

AFDT

Proficiency Testing Program

Report

Prepared by Dod Stewart

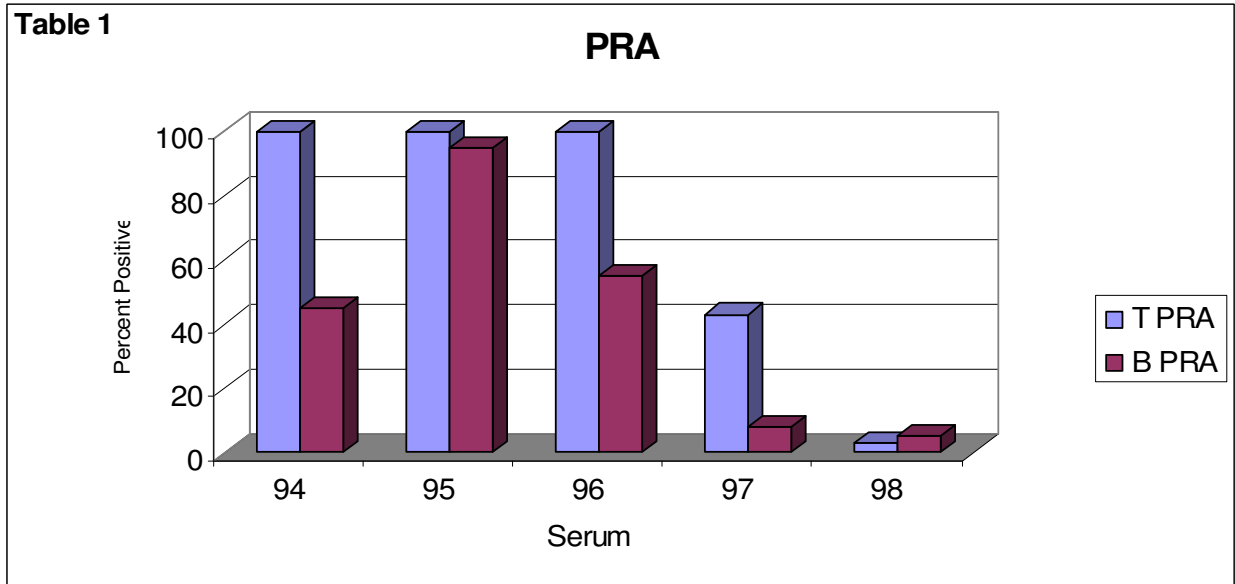
Reviewed by Jean Heneghan

AFDT Proficiency Testing Results –September 10, 2007

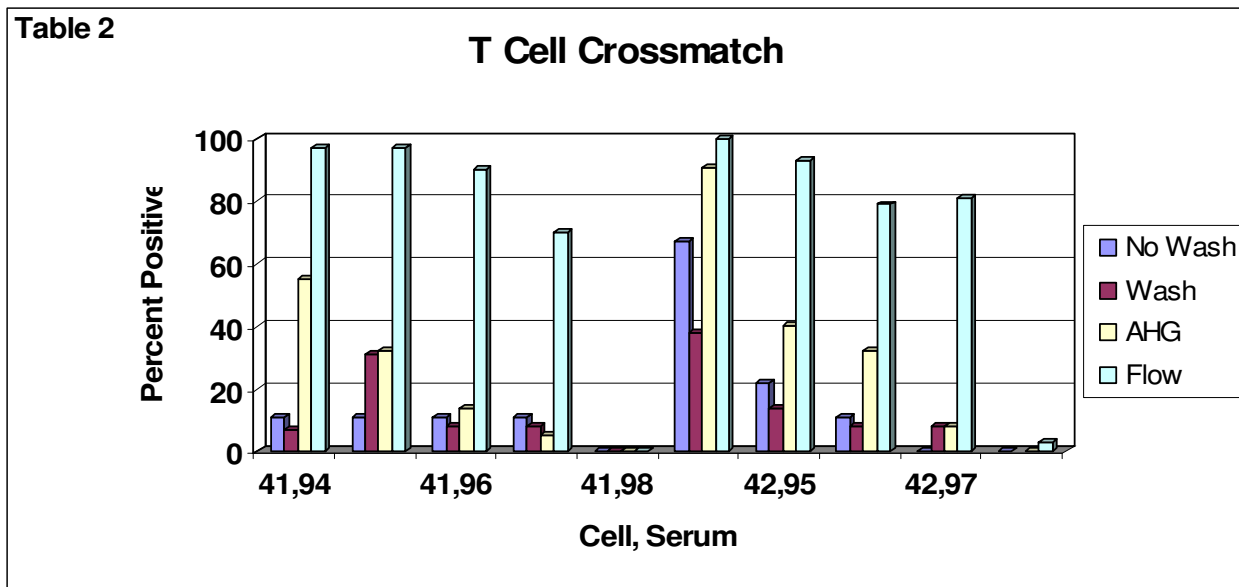
SUMMARY REPORT:

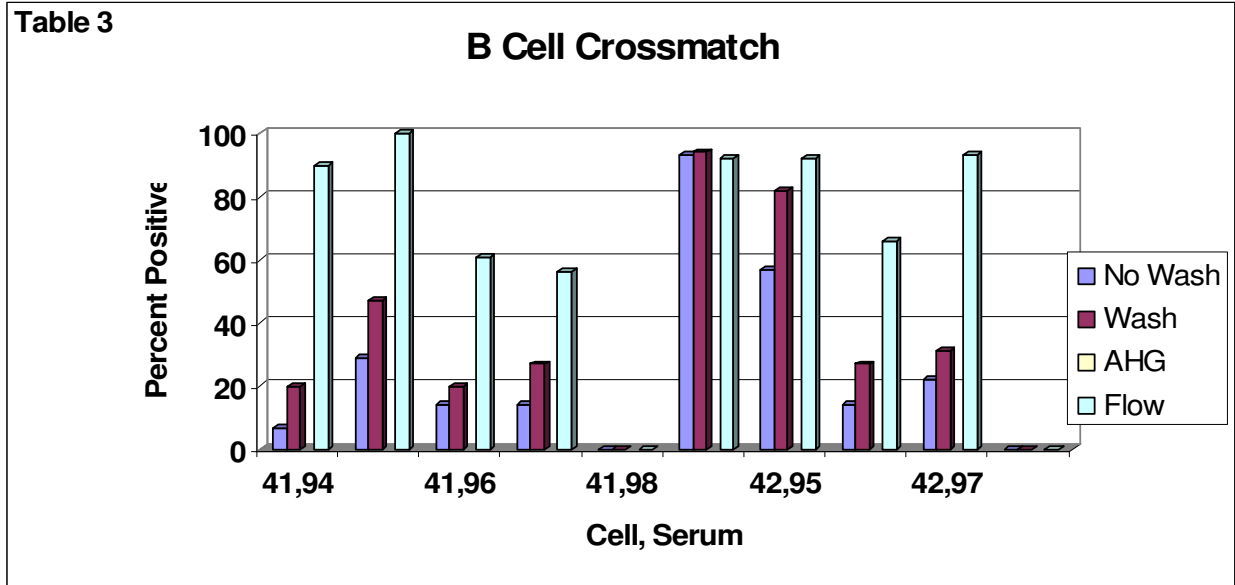
The September 10, 2007 Crossmatch / PRA exchange is the final crossmatch send-out for 2007. Kathie Nelson, Pauline Lai-Kwan and Patricia Kopko, MD and the staff at Blood Source in Sacramento, CA very capably provided all of the testing materials for this interesting exchange. AFDT owes a great debt to this lab for being a sendout lab. The mission and goals of AFDT Proficiency Testing is to provide cells and sera that approximate, as closely as possible, those clinical samples that are tested on a routine basis in most labs. This more accurately predicts how a lab functions clinically on a day-to-day basis. We feel that these AFDT Proficiency Testing Samples are more relevant and indicative of actual clinical situations and therefore more appropriate to meet the intent of CLIA, UNOS, CAP and ASHI guidelines and standards. The May 2007 send-out included five sera with complicated specificities. Some of these specificities were undetectable by standard CDC serological testing methods, and therefore significantly more difficult to detect by using serology alone. The results reported by most labs using techniques other than the standard CDC indicate that these sera do indeed contain Class I and Class 2 antibodies. Consensus has been changed from 85% to 80% this year. At the request of participants, for the second time Luminex results have been separated and analyzed separately from flow results. As we have seen in the previous surveys, the results from this survey were most interesting and informative.

A summary of PRA'S can be seen in Table 1.



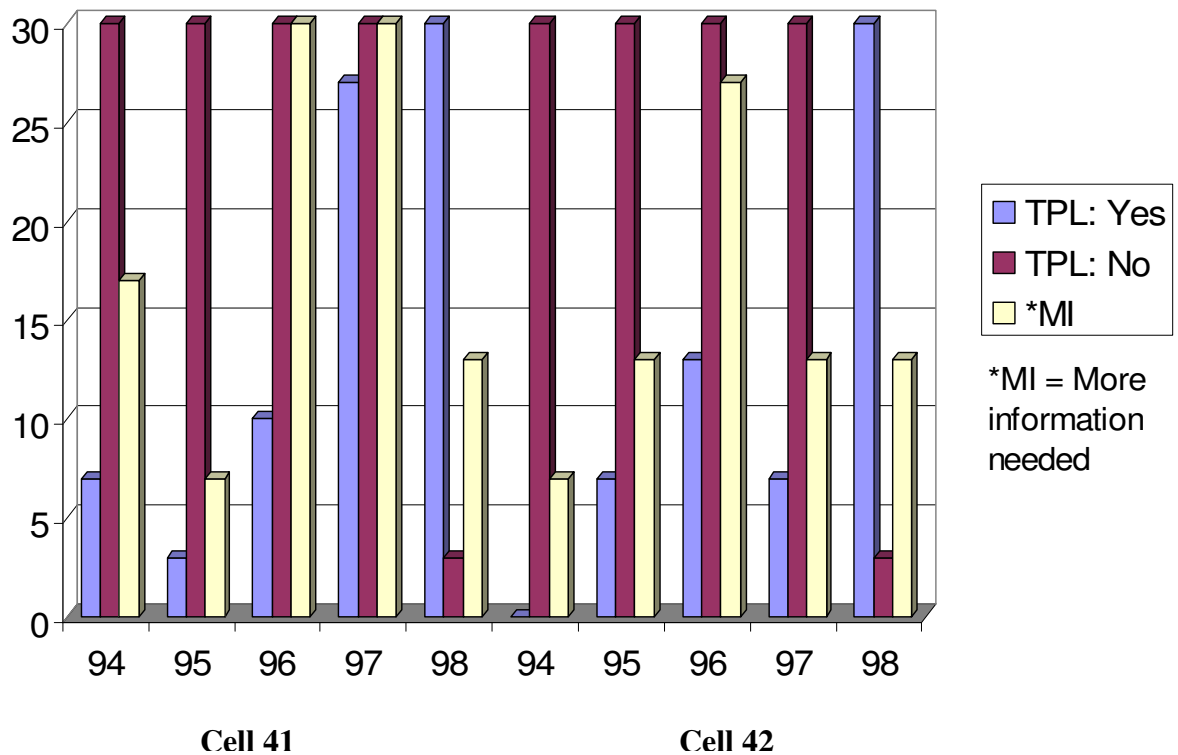
Crossmatching was performed and analyzed by the cell type and the various methods and techniques reported. (See Tables 2 and 3).





As a final question, each lab was also asked to indicate either whether a particular crossmatch pair would be transplanted or not at their respective centers, or if more information is needed. The results are summarized after each analysis in table 4. (Several laboratory directors commented that this question should always be answered “more information needed”, and is no longer pertinent since many centers now use desensitization and rescue protocols.)

Table 4

Transplant: Yes/No**September 2007 Crossmatch/PRA****Cells: Race: Phenotype:**

CC41: Cauc: HLA: A*11, A*24; B*51, B*40 (61); Bw4, Bw6; Cw*02, Cw*15; DRB1*0701, DRB1*11; DRB3* (DR52), DRB4*(DR53); DQB1*02, DQB1*0301(DQ7)

CC42: Cauc: HLA: A*02, A*03; B*37, B*15 (B62); Bw4, Bw6; Cw*06, Cw*03(Cw9); DRB1*10, DRB1*11, DRB3*03 (DR52), -; DQB1*05, DQB1*03 (DQ7)

Sera / Reported Specificities:

Bolded specificities without () indicates 80% or more labs reported this result, therefore consensus was reached. Specificities with () indicate that the

majority (50% or more labs) reported this result. Luminex results are written in *italics* and **bolded text** indicates 80% or more labs reported these results and 50% of the labs reported those in *italics and parentheses*.

CS94 - Anti - Class 1:

B5Br,B15Br,B17Br,B21Br,B35,B53,(B12Br),(B45),(B49),(B50),(B51),(B52),(B56),(B57),(B58),(B62),(B63),(B70Br),(B75)
B5Br,B12Br,B15Br,B17Br,B21Br,B35,B53,B70Br,(B15),(B46),(B49),(B50),(B51),(B52),(B56),(B57),(B58),(B62),(B63),(B71),(B75),(B78)

Class 2: Negative *Negative*

CS95 - Anti - Class 1: **A1,A3,A9Br,A11,A36**,(A23),(A24),(A80),

(B7),(B7Br),(B8),(B12Br),(B15Br),(B18),(B35),(B45),(B62),
A1,A3,A9Br,A11,A36,A80,(A23),(A24),(B8),(B12Br),(B15Br),(B18),(B35),(B42),(B76)

Class 2:

DR3Br,DR5Br,DR6Br,DR7,DR8,DR17,(DR11),(DR12),(DR13),(DR14),(DR18),**DR52**,(DQ2),
DR3Br,DR5Br,DR6Br,DR7,(DR8),(DR11),(DR13),(DR14),(DR17),(DR18),**DR52**

CS96 - Anti - Class 1:

A19Br,A33,(A9Br),(A10Br),(A25),(A28Br),(A32),(A33),B14Br,B18,(B5Br),(B8),(B12Br),(B14),(B15Br),(B16Br),(B21Br),(B35),(B49),(B62),
(A10Br),(A19Br),(A28Br),(A32),(A33),**B15Br,B18,B35**,(B5Br),(B8),(B12Br),(B13),(B14Br),(B16Br),(B21Br),(B40Br),(B53),(B62),(B70Br),(B78)

Class 2: DR4, **DR4**

CS97 - Anti - Class 1:

A2,A2Br,A9Br,A28Br,(A23),(A24),(A68),(A69),**B13,B17Br,B27**,(B12Br),(B16Br),(B37),(B38),(B44),(B47),(B57),(B58),
A2,A2Br,A9Br,A28Br,(A23),(A68),**B13,B17Br,B27**,(B12Br),(B16Br),(B37),(B38),(B40Br),(B47),(B53),(B57),(B58),(B59)

Class 2: Negative *Negative*

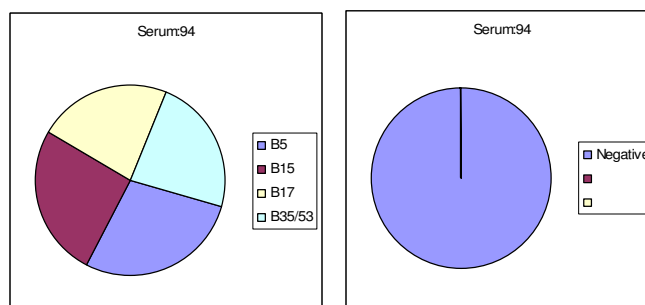
CS98 - Anti - Class 1: Undetermined *Negative*

Class 2: Undetermined *Negative*

RESULTS: SERUM CS94

Antibody Analysis CS94

Class 1: **B5Br, B15Br, B17Br, B21Br, B35, B53, (B12Br), (B45), (B49), (B50), (B51), (B52), (B56), (B57), (B58), (B62), (B63), (B70Br), (B75)**



B5Br, B12Br, B15Br, B17Br, B21Br, B35, B53, B70Br, (B15), (B46), (B49), (B50), (B51), (B52), (B56), (B57), (B58), (B62), (B63), (B71), (B75), (B78)

Class 2: Undetermined *Negative*

PRA Results

CS94 is a highly reactive serum containing predominantly Class 1, (and possibly Class 2) antibodies. All (100%) labs reported Class 1 reactivity. The range for T-cell/ Class 1 PRA was 0- 85%. The table below has the complete breakdown by methods. As expected solid phase assays (Flow, Luminex and ELISA) gave the most sensitive results. Additionally, labs using Wash techniques reported B8 antibodies. B8 was not detected by solid phase assays either.

Methods CS94	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	2	Positive	0 -22	11	Undetermined
Wash-T	4	Positive	0 -12	6	B8
AHG-T	13	Positive	0 -68	34	B5, B15, B17, B21, B35, B53
Flow Class I	26	Positive	1-85	43	B5, B15, B17, B21, B35, B53
ELISA Class 1	13	Positive	42-78	60	B5, B15, B17, B21, B35, B53
Luminex 1	17	Positive	NR	NR	<i>B5, B12, B15, B17, B21, B35, B53, B70</i>

Forty- Five percent (45%) of the labs reported Class 2 reactivity, somewhat less than the majority of the labs reporting. CS94 B-cell / Class 2 screening PRA values ranged from 0- 43%, with the highest PRA assigned by ELISA methods. Labs were unable to assign specificities, although DQ2 was reported by some flow labs. The complete breakdown is as follows:

Methods CS94	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	2	Negative	0-0	0	Negative
Wash-B	8	Positive	0-23	12	Undetermined
AHG-B	0	NT			NT
Flow Class 2	26	Positive	0 - 43	22	DQ2
ELISA Class 2	11	Positive	0 -13	7	Undetermined
Luminex 2	12	Positive	0	0	Undetermined

Crossmatching Results: CS94 vs. CC41 This cell-serum combination should have produced positive crossmatches due to the strong B5 and B15 CREG antibodies and the corresponding types found on Cell CC41. For labs using B-cells, all methods, with the exception of flow, did reach Negative consensus for CS94/CC41.

Note: The inconsistencies in the total number of labs for T-cell and B-cell results are because not all labs reported all methods each time. The actual number of lab responses is in the column "No Labs Total".

Crossmatch Consensus Results – CS94/CC41

Methods CS94/CC41	No Labs Total	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	1	8	89	Negative	1	13	93	Negative
Wash	13	1	12	93	Negative	3	12	80	Negative
AHG	22	10	12	45	Inconclusive	NT			Not Tested
Flow	30	29	1	97	Positive	26	3	90	Positive
ELISA	NT				Insufficient	NT			Insufficient

Transplant? Yes: 7 No: 77 More information needed: 17

CS94 Vs. CC42

Crossmatch Consensus Results – CS94/CC42

Results submitted for CS94 and CC42 were similarly unpredictable and unusual for T-cells in the results submitted by labs. T-cell crossmatches only reached Positive

consensus by Flow and AHG. The majority of the labs (67%) indicated positive using No-Wash methods.

Methods CS94/CC42	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	3	6	67	Inconclusive	5	9	36	Inconclusive
Wash	13	5	8	38	Inconclusive	14	1	93	Positive
AHG	22	14	9	91	Positive				NT
Flow	30	30	0	100	Positive	27	0	100	Positive
ELISA	0				NT				NT

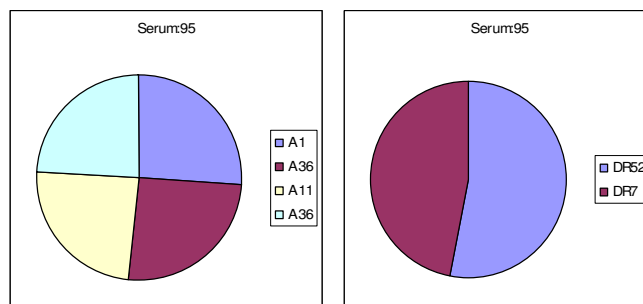
Transplant? Yes: 0 No: 93 More information needed: 7

SERUM CS95

Antibody Analysis:

Anti -Class 1: **A1,A3,A9,A11,A36**
A1,A3,A9,A11,A36,A80

Class 2: **DR3, DR5, DR6, DR7, DR8,**
DR17, DR52
DR3,DR5,DR6,DR7



PRA Results

All labs (100%) assigned a T-cell / Class1 PRA to CS95. The range was 0 - 97%. A1 CREG was the predominant antibody reported by all methods. B8 and B18 were reported by labs using AHG and Flow, but did not reach consensus. Some labs, specifically those using Luminex, also reported A80 antibodies. The complete results are below:

Methods CS95	No Lab s	Consensus	% PRA Range	Medi an PRA	Specificity
No-wash T	4	Positive	0 - 23	12	A1,A11,A36, A66
Wash-T	6	Positive	0 - 30	5	A1, B8
AHG- T	16	Positive	5 - 44	25	A1, A36, B 8
Flow Class I	28	Positive	50- 96	73	A1,A3,A9,A11,A36, B8, B18

ELISA Class 1	13	Positive	17- 97	57	A1,A3,A9,A11,A36
Luminex 1	12	Positive			A1,A3,A9,A11,A36,A80

Almost all labs (95%) reported Class 2 reactivity by all methods, especially Elisa, flow and Luminex. Antibodies to DR17 were reported by Wash B methods solid phase assays all reported DR3, DR5, DR6, DR7, DR8 and DR52. All reached consensus by most methods. The PRA ranged from 4 -94% with Flow having the highest (69%).The complete results are below:

Methods CS95	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	2	Positive	18-18	18	Undetermined
Wash-B	8	Positive	4-43	24	DR17
AHG-B	0	NT			Undetermined
Flow Class 2	26	Positive	44-94	69	DR3,DR5,DR6,DR7
ELISA Class 2	11	Positive	31-81	56	DR3,DR5,DR6,DR7,DR8,DR17,DR52
Luminex 2	12	Positive			DR3,DR5,DR6,DR7,DR52

Crossmatching Results: CS95 Vs. CC41

Crossmatch Consensus Results – CS95/CC41

Similarly inconsistent patterns were observed in this combination. Serological methods were somewhat less sensitive and produced the inconclusive patterns below seen in T cell subsets below. Class 2 reaction patterns reported by all labs by all methods were much like those reported in Class 1. Flow results were the only method that reached consensus in this combination.

Methods CC41/CS95	No Labs Total	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	1	8	89	Negative	4	10	71	Inconclusive
Wash	13	11	9	69	Inconclusive	7	8	53	Inconclusive
AHG	2	15	7	68	Inconclusive	NT			NT
Flow	30	29	1	97	Positive	29	0	100	Positive
ELISA	0				Insufficient				Insufficient

Transplant: Yes:3 No: 90 More Information needed: 7

Crossmatch Consensus Results – CS95/CC42

Similar inconsistent patterns were observed in this combination (CS95/CC42), as in the previous combination (CS95/CC41). There appears to be Class 2 antibodies directed against this cell, but the results reported positive B-Cell/ Class 2 reactions as seen below. Serological methods were not sensitive enough to detect these positive reactions, however No-Wash B was reported by (79%) of the labs, almost reaching the 80% consensus mark. T and B cell subsets are below:

Methods CC42/CS9 5	No Lab s Tota l	T-cell #	T-cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Con s		Pos	Neg	Con s	
No-wash	9	2	7	78	Inconclusive	3	11	79	Inconclusive
Wash	13	2	11	86	Negative	6	9	40	Inconclusive
AHG	22	9	13	60	Inconclusive	NT			NT
Flow	30	28	2	93	Positive	27	1	96	Positive
ELISA	NT				Insufficient				Insufficient

Transplant? Yes: 7 No: 80 More information needed: 1

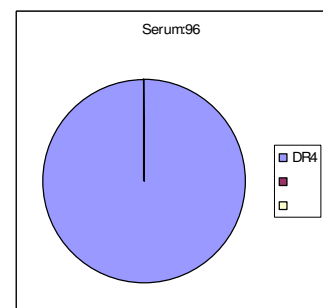
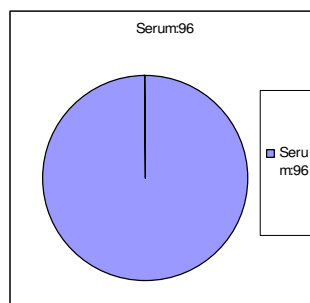
SERUM CS96

Antibody Analysis:

Anti – Class 1 : **A19Br, A33,
B15Br,B18,B35,B14**

Class 2: **DR4, DR4**

PRA Results:



All of the labs were able to determine whether there was Class 1 reactivity or not. B14 was detected by all methods and reached consensus by all methods. B18 and A9 were only detected by labs using solid phase assays. Luminex labs also reported reactivity to B15 and B35. This was in the majority although it did not reach consensus. The Class 1/ T-cell PRA range was 0 - 99%. Class 1 PRA did reach positive consensus by any methods. The results are seen below:

Methods CS96	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	0 -14	7	B14
Wash-T	6	Positive	0 - 12	6	B14
AHG-T	16	Positive	0 - 50	25	B14
Flow Class I	28	Positive	45-99	72	B14, B18, A9
ELISA Class 1	13	Positive	20-77	49	B14, B18
Luminex 1	12	Positive			<i>B14,B15,B18,B35</i>

The majority of the labs (55%) reported a B- cell/ Class 2 PRA, but not enough to reach consensus by any methods, including Luminex. The range was 0 - 90%. DR4 did reach consensus by solid phase assays, in addition to finding antibodies to DR7 and DR53 by Flow only. The breakdown by methods is as follows:

Methods CS96	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Negative	0	0	None
Wash-B	10	Inconclusive	0 - 21	11	Inconclusive
AHG-B	0	NT			
Flow Class 2	26	Positive	0 - 90	45	DR4, DR7,DR53
ELISA Class 2	11	Positive	0-18	9	DR4
Luminex 2	12	Positive			DR4

Crossmatching Results: CS96 Vs. CC41

Crossmatch Consensus Results – CS96/CC41

T-cell and B-cell crossmatches were quite concordant, by all methods based on the antibodies reported and the phenotype of CC41. All methods reached a negative consensus, with the exception of B-cell flow.

Method CS96/CC41	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	1	8	89	Negative	2	12	86	Negative
Wash	13	1	12	92	Negative	1	15	93	Negative
AHG	23	3	19	86	Negative	NT			Insufficient
Flow	29	3	26	90	Negative	17	11	61	Inconclusive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 10 No: 57 More information needed: 33

CS96 Vs. CC42

Crossmatch Consensus Results – CS96/CC42

T-cell/ Class 1 and B-cell/ Class 2 crossmatches were again quite inconsistent consensus negative by most methods, except by Flow, which was almost (79%) consensus positive.

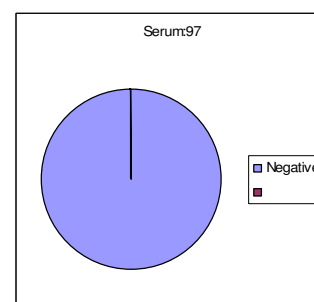
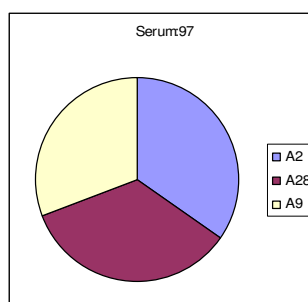
Method CS96/CC42	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	1	8	89	Negative	2	12	86	Negative
Wash	13	1	12	92	Negative	4	11	73	Inconclusive
AHG	22	7	15	68	Inconclusive	NT			NT
Flow	29	23	6	79	Inconclusive	18	9	66	Inconclusive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 13 No: 60 More information needed: 27

SERUM CS97

Antibody Analysis:

Anti - Class 1: **A2, A2Br, A9Br, A28Br,**
(A23), (A24), (A68),
(A69), **B13, B17Br, B27,**



(B12Br), (B16Br), (B37), (B38), (B44), (B47),
 (B57),(B58),
A2,A2Br,A9Br,A28Br,(A23),(A68),B13,B17Br,B27,(B12Br),(B16Br),
(B37),(B38),(B40Br),(B47),(B53),(B57),(B58),(B59)

Class 2: **Negative** **Negative**

PRA Result:

T-cell/ Class 1 PRA's ranged from 0 - 82%. Some of the labs (43%) reported Class 1 reactivity, and enough labs B13, B17 and weak A2 were consensus positive by most methods. A-locus antibodies were reported by most sensitive methods, and some did reach consensus: A2. Additional A locus antibodies (A28, A9) were reported by Luminex. The labs reported the following results:

Methods CS97	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	0-23	12	B13, B17
Wash-T	6	Positive	0 - 24	12	B13
AHG-T	16	Positive	0 - 50	25	B13, B17, A2
Flow Class I	28	Positive	35 -82	59	B13, B17, B27, A2
ELISA Class 1	17	Positive	30- 82	56	B13, B27, B17
Luminex 1	12	Positive			A2,A9,A28,B13,B17,B27

B-cell screening PRA values ranged from 0 to 38% depending on the technique used. Only 8% of the labs reported positive B cell/ Class 2 antibodies, by all methods. The breakdown by technique is as follows:

Methods CS97	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Negative	0 -0	0	None
Wash-B	8	Undetermined	0-38	19	None
AHG-B	0	NT			
Flow Class 2	26	Negative	0-5	3	None
ELISA Class 2	11	Negative	0-5	3	None
Luminex 2	12	Undetermined			None

Crossmatching Results: CS97 Vs CC41**Crossmatch Consensus Results – CS97/CC41**

T-cell cross matches were predicted to be consensus negative. B-cell crossmatches were all predicted to be negative as well. The complete inconsistent breakdown is below.

Methods CS97/CC41	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	1	8	89	Negative	2	12	86	Negative
Wash	13	1	12	92	Negative	4	11	73	Inconclusive
AHG	22	1	21	95	Negative	NT			NT
Flow	30	21	9	70	Inconclusive	16	13	56	Inconclusive
ELISA	0				Insufficient				

Transplant? Yes: 83 No: 3 More Information needed: 13

CS97 Vs. CC42**Crossmatch Consensus Results – CS97/CC42**

T-cell crossmatches were all majority positive by all methods except by flow. B-cell crossmatches were less consistent, but were consensus positive by flow methods. The complete breakdown is below.

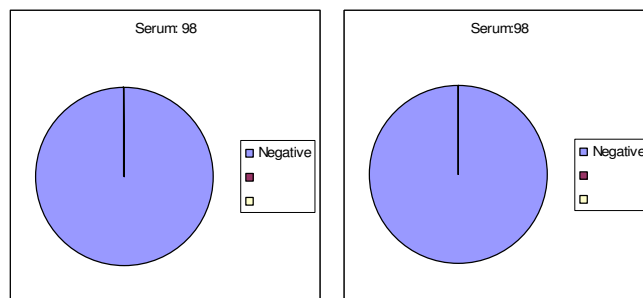
Methods CS97/CC42	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	2	7	78	Inconclusive	4	10	71	Inconclusive
Wash	3	4	9	69	Inconclusive	15	10	67	Inconclusive
AHG	22	13	9	51	Inconclusive	NT			NT
Flow	30	30	0	100	Positive	26	2	93	Positive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 10 No: 77 More information needed: 13

SERUM CS98**Antibody Analysis :**

Anti - Class 1: Negative

Class 2: Negative

**PRA Results:**

T-cell/ Class 1 PRA results ranged from 0 - 26%. All methods were consensus Negative for this serum. . The labs reported the following results:

Methods CS93	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Negative	0 - 9	5	Inconclusive
Wash-T	6	Inconclusive	0 - 26	13	Inconclusive
AHG-T	17	Inconclusive	0 - 7	4	Inconclusive
Flow Class I	33	Inconclusive	0 - 13	7	Inconclusive
ELISA Class 1	16	Inconclusive	0 -17	9	Inconclusive
Luminex 1	9	Negative			Negative

B-cell / Class 2 screening PRA values ranged from 7-97% depending on the techniques used. Positive consensus was observed in many of the methods tested. The breakdown, by technique is as follows:

Methods CS98	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Negative	0	0	None
Wash-B	10	Positive	0- 21	11	None
AHG-B	0	NT			NT
Flow Class 2	32	Negative	0-2	1	None

ELISA Class 2	15	Inconclusive	0-17	9	Inconclusive
Luminex 2	9	Negative			None

Crossmatching Results: CS98 Vs. CC41

T-cell and B-cell crossmatches were consensus negative by all methods,. due to the cell phenotypes and the reported antibodies.

Crossmatch Consensus Results – CS98/CC41

Methods CS98/CC41	No Labs Total	T-cell #	T-cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
		Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	0	9	100	Negative	0	14	100	Negative
Wash	13	0	13	100	Negative	0	15	100	Negative
AHG	23	0	23	100	Negative	NT			NT
Flow	28	0	28	100	Negative	27	0	100	Negative
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 83 No: 3 More information needed: 13

CS98 Vs. CC42

Crossmatch Consensus Results – CS98/CC42

T-cell and B-cell crossmatches were predicted to be negative, which they were.
The breakdown is below.

Methods CS98/CC42	No Labs Total	T- cell #	T- cell #	%T- cell	Result	B- cell #	B- cell #	%B- cell	Result
		Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	9	0	9	100	Negative	0	14	100	Positive
Wash	12	0	12	100	Negative	10	9	53	Inconclusive
AHG	23	0	23	100	Negative	NT			NT
Flow	30	0	30	100	Negative	27	0	100	Positive

ELISA	0				Insufficient				
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Transplant? Yes: 83 No: 3 More information needed: 13

Conclusions:

As in the past, labs using enhanced serological methods (AHG) and those labs using solid phase assays (Flow, Luminex and ELISA) reported significantly more antibody specificities than labs using less sensitive serological methods. Luminex and Flow results were separated again this time, for the second time for the analysis. With regard to antibody analysis, Luminex technology appears to be quite sensitive and possibly somewhat more sensitive than flow cytometry. Currently there are neither ELISA nor Luminex crossmatch techniques available, and no results reported to correctly compare crossmatch results with their respective antibody specificities reported. We encourage any labs using Luminex techniques to submit the results separately from their other solid phase methods. All solid phase methods are more sensitive than CDC, with the exception of AHG enhanced CDC. The next cell send-out will be November 5, 2007.