

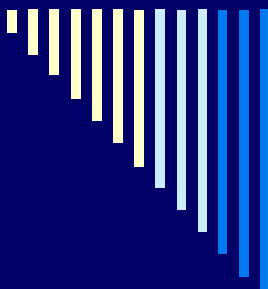
Use of Dithiothreitol (DTT) in Routine and Post Transplant Antibody monitoring using LabScreen SA beads

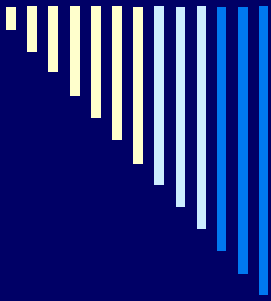
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Royal London Experience
Leicester 25th July 2011



Background

- Alloantibodies play an important role in all types of solid organ rejection; the presence of donor-specific antibodies (DSA) identified pre-transplant is associated with an increased risk of antibody mediated rejection (AMR)
 - To facilitate a successful transplant in a highly sensitised patient, accurate ID is essential in minimising likelihood of a positive crossmatch thereby reducing cold ischemia time
 - Post transplant monitoring of DSA is important in diagnosis and treatment AMR
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- SA test is relatively accurate in assignment of Abs especially in highly sensitised patients and can easily be applied in Ab monitoring pre and post transplant
 - Presence of IgM can inhibit the binding of specific IgG to their corresponding antigens on SA beads possibly due to idiotypic net formation or IgM link to IgG resulting in misleadingly weak or negative results for HLA antibodies
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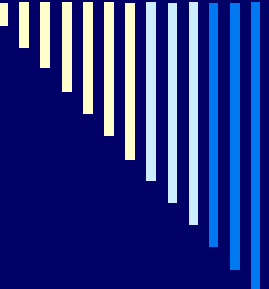
- Luminex SA assay can be modified for HLA CI and CII specific antibody screening by pre-treatment of sera with DTT

- DTT treatment of the patient sera can reveal many strong HLA IgG antibodies which are not detected in routine SA testing (-DTT) in pre and post transplant samples for DSA



Royal London Protocol

- All patients on transplant waiting list are screened every 2-3 months by LabScreen LSM/SA and CDC (panel of 45 cells)
 - All New patients entering tx list or being worked up for a LR donor transplant are tested by CI/CII SA regardless of being negative by LSM.
 - All deceased donor transplanted recipients are also tested by CI/CII SA at D0 regardless of being tested negative by LSM.
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Case Study 1- Patient LM, 38 years
HLA Type: A2,A31/B44,B51/Cw5,Cw15/
DR4,DR4/DR53/DQ7,DQ8

Activated on Renal transplant waiting list on 24.01.11

Antibody Screening: Luminex SA CI

A1,A3,A11,A25,A*3002,A36,A80,B7,B8,B27,B46,B54,B57,B58,B73B76,
B78,Cw1,Cw7,Cw8,Cw9,Cw10,Cw12,Cw14,Cw16,Cw18

CDC Screen using panel of 45 cells +/-DTT : A11, A23, A24
(CDC +DTT reactions stronger than -DTT)

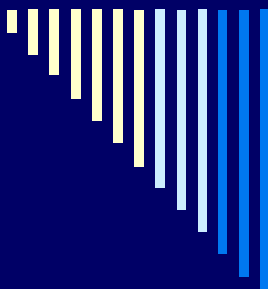
**+DTT: A1,A3,A11,A23,A24,A25,A*3002,A32,A36,A80,B7,B8,B27,
B35,B39,B42,B46,B54,B55,B57,B58,B61,B67,B73,B*1502,B76,B78,B8
2,Cw1,Cw7,Cw8,Cw9,Cw10,Cw12,Cw14,Cw16,Cw18**

Case Study 1 contd.

Specificity	Normalised MFI (-DTT)	Normalised MFI (+DTT)
A*23:01	390	13351
A*6601	342	142
B*51:01	335	297
A*6601	216	20
A*31:01	24	101
A*2402	0	13658
A*2403	0	12675

CDC +/-DTT defined specificities: A11, A23, A24

Raised concern for other patients on the transplant waiting list



Case Study 2- Patient MN, 47 years
HLA Type: A23,A66/B7,B8/Cw7,Cw7/
DRB1*01,DRB1*13/DQB1*05,DQB1*06, DQA1*0103,0101/04/05

Antibody Screening: Luminex CII SA

-DTT: DQB*0201/02,DQB*0401/402,DQA1*0301, ?DQB1*0302

LRD work up - donor type: A2,A36/B53,B57/Cw4,Cw6

DRB1*13,DRB1*15,DQB1*06,**DQB*0301**,DQA1*0102

CDC B cell XM +/-DTT strongly pos with **DQB1*0301** (MM specificity)

CII SA +DTT:

DQB1*0201/0202,DQB1*0401/0402,**DQB1*0301,DQB*0302,DQB*0303**

bead DQA*0301/**DQB1*0301**(MFI 2981), +DTT MFI 17341

bead DQA*0301/**DQB1*0302**(MFI 6921), + DTT MFI 12006

bead DQA*0301/**DQB1*0303**(MFI 3710), +DTT MFI 11877

Case study 2 contd..

Specificity	Normalised MFI -DTT	Normalised MFI DTT
DQA1*0301,DQB1*0301	2981	13376
DQA1*0201,DQB1*0301	2499	13719
DQA1*0502,DQB1*0301	408	14599
DQA1*0601,DQB1*0301	0	16341
DQA1*0503,DQB1*0301	0	14679
DQA1*0302,DQB1*0302	449	15468
DQA1*0302,DQB1*0303	515	14319
DQA1*0201,DQB1*0303	121	15230



Case Study 3: MB, Age 42

HLA Type: A1,A24/B8/Cw7/DR4,DR7/DQ2,DQ7

History: 1st Tx 05.05.1989 (Tx MM B60) Tx failed on 01.07.1998

Re-activated on the Tx list on 09.01.2000

Highly Sensitised Patient (CRF 90%)

Antibody Screen:

➤ CDC: Multispecific

➤ Luminex CISA –DTT:

A*0203,A*0206,A11,allelicA*2403,A29,A30,A31,A32,A74,A80,B18,B38,B42,B44,B46,B47,B*5101,B52,B54,B55,B56,B57,B58,B59,B63,B64,B65,B71,B75,B77,B78,Cw1,Cw4,Cw5,Cw8,Cw9,Cw10,Cw12,Cw14,Cw16,Cw18

Patient clinically urgently requires a transplant, running out of access



Case Study 3: MB, Age 42, A1,A24/B8/Cw7/DR4,DR7/DQ2,DQ7

June 2010 – Living related donor work up

- Donor MM – B*5101, C*0202
- CDC crossmatch positive, DSA C*0202, B51*01

Antibody Removal:

- Patient received Ritux followed by 7 rounds of IA/IvIg
 - DSA titres not sufficiently depleted.
 - Desensitisation unsuccessful
 - Patient not transplanted
-



Case Study 3: MB, Age 42

HLA Type: A1,A24/B8/Cw7/DR4,DR7/DQ2,DQ7

2nd Tx 04.11.10 Deceased HB kidney donor: MM A*0206, B7

- CDC Crossmatch +/-DTT Negative, FCXM T/B positive
- Luminex DSA wkB7
- Implant biopsy - severe acute injury with microthrombi
- Patient transplanted: D0 - Induction with ATG, Tac, MMF and Prednisolone (Immunosuppression regime for a HSP)

Post tx:

- Patient became dialysis dependent
 - Creatinine levels stuck ~700-900µmol/L
 - At 7 day Histopathology – ATN, humoral rejection, C4d positive
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Case Study 3: MB, Age 42
 HLA Type: A1,A24/B8/Cw7/DR4,DR7/DQ2,DQ7

Specificity –DTT (Pre-Tx)	Specificity +DTT (post Tx)
A*203,A11,A*2403,A29,A30,A31,A32,A*6602,A74,A80, B7wk ,B18,B*2708,B35,B38,B39,B41,B42,B44,B45,B46,B47,B51,B52,B53,B54,B55,B56,B57,B58,B59,B61,B62,B63,B64,B65,B67,B71,B72,B75,B76,B77,B78,B81,Cw1,Cw2,Cw4,Cw5,Cw6,Cw8,Cw9,Cw10,Cw12,Cw14,Cw16,Cw17,Cw17,Cw18	A*0203,A11,A*2403,A29,A30,A31,A32,A*6602,A74,A80, B7(DSA) , B13 ,B18,B27,B35, B37 ,B38,B39,B41,B42,B44,B45,B46,B47, B48 , B49 , B50 ,B51,B52,B53,B54,B55,B56,B57,B58,B59, B60 ,B61,B62,B63,B64,B65,B67,B71,B72, B73 ,B75,B76,B77,B78,B81,B82,Cw1,Cw2,Cw4,Cw5,Cw6,Cw8,Cw9,Cw10,Cw12,Cw14,Cw15,Cw16,Cw17,Cw18

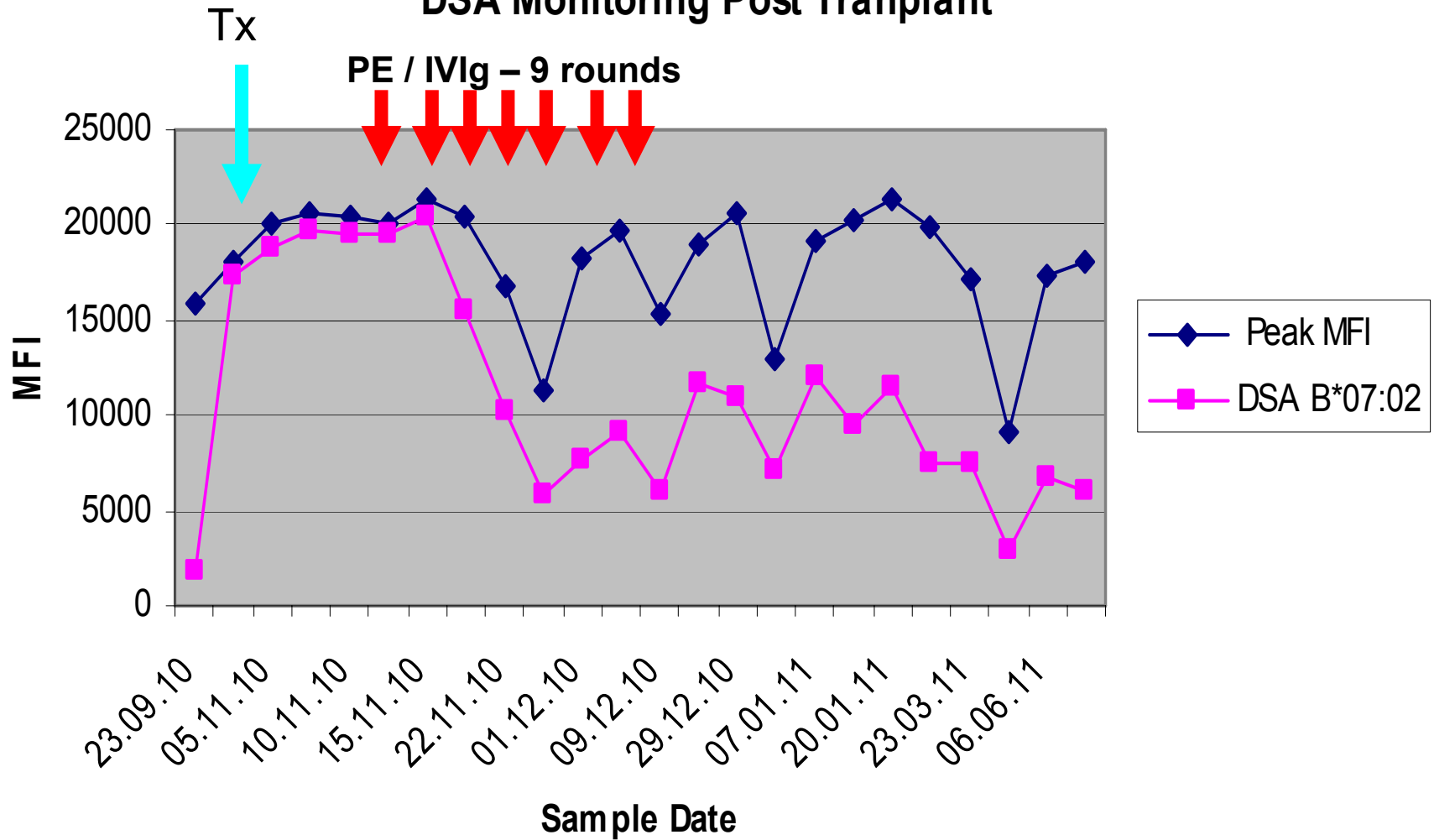


Case Study 3 MB contd...

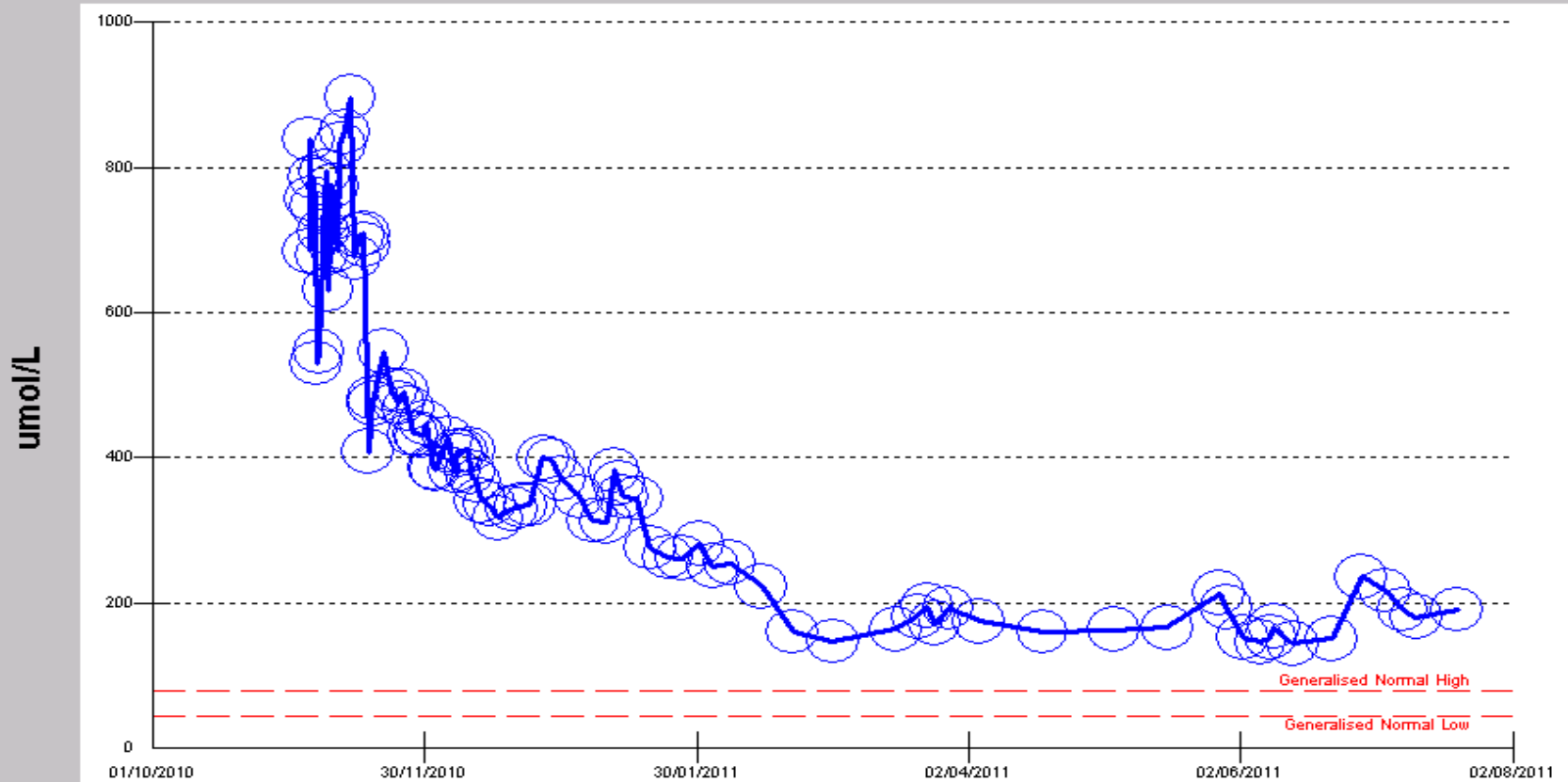
Specificity	Normalised MFI -DTT	Normalised MFI +DTT
B7 (DSA)	1838	17366
B13	346	17529
B*2705	618	16422
B2708	1114	16862
B37	574	15123
B60	0.0	17068
B73	823	16328
B81	1527	15686

CI SA +DTT - revealed specificities are all part of the same CREG

DSA Monitoring Post Transplant



Creatinine Serum



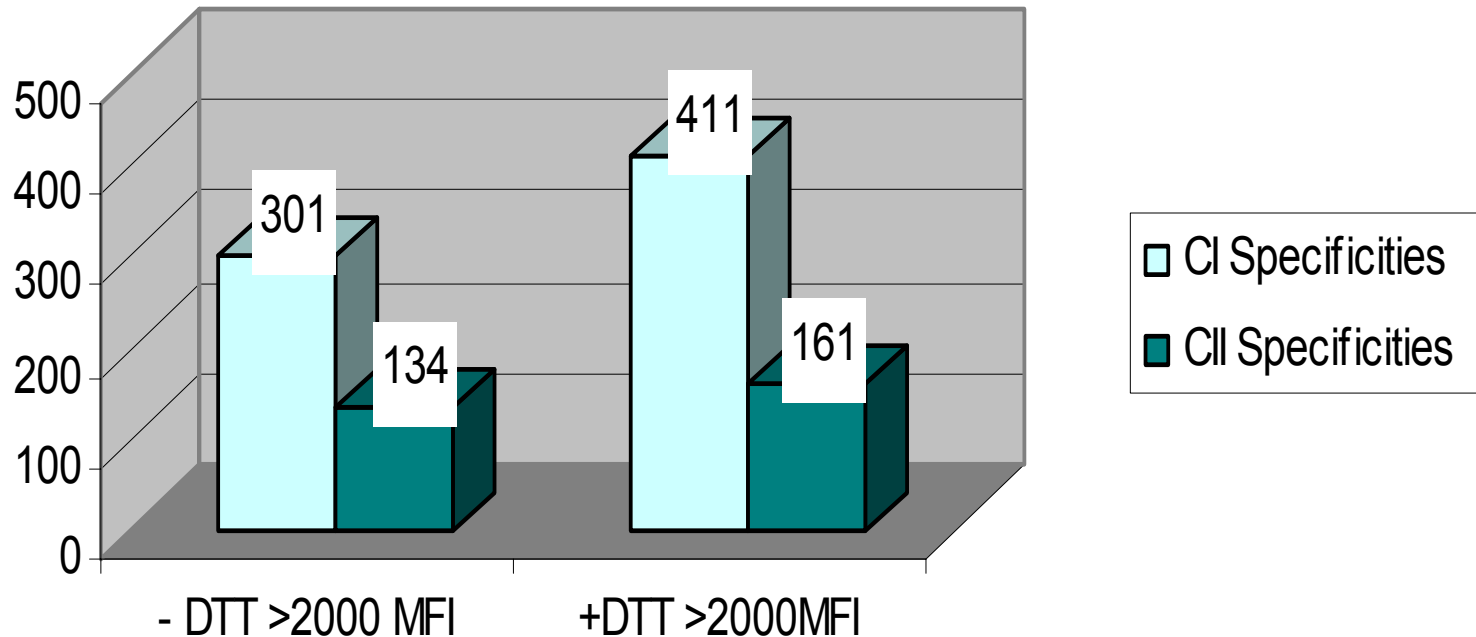
Post Tx day 17: C4d negative correlated with lower levels of DSA
8 months post tx - serum creatinine 191 $\mu\text{Mol/L}$



Royal London Experience

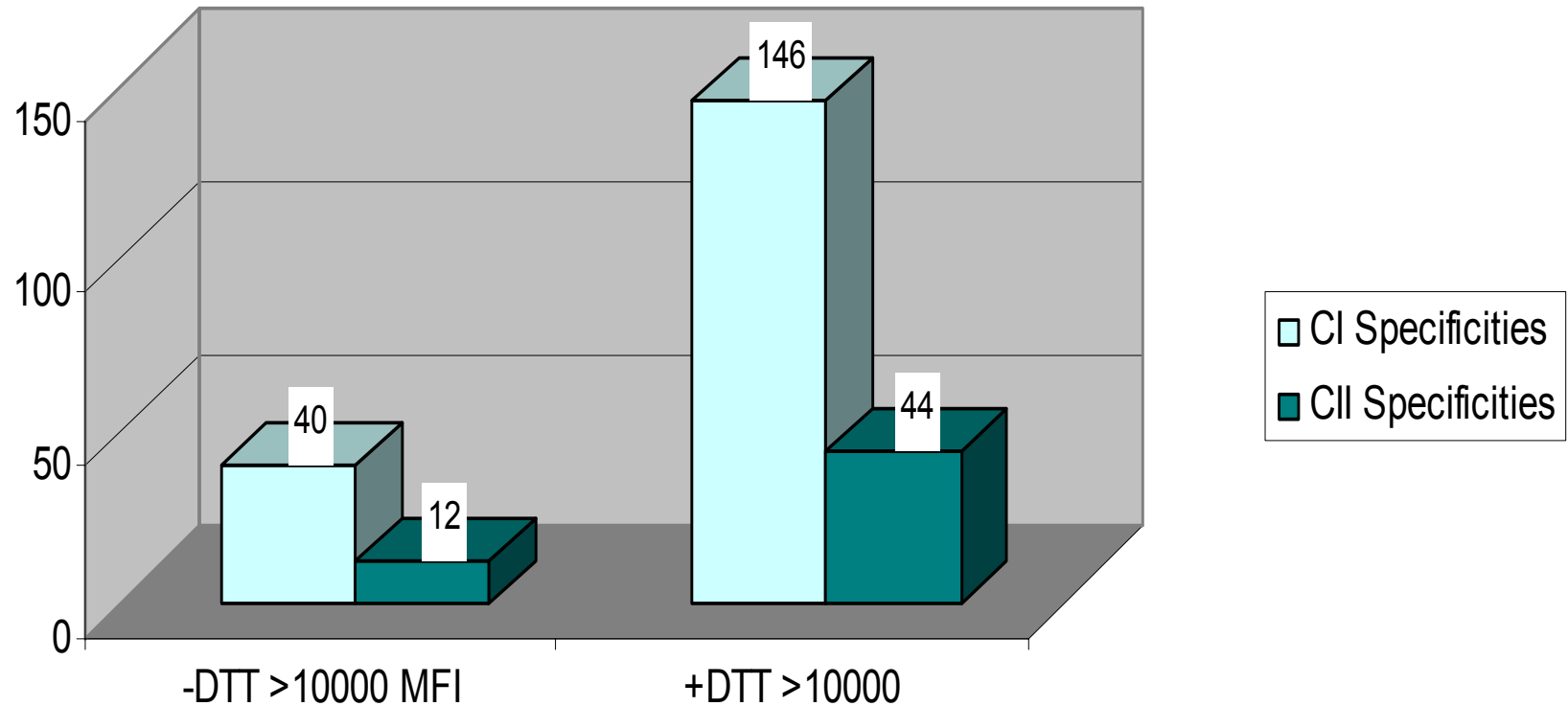
- Number of selected sera from patients on tx list and from post tx patients were tested -/+ DTT
 - Sera incubated with 0.077M DTT (10%) for 30 mins at RT
 - HLA specific IgG binding -/+DTT compared (no diluting effect)
 - MFI ≥ 2000 deemed positive, MFI ≤ 1000 considered particularly in post tx monitoring
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Number of Specificities Identified -DTT vs +DTT by CI and CII SA (n=31)



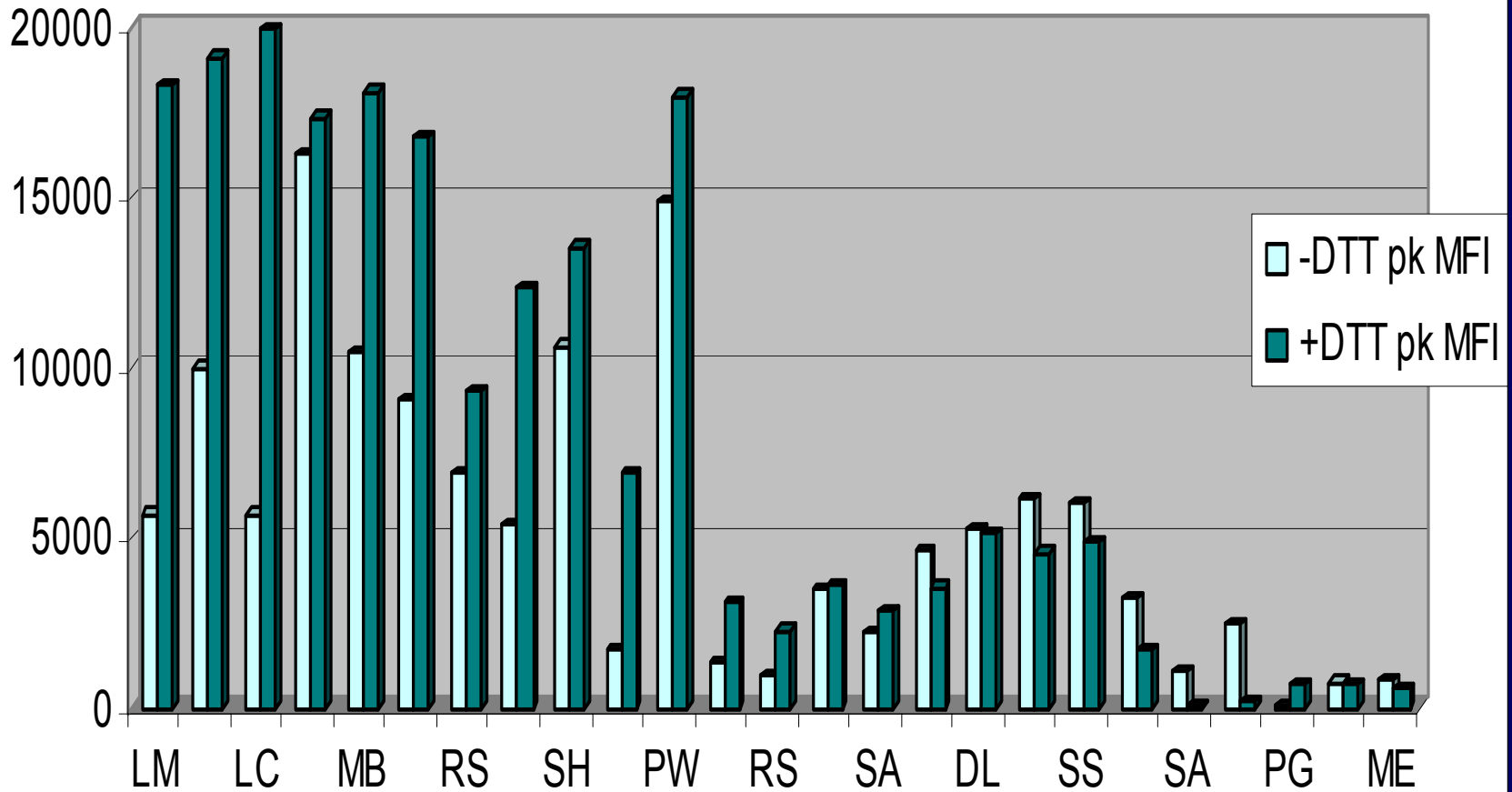
Significant increase in total no. of HLA CI/CII specificities identified +DTT
for MFI >2000

Specificities Identified -DTT vs +DTT (MFI >10,000)



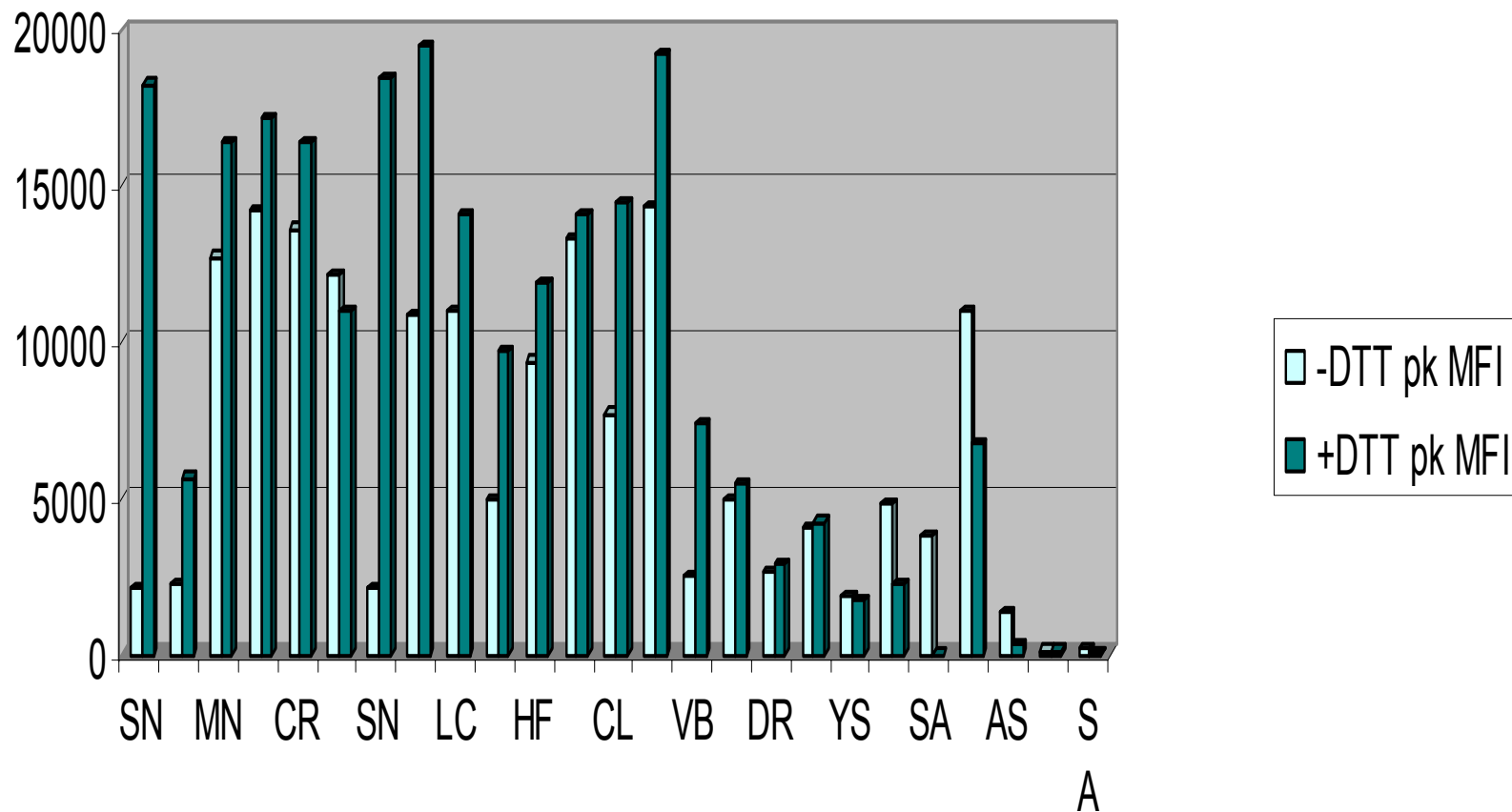
Significant increase in no. of HLA CI/CII specificities identified +DTT for MFI >10,000

CI SA Normalised Peak MFI -DTT vs +DTT



CI SA +DTT significant increase in peak MFI for many patients

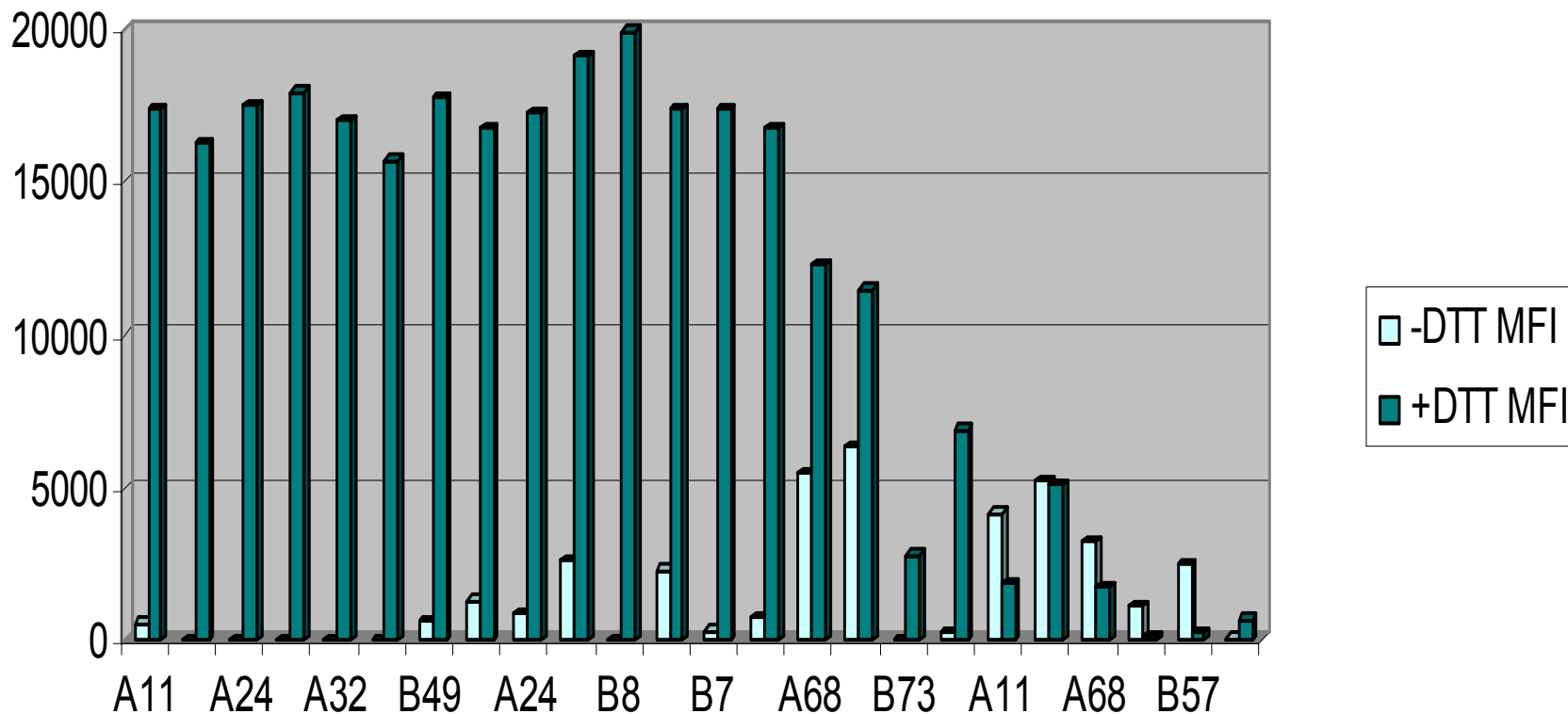
CII SA Normalised peak MFI -DTT vs +DTT



CII SA +DTT also significant increase in peak MFI for many patients

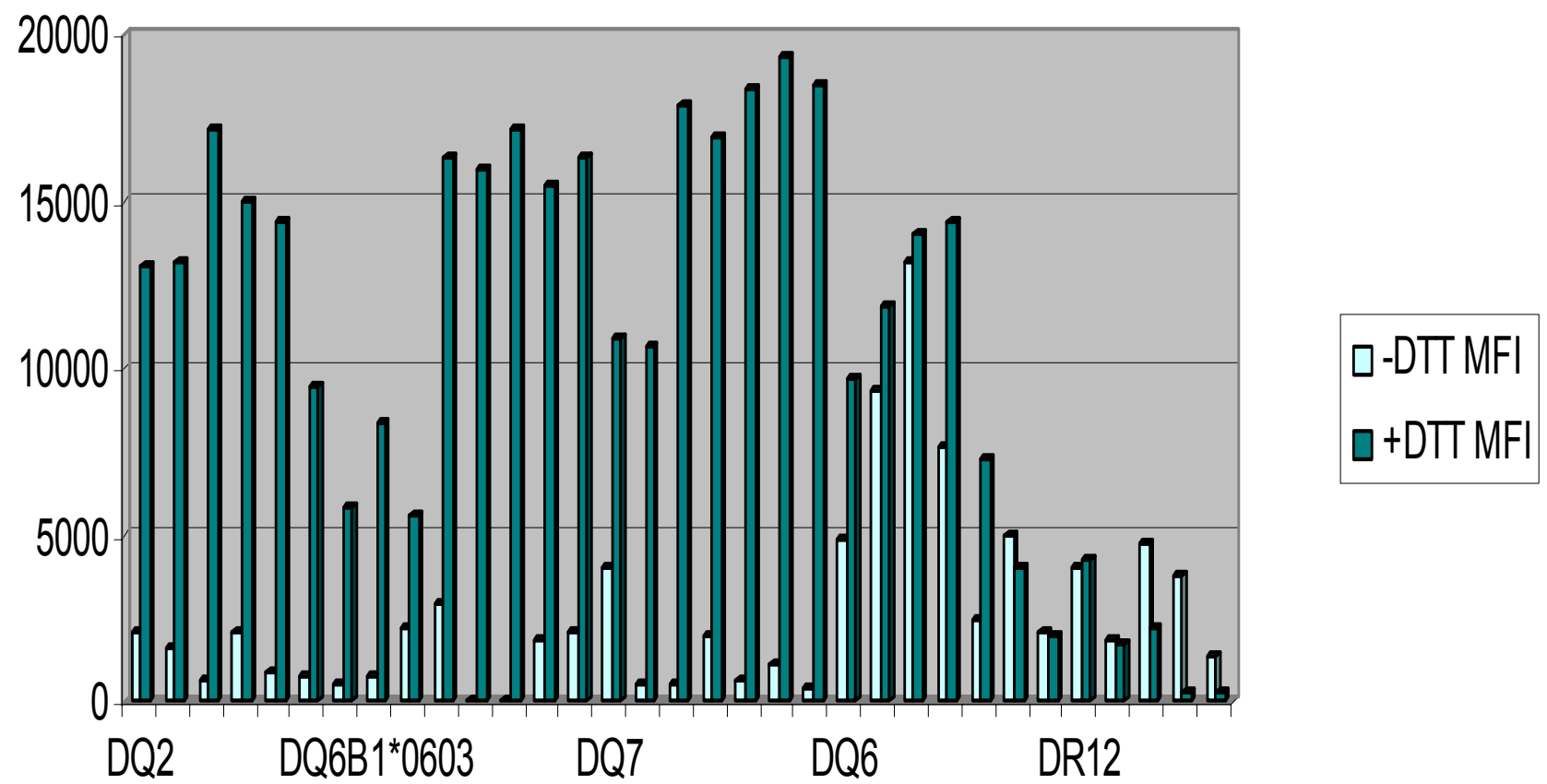
Specificities Identified by CI SA -DTT vs +DTT

n=25



+DTT many CI specificities revealed >10- 15,000 MFI (0 or v low MFI -DTT)

HLA CII Specificities Identified -DTT vs +DTT



CII +DTT also many specificities revealed >10 -15,000 MFI (0 or v low MFI -DTT)



Effect of DTT on CI/CII SA Results

- For CI and CII SA tests (n=50), approximately 34% (n=17) of the samples had their peak MFI increased between 5000 to 10000 mainly in Highly Sensitised Patients (HSP)
 - Normalised peak MFI for 46% (n=23) samples were in concordance +/-DTT
 - Interestingly, for 20% (n=10) peak MFI was lower in +DTT group than -DTT (cf. to HSPs where MFI increased +DTT)
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SA CI/CII Specificities revealed +DTT

- Impact of DTT apparent for both HLA CI and CII SA specificities
- In some cases, specificities revealed +DTT were part of a CREG that was detected –DTT especially for CII
- In HSP sera, effect of +DTT more apparent, resulting much greater increase in MFI to many CI and CII specificities mainly DQ
- DTT treatment revealed IgG HLA Abs to both CI and CII specificities including: A1, A2, A23, A24, A32, A68, B7, B8, B49, DR8, DQ2, DQ5, DQ6, DQ7, DQ8 and DQ9
- Weaker specificities knocked off +DTT included: A1, A11, B57, DR4, DR15



CI/CIISA specificities +DTT weaker or negative

Specificity	Normalised MFI - DTT	Normalised MFI +DTT
A1 (DSA)	1095	92
A11	4144	1861
B57	2500	200
DR4 (DSA)	1341	284
DR15	4075	1970



Implications for future Luminex SA testing

- ?do we routinely test all samples +DTT?
 - ?weak IgG Abs get knocked off +DTT
 - ?test only HSPs samples +DTT
 - Establish the extent of the problem ???????
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