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Histocompatibility and Immunogenetics in Transplantation

Characterising Donors and Recipients

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ODT September 2019

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Overview:

- What is HLA?
- Why is HLA important in Transplantation
  - HLA typing
  - Sensitisation to HLA and Unacceptable Antigen Definition
- HLA and the Patient Pathway
  - Incorporation of UKLDKSS

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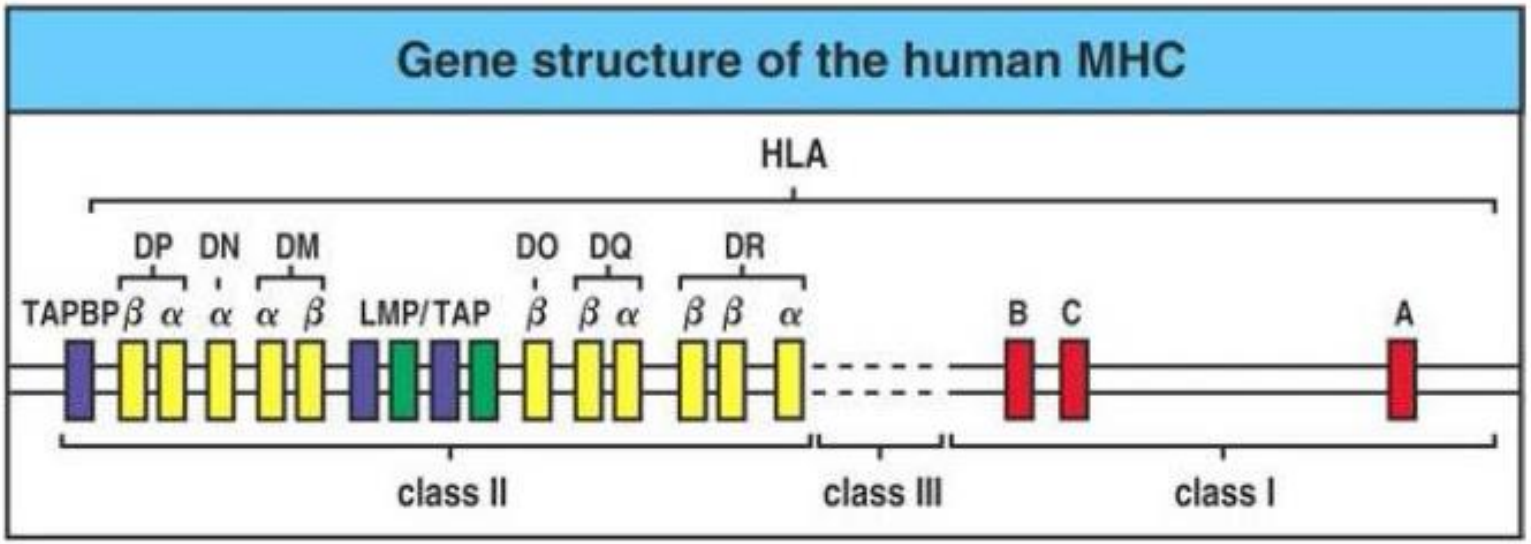
What is HLA?

- HLA (used to) = **H**uman **L**eukocyte **A**ntigen.
- Now just HLA – as they are expressed on more cells that Leukocytes.
- Encoded by the **M**ajor **H**istocompatibility **C**omplex – MHC.
- In Humans - Genetic complex found on chromosome 6.
- Made of 3 regions – Class I, Class II and Class III.



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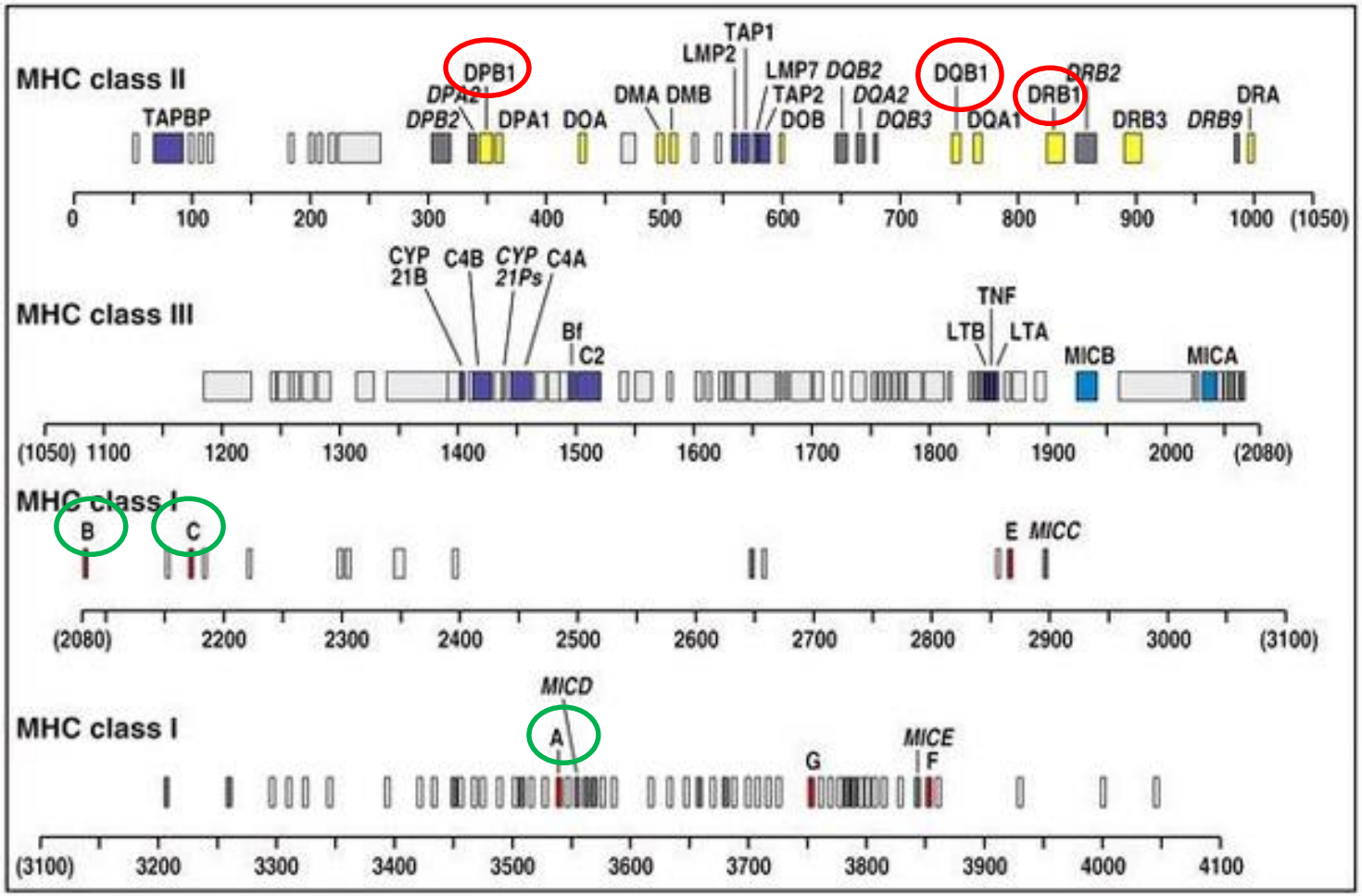
- Routine transplantation and tissue typing concentrates on ‘Classical’ Class I (A, B and C) and II (DR, DQ, DP) only.



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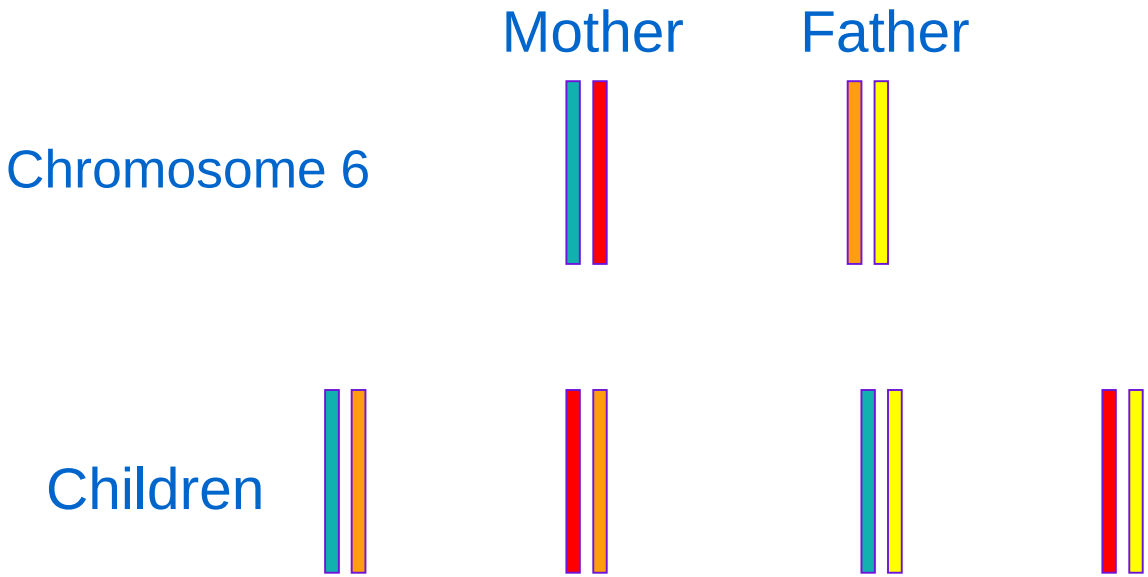
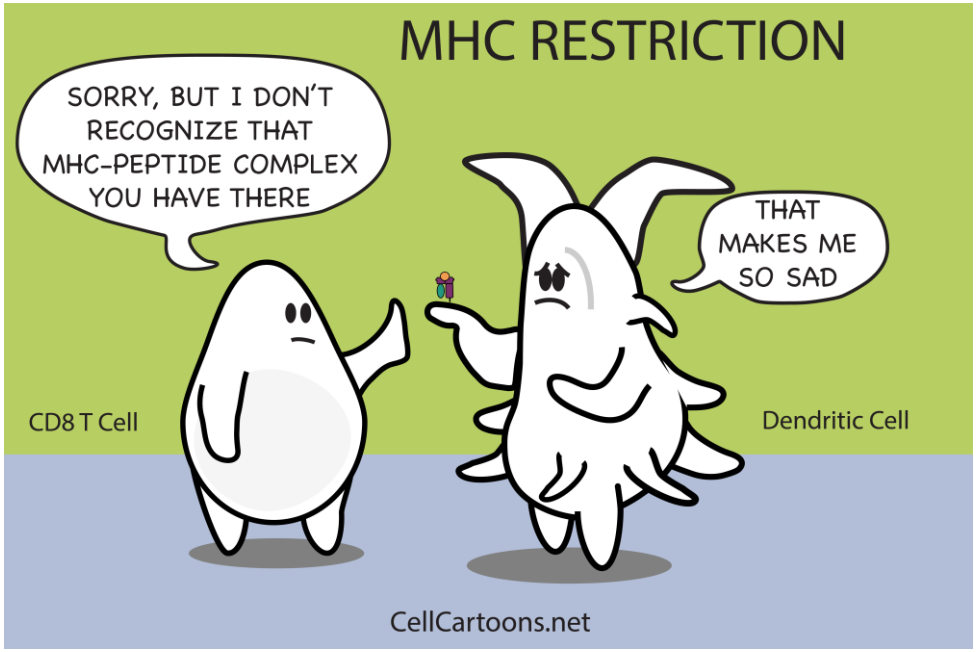
HLA Inheritance:

- The HLA genes come as an associated set of HLA –A, B, C, DR, DQ and DP – known as a haplotype
- Everybody carries two haplotypes – generally different ones (heterozygous) but sometimes they are the same (homozygous)
- Each individual inherits one haplotype from each parent.
- Generally from two parents there are four possible combinations that can be inherited, so you have a 1:4 chance of having the same HLA type as your sibling.



What Do HLA Molecules do?

- Control and dictate your Immune Response.
- Present peptides to responder cells in the immune system – self or pathogen derived.
- Normally cells will only respond if they recognise both the HLA molecule (as self) and the peptide being presented.



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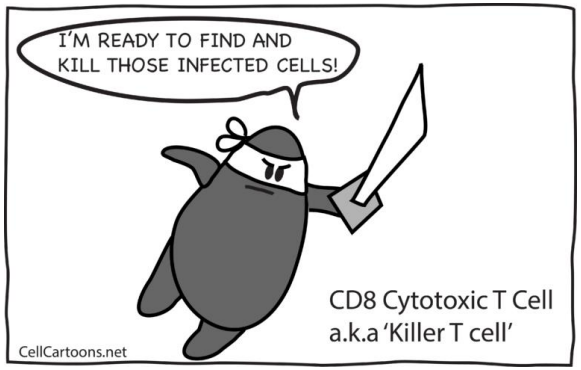
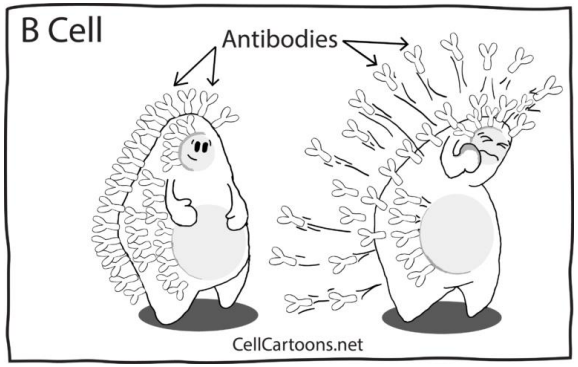
Relevance to Transplantation:

- HLA is **very** variable between people.
- In transplantation the HLA molecules on the new organ are recognised by the immune system as being non-self (you) and treated like an infection to fight.





- This can cause damage to the organ and rejection.
- The more similar the HLA of the recipient and donor the less the immune system recognises the organ as being different.
- A very well HLA matched deceased donor kidney gives a better outcome
- For living donors HLA is matching less important – fresh kidney with little time out of the body

- **Three important HLA in Transplantation** - HLA-**A**, **B** and **DR**

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HLA Diversity:

- Most diverse system in the Human genome.
- As of September 2019 within the worldwide population -

Loci	Different Genes	Different Protein Products	Serological Specificities
HLA A	5266	3552	28
HLA B	6537	4494	60
HLA C	5140	3359	16
Class I Total	16943	11405	104
HLA DRB	3171	2226	21
HLA DQB	1718	1151	9
HLA DPB	1449	960	None
Class II Total	6338	4337	30

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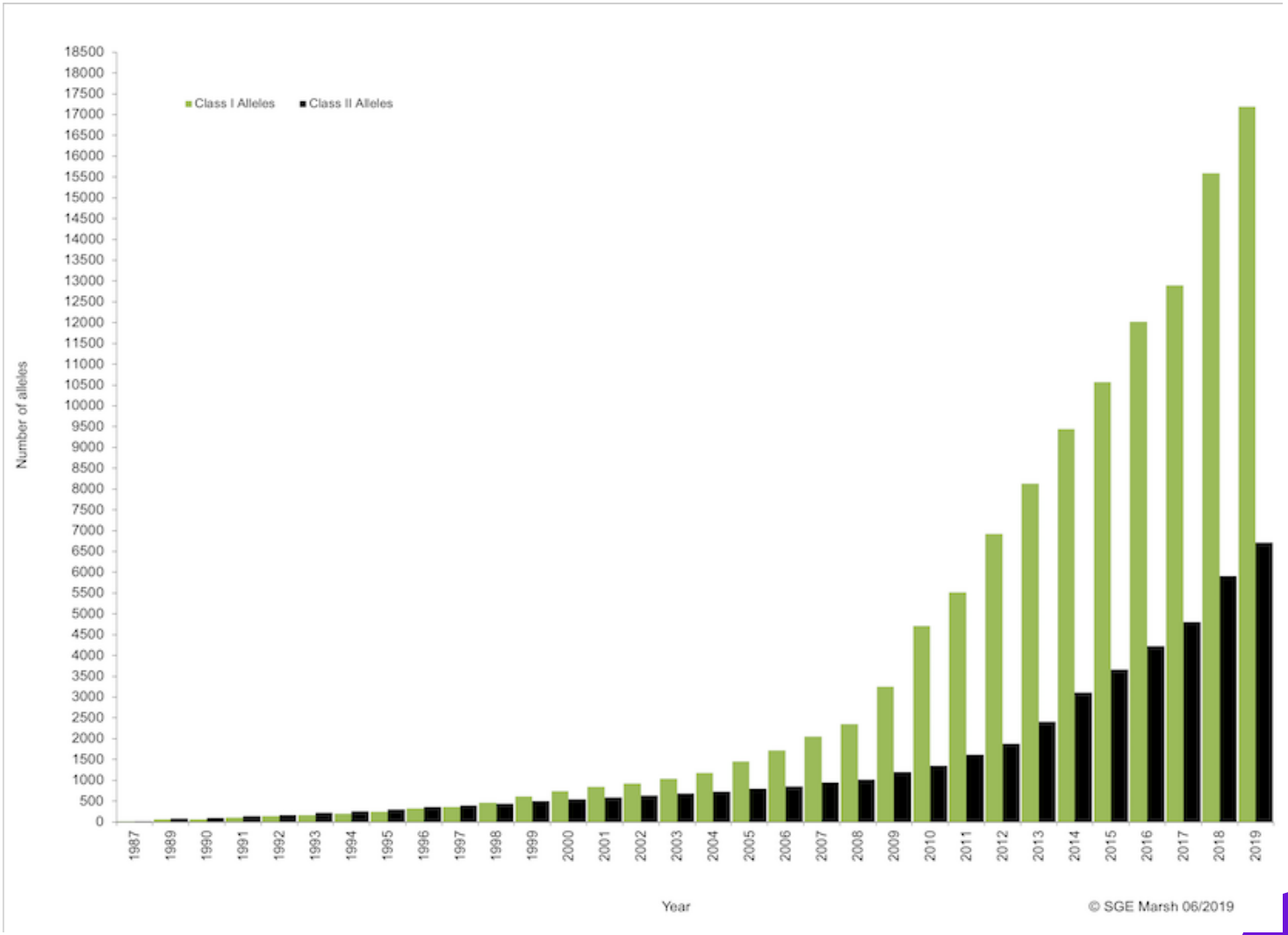
Rapidly increasing variation identified:



http://hla.alleles.org

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Tissue Typing:

- Term describing the identification of an individuals HLA molecules.
- Ideally to define which HLA molecules are expressed on the surface of a cell.
- All donors and recipients are HLA typed.
- Serological Typing – detection of HLA antigens on the surface of an individuals cells.
- Molecular Typing – detection of genes encoding the HLA type of an individual.
- Molecular typing is more sensitive and reliable.

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Serological HLA Typing:

- Provides low resolution typing and is rarely a front line method.
- Patient cells are reacted against a panel of serum containing known antibodies to HLA molecules.

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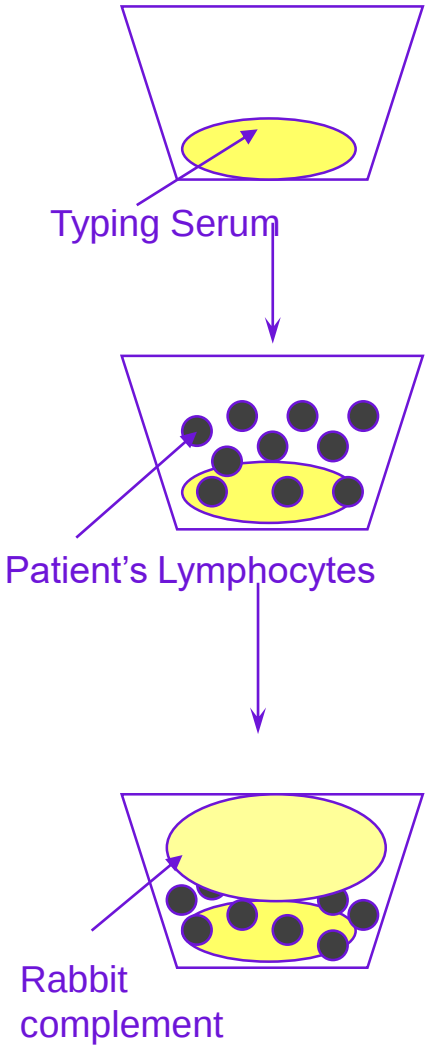
For Example:

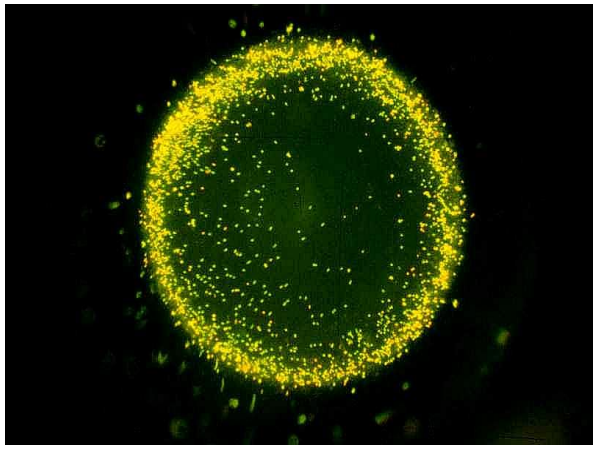
- Patient cells are mixed with the panel of known serum.
- If a patient is HLA A2, these molecules are on the surface of their cells.
- If the serum contains antibody directed to HLA A2, this antibody will bind to the A2 molecules on the cell.
- Complement is then introduced.
- This will bind to antibody bound to the cells and cause cell death
- A stain is then added –

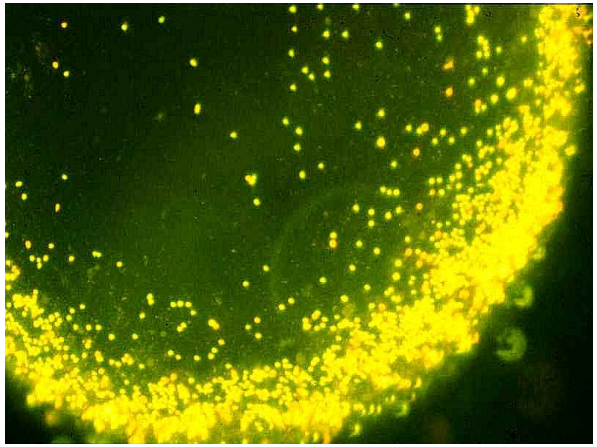
  Live cells appear green

  Dead cells appear red

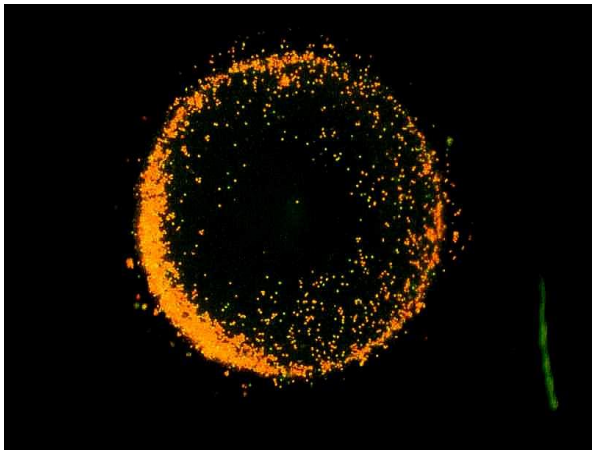


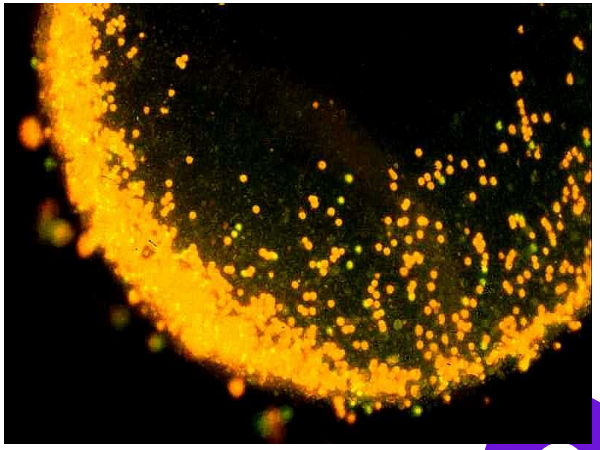
No antibody bound
Cells alive: Type NOT HLA-A2





Antibody bound
Cells dead: Type = HLA-A2





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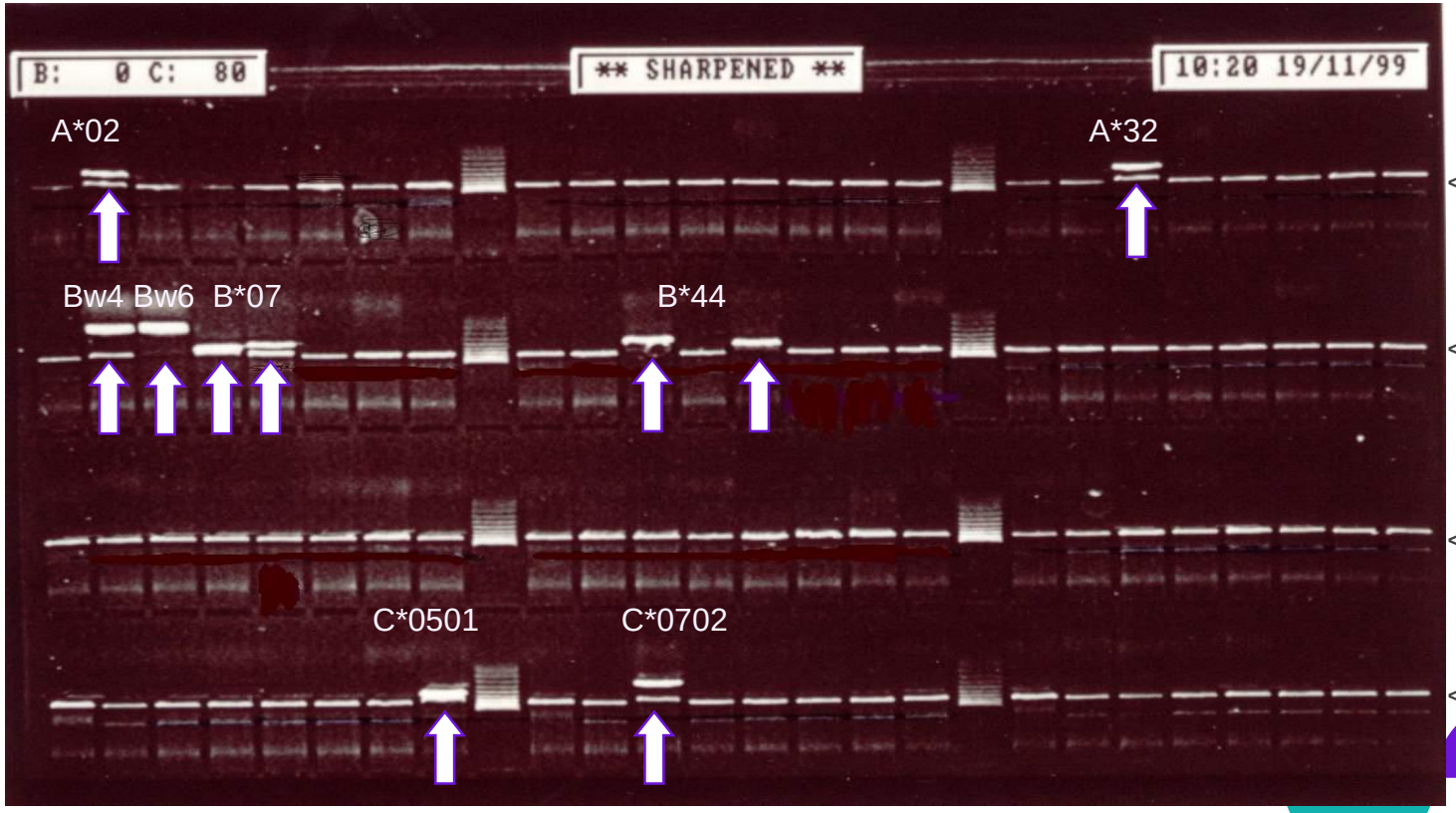
Molecular (DNA) Typing:

- The majority of labs worldwide now use a variety of molecular HLA typing methods – Genotyping.
- Genotyping – identifies the alleles present at a given loci.
  - From this we infer the HLA molecules being presented
- Depending on the method can give low, medium or high resolution HLA typing.
- DNA extracted from nucleated cells in peripheral blood or spleen/lymph node
- Rapidly changing technology providing ever more high resolution HLA types - ?Not currently needed for solid organ transplantation.
- PCR-SSP, Luminex based PCR-SSO, RT-PCR, Sanger sequencing, NGS and more recently 3rd generation sequencing.
- Routinely assess HLA A, B, C, DRB 1/3/4/5, DQB, DQA and DPB

HLA typing by PCR-SSP

1. DNA amplification using sequence specific primers
2. Detection of amplified DNA by gel electrophoresis
3. Interpretation of results

HLA A*02, 32 B*07, 44 Bw4, 6 Cw*0501, 0702



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HLA Nomenclature: 2010

HLA-A*	Identifies gene as belonging to HLA-A locus
HLA-A*03:	First field describes the allele family, often corresponds to the serological antigen
HLA-A*03:01:	Second field refers to the allele- assigned in order sequences were determined
HLA-A*03:01:01	Third field refers to a synonymous nucleotide substitution
	"N" refers to non-expressed "null" genes
	"L" refers to genes with low expression

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National Transplant Database

• To aid easier allocation matching is performed on the serological HLA types.

•Serological equivalents must be assigned to molecular HLA types

Loci	Different Genes	Different Protein Products	Serological Specificities
HLA A	5266	3552	28
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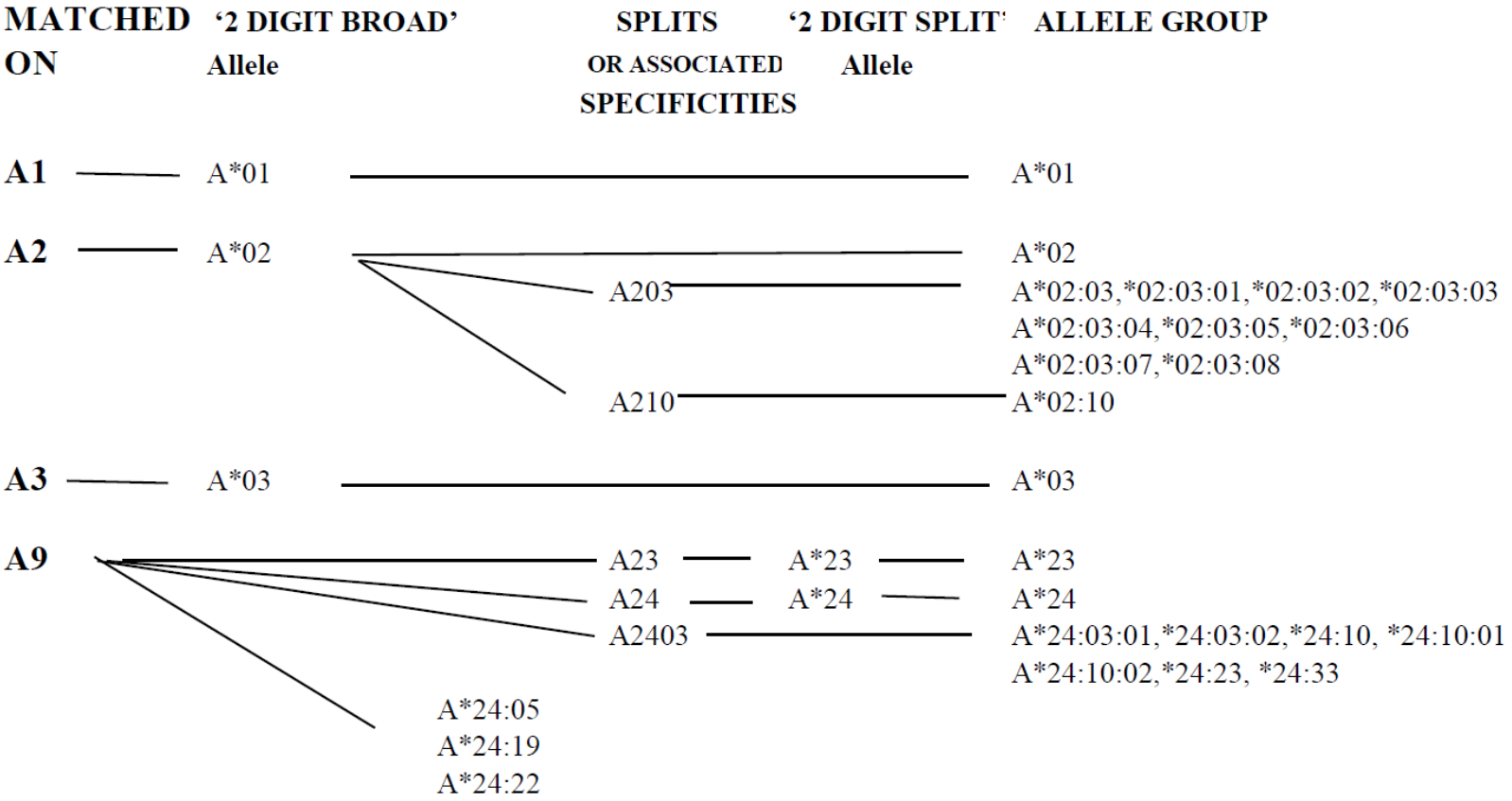
HLA Conversion Chart for Organ Allocation

Version 09/ 2019

NATIONAL TRANSPLANT DATABASE
HLA CONVERSION CHART FOR ORGAN ALLOCATION

HLA Dictionary 2008 NOMENCLATURE April 2010



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HLA Matching/Mismatching:

- Deceased donor organ allocation in the UK is based on low resolution matching at HLA A, B and DRB.
- In part because these loci contribute the majority of the polymorphism seen.
- For each locus there can be 0, 1 or 2 mismatches – with 0 denoting matched and 2 denoting completely unmatched.
- The best match is a 000. The worst match is a 222.
- In general the better the match, the better the long term graft survival.

Donor

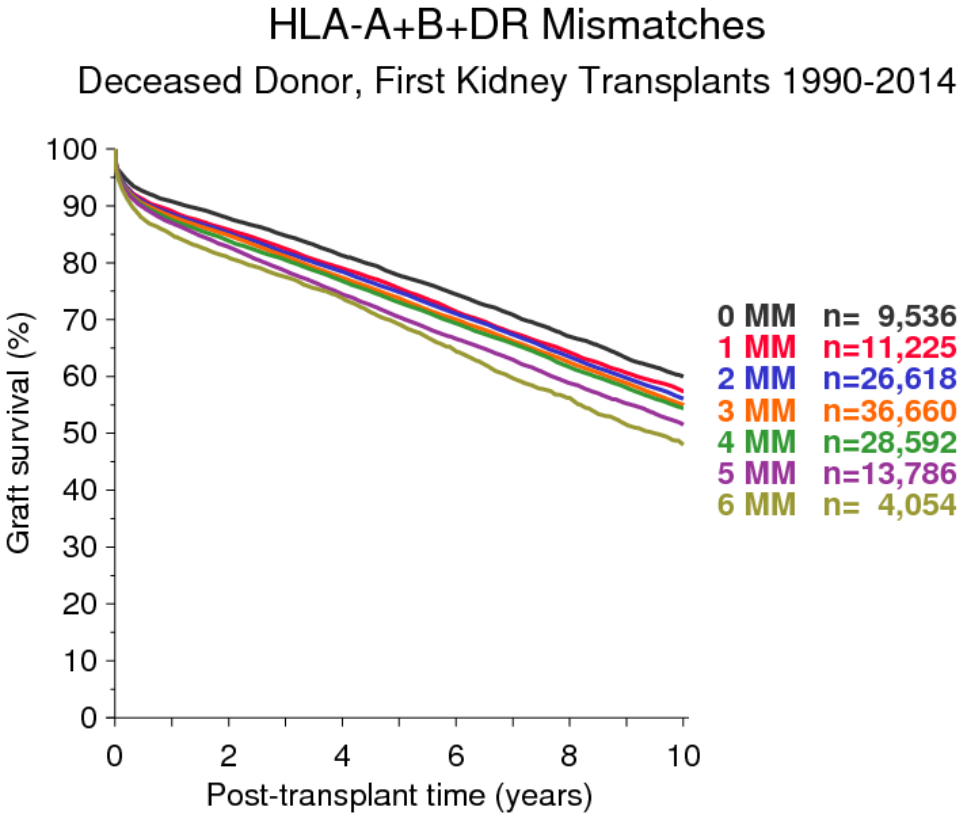
HLA-A1, A2, B7, B8, DR3, DR4

Recipient

A	HLA-A1, A2, B7, B8, DR3, DR4	000
B	HLA-A1, A3, B7, B8, DR3, DR4	100
C	HLA-A1, A9, B5, B8, DR3, DR4	110
D	HLA-A3, A9, B5, B8, DR3, DR7	211

Human Leukocyte Antigens – HLA

- HLA matching between donor and recipient improves kidney transplant survival



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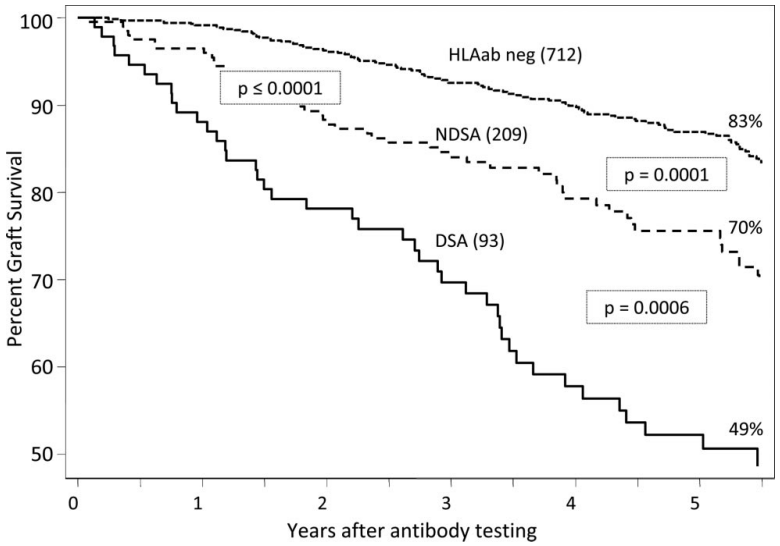
HLA Specific Antibodies:

- HLA specific antibodies are antibodies directed at non-self HLA.
- Can be directed at HLA Class I and II.
- HLA specific antibodies linked to rejection in Kidney, Pancreas, Heart, Lung and more recently Small Bowel and possibly also the Liver.
- The presence of donor HLA specific antibody is associated with poorer long term transplant survival.



Lachmann et al, Transplantation 2009

- Where possible we avoid transplantation of a donor organ expressing HLA to which a recipient produces Ab.
- This is through the registration of 'unacceptable antigens'.

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Routes to sensitsation:

- Prior exposure via Transplant, Pregnancy, Blood transfusion, Cage Fighting, First responders…
- Transplantation – rejection of a previous allograft can lead to generation of long-lived antibody response in >70% cases.
- Pregnancy - @20% of parous women produce HLA specific antibody.
- Transfusion – Red blood cell and platelet - reports of up to 40% of patients receiving multiple blood transfusion becoming sensitised.
- Approximately 50% of the waiting list have some antibody.
- Strength and breadth can vary over time so regular testing highly recommended.
- BTS Guidelines – Test every 3 months as a minimum, plus post sensitisation event.

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HLA specific Antibody Detection:

- We use sensitive methods analysed in the context of patient history, including previous sensitising events and vaccination history.
- Most centres now routinely use Luminex bead based.
- ‘Solid Phase’ assay - Intact HLA molecules solubilised or purified from cell membranes, or recombinant HLA antigens, from transfected cell lines are immobilised onto a polystyrene bead.
- Screening for yes/no, through to highly sensitive single antigen for fine definition of specificity.

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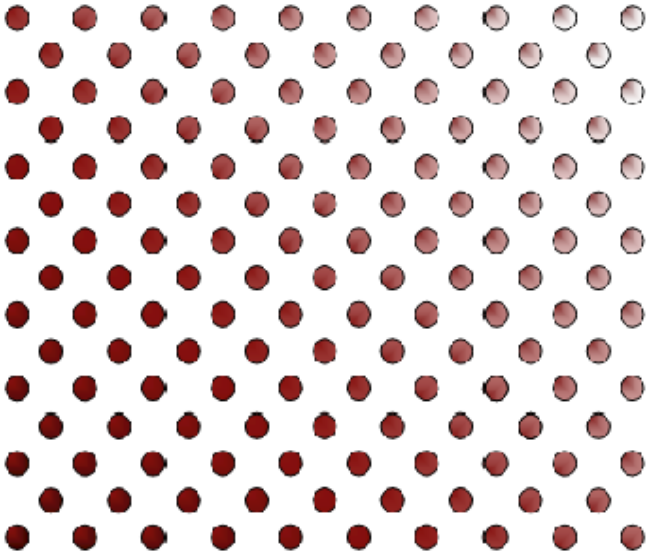
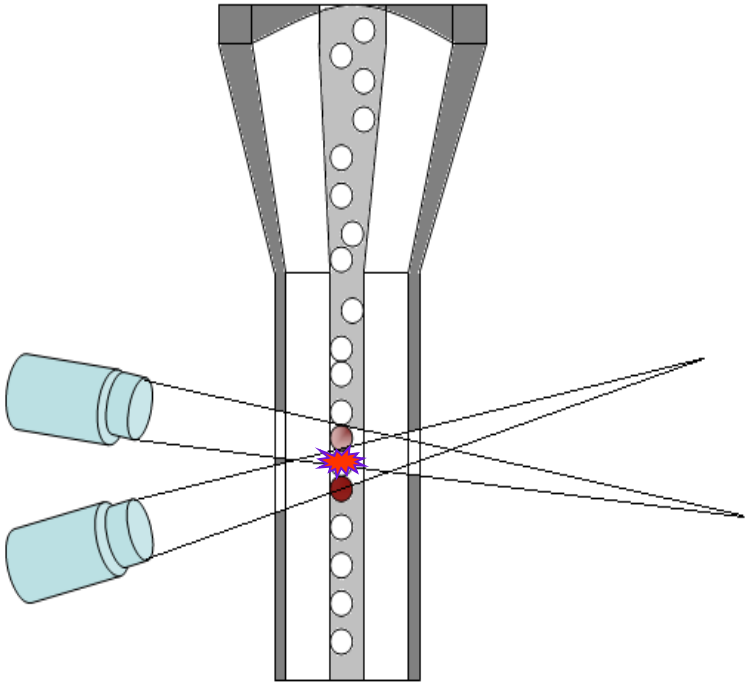
Luminex TECHNOLOGY





100 bead populations, uniquely identifiable by colouration with a combination of two dyes in different proportions

Bound antibody detected with a fluorescently labelled anti-human IgG antibody

Lasers excite internal dye and PE

HLA specific antibody binding reported as median fluorescence intensity (MFI) of the reporter signal

Fuggle & Martin, Transplantation 2008 86:384

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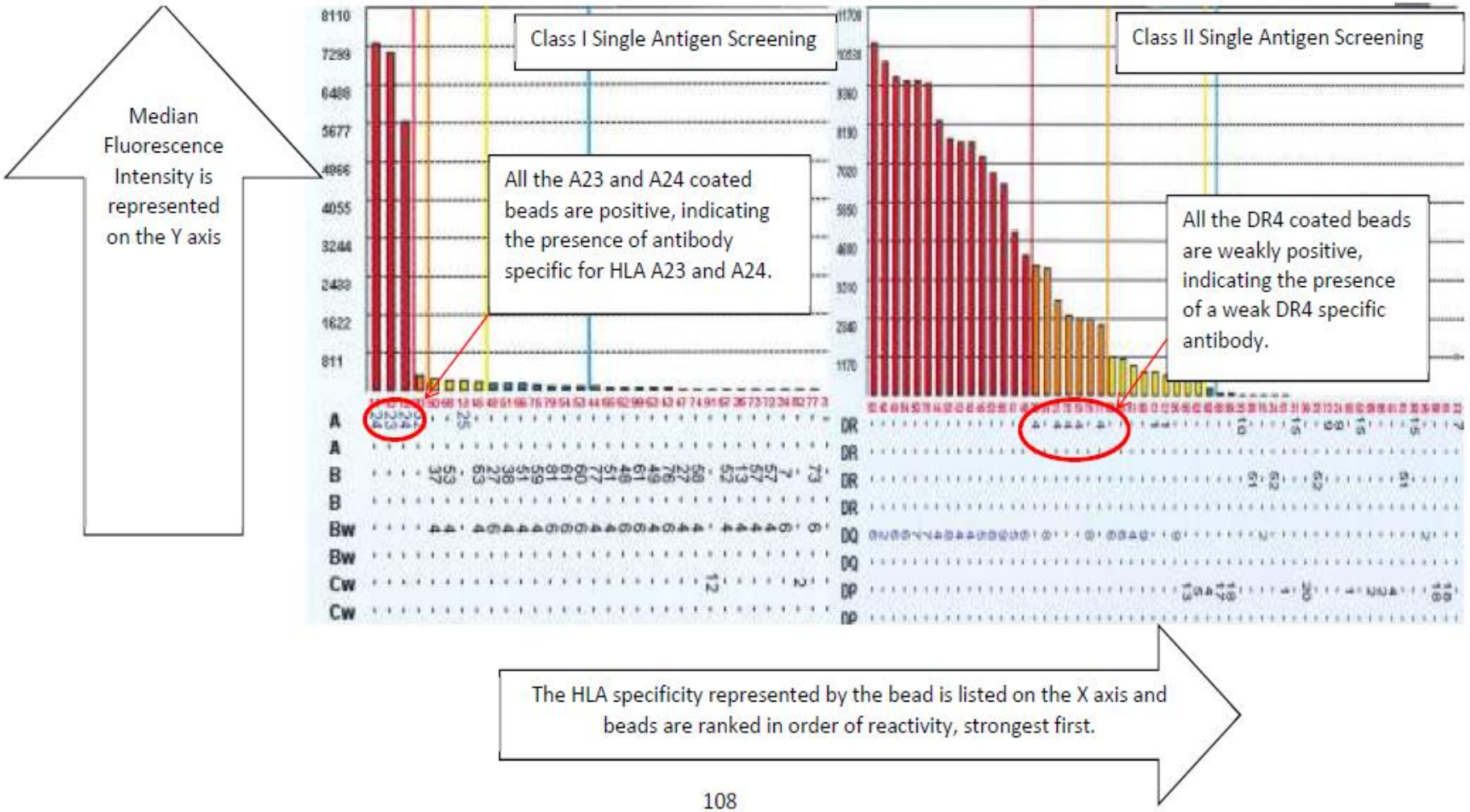
Single Antigen Beads



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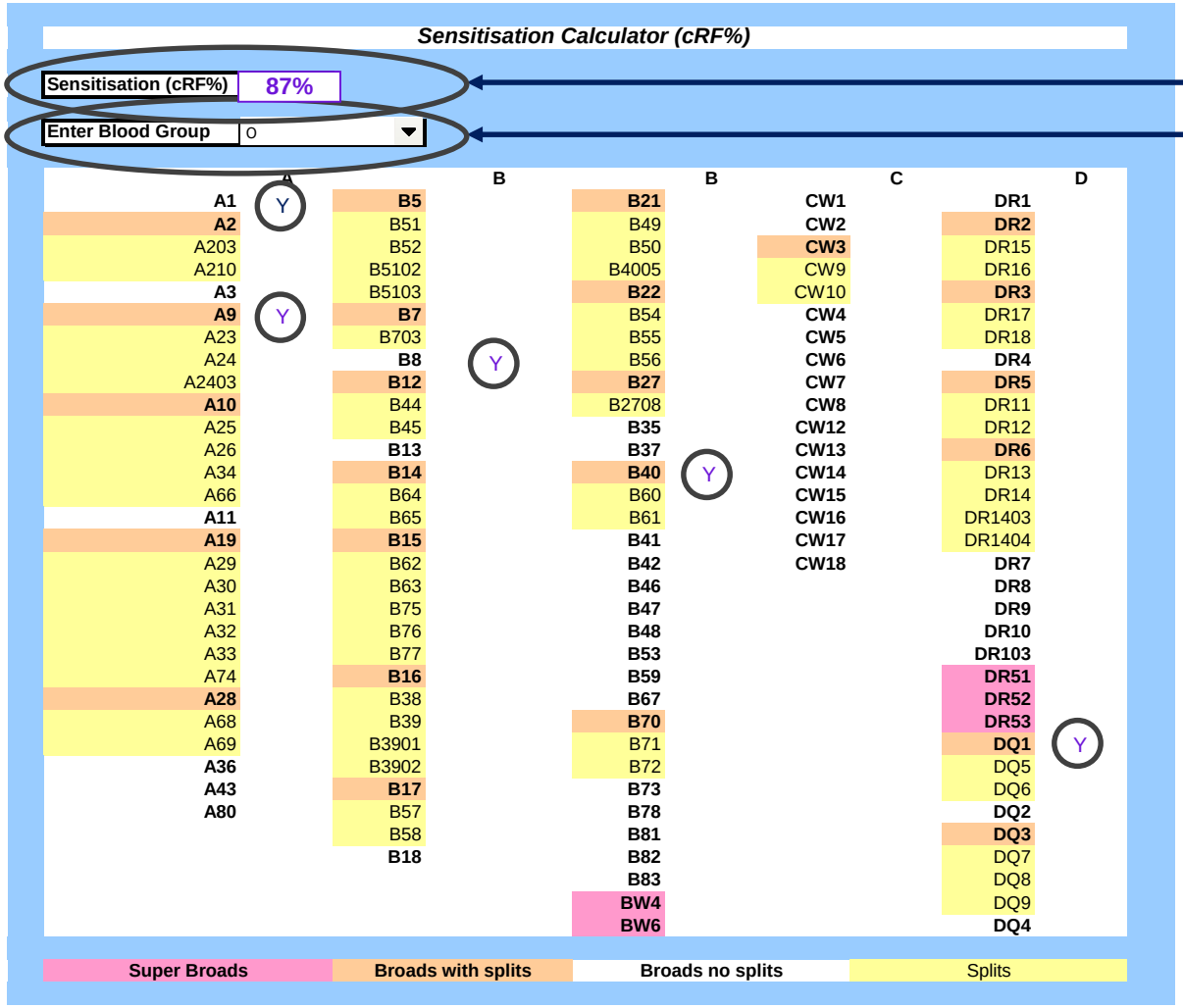
Unacceptable Antigens and Sensitisation:

- Generally these are HLA antigens to which a patient has antibody
- May also include previous mismatches without antibody or partners mismatches for example.
- Once defined they are registered on the national transplant database as unacceptable.
- Patients who produce HLA specific antibody are referred to as ‘sensitised’.
- A measure of sensitisation is the ‘calculated reaction frequency’ or %cRF.
- Calculates the % of deceased donors in the past 10000 with which we would expect a positive crossmatch.
- 0% cRF being unsensitised and 100% cRF being highly sensitised.

The cRF% Calculator

Calculated HLA antibody Reaction Frequency



Await cRF% result

Select ABO blood group

Add antibody specificities

Antibody can be a major barrier to transplantation.
Antibody detection and definition of unacceptable antigens is a balance between ensuring good outcomes and not limiting the chances of an offer.

Median wait to transplant for adult patients

NHS Blood and Transplant

2½ years

Calculated Reaction Frequency	Number of patients registered	Waiting time (days) Median	95% CI
0-84%	7917	963	942 - 984
85-94%	344	1577	1487 - 1667
95-99%	377	2138	1870 – 2406
100%	164	2424	2072 – 2776
TOTAL	**8802**	**1016**	**995 - 1037**

6½ years

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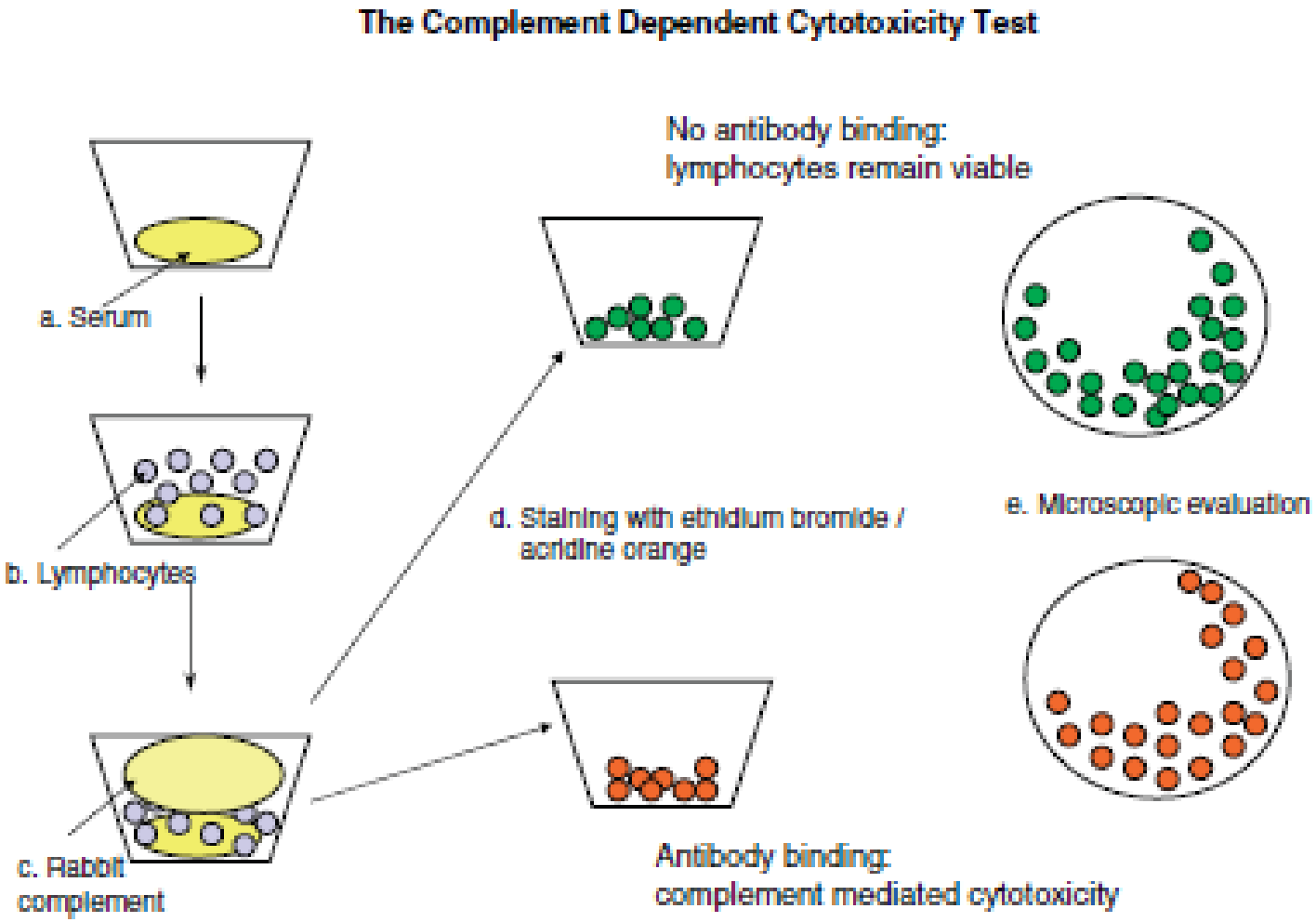
Crossmatching:

- This is the final compatibility test.
- One cell antibody screen
- Detects antibody in a patient specific to a given donor.
- Patient serum vs donor lymphocytes
  - Living donor PBLs
  - Deceased donor – PBL, Spleen or Lymph node
- Two main methods – CDC and Flow Cytometry

Complement Dependent Cytotoxicity Method



Fuggle SV, and Martin S. Transplantation 86 (3): 384-390

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Flow Cytometric Methods:

- Still cell based but reportedly up to 50 x more sensitive than CDC.
- Uses target donor derived lymphocytes
- Lymphocytes incubated with recipient sera.
- Ab bound to targets on test cells is detected through addition of a fluorescence labelled secondary Ab.
- This is then detected and quantified using a flow cytometer.
- Fluorescence in test compared to that in negative control and deviation from this assessed against predefined cut off to assign positive or negative result.
- Centre specific – based on clinical protocol, clinicians and historical transplant outcome data
- Screening methods now mean very few unexpected positive crossmatches.
- Important for deceased donors as reduces the cold ischaemia time.

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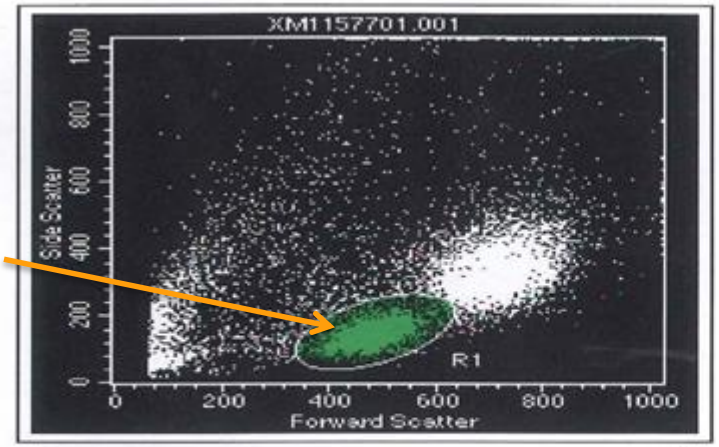
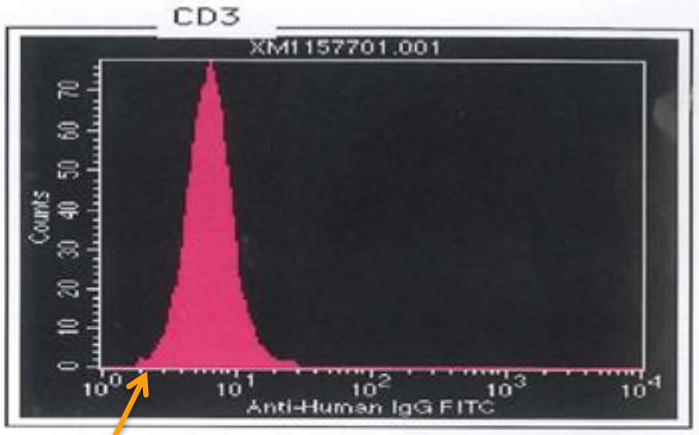
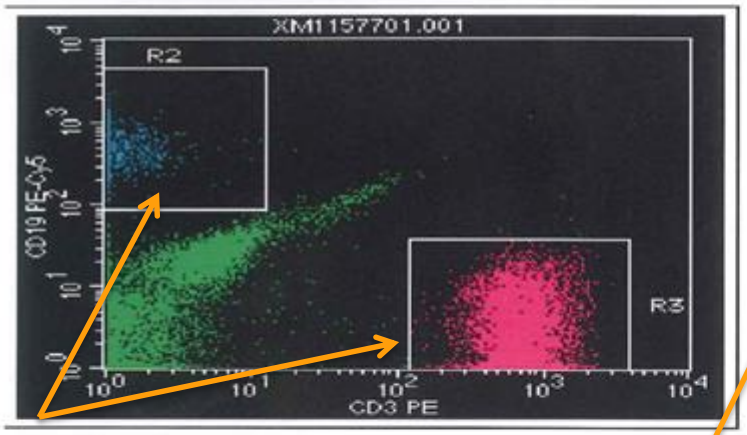
1. Cells identified on size





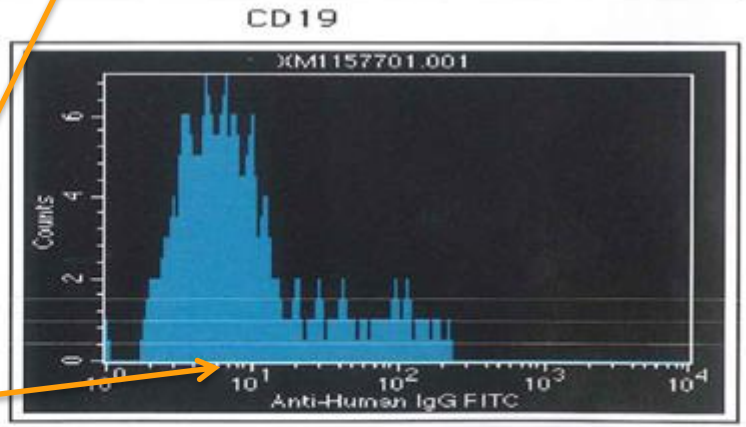
File: XM1157701.001
Gate: CD3
Acquisition Date: 12-Apr-13
Total Events: 58016

Events	Median	Peak Ch
5796	6.21	6



2. T cells (CD3+) and B cells (CD19+) identified on fluorescence

Negative Control



3. Ab binding identified by fluorescence.

File: XM1157701.001
Gate: CD19
Acquisition Date: 12-Apr-13
Total Events: 58016

Events	Median	Peak Ch
1097	6.61	6

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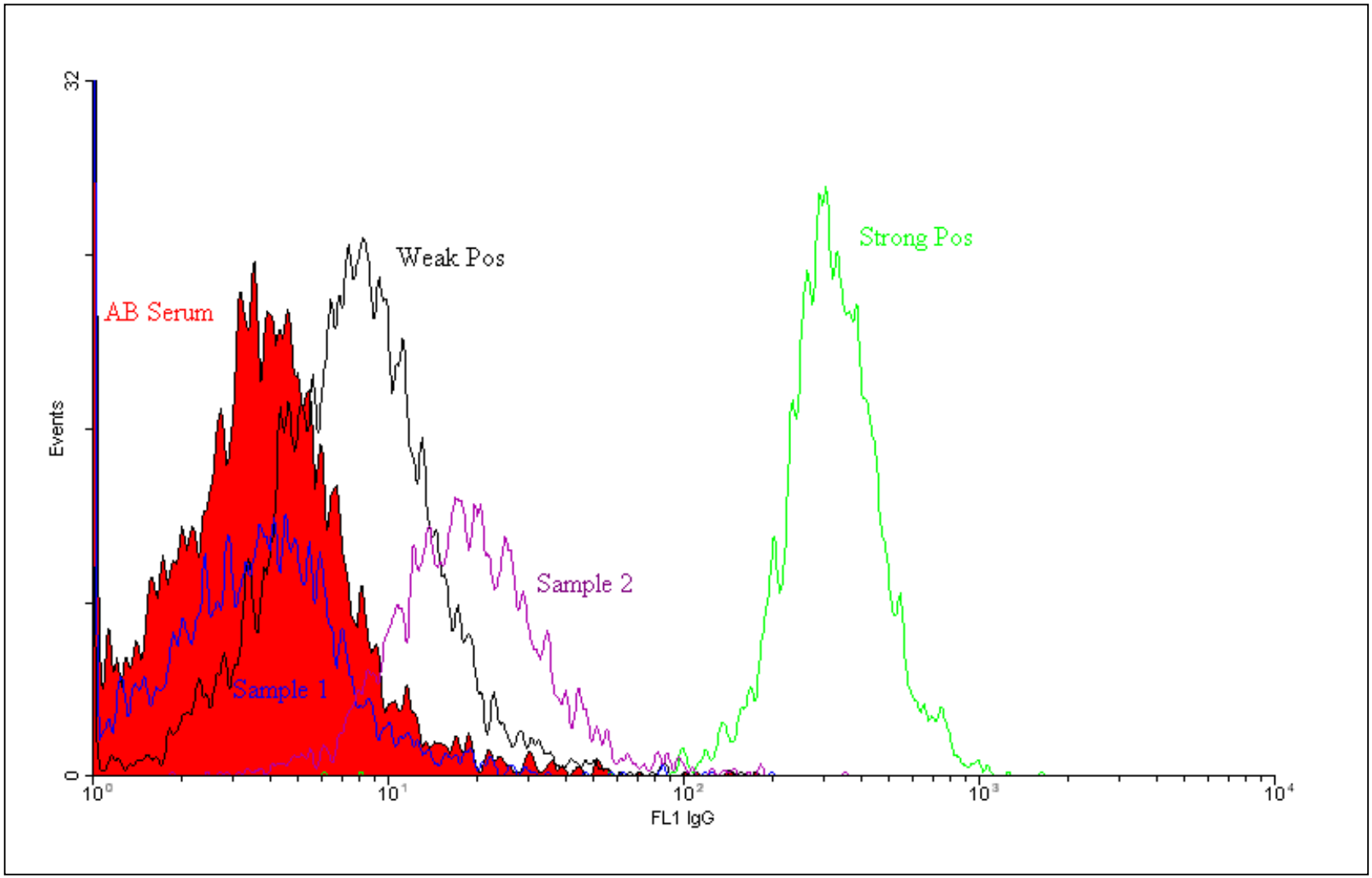

Sample 1 - Negative crossmatch
Sample 2 - Positive crossmatch

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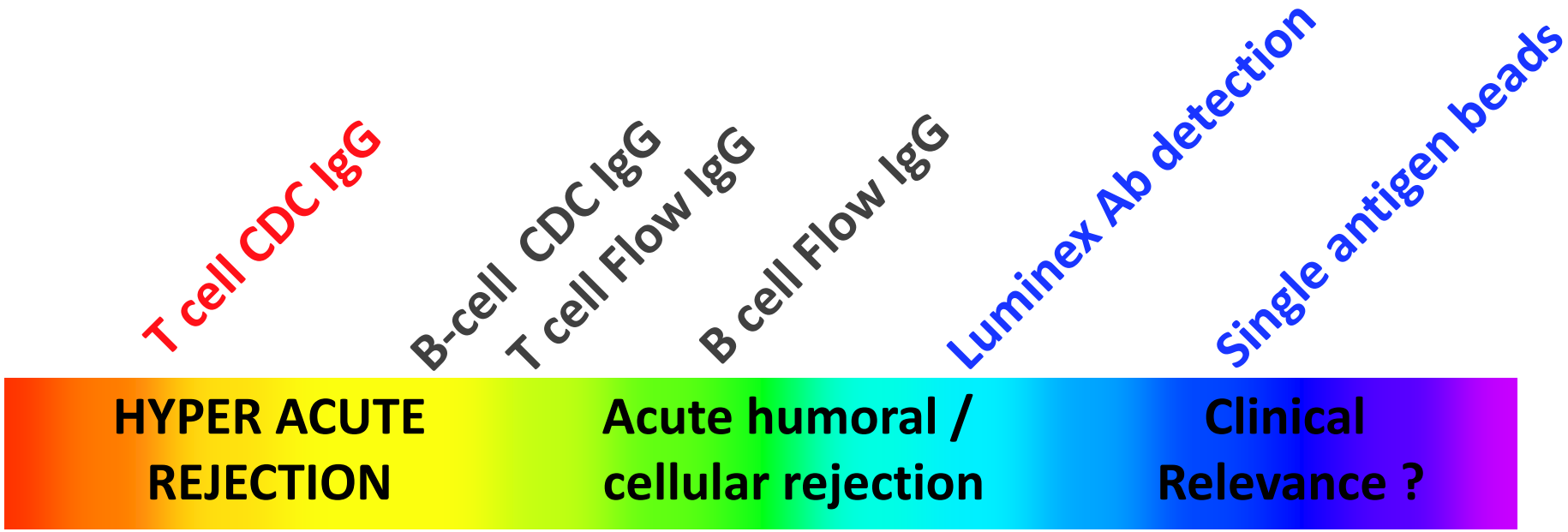
How does this define risk?

- Results of HLA typing, antibody screening history and crossmatching together help define the immunological risk of a potential transplant.
- Both current and historical results must be used together to aid assessment.



Based on - J Andrew Bradley, Craig Taylor, Cambridge,UK

Assessment Summary:

- Compatible blood group is necessary.
- HLA typing and matching – better the match the better the long term graft survival.
- HLA Antibody screening – avoid donors with HLA antigens to which there are pre-formed antibodies in the recipient.
- Crossmatching – avoid transplantation in the face of a positive crossmatch due to HLA specific antibody.
- These apply equally to deceased and living donation routes.
- For patients with incompatible but otherwise healthy living donors – what are the options?

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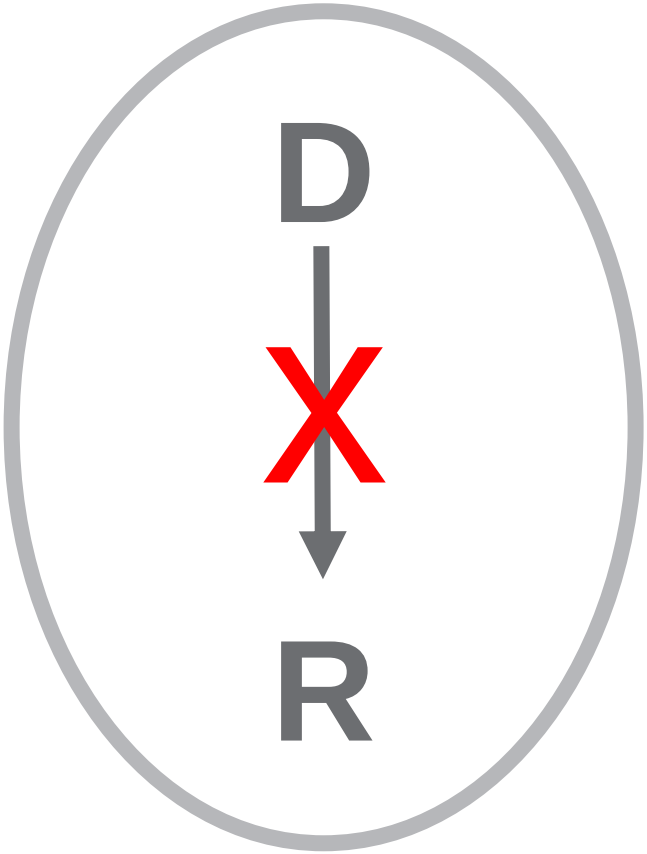
Living kidney donation
Potential Donor-Recipient pair



Approx 20-30% of possible living donor transplants in the UK are prevented due to -

Blood group incompatibility

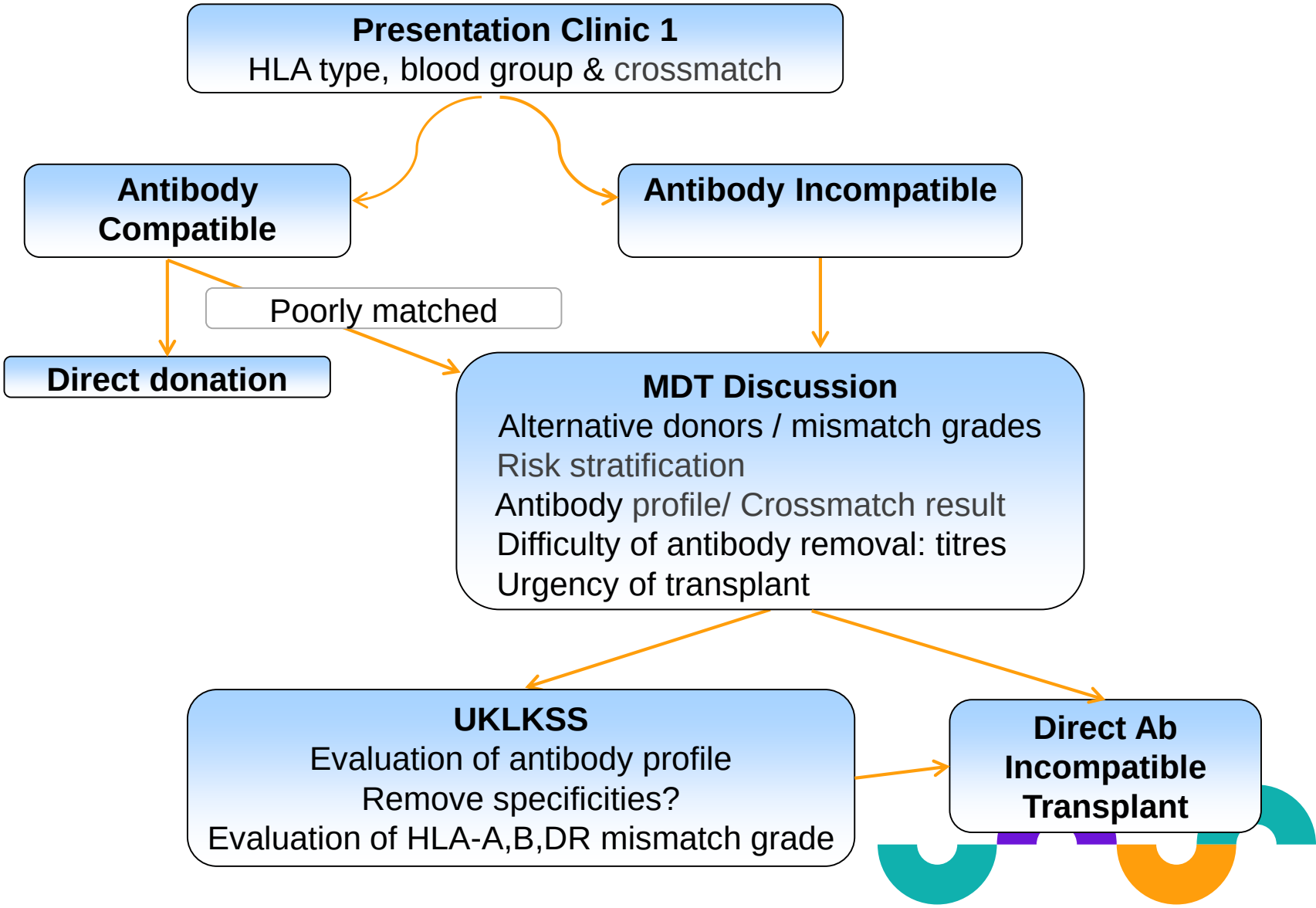
HLA antibody incompatibility

- Positive crossmatch

Poor HLA match

LKDSS now helps to improve the chances of transplant.

Multidisciplinary Team Flowchart



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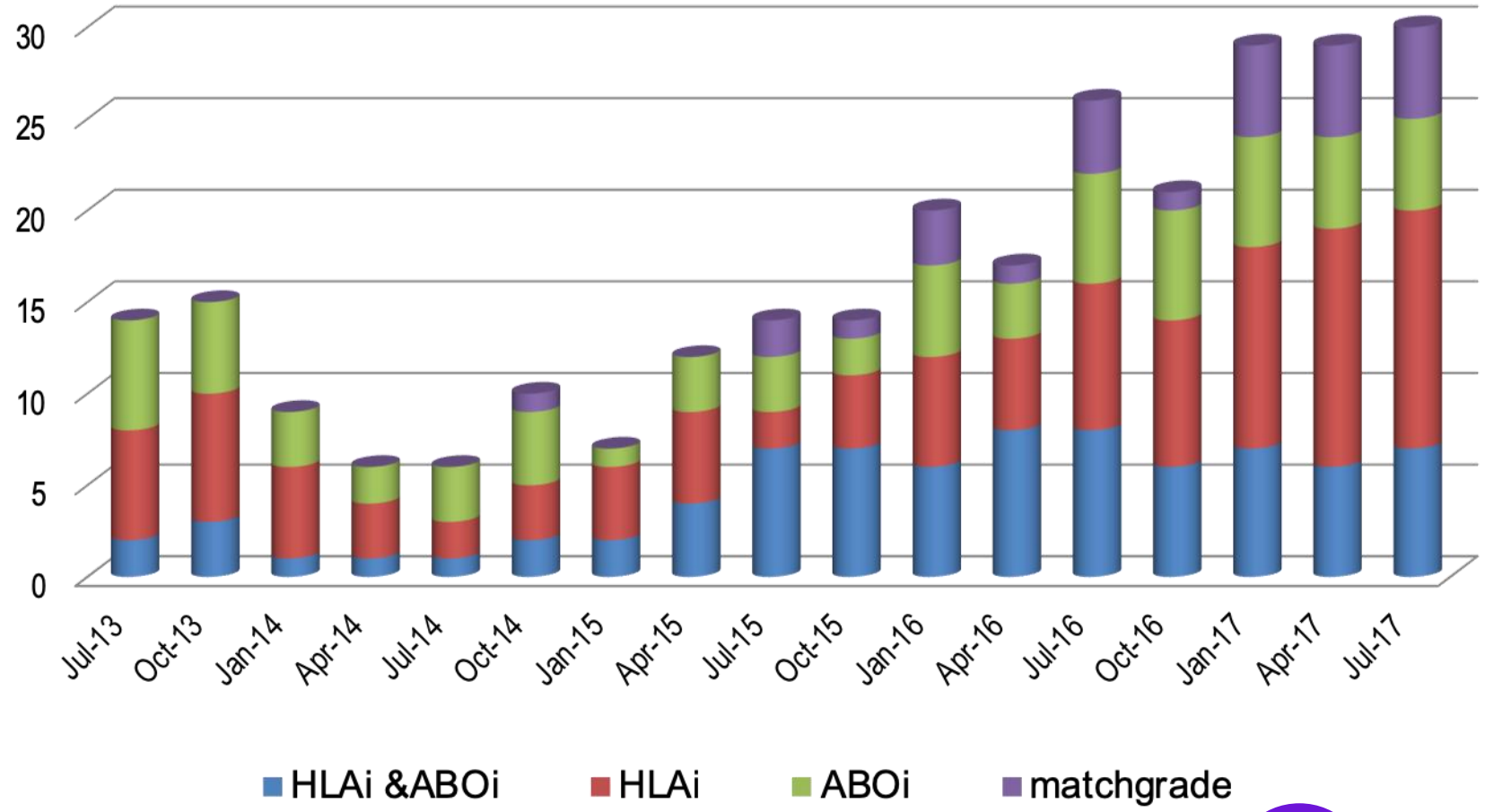
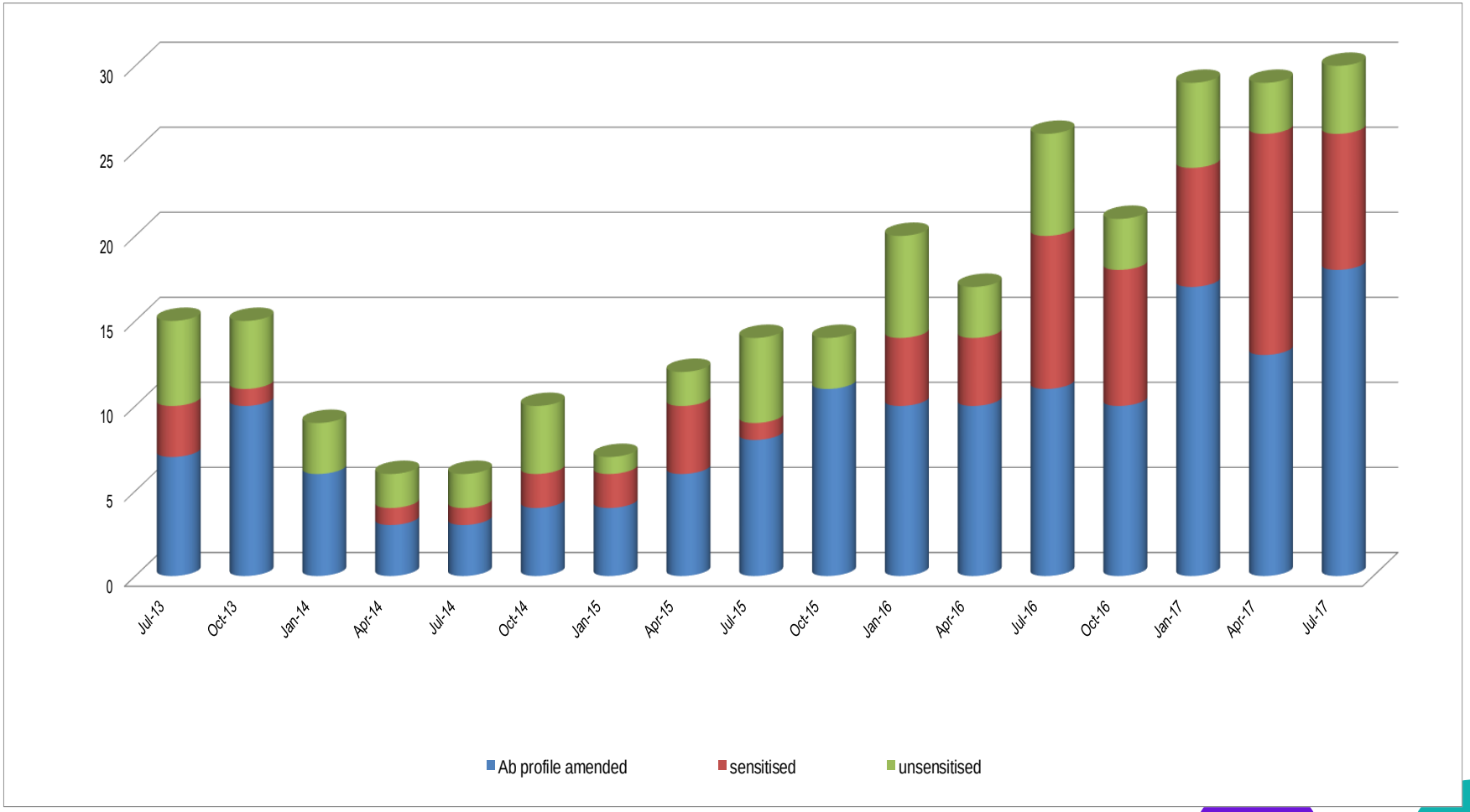
Patient/donor demographics per matching run:



Patient HLA Sensitisation status per matching run



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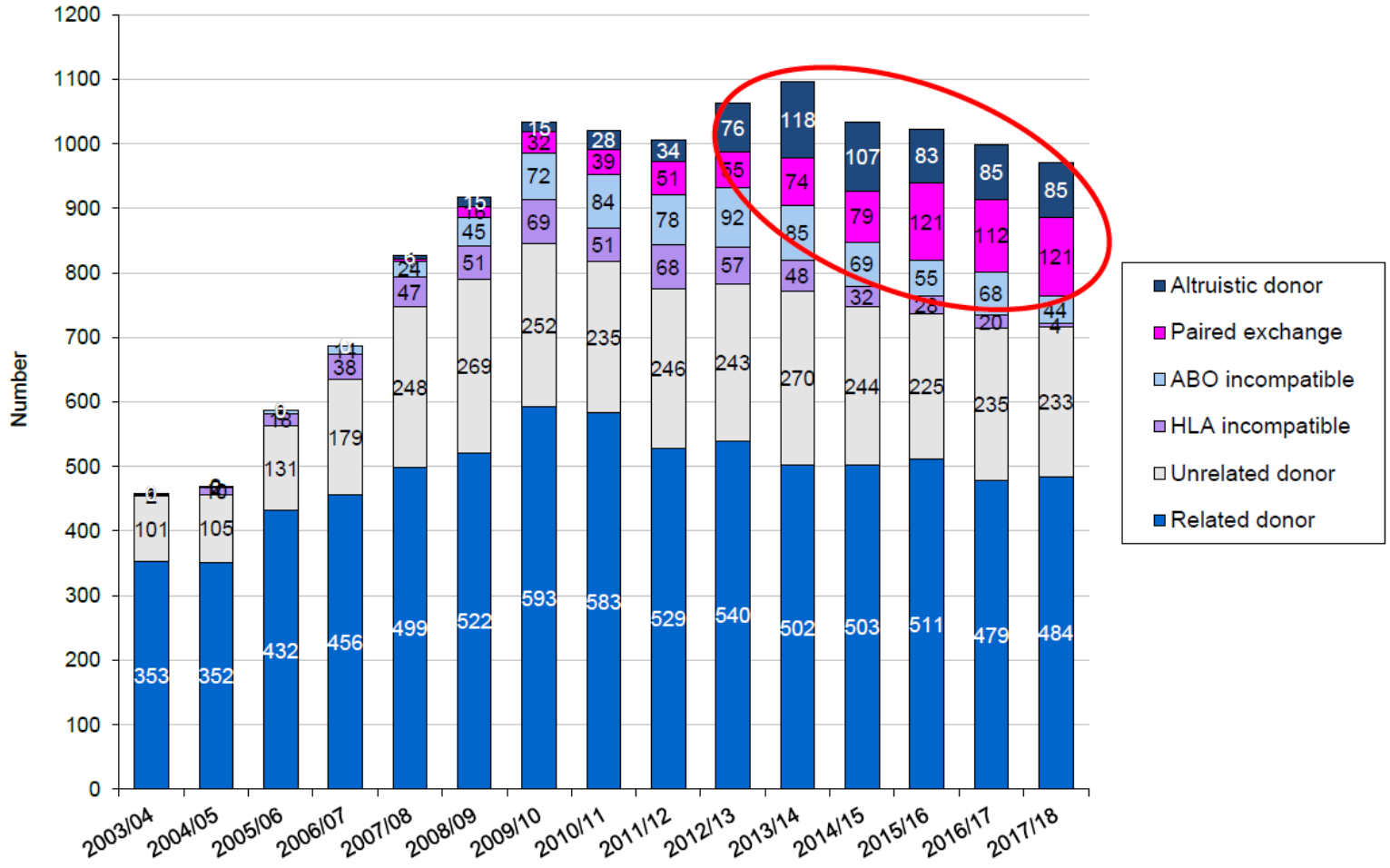
UK Living Kidney Transplants



Helping Patients Understand Their Chance of Transplant

Incompatible Pairs Living Donor Kidney Application

Variable	Select
Recipient Blood Group	A
Calculated Reaction Frequency	85-94
Donor Blood Group	O
ABOi TX with willing Donor†	Select
HLAi TX with willing Donor†	Select
Recipient Age	Select

Reset

NHS Blood and Transplant

Estimated Chance of Transplant

	Deceased Donor	NLDKSS	ABOi	HLAi
6 Months	<10%	41-50%	-	-
1 Year	11-20%	71-80%	-	-
3 Years	41-50%	>90%	-	-

-

Transplant Survival Rates

	Deceased Donor	NLDKSS	ABOi	HLAi
6 Months	-	-	-	-
1 Year	-	-	-	-
3 Years	-	-	-	-

Disclaimer: The information is provided for guidance only

†Low titre/Low DSA means acceptable for incompatible transplant. High titre/High DSA means unacceptable for incompatible transplant.

Note: NLDKSS chance of transplant is based on paired donation including short altruistic donor chains.

Chances of transplant through the NLDKSS could be increased by considering an antibody incompatible transplant within the scheme

For a more accurate estimate of waiting time for a deceased donor transplant based on more variables, please visit http://www.odt.nhs.uk/doc/chance_of_transplant.xls

Available at: http://www.odt.nhs.uk/transplantation/guidance-policies/tools/

viapath

The UKLKSS is a valuable addition to a living donor transplant programme

- increased opportunity for highly sensitised patients
- enables better HLA matching between donors and recipients

viapath

www.organdonation.nhs.uk

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Transplant centres and referring renal units

Kidney Advisory Group

LDKT 2020 Strategy Implementation Group
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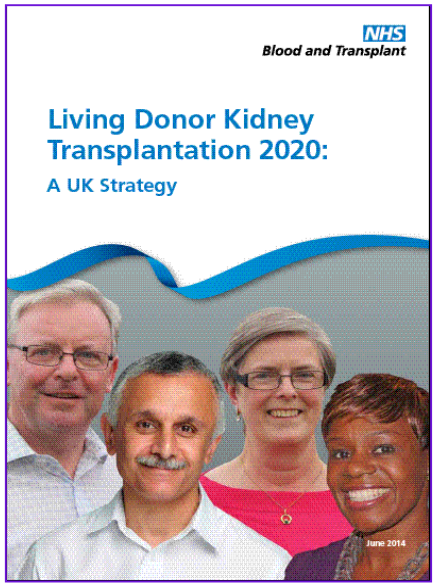

"To match world class performance in living donor kidney transplantation"