.

Manually adjusted full phenotype

Upload Donor HLA

Upload HML File — Evaluate HLA Typing — Correct Full Phenotype — 4 Verify & Save

	CIWD Genotype	Generated Full Phenotype	Manually Adjusted Full Phenotype
A	A*03:01, A*03:02 - A*11:01, A*11:02, A*11:05, A*11:12, A*11:29	A3, A11	A3 , A11
B	B*07:02, B*07:09, B*07:15 - B*40:02, B*40:03 - B*40:13	B7, B61 (40), B47	B7 , B61 (40)
C	C*02:02, C*02:07, C*02:10 - C*07:01, C*07:02, C*07:04, C*07:06, C*07:07, C*07:10, C*07:12, C*07:17, C*07:18, C*07:19, C*07:22, C*07:25, C*07:27, C*07:40, C*07:46, C*07:60	Cw2, Cw7	Cw2 , Cw7
DRB1	DRB1*15:01, DRB1*15:02, DRB1*15:03, DRB1*15:04, DRB1*15:06, DRB1*15:18 - DRB1*11:01, DRB1*11:04, DRB1*11:06, DRB1*11:08, DRB1*11:12, DRB1*11:15, DRB1*11:19, DRB1*11:24, DRB1*11:28, DRB1*11:29, DRB1*11:39, DRB1*11:43	DR15 (2), DR11 (5)	DR15 (2) , DR11 (5)
DRB345	DRB3*02:01, DRB3*02:02, DRB3*02:06, DRB3*02:11, DRB3*02:17 - DRB5*01:01, DRB5*01:02	DR52, DR51	DR51 , DR52
DQB1	DQB1*06:02, DQB1*06:03, DQB1*06:04, DQB1*06:09, DQB1*06:11 - DQB1*03:01, DQB1*03:09, DQB1*03:19	DQ6 (1), DQ7 (3)	DQ6 (1) , DQ7 (3)
DQA1	DQA1*01:01, DQA1*01:02, DQA1*01:03, DQA1*01:04, DQA1*01:05 - DQA1*05:01, DQA1*05:02, DQA1*05:03, DQA1*05:05, DQA1*05:09	DQA-01, DQA-05	DQA-01 , DQA-05
DPB1	DPB1*16:01 - DPB1*19:01	DP-10, DP-13	DP-10 , DP-13
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

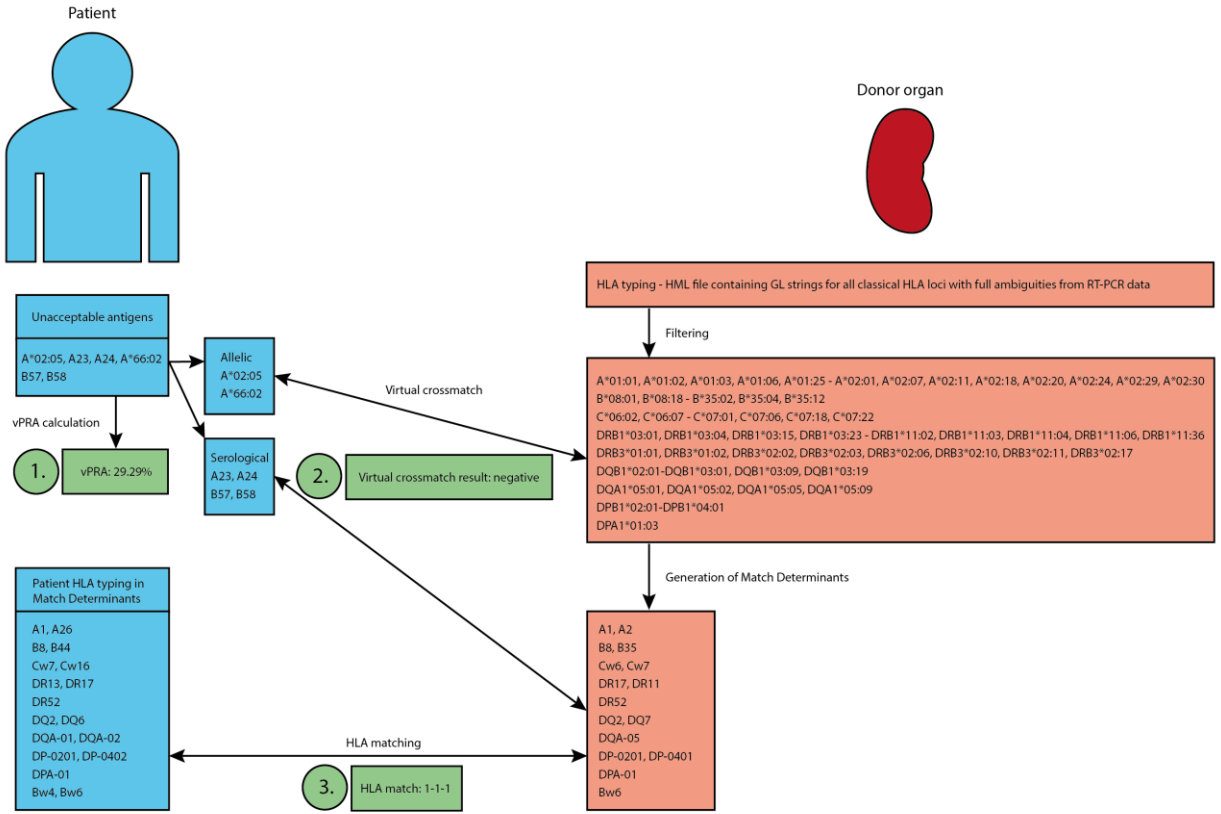
Verify the adjustment(s) to the Full Phenotype. If the changes are correct, press Save.

< Previous | Save | Cancel HLA Upload

Virtual crossmatch

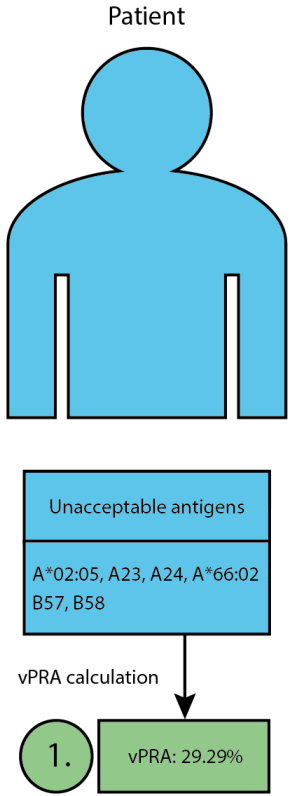
Unacceptable antigens	CIWD Genotype	Full phenotype
A*02:05 A3 A26 A34	A*02:01 A*02:04 A*02:11 A*02:24 A*02:30 A*02:151 A*03:01 A*03:33	A2 A3
B7	B*07:02 B*07:09 B*07:15 B*07:47 B*27:05 B*27:09 B*27:10 B*27:51	B7 B27 Bw4 Bw6
	C*02:02 C*07:02 C*07:10 C*07:17 C*07:25 C*07:27 C*07:46	Cw2 Cw7
	DRB1*03:01 DRB1*03:06 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

The virtual crossmatch process



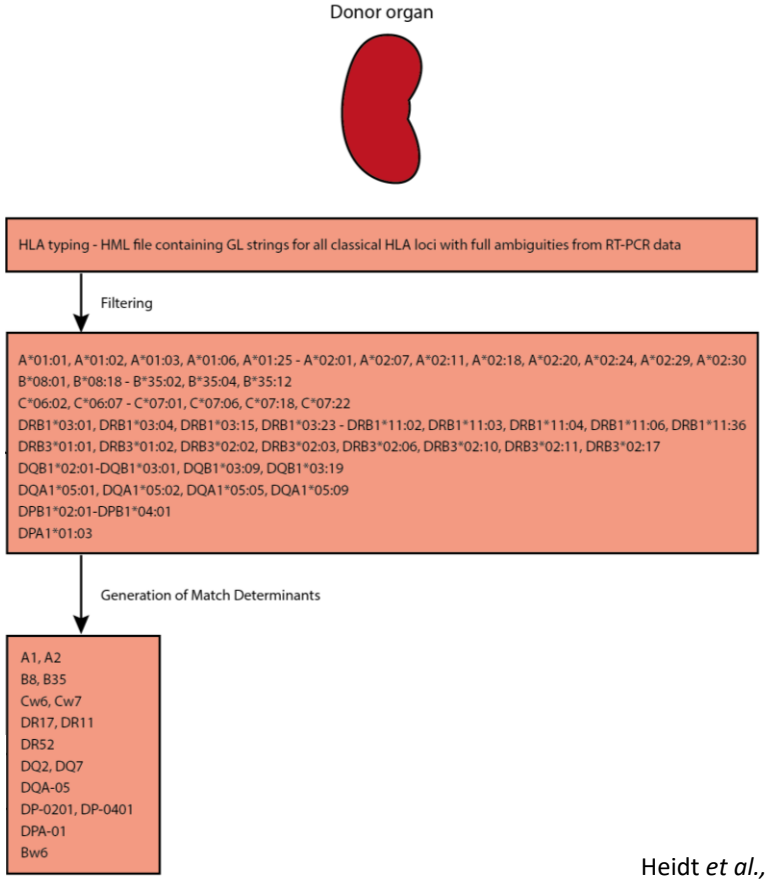
Heidt *et al.,* submitted

The virtual crossmatch process



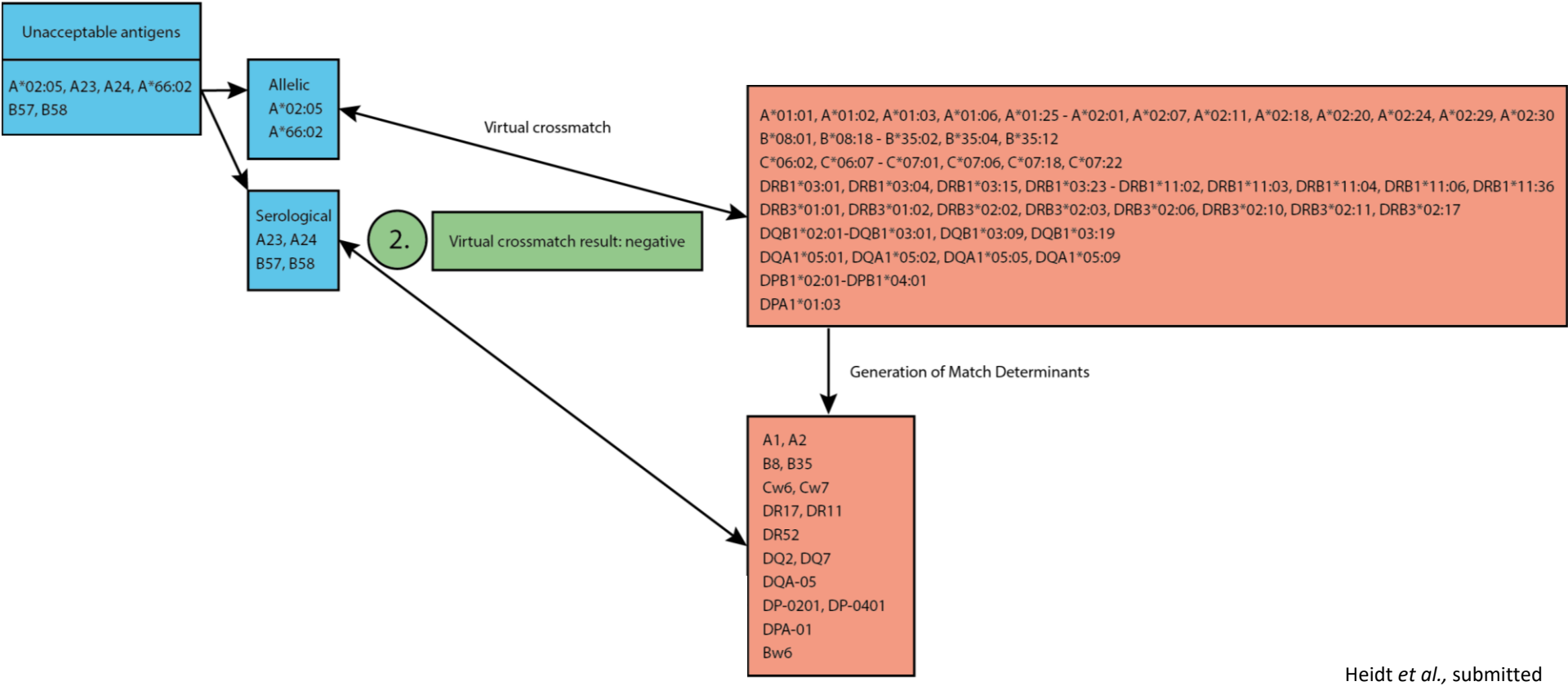
Heidt *et al.*, submitted

The virtual crossmatch process



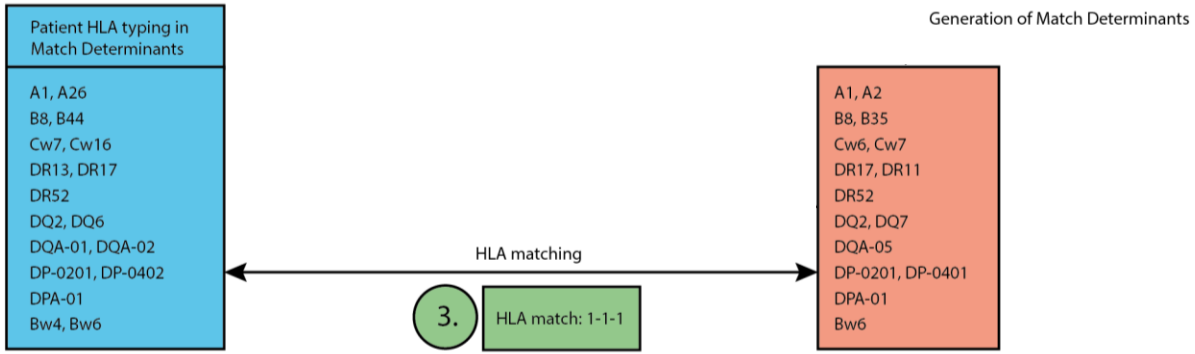
Heidt *et al.,* submitted

The virtual crossmatch process



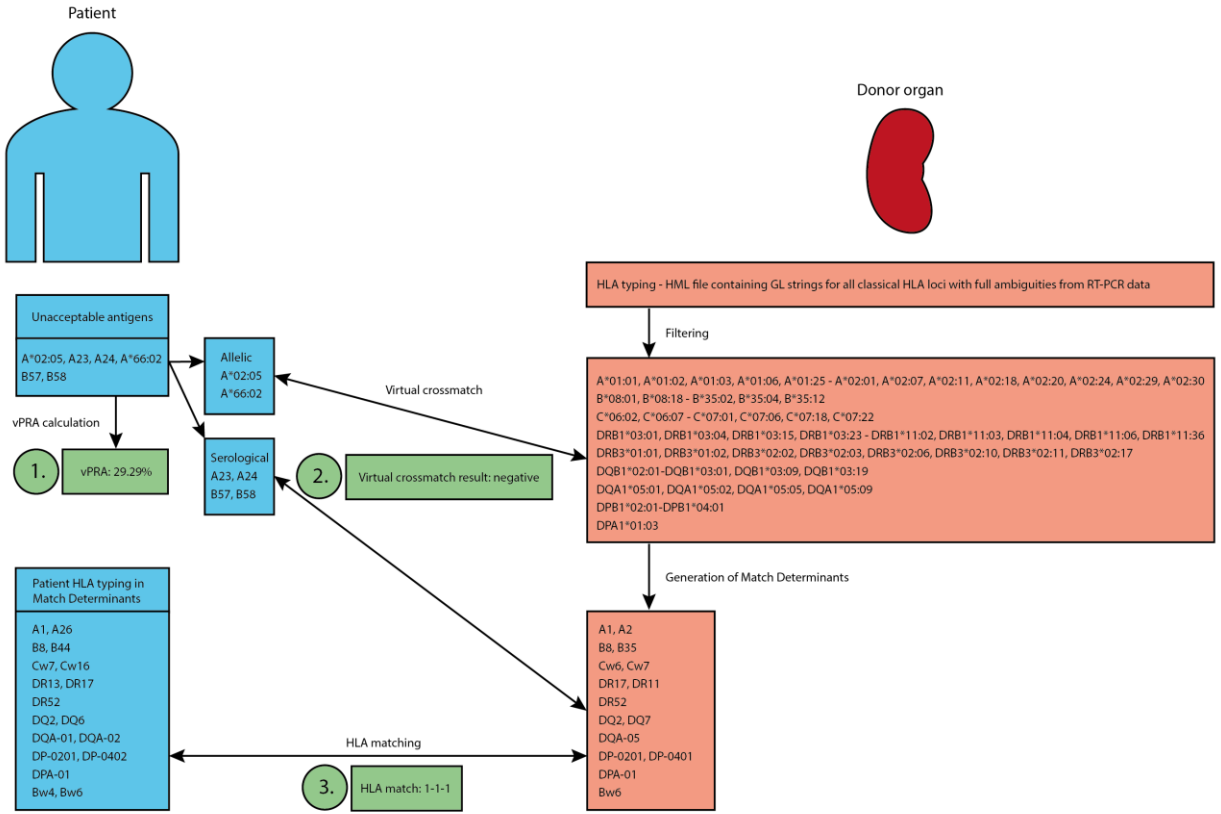
Heidt *et al.,* submitted

The virtual crossmatch process



Heidt *et al.,* submitted

The virtual crossmatch process



Heidt *et al.,* submitted

The virtual crossmatch process

- Virtual crossmatch process is applied for
  - AM
  - ETKAS
  - ESP*
  - EPAS*
  - EIAS currently only for Germany

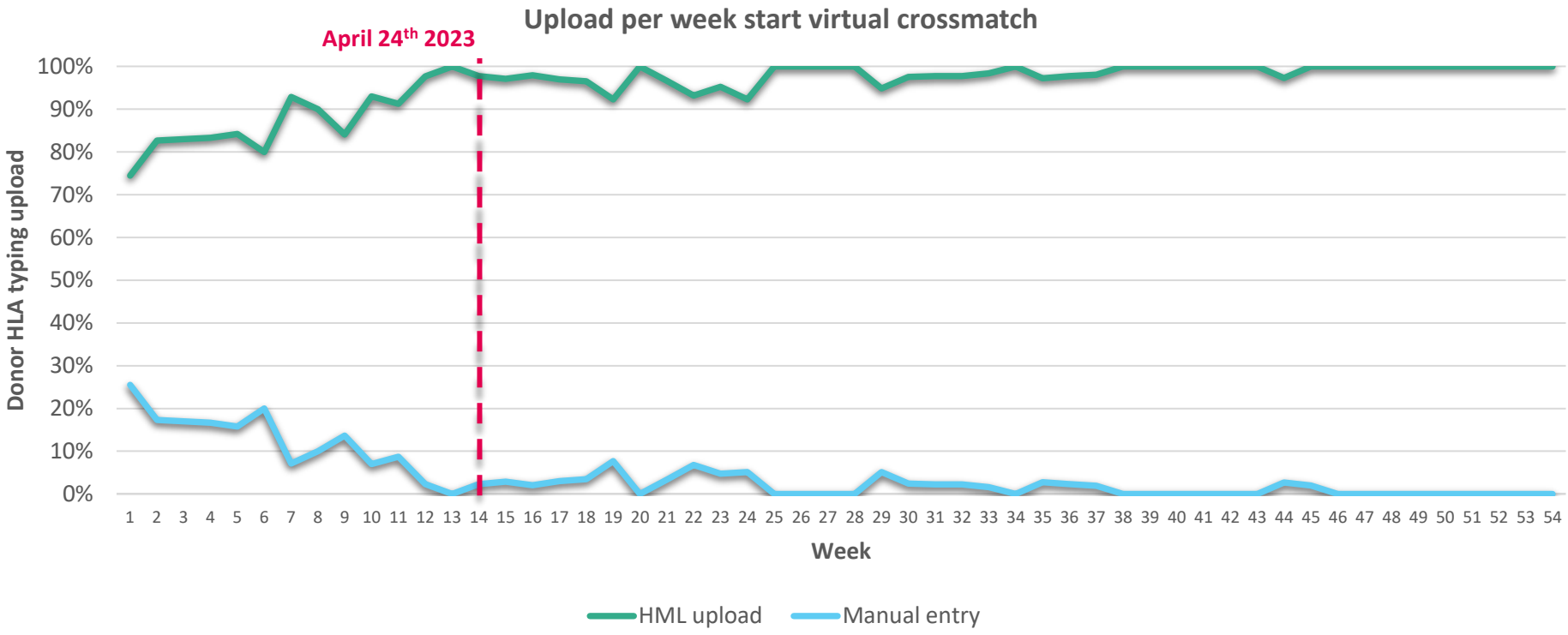
- Virtual crossmatch process is not applied for
  - ETHAS
  - ELAS

*When HLA typing is available before allocation

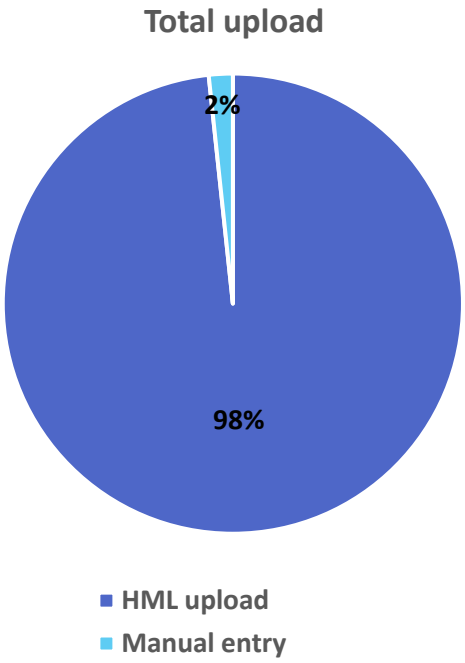
The virtual crossmatch first experience

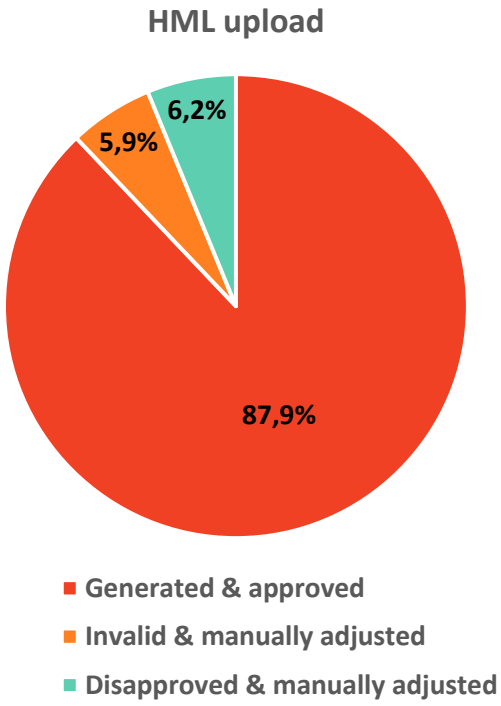
Donor HLA typing	**Monitoring of HML file uploads to ET immunology service**
Crossmatches	**Monitoring of recipient center crossmatches**

Donor HLA typing upload from January 24th, 2023 till January 31st, 2024



98% of donor HLA typing data is received through HML since April 24th, 2023 till January 31st, 2024





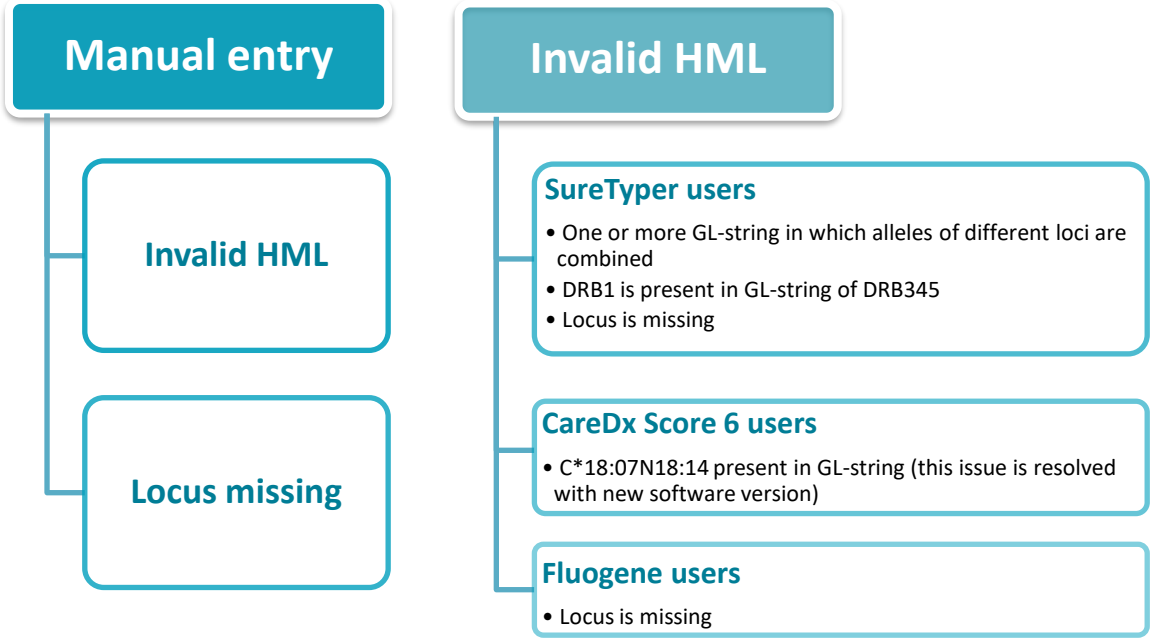
ETRL survey to request information on HLA typing kits and analysis software used during deceased donor procedure

Center	Survey replied	Number of techniques
AGATT	Yes	2
AIBTT	Yes	1
AOLTT	Yes	2
AWGTT	Yes	3
BBJTT	Yes	1
BBRTT	Yes	1
BLATT	Yes	1
BLGTT	Yes	1
BMETT	Yes	1
CRITT	Yes	2
CZATT	Yes	2
GBCTT	Yes	2
GERTT	Yes	2
GESTT	Yes	1
GFMTT	Yes	2
GFRTT	Yes	1
GHATT	Yes	2
GHBTT	Yes	1
GHOTT	Yes	2
GKMTT	Yes	2
GLUTT	Yes	2
GMLTT	Yes	1
GROTT	No	
HBUTT	Yes	1
NAWTT	Yes	1
NGRTT	Yes	1
NLBTT	Yes	1
NMSTT	Yes	1
NNYTT	Yes	1
NUTTT	No	
SLOTT	Yes	2
Grand Total	29/31	

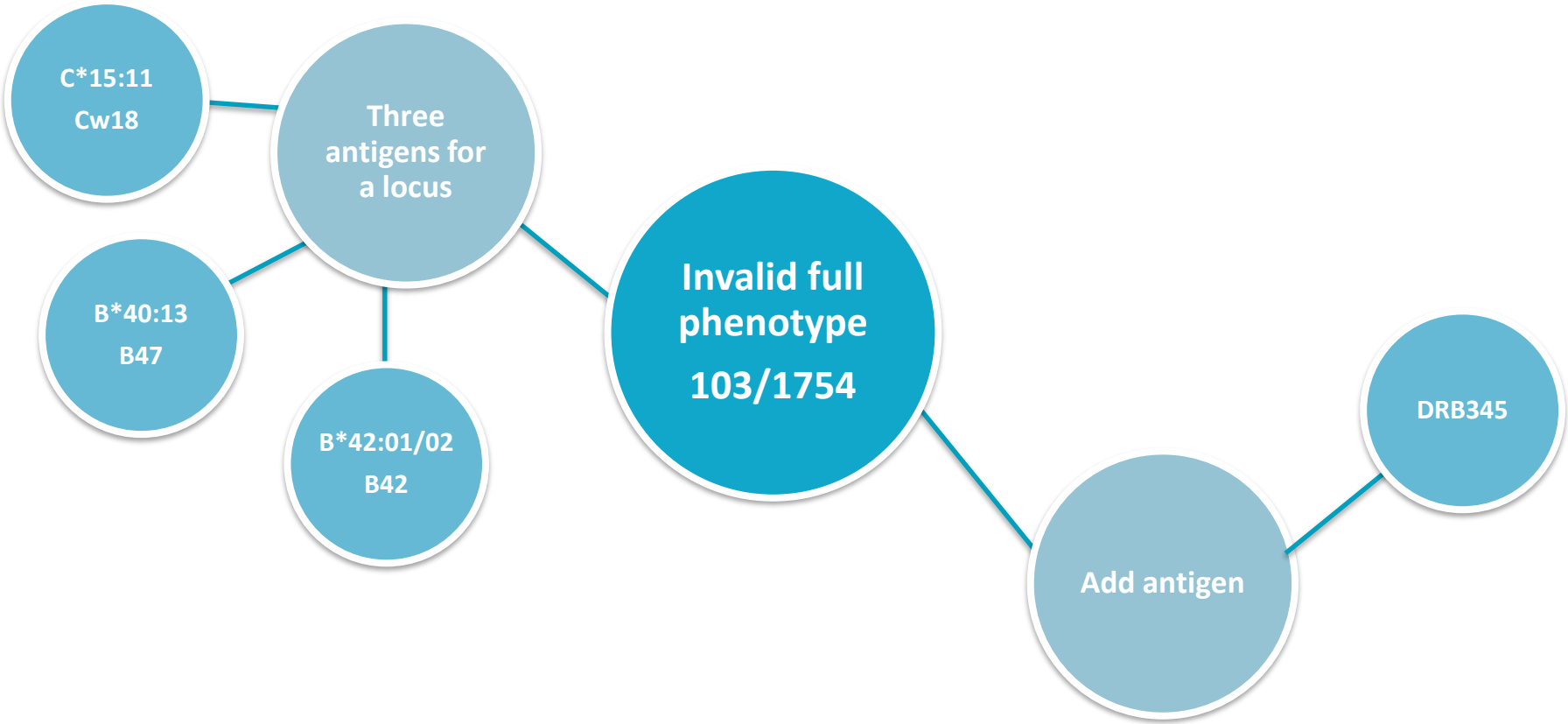
	Vendor						
Analysis Software + HLA typing kit	BAG Diagnostics	CareDx	Immucor	Inno-train	Protrans	Thermo Fisher Scientific	Grand Total
CareDx Score 6		12					12
Olerup Qtype 11		12					12
Fluogene				9			9
FluoVista				1			1
HLA-FluoGene 384 Match				2			2
HLA-FluoGeneNX Match				6			6
HELMBERG-SCORE		1					1
PCR-SSP (OLERUP)		1					1
MatchIt			1				1
Lifecodes HLA SSO Typing Kit			1				1
Other - Manual					1		1
PROTRANS HLA-A*/B*/C* + HLA-DRB1*/DQB1* Cyclerpla HLA-A,B,C and -DR,DQ SSP					1		1
PlexTyper 4	4						4
HistoType Rainbow	3						3
HistoType Rainbow QS6	1						1
PlexTyper 5.0	1						1
HistoType Rainbow	1						1
PlexTyper 5.2	1						1
HistoType Rainbow	1						1
SureTyper						13	13
LinkSeq HLA-ABCDRDQDP SABR 384 Kit						7	7
LinkSeq HLA-ABCDRDQDP+384 kit						6	6
Grand Total	6	13	1	9	1	13	43

Reasons for manual entry of donor HLA typing

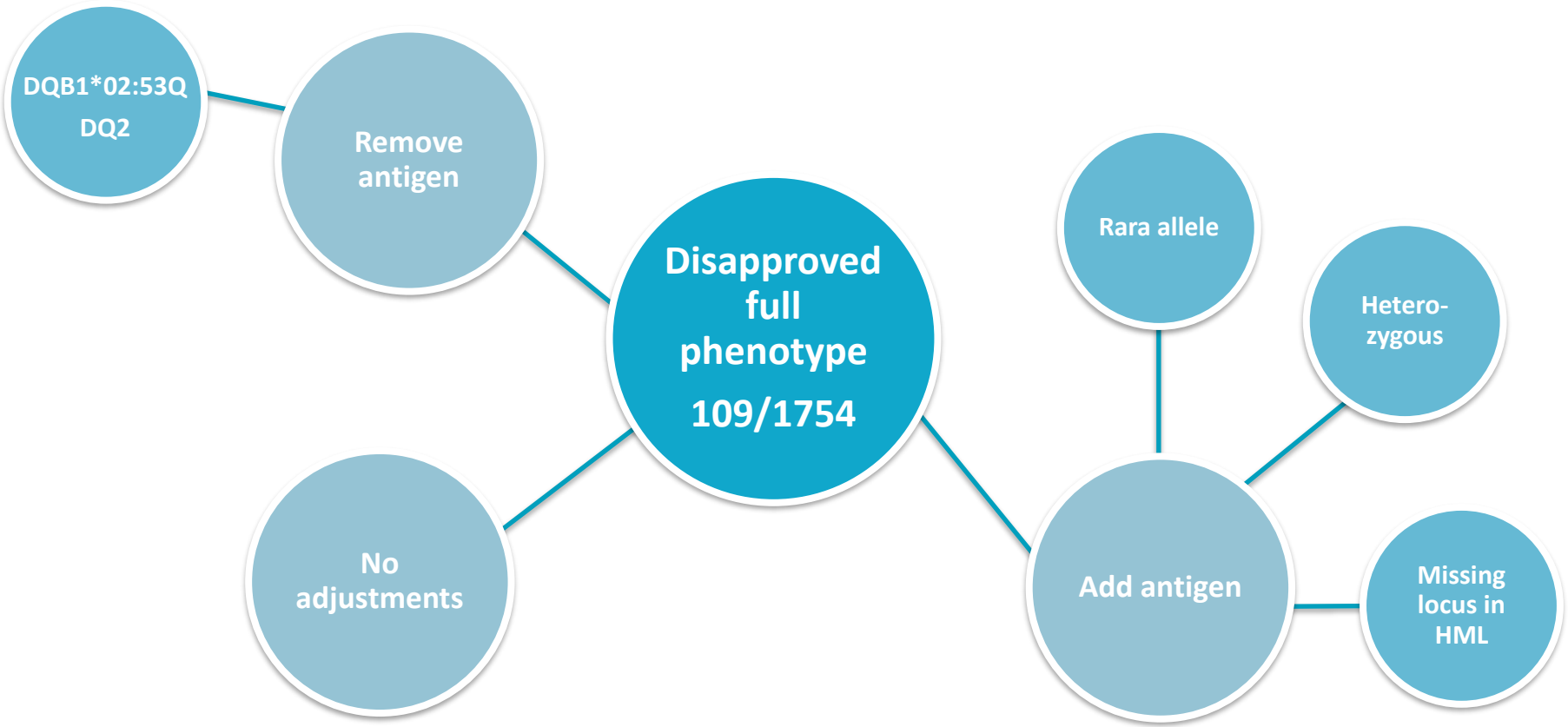
- ***Manual Enter HLA typing*** is available in rare occasion that center is unable to upload an HML file
- Majority of invalid HML file can be related to **analysis software settings**, which can be resolved
- Ultimate aim is to have **no manual entry** of donor HLA typing



Invalid full phenotype



Disapproved full phenotype



Electronically submission of donor HLA typing to ET immunology application

- The submission of donor HLA typing is a **success**, and only for small percentage of typing adjustments are necessary

- Currently, no changes will be made to the ET immunology application **software**
  - A document including the most adjustments made to full phenotype is being drafted

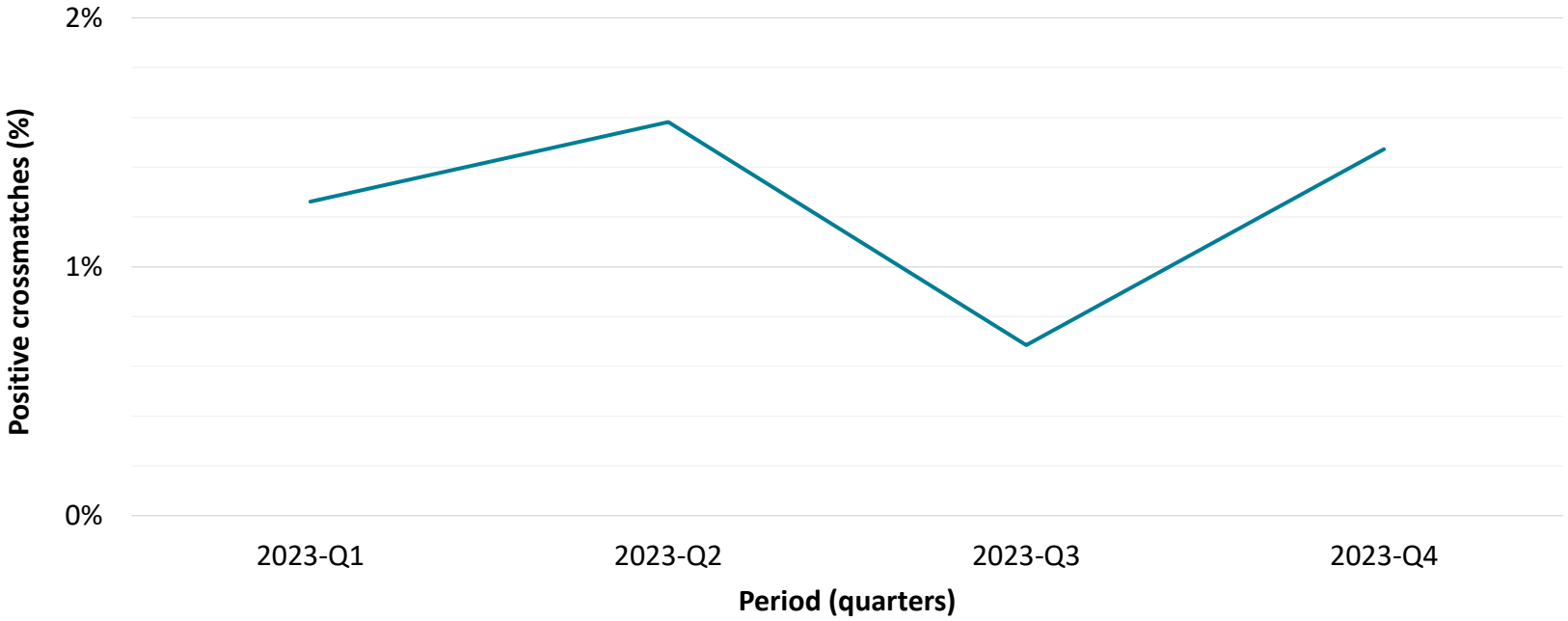
- When removing an antigen **carefully check** the whole full phenotype before saving it
  - For changes in HLA-B locus the associated Bw4/Bw6 should also be removed/checked

- Manually enter HLA typing option is only for **emergency** if HML file is not valid or available

- Once a donor HLA typing is submitted the **matching and allocation** process will start

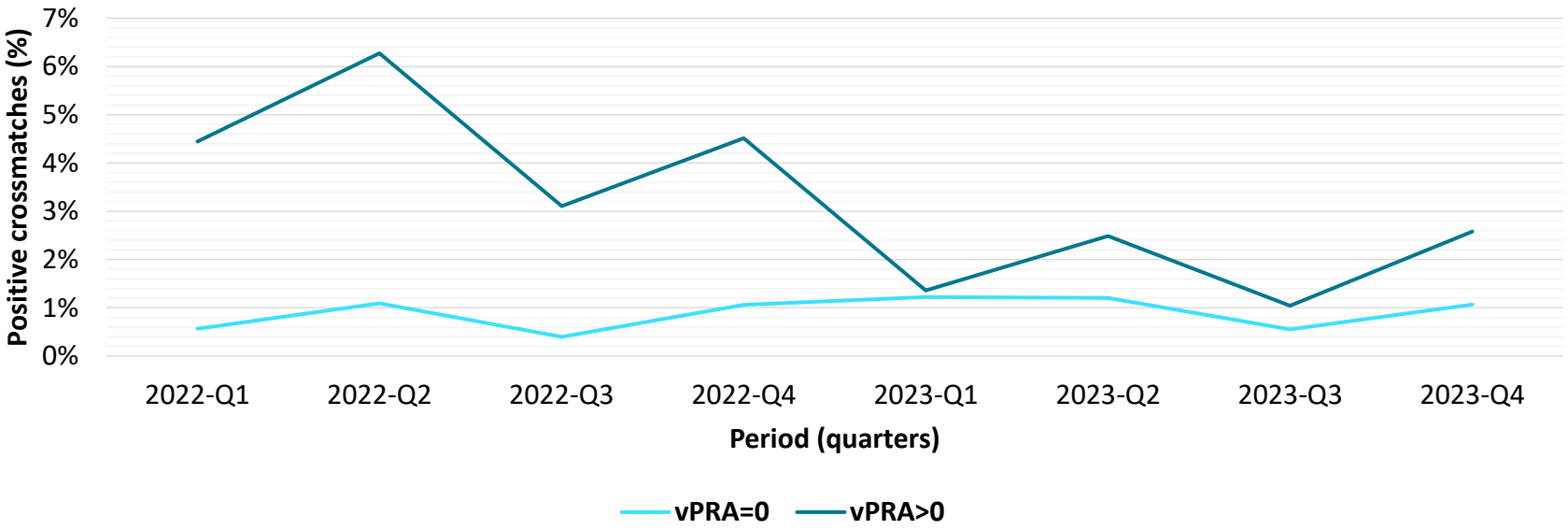
Relative positive recipient center crossmatches in 2023

Out of 5580 crossmatches performed 68 were reported as positive



Kidney & pancreas only

The positive crossmatches for vPRA>0% recipients approaches the same level as the vPRA=0% recipients



Kidney & pancreas only

Most positive crossmatches were reported for back-up recipients with vPRA=0%

	Shadow phase		Post-shadow phase		
	vPRA = 0%	vPRA > 0%	vPRA = 0%	vPRA > 0%	Total
Decisive crossmatches	2	3	3	17	25
Back-up crossmatches	10	2	24	7	43
Total	12	5	27	24	68

Summary

- Donor HLA typing submission via ET immunology application using HML file
- Virtual crossmatch implemented in Eurotransplant on January 24th 2023
  - Virtual crossmatch performed on allelic and antigen level
- Since April 24th 2023, donor center crossmatches are no longer performed
- Virtual crossmatch has not resulted in increase of positive recipient center crossmatches

Leiden University Medical Center

Eurotransplant Reference Laboratory

We would like to acknowledge and thank everyone involved in the implementation of virtual crossmatch!

Eurotransplant