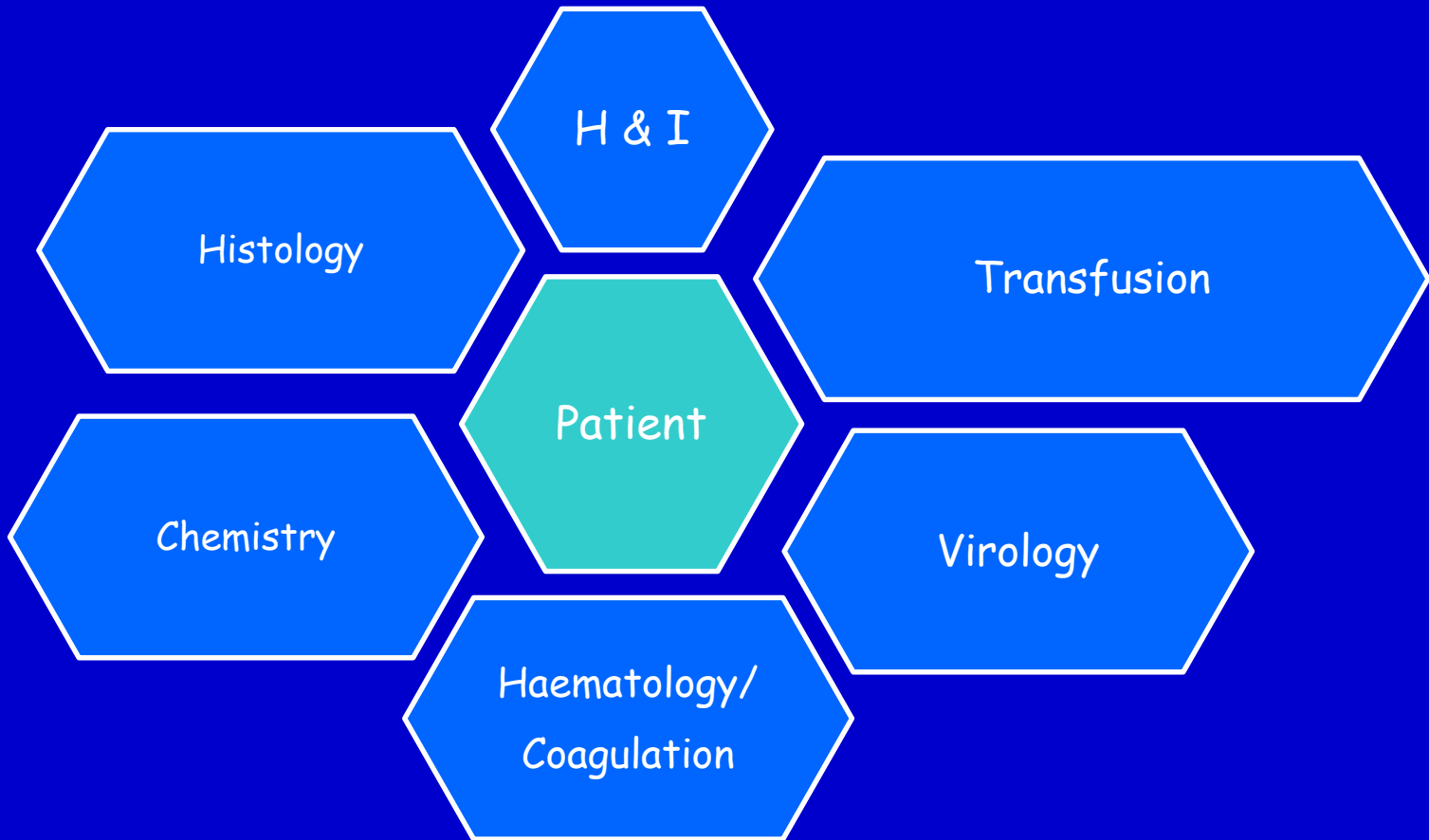


# Solid Organ Transplantation - How the Lab Contributes to Improved Patient Outcomes

Mary Keogan,  
on behalf of all in NHISSOT



# Solid Organ Transplantation



# H & I - Aims

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- To ensure that grafts are not allocated to immunologically unsuitable recipients.
- To provide additional information which may help to optimise outcome of transplantation
- Post-transplantation monitoring

# Overview of Talk

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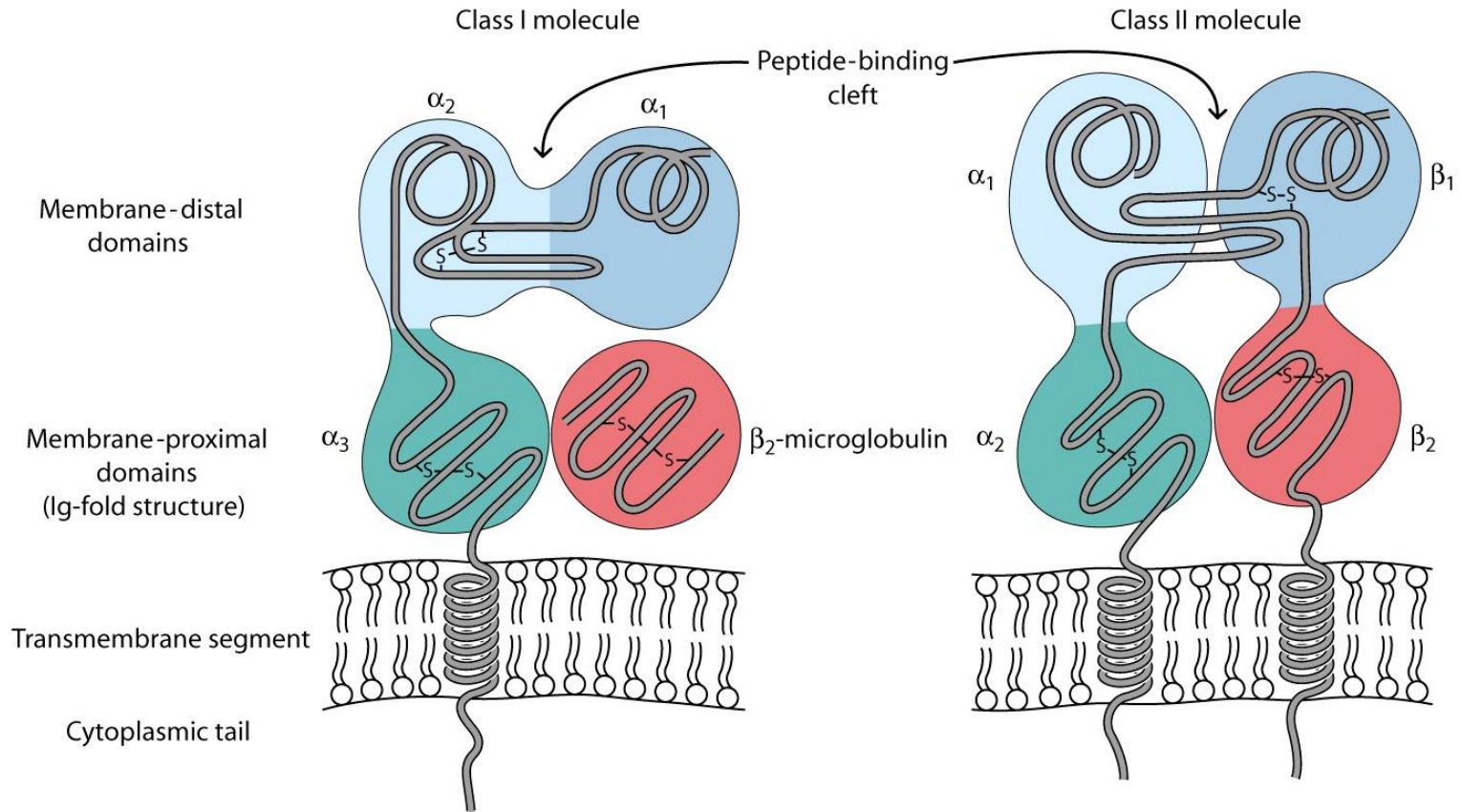
- What do we do?
- How does it fit together?
- Contribution to individual patient and programme outcomes.

# What do we do?

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- HLA Type
- Screen for anti-HLA antibodies
- Crossmatching

# HLA molecules



# HLA typing

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- HLA-A,B,C DR, DQ $\alpha$ , DQ $\beta$ , DP
- Recipients & Living Donors
  - SSO
  - Serology (CDC)
- Deceased Donors
  - SSP
  - Serology (CDC)

# Anti-HLA antibodies

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## Sensitisation through

- Transfusion
- Previous transplants
- Pregnancy
  
- Infection
- "naturally occurring antibodies"

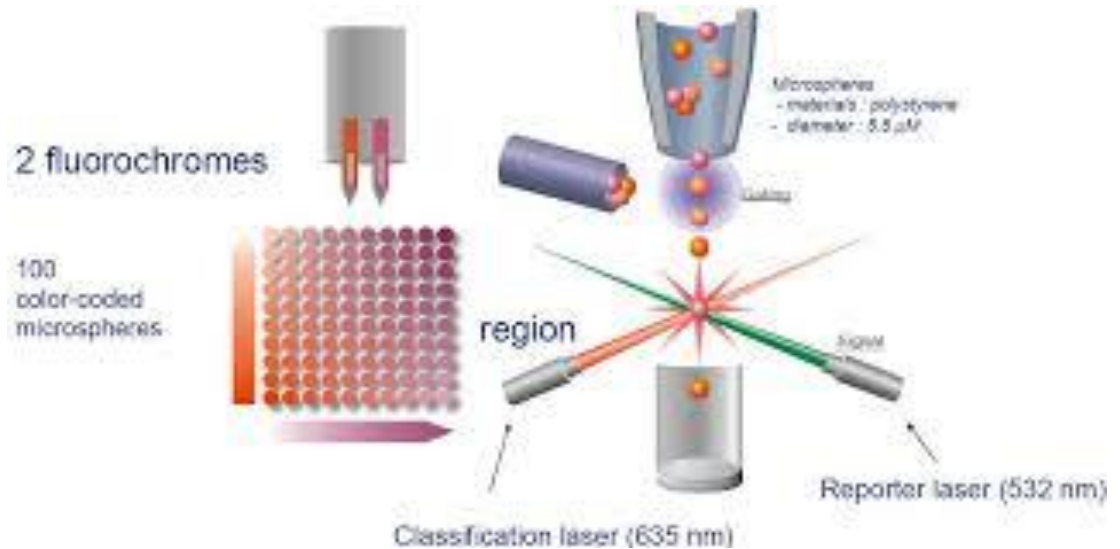


# Anti-HLA antibody screening

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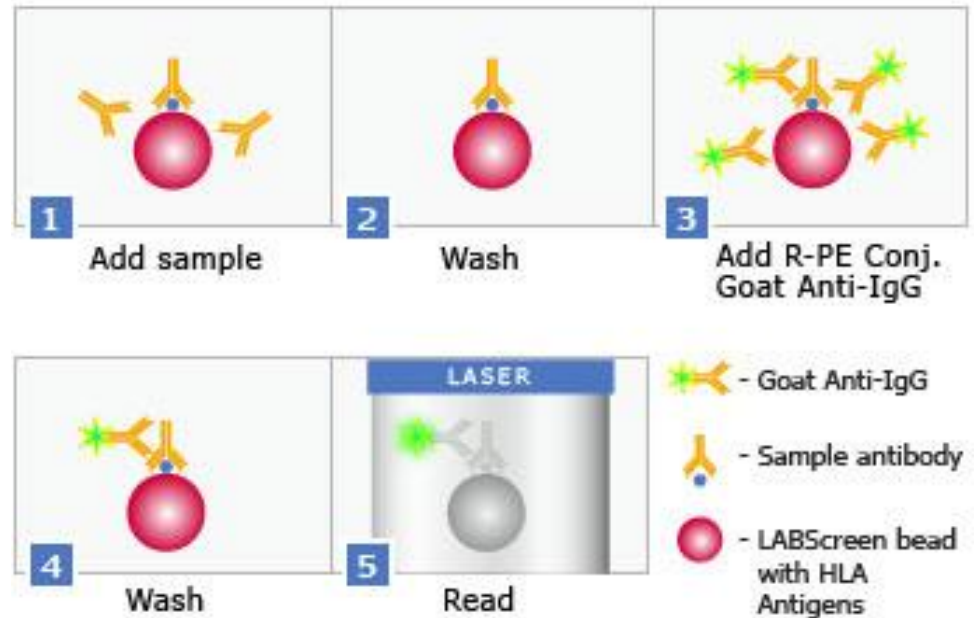
- Luminex single antigen beads
  - Total IgG
  - C1q - complement fixing Abs
- CDC (Complement Dependent Cytotoxicity)
  - now limited role

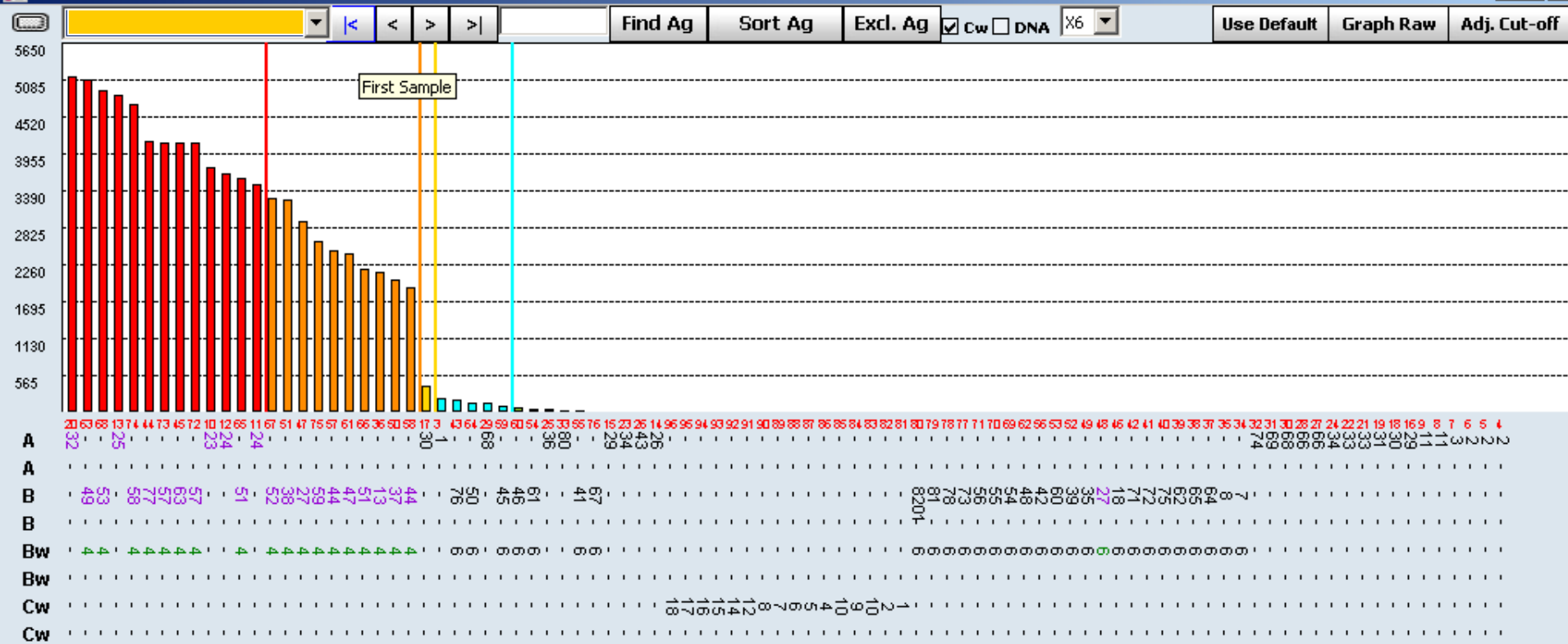
# Luminex Beads



# Staining

## Method





1C(10)				1C(19)				1C				2C				5C								7C				8C				12C				Bw																																																																																																																																											
A26(4)	A27(4)	A28(4)	A29(4)	A30(4)	A31(4)	A32(4)	A33(4)	A34(4)	A35(4)	A36(4)	A37(4)	A38(4)	A39(4)	A40(4)	A41(4)	A42(4)	A43(4)	A44(4)	A45(4)	A46(4)	A47(4)	A48(4)	A49(4)	A50(4)	A51(4)	A52(4)	A53(4)	A54(4)	A55(4)	A56(4)	A57(4)	A58(4)	A59(4)	A60(4)	A61(4)	A62(4)	A63(4)	A64(4)	A65(4)	A66(4)	A67(4)	A68(4)	A69(4)	A70(4)	A71(4)	A72(4)	A73(4)	A74(4)	A75(4)	A76(4)	A77(4)	A78(4)	A79(4)	A80(4)	A81(4)	A82(4)	A83(4)	A84(4)	A85(4)	A86(4)	A87(4)	A88(4)	A89(4)	A90(4)	A91(4)	A92(4)	A93(4)	A94(4)	A95(4)	A96(4)	A97(4)	A98(4)	A99(4)	A100(4)	A101(4)	A102(4)	A103(4)	A104(4)	A105(4)	A106(4)	A107(4)	A108(4)	A109(4)	A110(4)	A111(4)	A112(4)	A113(4)	A114(4)	A115(4)	A116(4)	A117(4)	A118(4)	A119(4)	A120(4)	A121(4)	A122(4)	A123(4)	A124(4)	A125(4)	A126(4)	A127(4)	A128(4)	A129(4)	A130(4)	A131(4)	A132(4)	A133(4)	A134(4)	A135(4)	A136(4)	A137(4)	A138(4)	A139(4)	A140(4)	A141(4)	A142(4)	A143(4)	A144(4)	A145(4)	A146(4)	A147(4)	A148(4)	A149(4)	A150(4)	A151(4)	A152(4)	A153(4)	A154(4)	A155(4)	A156(4)	A157(4)	A158(4)	A159(4)	A160(4)	A161(4)	A162(4)	A163(4)	A164(4)	A165(4)	A166(4)	A167(4)	A168(4)	A169(4)	A170(4)	A171(4)	A172(4)	A173(4)	A174(4)	A175(4)	A176(4)	A177(4)	A178(4)	A179(4)	A180(4)	A181(4)	A182(4)	A183(4)	A184(4)	A185(4)	A186(4)	A187(4)	A188(4)	A189(4)	A190(4)	A191(4)	A192(4)	A193(4)	A194(4)	A195(4)	A196(4)	A197(4)	A198(4)	A199(4)	A200(4)	A201(4)

**Statistics**

PC: (002)3433  
 NC: (001)41  
 PC/NC: 83.732

Cutoff	OLI	Current
X2	114	114
X4	415	415
X6	1912	1912
X8	3483	3483

ResultType: Default

**Excluded Antigen**

**Tail Analysis Results**

Spec.	TP	FP	TN	FN	R	% In
B57	2	21	71	0	0.26	100
B44	2	21	71	0	0.26	100
B51	2	21	71	0	0.26	100
A24	2	21	71	0	0.26	100
A25	1	14	71	0	0.24	100
B13	1	14	71	0	0.24	100
B59	1	14	71	0	0.24	100
B47	1	14	71	0	0.24	100

**Epitope Analysis Results**

Spec.	>= X6	< X6	Mean
A32	1	0	5187
B49	1	0	5164
B58	1	0	5022
B53	1	0	4998
A25	1	0	4918
B57	2	0	4435.5
B63	1	0	4415
B77	1	0	4214
A23	1	0	3792

**Final Assignment**

Spec.	T	R

Remove ^ Assign -ve

# Anti-HLA antibodies & Pgen

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- Recipients
- On listing (x2) & every 3 months
- Express as Pgen - % of Irish donors to whom recipient has antibodies
- Cumulative value
- How difficult will it be to find an antibody compatible donor

# Complex list

Pgen	<95%	95-99%	100%
Total	333 (72%)	44 (9%)	87 (19%)
Regrafts	21 (17%)	28 (22%)	76 (61%)
1st Grafts	312 (92%)	16 (5%)	11 (3%)

Regrafts: Ireland 30%; UK 22%

Pgen > 94%: Ireland 28%; UK 11%

# Donor Specific Antibodies

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- Anti-HLA antibodies specific for donor HLA
- Main focus once a donor identified
- Patients with very high Pgen (>94%) increased risk even if DSA -ve

# Why do antibodies matter?

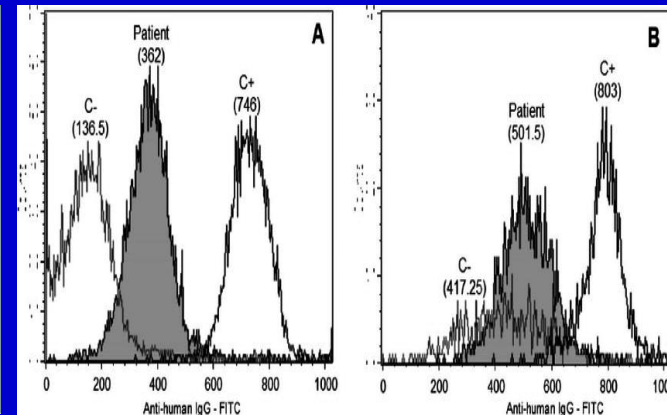
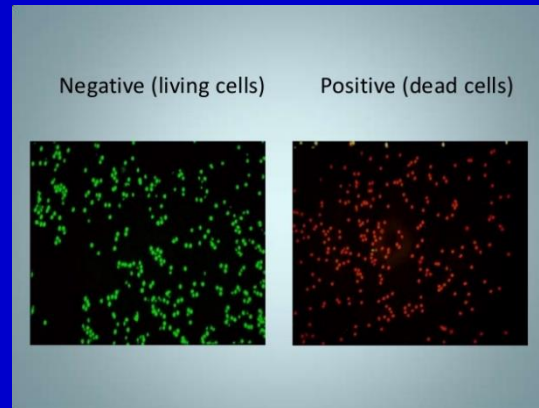
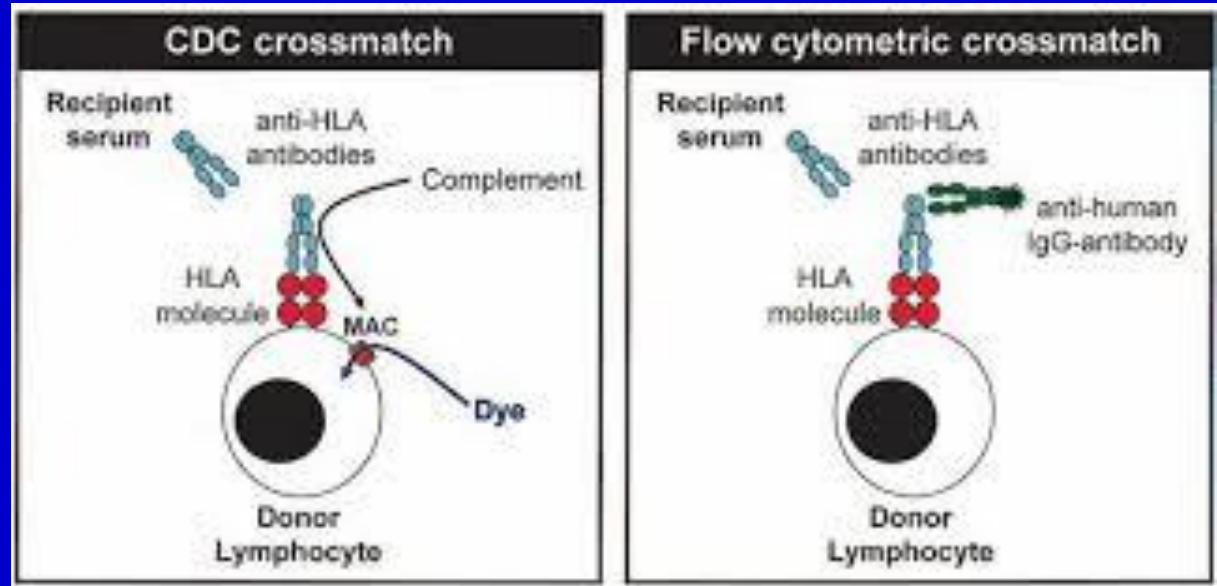
## Rejection

- Hyperacute
- Acute - Cellular  
Antibody mediated
- Chronic



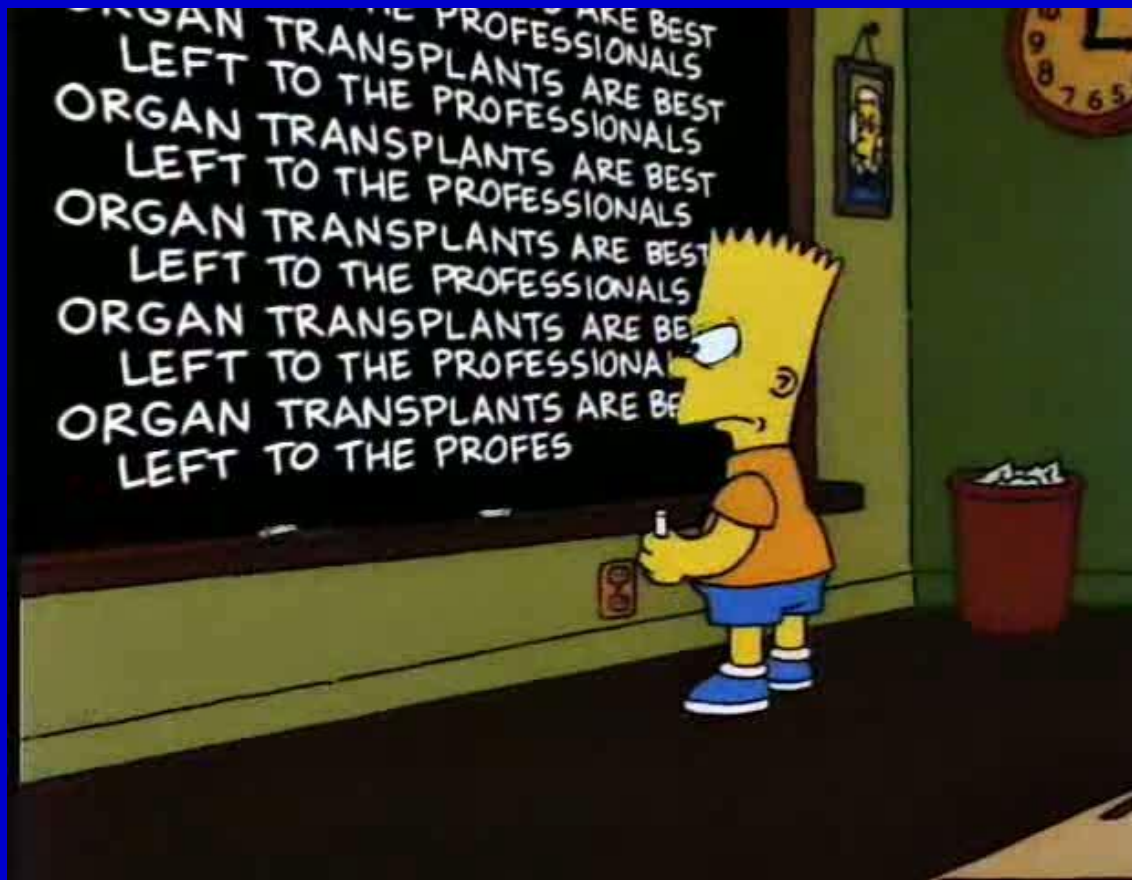
# Crossmatch

- CDC
- Flow Cytometry
- Virtual





# So what's the problem?



# Single National Centre

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- Lab extremely busy on donor night
- Renal unit - 2 transplants
- Cardiothoracic - sometimes 2
- When there are 2 or 3 donors.....

# Donor Nights

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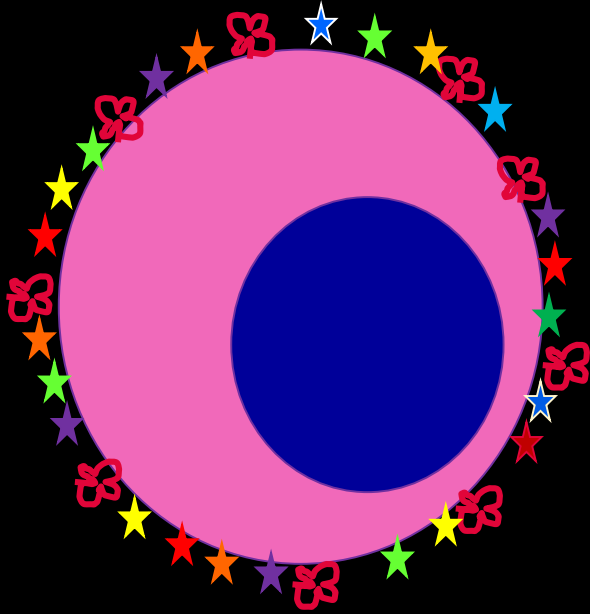
- Confirm blood group
- HLA type on peripheral blood
- Cross match up to 4 recipients on peripheral blood (cardiothoracic/pancreas)
- Virtual crossmatch entire renal list
- Prepare shortlist of pot renal recipients

## Post-Retrieval

- Confirm blood group & HLA type (spleen)
- Crossmatch 4 renal recipients

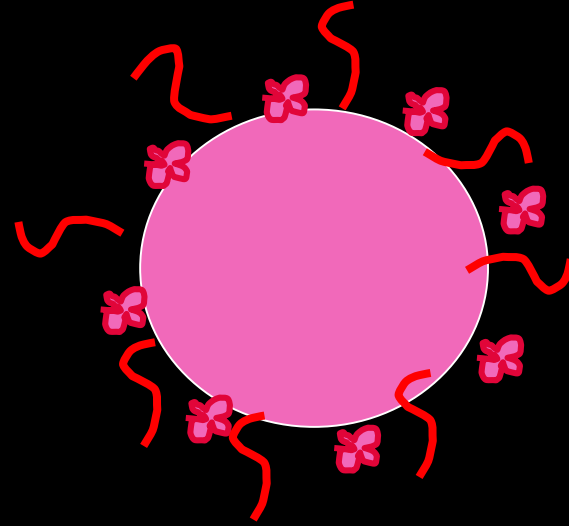
# No assay is perfect!

## Crossmatch



Thousands of proteins  
IgG Receptors  
Positive result if  
antibodies to any of them  
Only relevant if anti-HLA

## Luminex Beads



Covered with HLA molecules  
Some native, some denatured  
Amount of HLA varies  
between beads & batches  
Only relevant if to native  
HLA

# Risk Assessment

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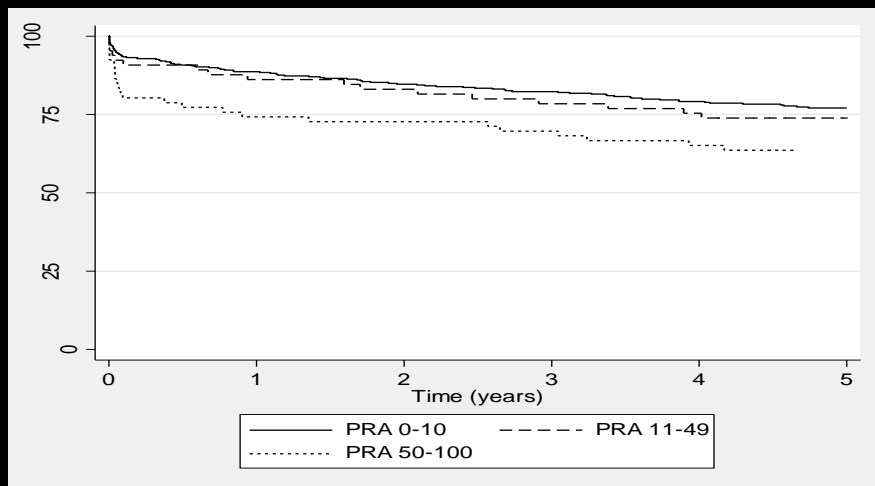
- Sensitisation history
- Pattern of sensitisation
- Crossmatch
- Concordance of results
- Technical aspects & QC weight results
- HLA reactivity or not?
- Can you mitigate with immunosuppression

# Initiative 1: Flow Cytometry

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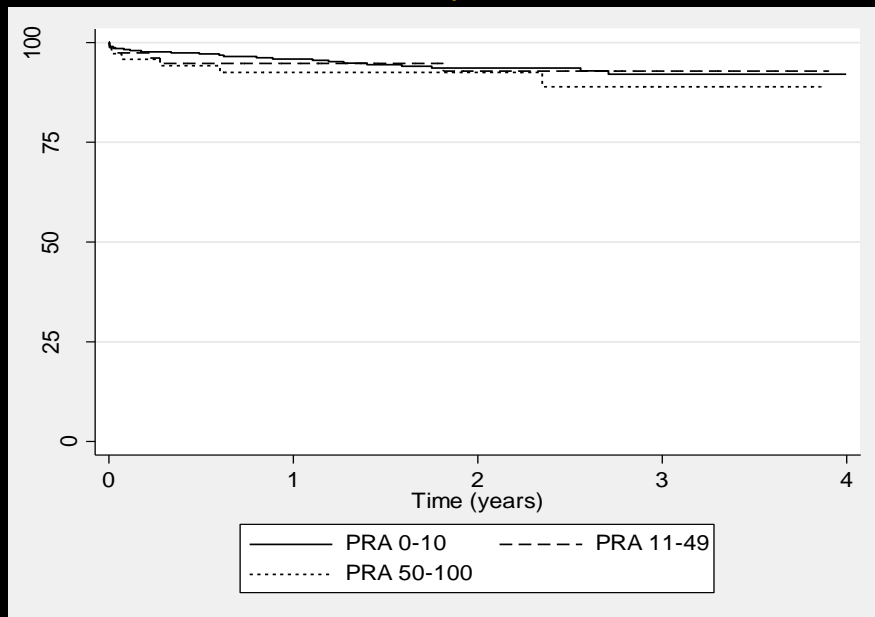
- No standardisation
- No consensus
- Introduction of clinically validated cut-offs

# Results by PRA; 1998 to 2001



PRA	3 yr
<10%	82%
11-49	78%
50+	70%

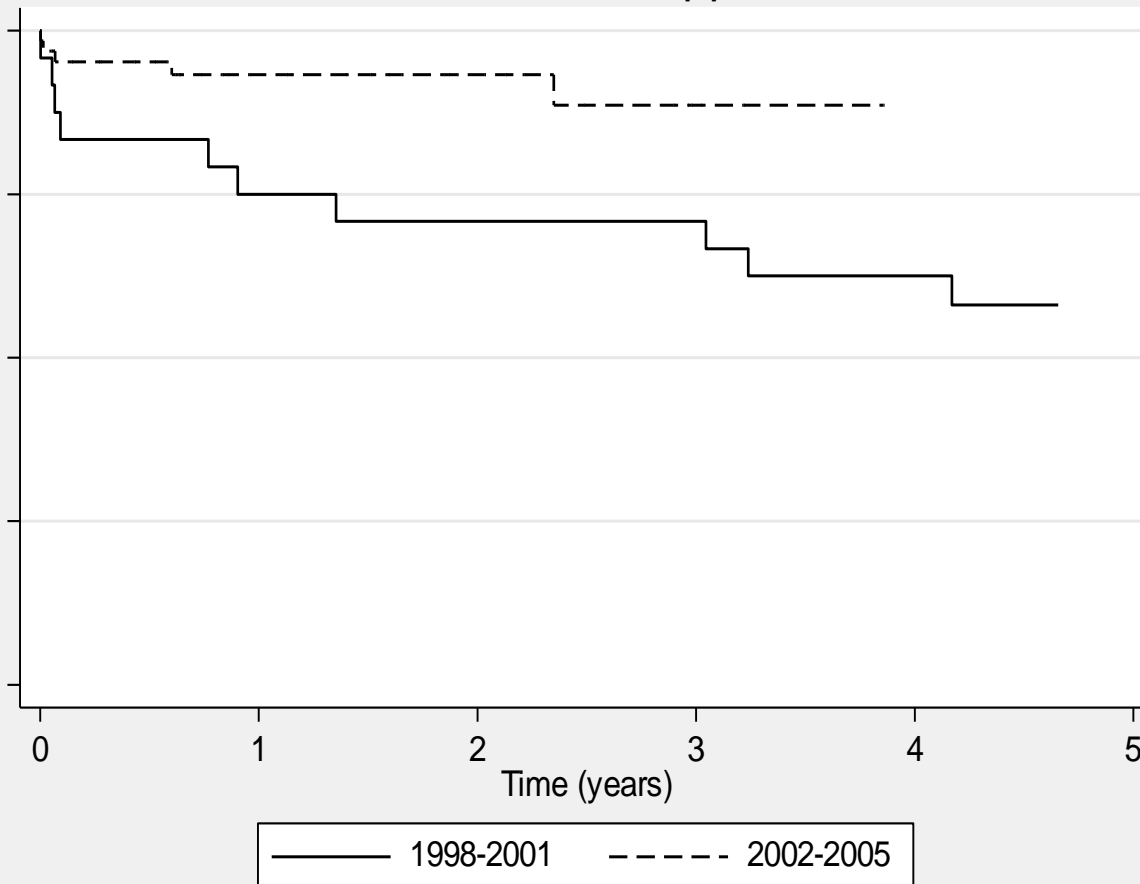
# Results by PRA; 2002 to 2005



PRA	3 yr
<10%	92%
11-49	93%
50%+	89%

# Clinically Validated Flow XM

PRA 50%-100% with Immunosuppression Fk/MMF/DC



## Issues

- Concerns re over sensitivity
- No consensus method or cut-off

## Impact

- Virtually abolished AMR
- Improved survival in sensitised pts



# Initiative 2: Luminex

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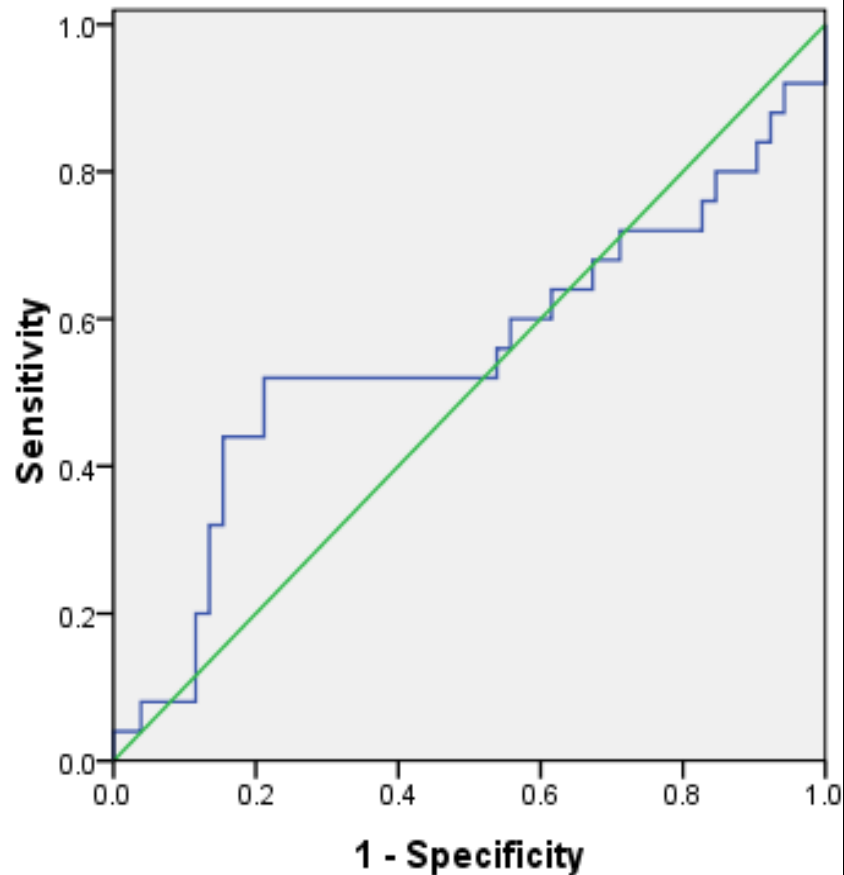
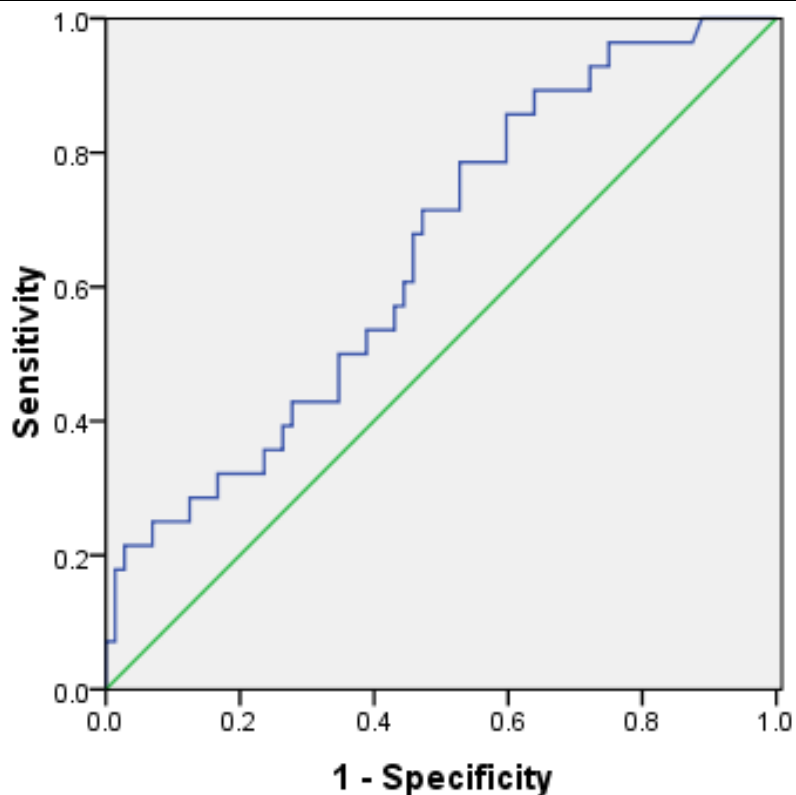
Which antibodies are clinically significant?

# Luminex - Issues

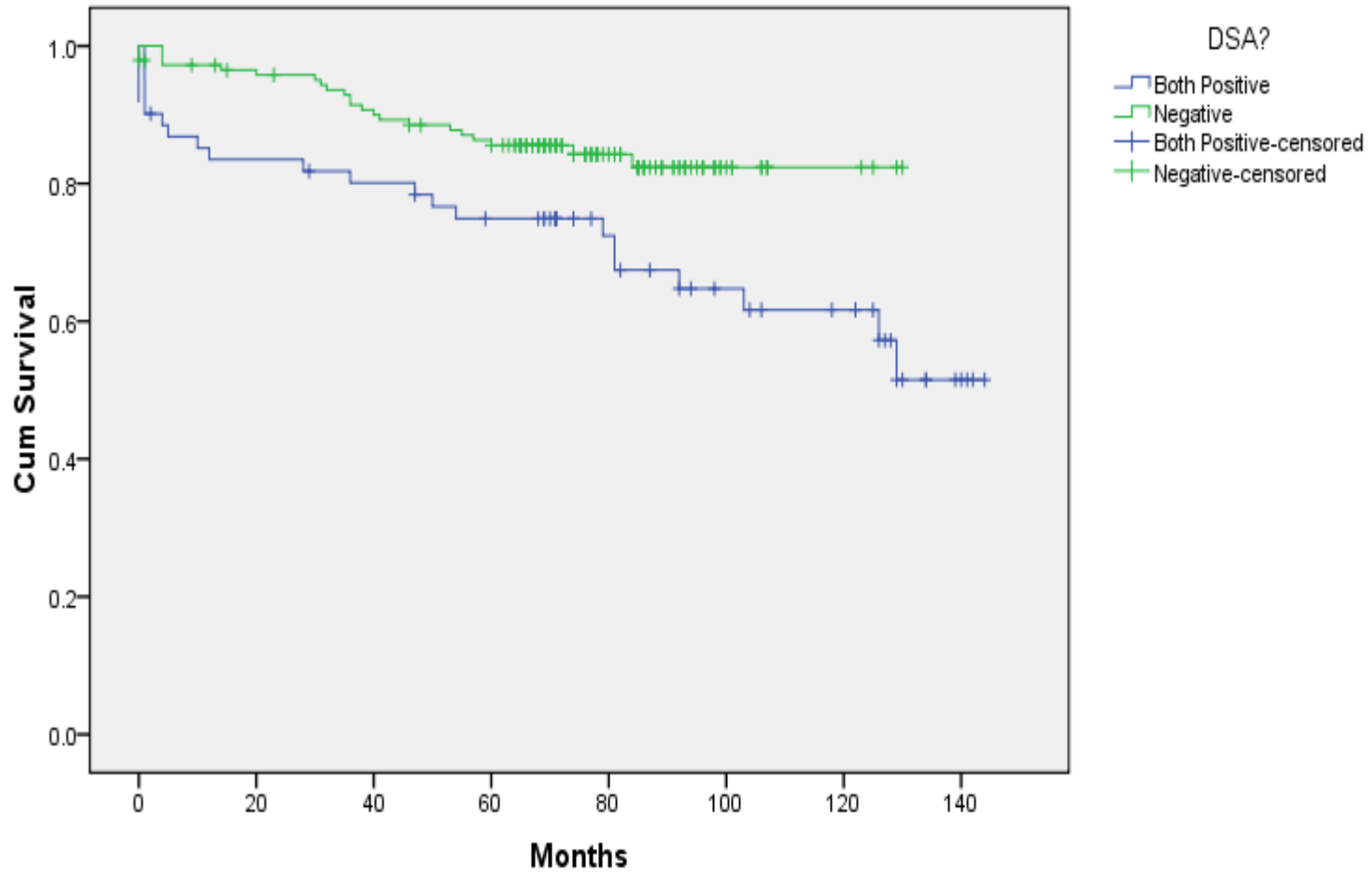
- Concerns re oversensitivity
- Lack of consensus re cut-off
- Reproducibility
- Antigen loading on beads
- Specificity - denatured antigen
- Batch to batch variation
- Temperature effect
- Prozone effect



# Luminex SAB - ROC curves



# Single vs Dual Technique Abs



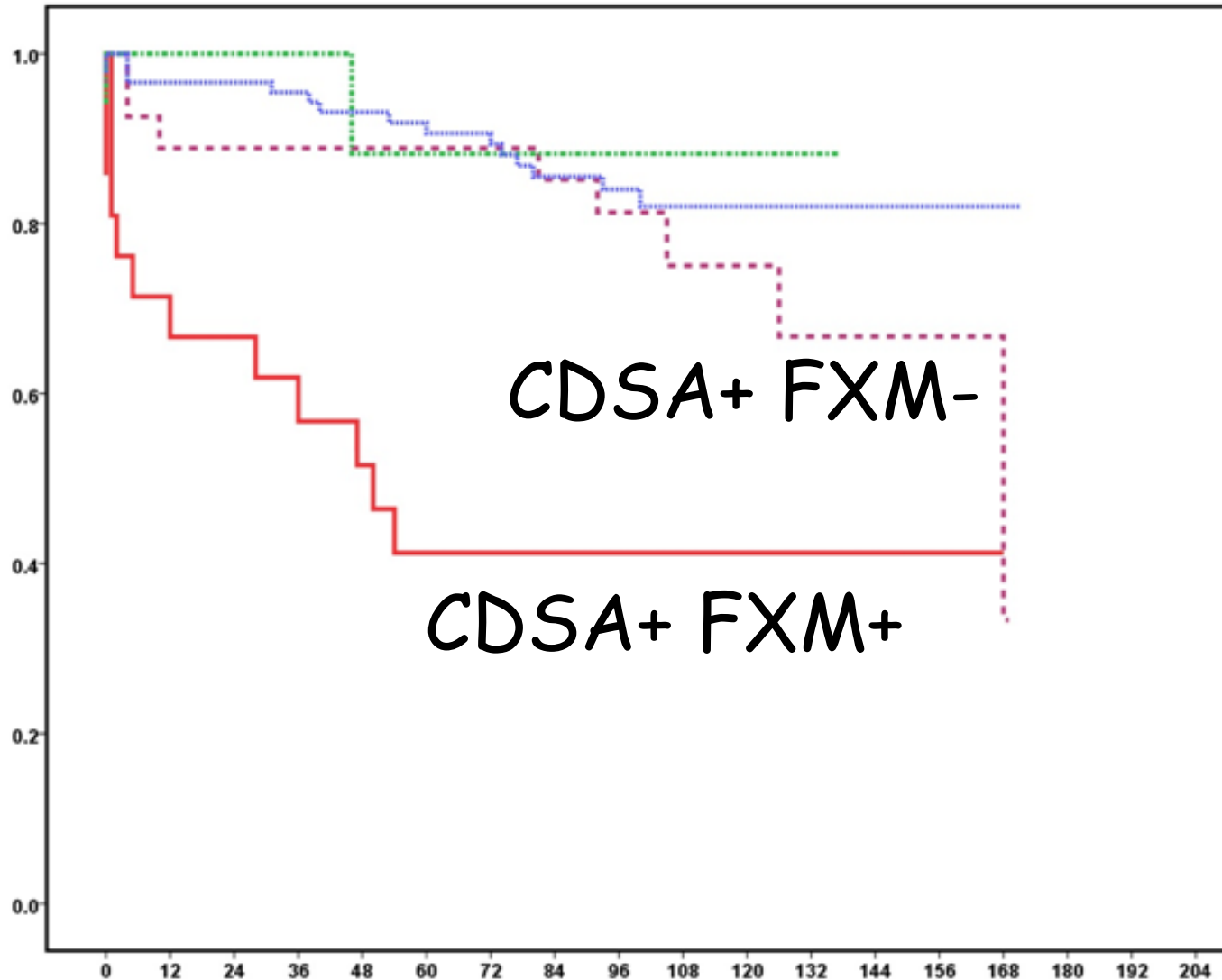


# Graft Survival

	3 months	1 year	3years	5years	10 years
DSA+ C1q+ n=15	78	71	64	64	55
DSA+ C1q- n=46	91	85	82	76	67
No sig. Abs. n=145	98	97	94	86	80

% graft survival

# DSA-FCX in DSA positive patients



# Audit - Clinical Outcomes

	STA	DSA	Paired Kidney
Number	15	10	20
DGF	1	2	4
Rejection	1	2	1
Creatinine 3mths	115 (79-173)	110 (83-152)	121 (39-266)
Creatinine 1 yr	117	127	122

No AMR or graft failure



# Initiative 3: Virtual crossmatch

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- Reduce cold ischaemia time
- Need excellent sensitisation history
- Full 6 locus type
- Up to date Luminex screening
- Patient safety paramount

# Crossmatches - not perfect either

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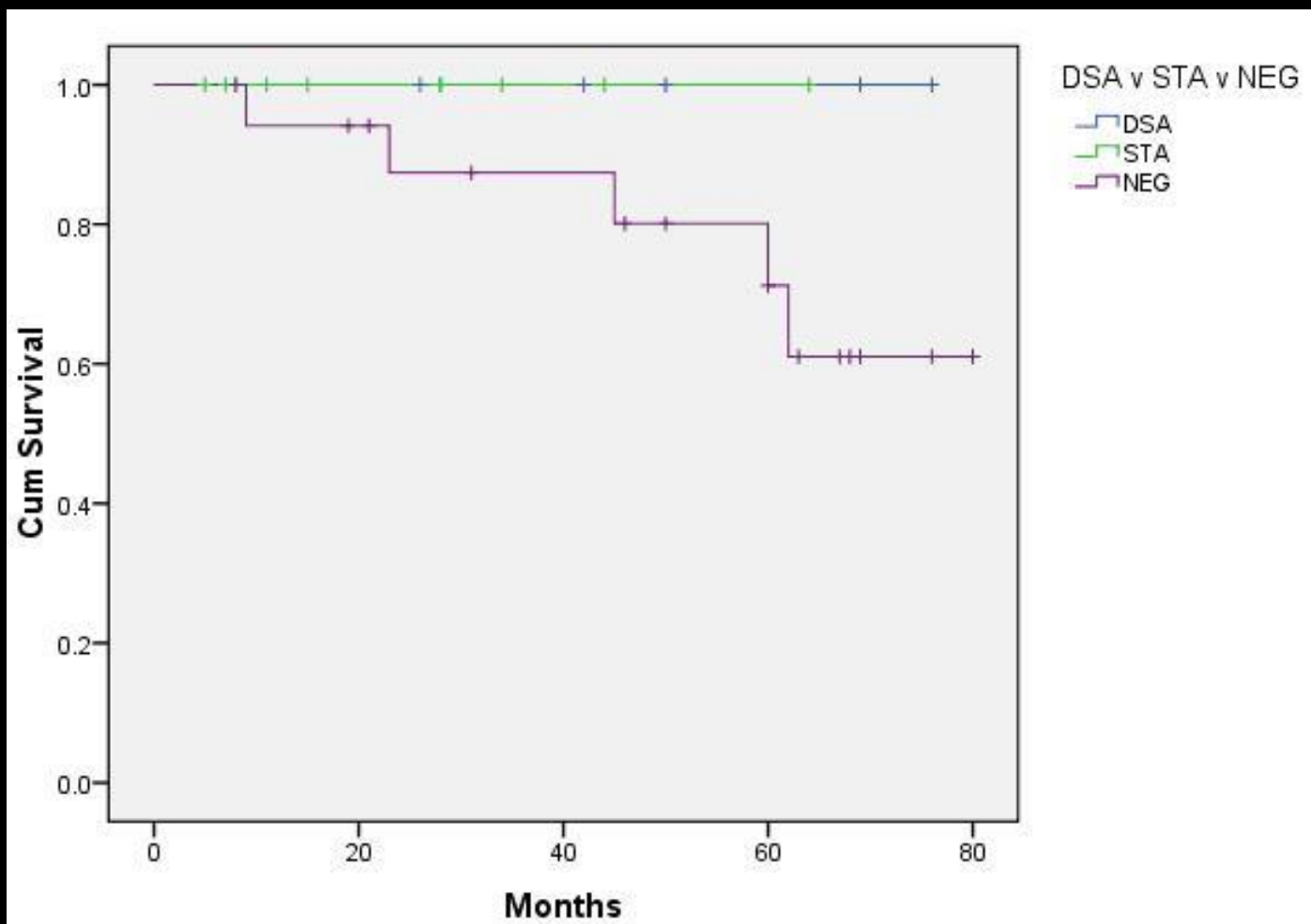
- Multiple polymorphic proteins on cell surface
- Only anti-HLA matter
- Low avidity antibody binds well in cold
- Multiple false positives
- In Beaumont - triggers senior review
- Proceed unless cross-match deemed to be due to anti-HLA antibodies

# Cardiothoracic Transplantation

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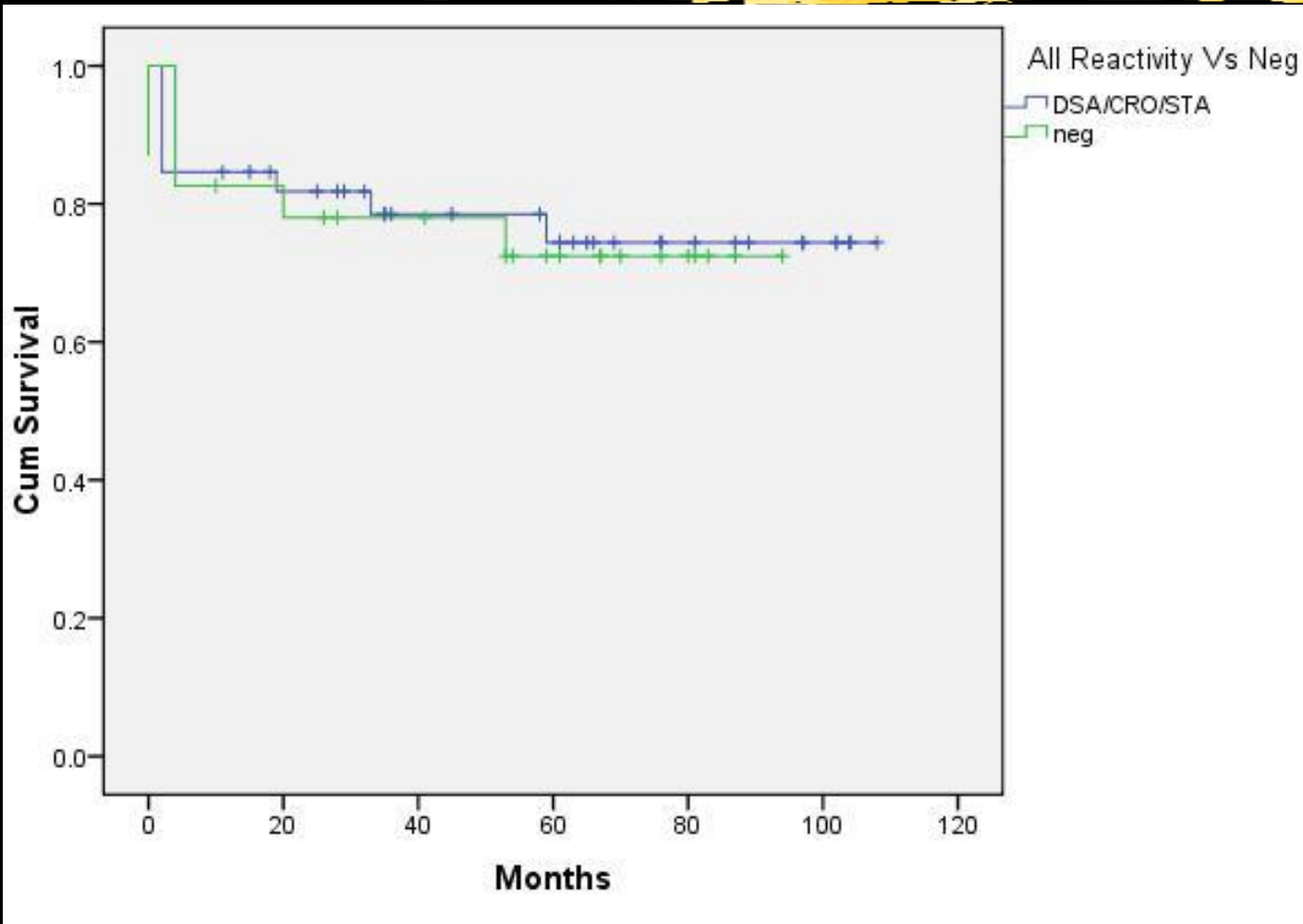
- Need to size match
- Difficult to avoid antibodies in small donor pool
- Time constraints very tight
- CTAG guidelines: Cumulative MFI
  - 2000-5000 - risk, consider antibody removal
  - >5000 - only Tx in exceptional circumstances

# Lung Recipients: DSA Vs STA Vs Negative



p=0.29 (NS)

# Cardiac Recipients: Antibody Pos Vs Neg

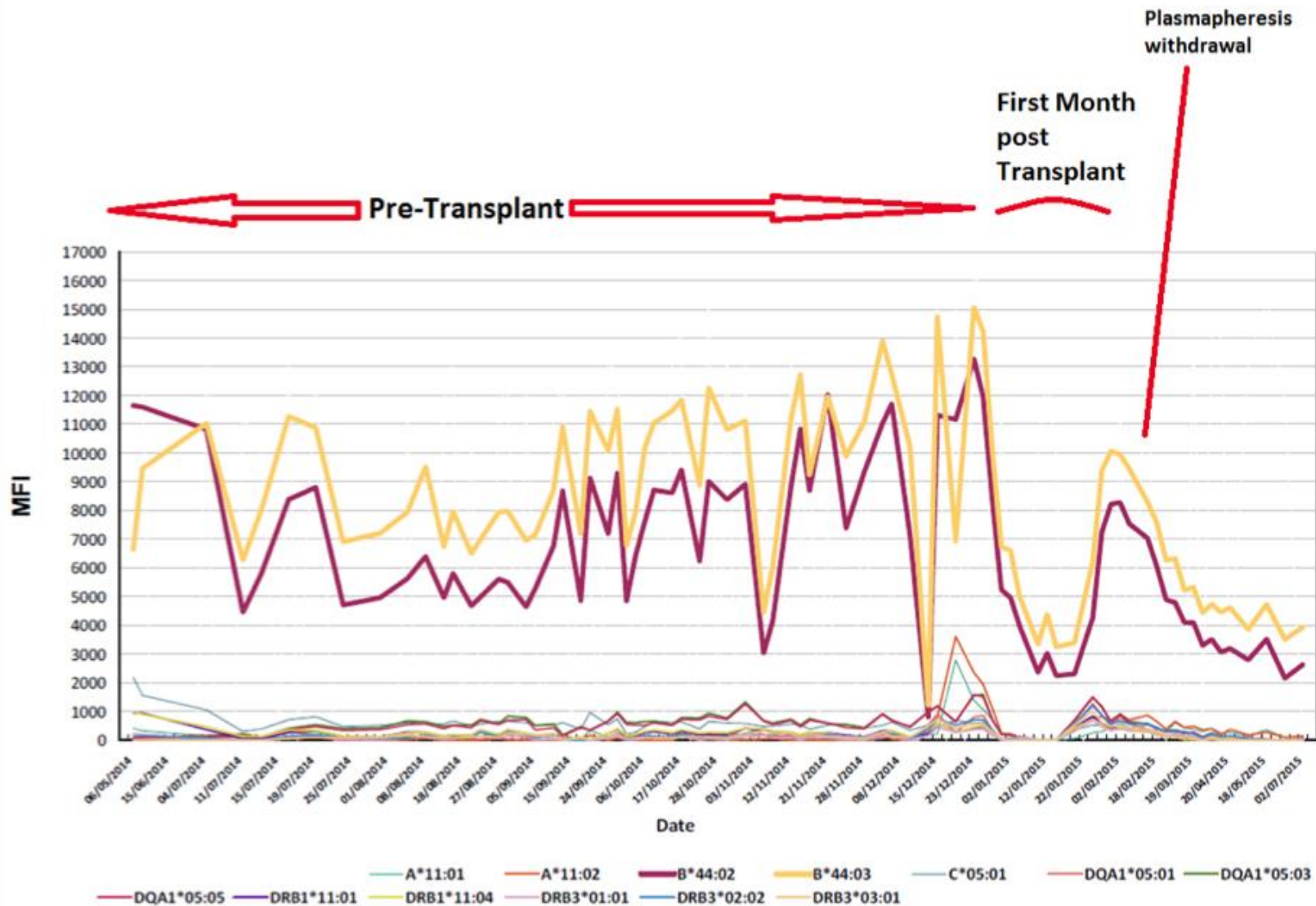


$p=0.837$  (NS)

# Initiative 4

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- When all else fails - antibody removal
- 48yo woman
- Severe cardiac failure (dilated cardiomyopathy)
- Pgen 100%



# Summary

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- Excellent laboratory medicine essential to all transplant programmes
- H & I focus on history, HLA typing, antibody screening & crossmatching
- Good clinical science has brought about real improvement in outcomes



# Acknowledgements

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Flow: All staff in NHISSOT

STA vs DSA:

David Keegan

C1q antibodies:

Cath Taylor

Audit:

Dervla Connaughton

IT:

Ian O'Neill

Statistics:

Patrick O'Kelly

Cardiac Case:

Niall Mahon

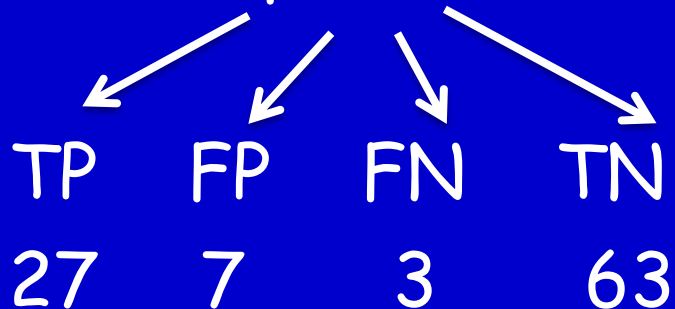
Jonathon McGuinness

Transplant Surgeons, Co-ordinators

Donor Families

# Flow XM - 90% sens & spec

30% Population DSA+

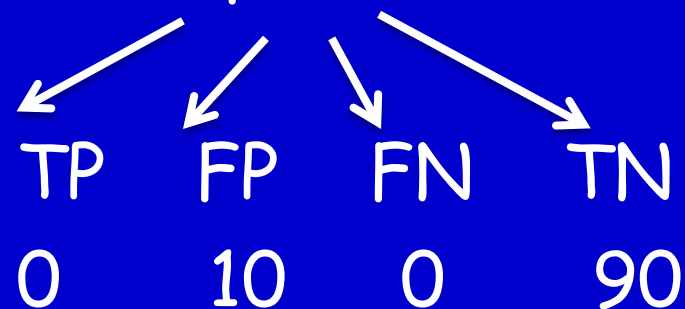


34 +ve

PPV = 80%

NPV = 90%

0% Population DSA+



10 +ve

PPV = 0%

NPV = 100%

# Positive Flow, Negative Virtual XM

