

SEOPF

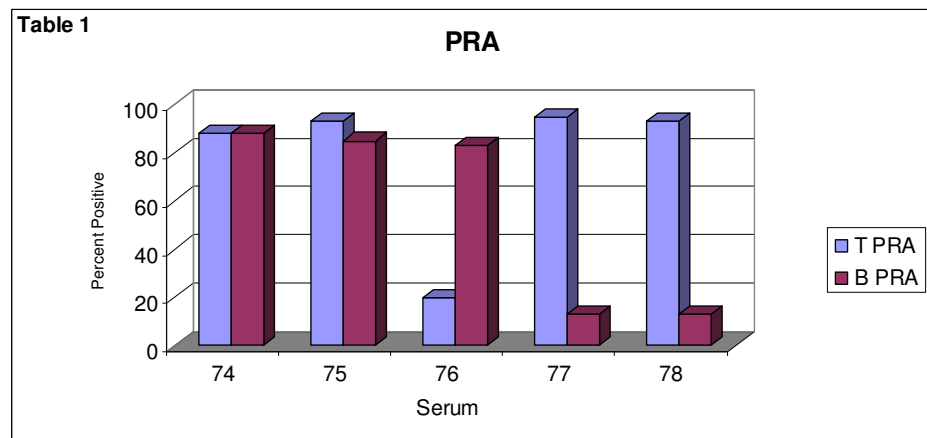
Proficiency Testing Program Report

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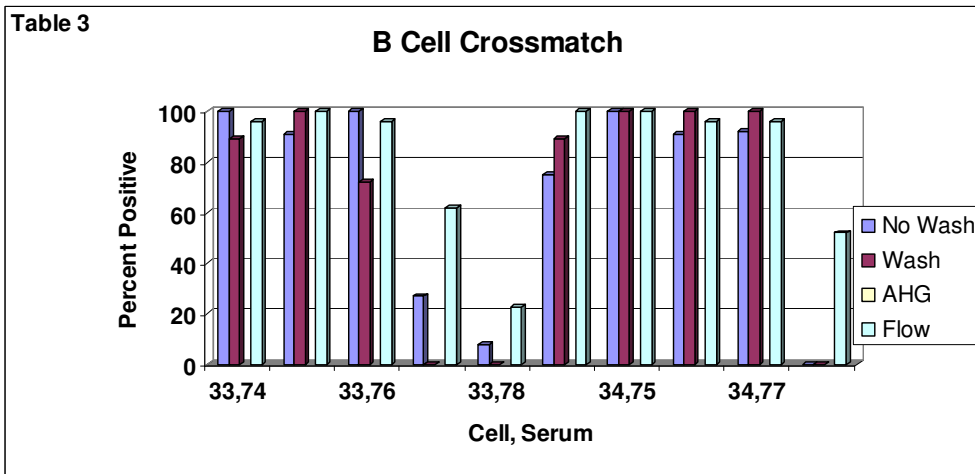
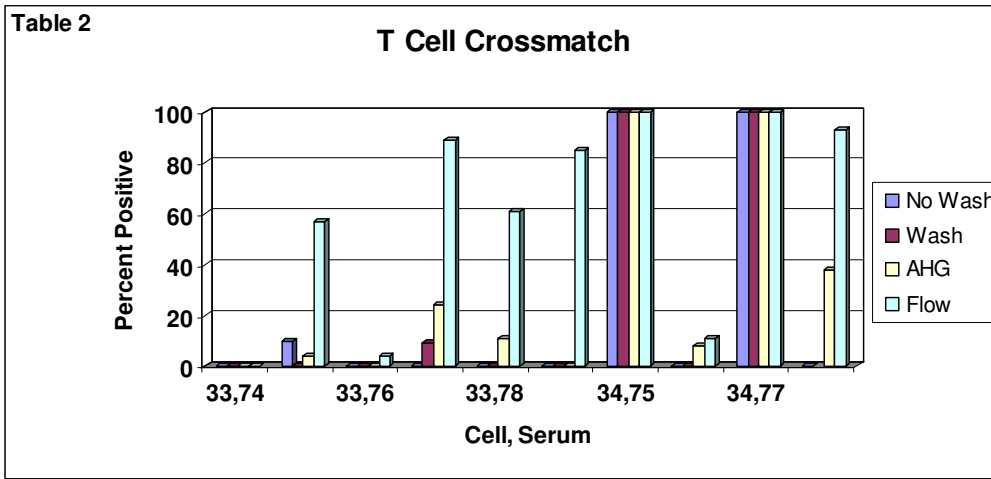
SEOPF Proficiency Testing Results - November 2005

SUMMARY REPORT:

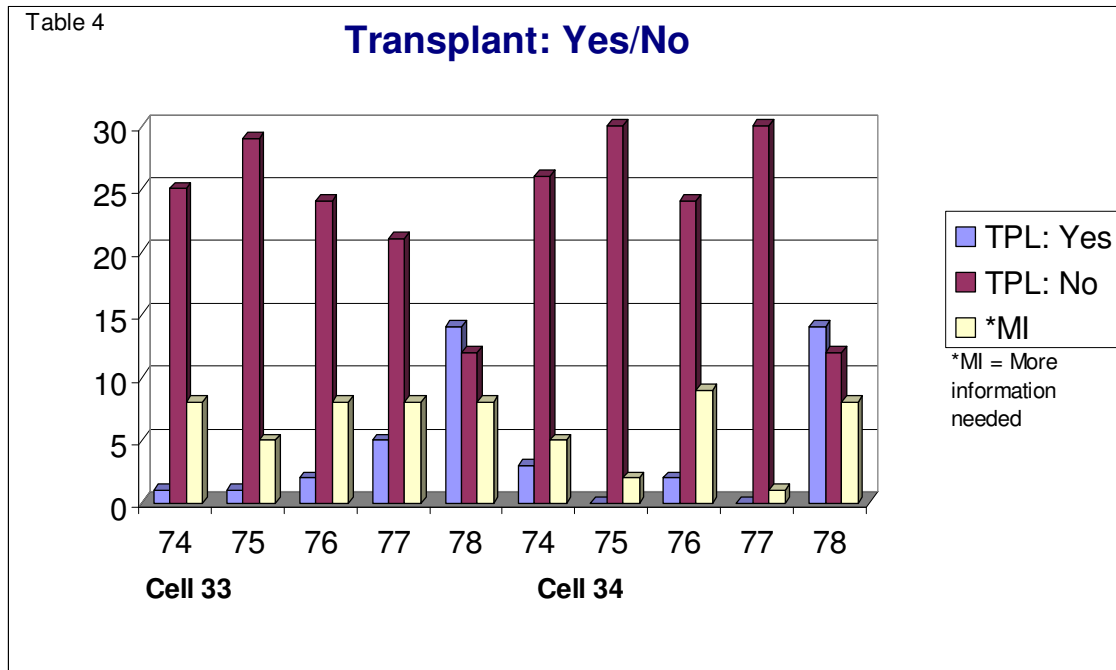
The November 2005 Crossmatch / PRA is the last send out using the SEOPF name. SEOPF has officially changed its name to American Foundation for Donation and Transplantation (AFDT) . Please note these changes and notify appropriate individuals at your institutions of this name change. The mission of the proficiency testing programs will remain the same however, even after the name changes in 2006. AFDT Proficiency Testing will continue to send out admixtures of both Class I and Class 2 antibodies. The report below is a summary of the November 2006 send out. The goal of AFDT Proficiency Testing will remain the same as SEOPF's which 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 SEOPF Proficiency Testing Samples are more relevant and indicative of actual clinical situations and therefore more appropriate to meet the intent of CLIA, UNOS and ASHI standards. This 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 I and Class 2 antibodies. 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 reported. (See tables 2 and 3).



As a final question, each lab was also asked to indicate whether or not each particular crossmatch pair would be transplanted or not, or whether 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 de-sensitization and rescue protocols.)



November 2005 Crossmatch/PRA

Cells: Race: Phenotype:

**CC33: Cauc: HLA: A*02,*33; B*08, *1516; Bw4, Bw6; Cw*07,*14
DRB1*01,*07; DRB*4; DQB1*05, 0302/05**

**CC34: Cauc: HLA: A*02,*11; B*13, *44; Bw4; Cw*05, *06
DRB1*01, *07; DRB4*; DQB1*05, *02**

Sera / Reported Specificities:

Specificities without () indicates 85% or more labs reported this result, therefore consensus was reached. Specificities with () indicate that the majority (50% or more labs) reported this result.

**CS74 - Anti - Class 1: B7, (B27), (B40)
Class 2: (DR7), (DR9)**

**CS75 - Anti - Class 1: B13, (B40)
Class 2: DR7, (DR9), (DQ2)**

**CS76 - Anti - Class 1: Undetermined
Class 2: (DR1,103), (DR10), (DR4)**

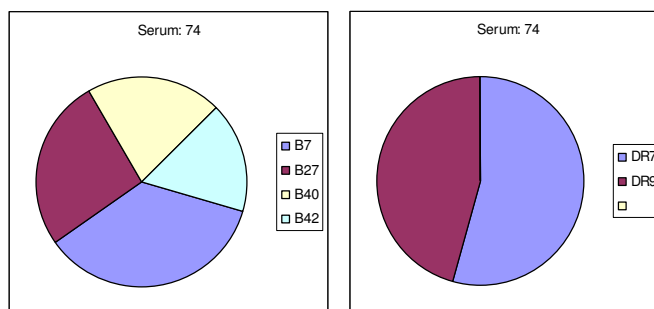
**CS77 - Anti - Class 1: (A3), (A11), (B44), (B45)
Class 2: Undetermined**

**CS78 - Anti - Class 1: B7, (B27), (B40), (A3), (A11)
Class 2: Undetermined**

RESULTS: SERUM CS74

Antibody Analysis

CS74 - Anti - Class 1: B7, (B27), (B40)
Class 2: (DR7), (DR9)



PRA Results

The range for T-cell/ Class 1 PRA was 0 - 86 %. The table below has the complete breakdown by methods. As expected solid phase assays (Flow, Luminex and ELISA) gave the most sensitive results. Flow and Luminex results were combined for the evaluations in this exchange. CDC methods detected B7, B27, B40 and B81, but only B7 reached consensus by all methods. B67, 42 were also reported by labs using solid phase methods. It should be noted that all methods reached a T-cell/ Class 1 consensus positive. Bolded text indicates specificities that reached consensus.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	0 - 3	B7 ,(27,61,81)
Wash-T	6	Positive	0 - 3	B7 , (27,61,81)
AHG-T	17	Positive	0 - 18	B7 ,(27)
Flow Class I	33	Positive	2 - 86	B7 , (27,40,67,81,42)
ELISA Class 1	16	Positive	0 - 64	B7 ,(42,67,81)

B-cell/ Class 2 screening PRA values ranged from 0 to 55%. The predominant specificities reported by all methods were DR7 (80%) and DR9 (68%). The breakdown is below.

The breakdown is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	3	Positive	0 - 29	(DR7,9)
Wash-B	10	Positive	0 - 38	(DR7,9)
B-cell AHG	0	NT		Insufficient
Flow Class 2	32	Positive	27 - 51	(DR7,9) 53
ELISA Class 2	15	Positive	22 - 54	(DR7,9) 53

Crossmatching Results: CS74 vs CC33 This cell-serum combination should have been negative based on the cell phenotype and the reported antibody specificities. All methods reached negative consensus as predicted. B-cell crossmatches were predicted to be positive due to the DR7 antibody identified and the DR7 on cell CC33. All methods, except flow, reached consensus positive. 79% of the labs reported flow positive crossmatches.

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 – CS74/CC33

Methods	No Labs Total	T-cell		%T-cell	Result	B-cell		%B-cell	Result
		#	#			#	#		
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	12	0	10	100	Negative	12	0	100	Positive
Wash	18	0	11	100	Negative	16	2	89	Positive
AHG	26	0	22	100	Negative	NT			Insufficient
Flow	28	0	5	100	Negative	21	7	75	Inconclusive
ELISA	NT				Insufficient	NT		Insufficient	Insufficient

Transplant? Yes: 1 No: 25 More information needed: 8

CS74 Vs. CC34

Crossmatch Consensus Results – CS74/CC34

CS74 and CC34 were expected to be T-cell negative, which they were. The B-cell crossmatches were predicted to be positive. The no-wash T-cell was inconclusive with 75% of the labs reporting positive results. All other methods reached positive consensus as expected.

Methods	No Labs Total	T-cell		%T-cell	Result	B-cell		%B-cell	Result
		#	#			#	#		
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	12	0	10	100	Negative	9	3	75	Inconclusive
Wash	18	0	11	100	Negative	16	2	89	Positive
AHG	26	0	26	100	Negative	NT			Insufficient
Flow	27	4	23	85	Negative	25	0	100	Positive

ELISA 0

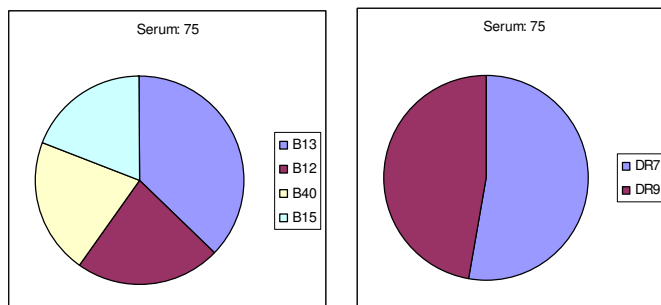
Insufficient

Transplant? Yes: 3 No: 27 More information needed: 5

SERUM CS75

Antibody Analysis:

Anti - Class 1: B13, (B40)
 Class 2: DR7, (DR9),
 (DQ2)



PRA Results

100% of the labs assigned a T-cell / Class1 PRA to CS75. The range was 0- 90%. B13 was reported by 88% of the labs. B12 (B44 and B45) was also assigned by 53% and 50% assigned B40. B15, and some of its splits, was also reported by labs using solid phase methods. The complete results are below.

Methods

	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	0 -14	B13
Wash-T	6	Positive	0 - 13	B13
AHG-T	17	Positive	4 - 30	B13, 62
Flow Class 1	33	Positive	4 - 80	B13,15,(40,12)
ELISA Class 1	16	Positive	23 - 90	B13,(12,40)

All labs reported Class 2 reactivity. The specificities most often reported were DR7 (65%) and DR9 (58%) and DQ2 (60%). The B- cell/ Class 2 PRA's ranged from 0-97%. The PRA reached consensus Positive by all methods. The complete results are below.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	3	Positive	0 - 25	(DR7, DQ2)

Wash B	10	Positive	7 - 54	(DR7, DQ2)
B-cell AHG	0			
Flow Class 2	32	Positive	11 - 82	(DR7,9,12 DQ2,9)
ELISA Class 2	15	Positive	37 - 97	(DR52, 53)

Crossmatching Results: CS75 Vs. CC33

Crossmatch Consensus Results – CS75/CC33

Consensus negative was reached by all methods except for flow which reported 57% positive. CC33 is a B63 and the flow labs were probably detecting this in the crossmatch method. Solid phase results reported antibodies to B15, which includes B1516 (B63) present on CC33B63. The B-cell crossmatches were expected to be positive, which they all were.

Methods	No Labs Total T/B	T-cell		%T- cell	Result	B- cell		%B- cell	Result
		# Pos	# Neg	Cons		# Pos	# Neg	Cons	
No-wash	12	1	9	90	Negative	10	1	91	Positive
Wash	18	0	18	100	Negative	18	0	100	Positive
AHG	25	1	24	96	Negative	NT			Insufficient
Flow	28	16	12	57	Inconclusive	26	0	100	Positive
ELISA	0				Insufficient				Insufficient

Transplant: Yes: 1 No: 29 More Information needed: 5

Crossmatch Consensus Results – CS75/CC34

Methods	No Labs Total T/B	T-cell		%T- cell	Result	B- cell		%B- cell	Result
		# Pos	# Neg	Cons		# Pos	# Neg	Cons	
No-wash	12	10	0	100	Positive	11	0	100	Positive
Wash	18	11	0	100	Positive	18	0	100	Positive
AHG	25	25	0	100	Positive	NT			Insufficient

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

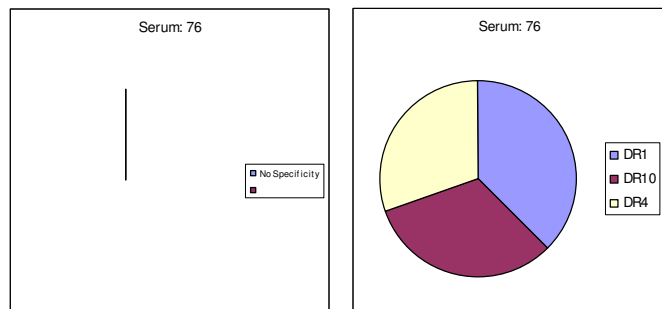
All T-cell techniques reached consensus positive, as expected due to the B13 antibody and CC34 has B13 in the phenotype. The B-cell results were also as predicted due to antibodies to DR7 and DQ2, both found on CC34. Consensus positive was reached by all methods.

Transplant? Yes: 0 No: 32 More information needed: 2

SERUM CS76

Antibody Analysis:

Anti - Class 1: Undetermined
 Class 2: (DR1, 103), (DR10), (DR4)



PRA Results

Not all of the labs assigned a T-cell/Class1 PRA to CS76. The Class 1/ T-cell PRA range was 0 - 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	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Inconclusive	0 - 3	-
Wash-T	6	Inconclusive		-
AHG-T	17	Inconclusive	0 - 17	-
Flow Class 1	33	Inconclusive	0 - 39	B8, 44
ELISA Class 1	16	Inconclusive	0 - 100	-

B-cell/ Class 2 PRA results did reach consensus positive. The range was 0-100%. Specificities reported were DR10 (68%) and DR1 (80%). The breakdown by methods is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	3	Positive	0 - 11	(DR1)
Wash B	10	Positive	7-21	(DR1, 103)
B-cell AHG	0			
Flow Class 2	32	Positive	17 - 80	(DR1, 103, 10)
ELISA Class 2	15	Positive	44 - 100	(DR1, 10, 103) 4

Crossmatching Results: CS76 Vs. CC33

Crossmatch Consensus Results – CS76/CC33

All T-cell crossmatches reached negative consensus. CC33 is a DR1 and antibodies to DR1 were reported for CS76. B-cell crossmatches should have been positive, and were for the most part. 72% of the labs reported B-cell positive using wash techniques.

Method	No Labs Total	T-cell #		%T-cell Cons	Result	B-cell #		%B-cell Cons	Result
		Pos	Neg			Pos	Neg		
No-wash	10	0	10	100	Negative	11	0	100	Positive
Wash	11	0	11	100	Negative	13	5	72	Inconclusive
AHG	25	2	23	92	Negative	NT			Insufficient
Flow	27	3	24	90	Negative	9	1	89	Positive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 2 No: 24 More information needed: 8

CS76 Vs. CC34

Crossmatch Consensus Results – CS76/CC34

T-cell crossmatches were all consensus negative by all methods except for Flow where 35% (11/31) labs did report positive crossmatches. B-cell crossmatches were basically all consensus positive by all methods due to the DR1 on CC34

Method	No Labs Total T/B	T-cell #		%T-cell Cons	Result	B-cell #		%B-cell Cons	Result
		Pos	Neg			Pos	Neg		
No-wash	11	0	10	100	Negative	10	1	91	Positive
Wash	11	0	11	100	Negative	15	3	83	Inconclusive
AHG	25	2	23	92	Negative	NT			Insufficient
Flow	27	3	24	90	Negative	25	1	89	Positive
ELISA	0				Insufficient				Insufficient

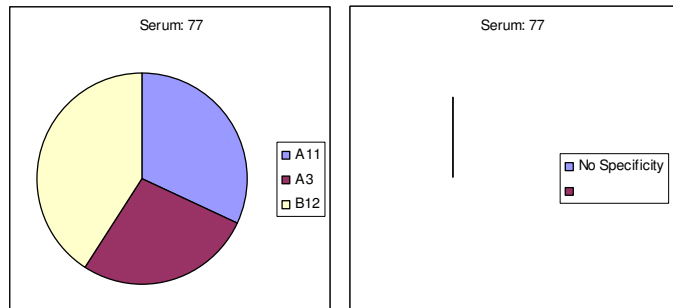
Transplant? Yes: 2 No: 24 More information needed: 9

SERUM CS77

Antibody Analysis:

Anti - Class 1: (A3), (A11),
(B44), (B45)
Class 2: Undetermined

PRA Results



T-cell/ Class 1 PRA's ranged from 7- 100%. B12 (83%) of the labs reported this specificity, which is very close to the 85% consensus cutoff. Solids phase methods resulted in A3 (53%) and A11 (45%) being reported. The labs reported the following results:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	17 - 24	(B44)
Wash T	6	Positive	14 -18	(B44,45)
AHG-T	17	Positive	7 - 73	(B12),13,27,37,21, A3,11
Flow Class 1	33	Positive	57 - 93	(B12), 13, 27, A3,11
ELISA Class 1	16	Positive	15 - 100	(B12) , 13, 27, A3,11

B-cell screening PRA values ranged from 0 to 58% depending on the technique used. Almost all labs reported negative B cell/ Class 2 antibodies, however, which did reach consensus.

The breakdown, by technique is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash	3	Negative	0 - 18	-
Wash B	10	Negative	0 - 58	DR7
B-cell AHG	0	Insufficient		
Flow Class 2	32	Negative	0 - 3	-
ELISA Class 2	15	Negative	0 - 28	-

Crossmatching Results: CS77 Vs CC33

Crossmatch Consensus Results – CS77/CC33

All T-cell cross matches were consensus negative except for the labs using flow, where 24% reported positive results. B-cell crossmatches were all inconsistent. The only method to reach negative consensus was the wash method.

Methods	No Labs Total	T-cell # Pos	T-cell # Neg	%T-cell Cons	Result	B-cell # Pos	B-cell # Neg	%B-cell Cons	Result
No-wash	11	0	10	100	Negative	3	8	27	Inconclusive
Wash	11	1	10	91	Negative	0	18	100	Negative
AHG	25	6	19	24	Inconclusive	NT			Insufficient
Flow	28	25	3	90	Positive	16	10	62	Inconclusive
ELISA	0				Insufficient				

Transplant? Yes: 5 No: 21 More Information needed: 8

CS77 Vs. CC34

Crossmatch Consensus Results – CS77/CC34

T-cell crossmatches were all consensus positive due to the B44 antibody reported and the B44 phenotype of CC34. B-cell crossmatches were consensus positive as well.

Methods	No Labs Total	T-cell			Result	B-cell			Result
		#	#	%T-cell		#	#	%B-cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	12	10	0	100	Positive	11	1	92	Positive
Wash	18	10	0	100	Positive	18	0	100	Positive
AHG	25	25	0	100	Positive	NT			Insufficient
Flow	27	27	0	100	Positive	24	1	96	Positive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 0 No: 33 More information needed: 1

SERUM CS78

Antibody Analysis

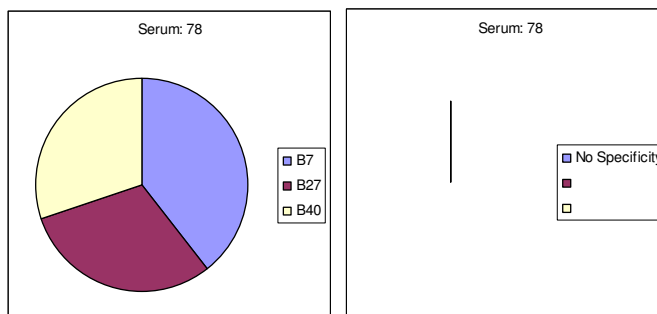
Anti – Class 1: B7, (B27),
(B40), (A3), (A11)
Class 2: Undetermined

PRA Results

T-cell/ Class 1 PRA results ranged from 2 -100%. B7 did reach consensus. Other specificities reported were A3 (53%), B27 (65%), B40 (65%) and B81 (54%).

The labs reported the following results:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	2 – 17	B7 , 81
Wash T	6	Positive	7 - 8	B7 , 81
AHG-T	17	Positive	0 - 50	B7 , 81, 48
Flow Class 1	33	Positive	58 - 100	B7 , 22, 40, 41
ELISA Class 1	16	Positive	7 - 93	B7 , 81, 40, +



B-cell / Class 2 screening PRA values ranged from 0 to 54% depending on the technique used. Some labs reported DR15 and DR14 by enhanced serological methods, but solid phase methods were consensus negative. This antibody is probably an IgM antibody, which is not detectable by solid phase methods that are IgG specific.

The breakdown, by technique is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	3	Inconclusive	0 - 32	DR15
Wash B	10	Inconclusive	0 - 54	DR14, 15
B-cell AHG	0	Insufficient		
Flow Class 2	32	Negative	0 - 10	-
ELISA Class 2	15	Negative	Negative	-

Crossmatching Results: CS78 Vs. CC33

Both T-cell and B-cell crossmatches were consensus negative by all methods except for flow. 61% of the labs reported positive T-cell results for CC33. 23% of the labs also reported positive B-cell results.

Crossmatch Consensus Results – CS78/CC33

Methods	No Labs Total	T-cell # Pos	T-cell # Neg	%T-cell Cons	Result	B-cell # Pos	B-cell # Neg	%B-cell Cons	Result
No-wash	10	0	10	100	Negative	1	11	91	Negative
Wash	11	1	10	91	Negative	0	18	100	Negative
AHG	26	3	23	88	Negative	NT			Insufficient
Flow	28	17	11	61	Inconclusive	6	20	23	Inconclusive
ELISA	0				Insufficient				

Transplant? Yes: 14 No: 12 More information needed: 8

CS78 Vs. CC34**Crossmatch Consensus Results – CS78/CC34**

T-cell crossmatches by less sensitive serological methods were all consensus negative. 10/26 (38%) reported positive AHG crossmatches. Flow T-cell crossmatches reached 100% consensus. Flow labs also reported antibodies to A11, which is on cell CC34. B-cell/ Class 2 crossmatches reached negative consensus only by serological methods. Solid phase assays were less consistent. 52% of the flow labs did report positive results. The breakdown is 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	0	10	100	Negative	0	12	100	Negative
Wash	11	0	11	100	Negative	3	15	16	Inconclusive
AHG	26	10	16	38	Inconclusive	NT			
Flow	27	25	2	93	Positive	13	12	52	Inconclusive
ELISA	0								

CS78 vs. CC34

Transplant? Yes: 14 No: 12 More information needed: 8

Conclusions: Labs using enhanced serological methods (AHG) and labs using solid phase assays like Flow, Luminex and ELISA reported significantly more antibody specificities than labs using less sensitive serological methods. Luminex and Flow results were combined for the specificity analysis. ELISA results are separate. The same pattern was seen with crossmatch results. Flow methods were more sensitive than CDC. The next cell/ serum send out will be in May 2006. The consensus for 2006 will be lowered from 85 % to 80%, at that time. This will be more consistent with CLIA, ASHI and CAP.