

## **AFDT**

### **Proficiency Testing Program Report**

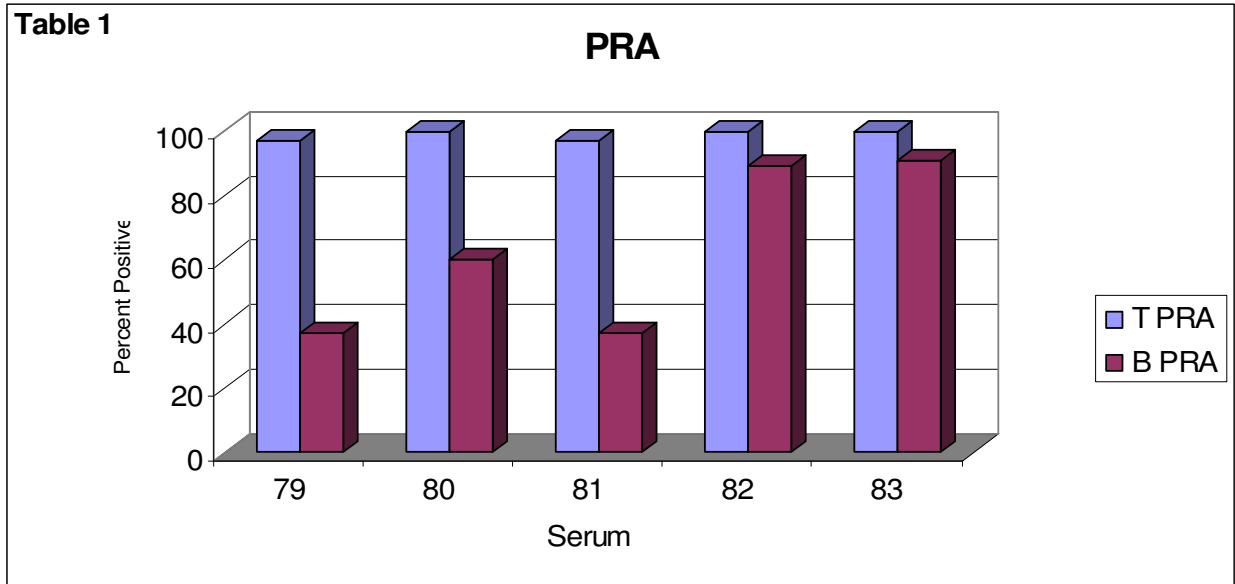
Prepared by Dod Stewart  
Reviewed by Jean Heneghan

## **AFDT Proficiency Testing Results - May 2006**

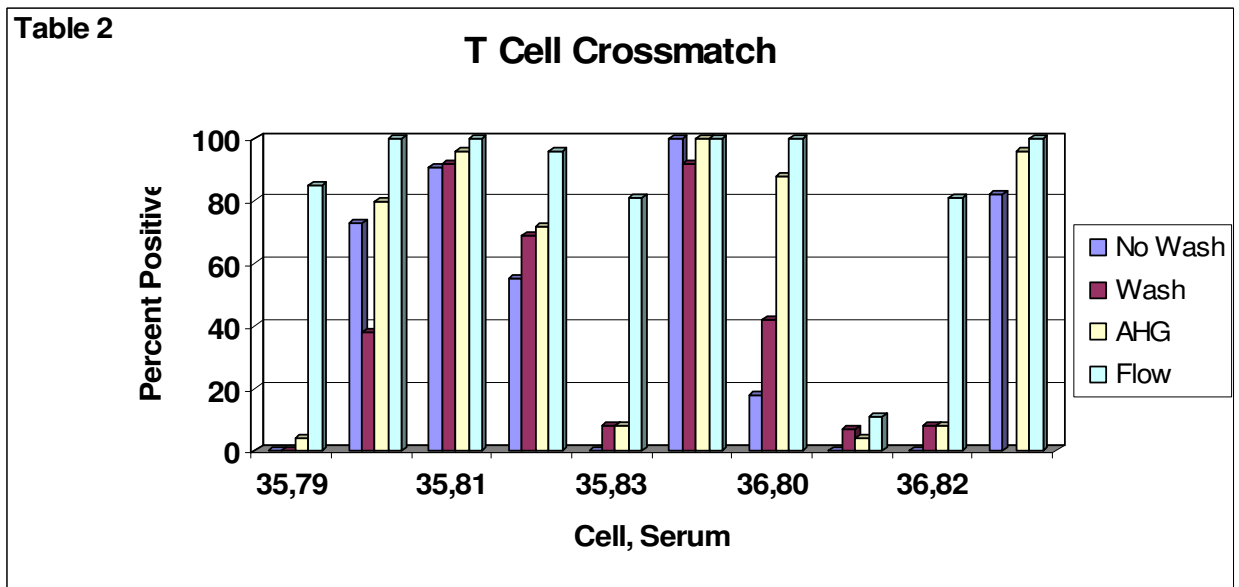
### **SUMMARY REPORT:**

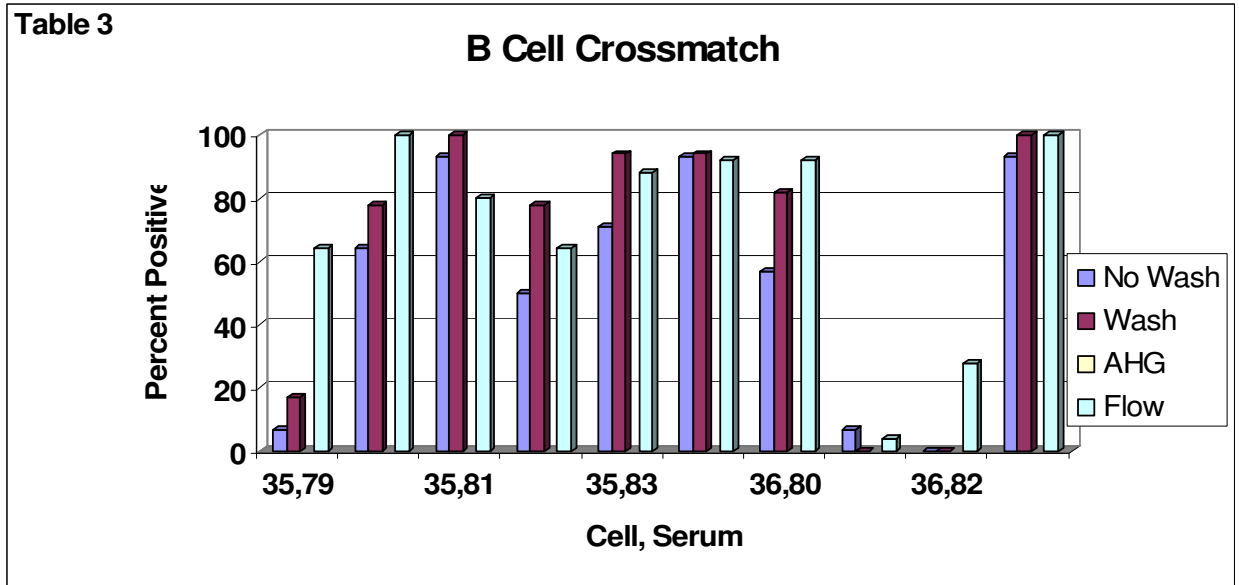
The May 2006 Crossmatch / PRA exchange is the first for 2006. The mission and goals of AFDT Proficiency Testing is to provide cells and sera that approximate, as closely as possible, those clinical samples that are tested on a routine basis in most labs. This more accurately predicts how a lab functions 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 2006 send-out included five sera of known specificity. Several of these sera were relatively weak by standard CDC serological testing methods, and therefore more difficult to detect by serology. The results reported by most labs using techniques other than the standard CDC indicate that these sera do indeed contain Class 1 and Class 2 antibodies. Consensus has been changed from 85% to 80% this year. At the request of participants, for the first 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.

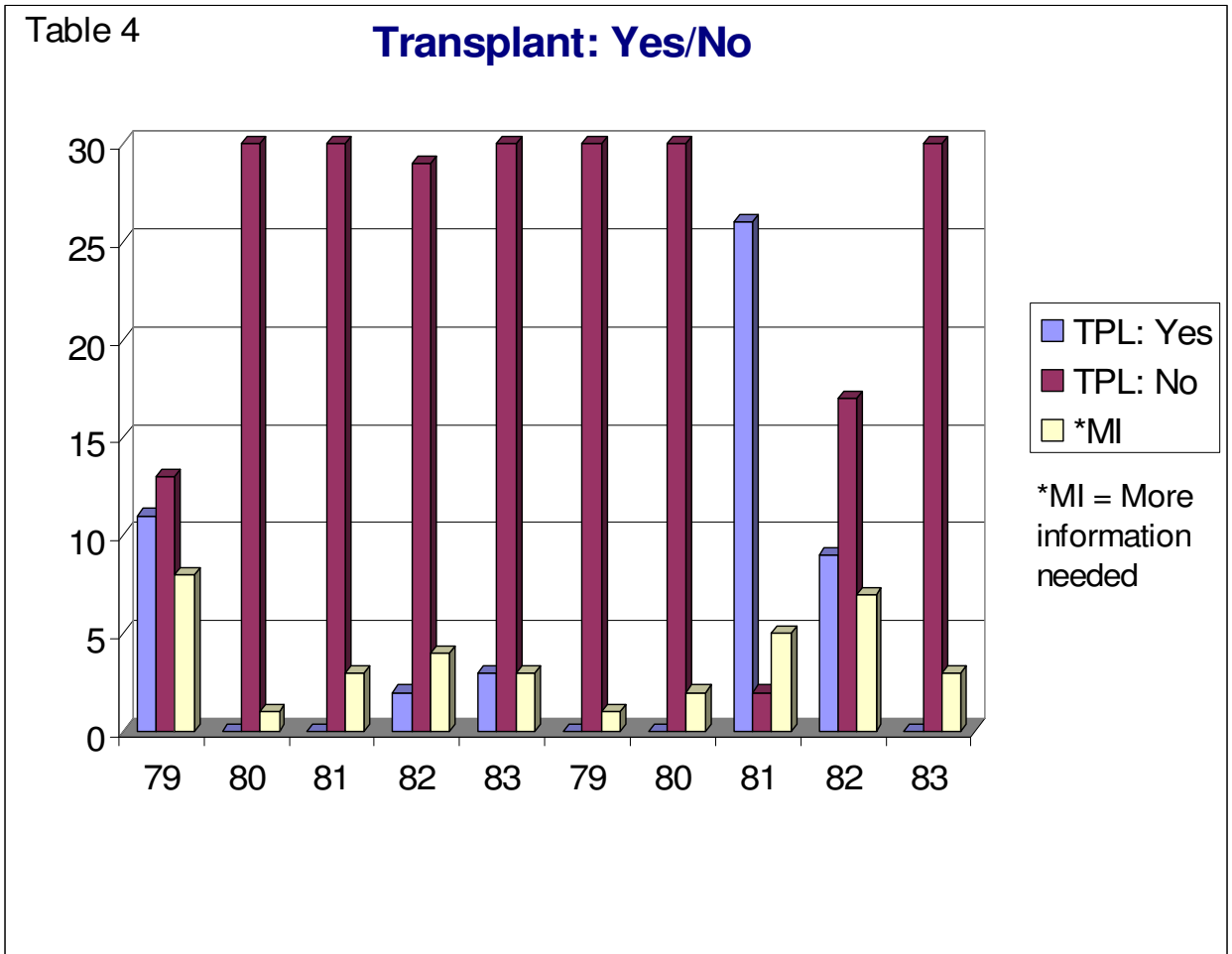


Crossmatching was performed and analyzed by the cell type and the various methods and techniques reported. (See Tables 2 and 3).





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.)



## May 2006 Crossmatch/PRA

**Cells: Race: Phenotype:**

**CC35: Cauc: HLA: A\*01, \*29; B\*35, \*44; Bw4, Bw6; Cw\*04, \*16  
DRB1\*07, \*14; DRB\*3, 4; DQB1\*02, 05**

**CC36: Cauc: HLA: A\*02, \*03; B\*07, X; Bw6; Cw\*07, X  
DRB1\*0301/04, \*15; DRB3, 5\*; DQB1\*02, \*06**

**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.

**CS79** - Anti - Class 1: **B7**, (B27, B40, B42, B48, B60), (A3, A11, A19)

Class 2: Negative

**CS80** - Anti - Class 1: **B40**, (B7, B27, B12, B13, B41, B60), (A2, A28, A29, A33)

Class 2: Positive (Undetermined)

**CS81** - Anti - Class 1: **B5, B35**, (B15, B17, B18, B21, B70, B53)

Class 2: (Undetermined)

**CS82** - Anti - Class 1: (A3, A19)

Class 2: **DR1**, (DR10, DR103)

**CS83** - Anti - Class 1: **B7, B27, B40**, (B8, B13, B41, B42, B47, B48, B81)

Class 2: (DR2), (DQ1)

## RESULTS:

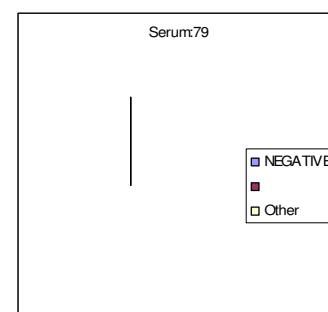
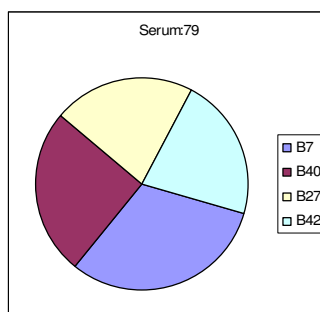
### SERUM CS79

#### Antibody Analysis

**CS79** - Anti –

Class 1: **B7**, (B27, B40, B42, B48, B60), (A3, A11, A19)

Class 2: Negative



#### PRA Results

The range for T-cell/ Class 1 PRA was 2 - 100 %. The table below has the complete breakdown by methods. As expected solid phase assays (Flow, Luminex and ELISA) gave the most sensitive results. At the request of several participants, Flow and Luminex results are separated (for the first time), in this exchange. CDC methods detected B7 and B81, but only B7 reached consensus by all methods. B27 (67%), B40 (78%) and B42 (67%), B48 (64%) were also reported by labs using enhanced CDC assays and solid phase methods. A3 (61%), A10 (44%), A11 (50%) and A19 (53%) were also reported by these participants. It should be noted that all methods reached a T-cell/ Class 1 consensus positive. Bolded text indicates specificities that reached consensus.

Methods CS79	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	2	Positive	12 -19	16	<b>B7</b> ,(B81)

Wash-T	5	Positive	2 - 38	20	<b>B7</b> , (B27,B61,B81)
AHG-T	13	Positive	10 -100	55	<b>B7</b> ,(B27) (A3,11)
Flow Class I	25	Positive	50 -100	75	<b>B7</b> , (B27,40,67,81,42) (A3,10,11,29,31,19)
ELISA Class 1	14	Positive	25 - 96	61	<b>B7</b> ,(B42,67,81) (A3,10,11,29,31,19)
Luminex 1	9	Positive			<b>B7,27,40</b> (B22,42,4748,81) (A3,10,11,19)

B-cell/ Class 2 screening PRA values ranged from 0 to 22 % with consensus negative being reached by all methods.

The complete breakdown is as follows:

Methods CS79	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	0	NT	-	-	None
Wash-B	5	Negative	4 - 22	13	None
AHG-B	0	NT			Insufficient
Flow Class 2	27	Negative	0 - 9	5	None
ELISA Class 2	14	Negative	0 - 3	2	None
Luminex 2	9	Negative			None

**Crossmatching Results: CS79 vs. CC35** This cell-serum combination should have been negative based on the cell phenotype and the “predominant” reported antibody specificities. All methods, except Flow, reached negative consensus. Labs performing flow crossmatches all reported positive crossmatches, in contrast to the other methods. The positive crossmatch is due to the antibody to A29, on the CS79, also reported by labs using solid phase assays. A similar pattern was seen with B cells; 38% of the labs also reported positive results for B cell flow, which probably was the Class 1 antibody in this sample, being detected by B cells.

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

**Crossmatch Consensus Results – CS79/CC35**

Methods	No Labs Total	T-cell	T-cell	%T-cell	Result	B-cell	B-cell	%B-cell	Result
		#	#	cell		#	#	cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	10	0	10	100	Negative	0	13	100	Negative
Wash	12	1	11	92	Negative	3	14	82	Negative
AHG	24	1	23	96	Negative	NT			Insufficient
Flow	26	22	4	85	Positive	9	15	38	Inconclusive
ELISA	NT				Insufficient	NT		Insufficient	Insufficient

Transplant? Yes: 11 No: 13 More information needed: 8

**CS79 Vs. CC36****Crossmatch Consensus Results – CS79/CC36**

CS79 and CC36 were predicted to be both T-cell and B-cell positive, because of the strong antibody to B7 and the apparent homozygosity of B7 on cell CS36. All methods reached the predicted positive consensus for this cell-serum combination.

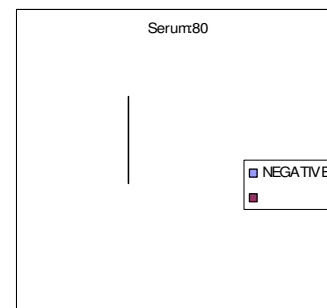
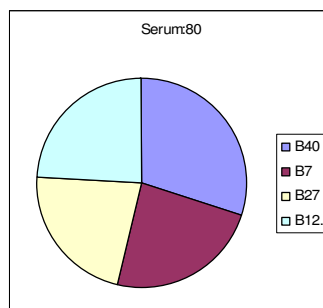
Methods	No Labs Total	T-cell	T-cell	%T-cell	Result	B-cell	B-cell	%B-cell	Result
		#	#	cell		#	#	cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	10	10	0	100	Positive	12	1	92	Positive
Wash	12	11	1	92	Positive	16	1	94	Positive
AHG	24	24	0	100	Positive	NT			Insufficient
Flow	27	27	0	100	Positive	23	2	92	Positive
ELISA	0				Insufficient				

Transplant? Yes: 0 No: 32 More information needed: 1

**SERUM CS80****Antibody Analysis:**

Anti - Class 1: B40 (B7,27,12,13,41,60)  
(A2,28,29,33)

Class 2: Undetermined



## PRA Results

All (100%) of the labs assigned a T-cell / Class1 PRA to CS80. The range was 22 - 100%. B40 (B60 and B61) (86%) reached consensus by all methods. B13 (64%), B41 (56%), B12 (B44 and B45) (69%) ,B7 (67%),B27 (64%),were also reported by almost all methods. Additional A locus antibodies ( see chart below) were also reported by labs using enhanced CDC and solid phase assays: A2 (61%), A28(64%), A33 (50%), A19(64%). The complete results are below:

Methods CS80	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	22-37	30	<b>B40</b> , (13)
Wash-T	8	Positive	22-58	40	<b>B40</b> , (B7, 27, 12,13,41,60)
AHG-T	16	Positive	27-88	58	<b>B40</b> ,(27) (A33,A2,28,19))
Flow Class I	28	Positive	63-100	82	<b>B40</b> , (B7, 27, 12,13,41,60) (A2,28,33,19)
ELISA Class 1	17	Positive	60-100	80	<b>B40</b> , (B7, 27, 12,13,41,60) (A2,28,19,33)
Luminex 1	9	Positive			<b>B12</b> , (B13,21,27,40,47,7) (A2,28,19,33)

Almost all labs reported Class 2 reactivity by all methods, reaching consensus positive for this serum. The specificities most often reported were DR7 (39%) and DR9 (22%) and DQ3 (22%). The B- cell/ Class 2 PRA's ranged from 0 -22%. The PRA reached consensus positive by all methods. The complete results are below.

Methods CS80	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Positive	15 -15	15	Undetermined
Wash-B	10	Positive	4 - 22	13	
AHG-B	0	NT			Insufficient
Flow Class 2	32	Positive	0 - 9	5	Undetermined
ELISA Class 2	15	Positive	0 - 3	2	Undetermined
Luminex 2	9	Positive			(DR7)

## Crossmatching Results: CS80 Vs. CC35

### Crossmatch Consensus Results – CS80/CC35

Very inconsistent patterns were observed in this combination. There appears to be a B12 antibody, detectable by enhanced and solid phase assays only. This explains the positive consensus reached by these crossmatch methods below. Serological methods were clearly less sensitive and produced the inconclusive patterns below seen in both T and B cell subsets below:

Methods	No Labs Total T/B	T-cell # Pos	T-cell # Neg	%T-cell Cons	Result	B-cell # Pos	B-cell # Neg	%B-cell Cons	Result
No-wash	10	3	7	30	Inconclusive	9	5	64	Inconclusive
Wash	13	7	5	42	Inconclusive	14	4	78	Inconclusive
AHG	24	20	4	83	Positive	NT			Inconclusive
Flow	27	27	0	100	Positive	25	0	100	Positive
ELISA	0				Insufficient				Insufficient

Transplant: Yes: 0 No: 32 More Information needed: 1

### Crossmatch Consensus Results – CS80/CC36

Similar inconsistent patterns were observed in this combination (CS80/CC36) as in the previous ones (CS80/CC35). There appears to be antibodies directed against A2 and B7 that are again detectable by enhanced and solid phase assays only. This explains the positive consensus reached by these crossmatch methods below. Serological methods were clearly less sensitive and produced the inconclusive patterns below seen in both T and B cell subsets below:

Methods	No Labs Total T/B	T-cell # Pos	T-cell # Neg	%T-cell Cons	Result	B-cell # Pos	B-cell # Neg	%B-cell Cons	Result
No-wash	10	2	8	20	Inconclusive	5	8	38	Inconclusive
Wash	11	6	5	55	Inconclusive	14	2	88	Positive
AHG	23	21	2	91	Positive	NT			Insufficient

Flow	27	27	0	100	Positive	23	2	92	Positive
ELISA	0				Insufficient				Insufficient

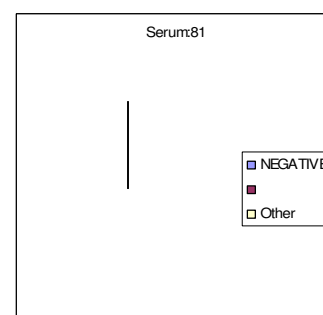
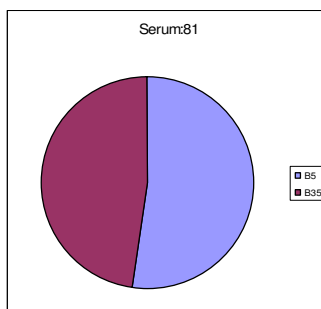
Transplant? Yes: 0 No: 31 More information needed: 2

## SERUM CS81

### Antibody Analysis:

Anti - Class 1: **B5, 35**  
(B15,17,18,21,70, 53)

Class 2: Undetermined



### PRA Results

Almost all of the labs assigned a T-cell/Class1 PRA to CS81. The Class 1/ T-cell PRA range was 22 -100%. Class 1 PRA did not reach positive consensus by any methods. B8 and B44 were reported by a few labs using flow. The results are seen below.

Methods CS81	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	33 - 41	37	<b>B5,35</b>
Wash-T	6	Positive	22 - 58	48	<b>B5,35</b> (B15,17,18,21,70,53)
AHG-T	17	Positive	27 - 88	61	<b>B5,35</b> (B15,17,18,21,70,53)
Flow Class I	33	Positive	63 -100	65	<b>B5,35</b> (B15,17,18,21,70,53)
ELISA Class 1	16	Positive	60 -100	66	<b>B5,35</b> (B15,17,18,21,70,53)
Luminex 1	9	Positive			<b>B5,35,53,21</b> (B15,17,18,70)

B-cell/ Class 2 PRA results did reach consensus by any methods reported, except Luminex which was consensus negative. The range was 0 - 62%. The breakdown by methods is as follows:

Methods CS81	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Inconclusive	44- 44	44	Inconclusive
Wash-B	10	Inconclusive	0 - 59	30	Inconclusive
AHG-B	0	Insufficient			Insufficient
Flow Class 2	32	Inconclusive	0 - 6	3	Inconclusive
ELISA Class 2	15	Inconclusive	0 - 62	31	Inconclusive
Luminex 2	9	Negative			None

### Crossmatching Results: CS81 Vs. CC35

#### Crossmatch Consensus Results – CS81/CC35

All T-cell crossmatches reached positive consensus. CS81 has very strong antibodies directed against B35 which is present on the phenotype of CC35. B-cell crossmatches, exhibited a similar pattern with this combination.

Method	No Labs Total	T-cell			Result	B-cell			Result
		#	#	%T-cell		#	#	%B-cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	10	1	91	Positive	12	1	92	Positive
Wash	13	12	1	92	Positive	17	0	100	Positive
AHG	24	23	1	96	Positive	NT			Insufficient
Flow	26	26	0	100	Positive	20	5	80	Positive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 0 No: 30 More information needed: 3

### CS81 Vs. CC36

#### Crossmatch Consensus Results – CS81/CC36

T-cell crossmatches were consensus negative by all methods. B-cell crossmatches were also consensus negative by all methods.

Method	No Labs Total	T-cell			Result	B-cell			Result
		#	#	%T-cell		#	#	%B-cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	10	0	10	100	Negative	1	12	92	Negative
Wash	11	1	11	92	Negative	0	17	100	Negative
AHG	24	1	23	96	Negative	NT			Insufficient
Flow	27	3	24	89	Negative	1	24	96	Negative
ELISA	0				Insufficient				Insufficient

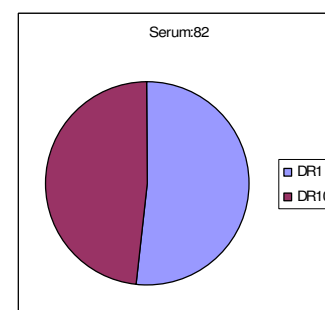
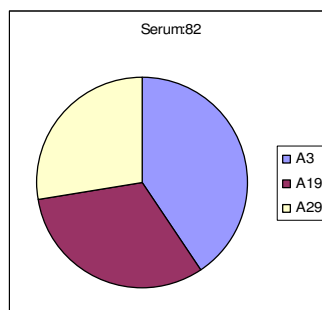
Transplant? Yes: 26 No: 2 More information needed: 5

## SERUM CS82

### Antibody Analysis:

Anti - Class 1: (A3,A19)

Class 2: **DR1** (DR10,103)



### PRA Results

T-cell/ Class 1 PRA's ranged from 0 - 98%. A locus antibodies were reported by most methods, but none reached consensus, except A3 by Luminex. Other specificities reported were A3 (78%), A19 (61%) A29 (53%), A31 (53%). Many of the labs using AHG reported B35 and B53. Labs using Luminex also reported A28 antibodies. The labs reported the following results:

Methods CS82	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	0 -21	11	Undetermined
Wash-T	6	Positive	0 -28	14	Undetermined
AHG-T	17	Positive	0 -24	12	Undetermined

Flow Class I	33	Positive	0 - 98	49	(A3,A19,29,31)
ELISA Class 1	16	Positive	0 - 98	49	(A3, A19, 29,31)
Luminex 1	9	Positive			<b>A3</b> , (A19,28,29,31,33)

B-cell screening PRA values ranged from 0 to 75% depending on the technique used. Almost all labs reported positive B cell/ Class 2 antibodies, except labs using the less sensitive "No Wash " cytotoxicity methods. The only specificity to reach consensus was DR1 (83%) and DR1 and DR10 by Luminex. Other specificities reported were DR10 (78%) and DR103 (64%).The breakdown by technique is as follows:

Methods CS82	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Negative	0	0	None
Wash-B	10	Positive	0 -28	14	<b>DR1</b>
AHG-B	0	NT			Insufficient
Flow Class 2	32	Positive	9 - 43	26	<b>DR1</b> (DR10,103)
ELISA Class 2	15	Positive	13 -75	44	<b>DR1</b> (DR10,103)
Luminex 2	9	Positive			<b>DR1, DR10</b> (DR103)

### Crossmatching Results: CS82 Vs CC35

#### Crossmatch Consensus Results – CS82/CC35

T-cell cross matches trended to be positive, but no consensus was reached, except by flow which was consensus positive. CS82 has an antibody to A29 which is on cell CC35. B-cell crossmatches were all inconsistent as well, indicating that the majority of labs found the test to be positive by most methods reported

Methods	No Labs Total T/B	T-cell # Pos	T-cell # Neg	%T-cell Cons	Result	B-cell # Pos	B-cell # Neg	%B-cell Cons	Result
No-wash	10	6	4	60	Inconclusive	7	6	54	Inconclusive

Wash	12	8	4	67	Inconclusive	14	3	82	Positive
AHG	25	18	6	75	Inconclusive	NT			Insufficient
Flow	26	25	1	96	Positive	16	9	62	Inconclusive
ELISA	0				Insufficient				

Transplant? Yes: 2 No: 29 More Information needed: 4

## CS82 Vs. CC36

### Crossmatch Consensus Results – CS82/CC36

T-cell crossmatches were all consensus negative by serological methods. Flow labs reported positive results which did reach consensus, and conformed with the presence of an antibody to A3 which is present on cell CC36 and detectable by flow. B-cell crossmatches were consensus negative by serological methods, but inconclusive by flow crossmatches with only 28% of the labs reporting positive results.

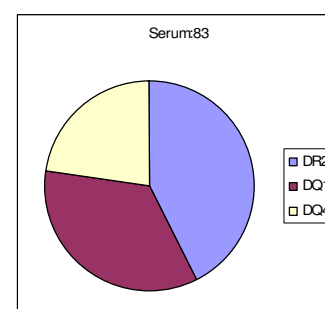
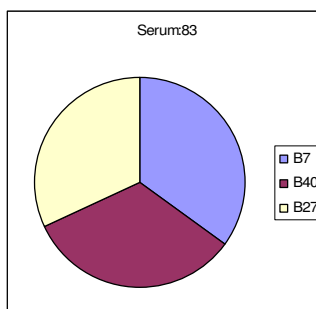
Methods	No Labs Total T/B	T-cell #		%T-cell	Result	B-cell #		%B-cell	Result
		Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	10	0	10	100	Negative	0	13	100	Negative
Wash	12	1	11	92	Negative	0	17	100	Negative
AHG	24	2	22	92	Negative	NT			Insufficient
Flow	27	22	5	82	Positive	7	18	28	Inconclusive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 9 No: 17 More information needed: 7

## SERUM CS83

### Antibody Analysis

Anti – Class 1: **B7, B27, B40** (B81,13,41,42,47,48)



Class 2: (DR2), (DQ1)

### PRA Results

T-cell/ Class 1 PRA results ranged from 16 -100%. All methods were consensus positive for this serum. B7 (94%), B27 (86), B40 (89%) did reach consensus by almost every methods. Luminex consensus was reached on B7, B13, B27, B40, B47, and B48. Other antibody specificities reported were B13 (75%), B42 (58%), B47 (56%),B48 (75%) B8 (53%) ,B60 (55%) and B61 (55%). The labs reported the following results:

Methods CS83	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash T	4	Positive	16 -30	23	(B7, B81,48,40)
Wash-T	6	Positive	28 -38	30	(B7,13, 27,40,48)
AHG-T	17	Positive	20 - 60	40	<b>B7</b> , (13,27,40,47,48)
Flow Class I	33	Positive	40 - 96	68	<b>B7</b> , (13,27,40,47,48,22)
ELISA Class 1	16	Positive	30 -100	65	<b>B7</b> , (13,27,40,47,48,22)
Luminex 1	9	Positive			<b>B7,13,27,40,47,48</b> (B5,8,15,41,81,42)

B-cell / Class 2 screening PRA values ranged from 0 to 94% depending on the technique used. Positive consensus was observed in all methods. DR2 was reported by 78% of the labs, so it almost met consensus . DQ1 (64%) and DQ4 (42%) and DR51 (39%) were the specificities most frequently reported by labs using solid phase assays. The breakdown, by technique is as follows:

Methods CS83	No Labs	Consensus	% PRA Range	Median PRA	Specificity
No-wash B	3	Positive	37- 37	37	None
Wash-B	10	Positive	0 - 50	25	(DR2,DR51,DQ1)
AHG-B	0	NT			Insufficient
Flow Class 2	32	Positive	19 -94	57	(DR2,DR51,DQ1,DQ4)
ELISA Class 2	15	Positive	10 -83	47	(DR2,DR51,DQ1,DQ4)
Luminex 2	9	Positive			(DR2,DR51,DQ1,DQ4)

### Crossmatching Results: CS83 Vs. CC35

T-cell crossmatches were consensus negative by all methods except for flow where 81% of the labs reported positive results. It is not clear why the flow crossmatches were positive in this combination. The majority of B cell crossmatches were positive by all methods.

#### Crossmatch Consensus Results – CS83/CC35

Methods	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	10	0	10	100	Negative	10	3	77	Inconclusive
Wash	12	1	11	92	Negative	16	1	94	Positive
AHG	24	2	22	92	Negative	NT			Insufficient
Flow	26	21	5	81	Inconclusive	21	3	88	Positive
ELISA	0				Insufficient				

Transplant? Yes: 3 No: 30 More information needed: 3

### CS83 Vs. CC36

#### Crossmatch Consensus Results – CS83/CC36

T-cell and B cell crossmatches were are predicted to be positive, which they were by all methods, because of the very strong antibody to B7 in CS83 and the B7 on cell CC36. The breakdown is below:

Methods	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	10	9	1	90	Positive	12	1	92	Positive
Wash	12	10	2	83	Positive	17	0	100	Positive
AHG	24	23	1	96	Positive	NT			Inconclusive

Flow	27	27	0	100	Positive	25	0	100	Positive
ELISA	0								

**CS83 vs. CC36**

**Transplant? Yes: 0 No: 30 More information needed: 3**

**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 this time for the first time and 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 serum send out will be September 11, 2006.