

SEOPF

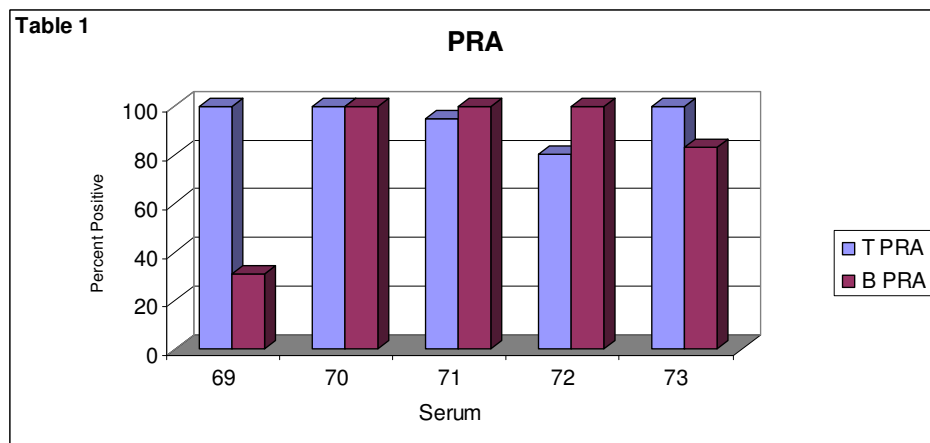
Proficiency Testing Program Report

Prepared by Dod Stewart
Reviewed by Jean Heneghan

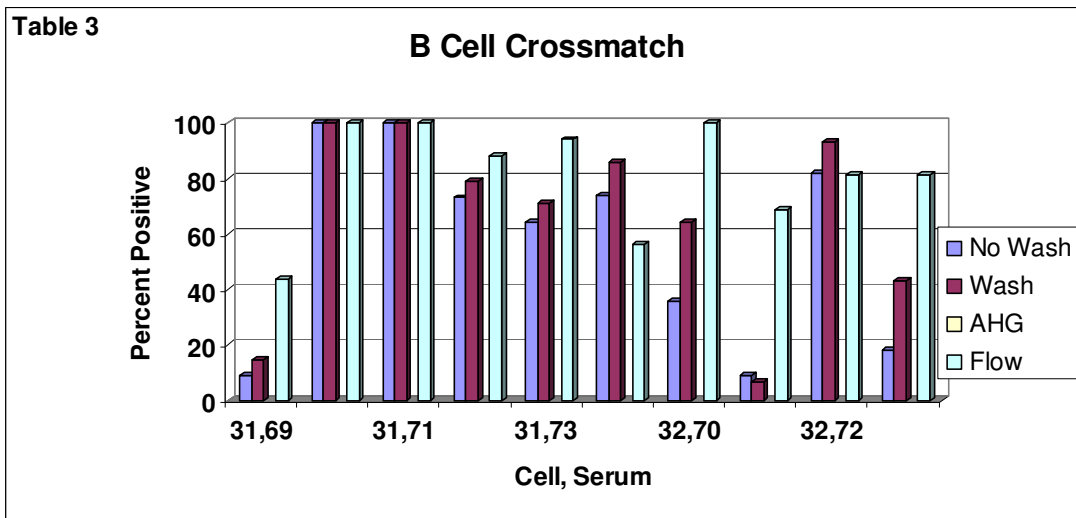
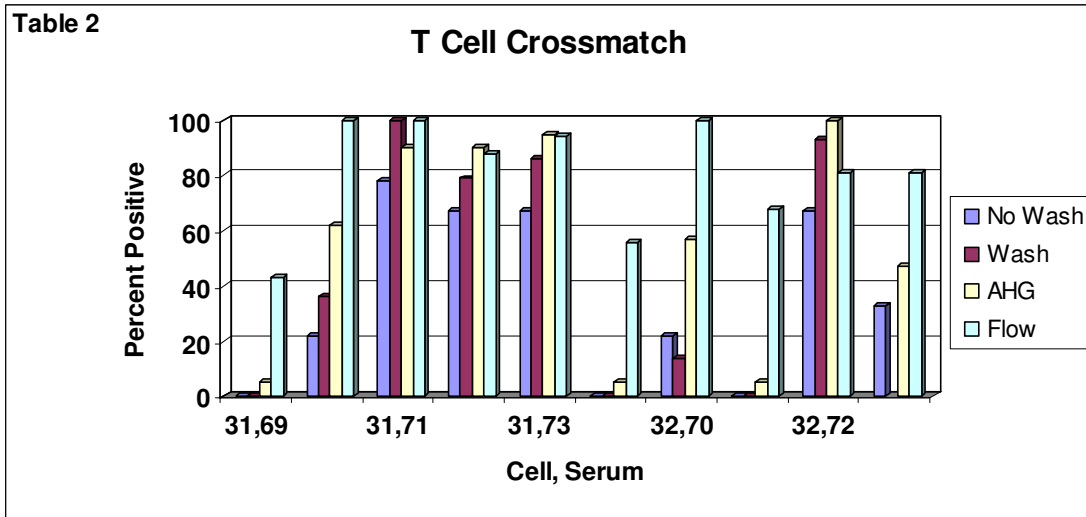
SEOPF Proficiency Testing Results - May 2005

SUMMARY REPORT:

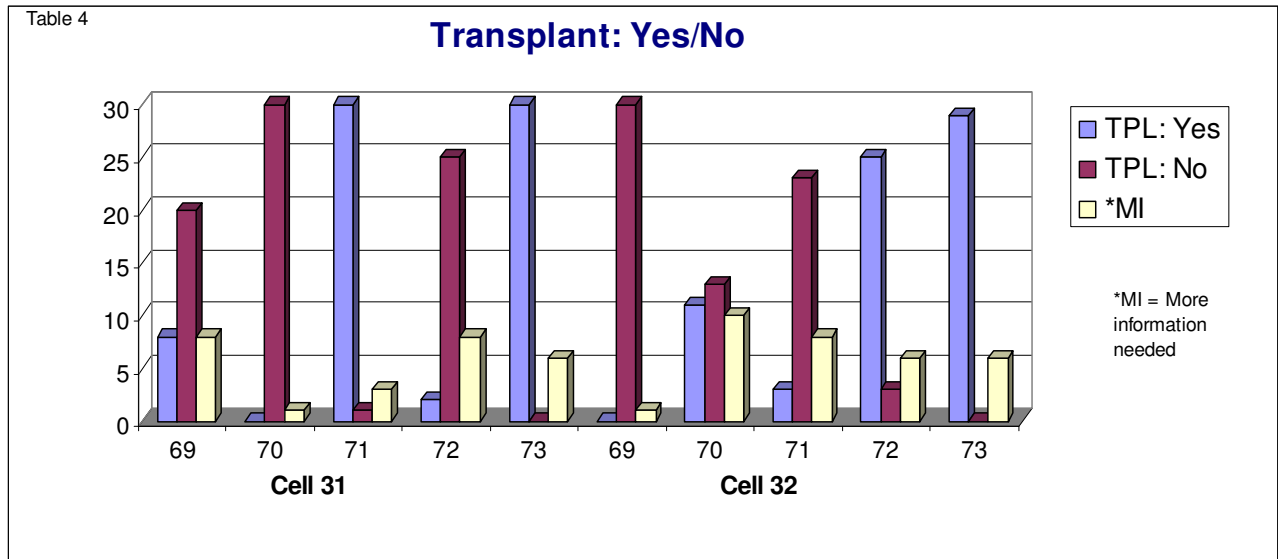
The May 2005 Crossmatch/PRA send out presented an admixture of both Class I and Class 2 antibodies within the same serum. 45 Laboratories participated in the exchange. The goal of the SEOPF Proficiency Testing Program and this Crossmatch/PRA Exchange is to provide cells and sera that approximate, as closely as possible, those clinical samples that are tested on a routine basis in most labs. This more accurately predicts how a la 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 or 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. These results are summarized after each analysis in Table 4.



Cells: Race: Phenotype:

**CC31: Cauc: HLA: A*01,*23; B*08,*44; Bw4, Bw6; Cw*07,*16
DRB1*0301/04,*07; DRB3*; DRB4*; DQB1*02**

**CC32: Cauc: HLA: A*02,*25; B*1402,*18; Bw6; Cw*08,*1203/06
DRB1*04,*15; DRB4*, DRB5*; DQB1*0301/04,*06**

Sera / Reported Specificities:

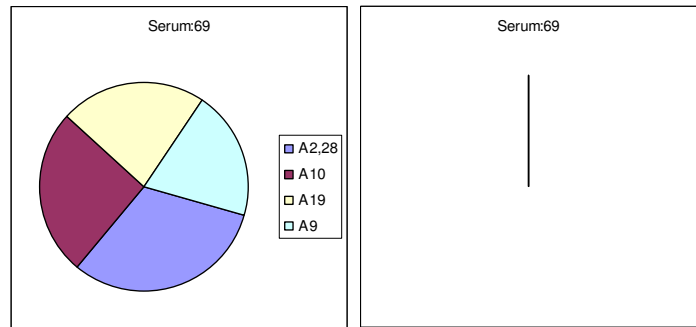
Specificities without () indicates 85% or more labs reported this result, therefore consensus was reached. Specificities with () indicate that the majority (50% or more labs) reported this result.

- CS69** - Anti - Class 1: A2, 28, (A9), (A10), (A19)
Class 2: Negative
- CS70** - Anti - Class 1: A1, (A11), (A19), (A9), (B5)
Class 2: (DR52), (DR8), (DR2, 51)
- CS71** - Anti - Class 1: A3, (A19)
Class 2: (DR1, 103), (DR10), (DQ1),(DQ4)
- CS72** - Anti - Class 1: (B17)
Class 2: DR7, (DR9), (DR11)
- CS73** - Anti - Class 1: A10, (A19), (A28)
Class 2: (DQ1), (DQ4)

RESULTS: SERUM CS69

Antibody Analysis

Anti - Class 1: A2, A28 (A10),
(A9), (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. Flow and Luminex results were combined for this exchange. CDC methods only detected A2, 28. Additional reactivity for A34 was seen in AHG-T results. Broad A10 and A19 groups were detected and assigned by labs using solid phase methods. It should be noted that all methods reached a consensus positive.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	36 -55	A2,28
Wash-T	7	Positive	2-50	A2,28
AHG-T	19	Positive	12-90	A2,28,34
Flow Class I	33	Positive	63 -100	A2,28,10,19
ELISA Class 1	20	Positive	22-97	A2,28,10,19

B-cell/ Class 2 screening PRA values ranged from 0 to 57%. CDC methods gave inconsistent patterns. "No-Wash" B results failed to detect any B-cell/ Class 2 antibodies and were consensus negative. Ten labs using "wash techniques" reported B-cell reactivity making this techniques consensus positive. No antibody specificities were reported by any of these serological methods. Labs using Flow/ Luminex and other solid phase assays reported a "weak" DR3 antibody. ELISA results were all negative. This antibody appears to be an IgM antibody based on the unusual results by the various methods.

The breakdown is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	1	Negative	0	None
Wash-B	10	Positive	0-57	None

B-cell AHG	0	NT		Insufficient
Flow Class 2	31	Inconclusive	0-33	Weak DR3
ELISA Class 2	19	Negative	0-9	None

Crossmatching Results: CS69 vs CC31 This cell-serum combination should have been negative based on the cell phenotype and the reported antibody specificities. Labs using Flow techniques reported 84% Negative, just below the 85% cutoff. The other methods did not reach Negative consensus as predicted. This is probably because this antibody is IgM isotype.

Crossmatch Consensus Results – CS69/CC31

Methods	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	2	9	18	Inconclusive	1	9	90	Negative
Wash	13	1	12	92	Negative	1	18	95	Negative
AHG	28	6	22	21	Inconclusive	NT			Insufficient
Flow	31	26	5	84	Inconclusive	19	7	73	Inconclusive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 8 No: 20 More information needed: 8

CS69 Vs. CC32

Crossmatch Consensus Results – CS69/CC32

Cell CC32 has A locus antigens of A2, A25 which are both antibodies assigned by labs for CS69. The crossmatches were all consensus positive as expected, except for No-wash T and B cell methods which came very close at 82% and 80%, just below the 85% cutoff for consensus.

Methods	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	9	2	82	Inconclusive	8	2	80	Inconclusive

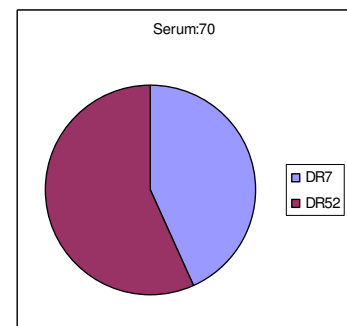
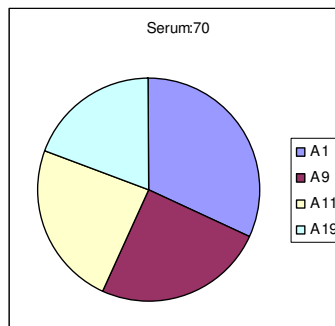
Wash	13	13	0	100	Positive	18	1	95	Positive
AHG	28	28	0	100	Positive	NT			
Flow	31	31	0	100	Positive	26	0	100	Positive
ELISA	0				Insufficient				

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

SERUM CS70

Antibody Analysis

Anti - Class 1: A1, (A11), (A19),
(A9), (B5)
Class 2:(DR52), (DR8),
(DR2, 51)



PRA Results

100% of the labs assigned a T-cell / Class1 PRA to CS70. The range was 0-83%. Reported specificities increased with the more sensitive methods. The complete results are below.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	12 - 51	A1, B51
Wash-T	7	Positive	3 - 15	A1, A80, B5
AHG-T	19	Positive	7 - 95	A1,11,9,19,80,B5
Flow Class 1	33	Positive	36 - 100	A1,11,9,19,10,80,B5
ELISA Class 1	20	Positive	35 - 96	A1,11,19,10,80, B5

All labs reported Class 2 reactivity. The B- cell/ Class 2 PRA's ranged from 0-100%. The PRA reached consensus Positive by all methods. The complete results are below.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	1	Positive	0 -31	DR11, 13, 14
Wash B	10	Positive	11 - 81	DR8, DR52, DQ4, DQ7

B-cell AHG	0	Positive		
Flow Class 2	31	Positive	67 -100	DR2, 8, 51, 52
ELISA Class 2	19	Positive	67 - 100	DR2, 51, 52

Crossmatching Results:**CS70 Vs. CC31****Crossmatch Consensus Results – CS70/CC31**

Consensus Positive was reached by all methods except the “No-Wash” technique which was 82% close to the 85% cut-off.

Method	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	9	2	82	Inconclusive	9	1	90	Positive
Wash	13	13	0	100	Positive	18	1	95	Positive
AHG	28	28	0	100	Positive	NT			Insufficient
Flow	31	31	0	100	Positive	26	0	100	Positive
ELISA	0				Insufficient				Insufficient

Transplant: Yes: 0 No: 34 More Information needed: 1

CS70 Vs. CC32**Crossmatch Consensus Results – CS70/CC32**

Methods	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	0	11	100	Negative	6	4	60	Inconclusive
Wash	13	0	13	100	Negative	4	15	21	Inconclusive
AHG	28	0	28	100	Negative	NT			Insufficient
Flow	31	1	30	97	Negative	22	4	84	Inconclusive
ELISA	0				Insufficient				Insufficient

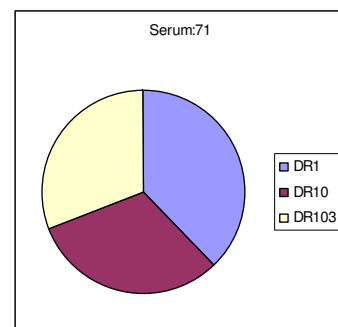
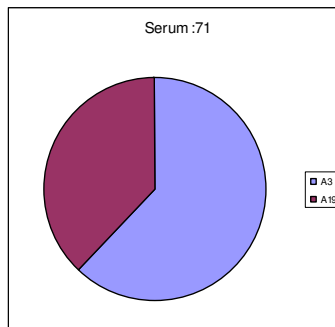
All T-cell techniques reached negative consensus. One lab did report a Positive T-cell Flow Crossmatch. Curiously the B-cell results were less consistent. 60% (6/10 labs) using the No-wash B-cell found Positive results and 4/19 labs (21%) using the "Wash" B-cell methods reported positive results. 84% (22/26 labs) reported Positive B-cell reactions by Flow.

Transplant? Yes: 11 No: 13 More information needed: 10

SERUM CS71

Antibody Analysis:

Anti - Class 1: A3, (A19)
 Class 2: (DR1, 103),
 (DR10), (DQ1),(DQ4)



PRA Results

100% of the labs assigned a T-cell/Class1 PRA to CS51. The Class 1/ T-cell PRA range was 0- 98%. Class 1 PRA did reach positive consensus as did the A3 specificity. As in other sera results, sensitive methods were able to detect the A3, A10 and A19 antibodies. The results are seen below.

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	0 -14	-
Wash-T	7	Positive	0 -25	-
AHG-T	19	Positive	0 - 43	A3
Flow Class 1	33	Positive	6 - 90	A3, 10, 19
ELISA Class 1	20	Positive	0 - 98	A3, 10, 19

B-cell/ Class 2 PRA results also reached 100% consensus. The range was 0-97%. The breakdown by methods is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash B	1	Positive	0 - 54	DR1, DQ1
Wash B	10	Positive	0 - 65	DR1, 103, DQ1
B-cell AHG	0	Insufficient		
Flow Class 2	31	Positive	38 -93	DR1, 103, 10, DQ4
ELISA Class 2	19	Positive	40 -97	DR1, 10, 103, DQ1, DQ4

Crossmatching Results: CS71 Vs. CC31

Crossmatch Consensus Results – CS71/CC31

All T-cell crossmatches reached negative consensus. As predicted all B-cell crossmatches combinations also reached negative consensus.

Method	No Labs	T-cell #	T-cell #	%T-cell Cons	Result	B-cell #	B-cell #	%B-cell Cons	Result
	T/B	Pos	Neg			Pos	Neg		
No-wash	11	0	11	100	Negative	0	10	100	Negative
Wash	13	1	12	92	Negative	0	19	100	Negative
AHG	28	1	27	96	Negative	NT			Insufficient
Flow	31	0	31	100	Negative	0	26	100	Negative
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 30 No: 1 More information needed: 3

CS71 Vs. CC32

Crossmatch Consensus Results – CS71/CC32

T-cell crossmatches were all consensus negative for all methods except for Flow where 35% (11/31) labs did report positive crossmatches. B-cell crossmatches were all consensus positive

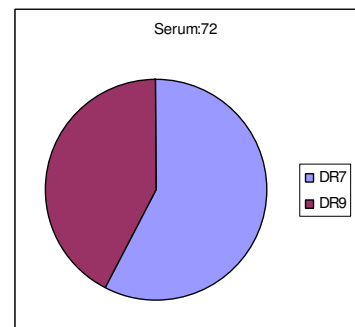
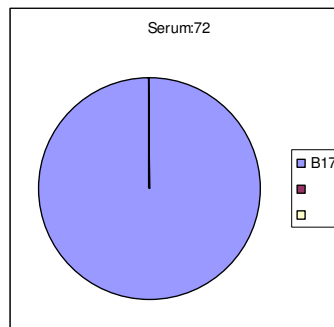
Method	No Labs	T-cell #	T-cell #	%T-cell Cons	Result	B-cell #	B-cell #	%B-cell Cons	Result
	T/B	Pos	Neg			Pos	Neg		
No-wash	11	0	11	100	Negative	8	2	80	Inconclusive

Wash	13	1	12	92	Negative	18	1	95	Positive
AHG	28	2	26	93	Negative	NT			Insufficient
Flow	31	11	20	35	Inconclusive	25	1	96	Positive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 3 No: 23 More information needed: 8

SERUM CS72

Antibody Analysis
 Anti - Class 1: (B17)
 Class 2: DR7, (DR9),
 (DR11)



PRA Results

T-cell/ Class 1 PRA's ranged from 0-53%. Labs using enhanced and solid phase methods reported a B17 specificity. Consensus was not reached by any of the methods. The labs reported the following results:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Inconclusive	0 -3	-
Wash T	7	Inconclusive	0 -14	-
AHG-T	19	Inconclusive	0 -20	B17
Flow Class 1	33	Inconclusive	0 -53	B17
ELISA Class 1	20	Inconclusive	0 -33	B17

B-cell screening PRA values ranged from 0 to 91% depending on the technique used. All labs reported B cell/ Class 2 antibodies.

The breakdown, by technique is as follows:

Methods	No Labs	Consensus	% PRA Range	Specificity
No-wash	1	Positive	0 -19	DR7
Wash B	10	Positive	6 -39	DR7
B-cell AHG	0	Insufficient		
Flow Class 2	31	Positive	8 -93	DR7, 9, 11, 12
ELISA Class 2	19	Positive	19 -91	DR7, 9, 11, 12

Crossmatching Results:**CS72 Vs CC31****Crossmatch Consensus Results – CS72/CC31**

All T-cell cross matches were consensus negative except for the labs doing flow. 13/31 labs (42%) reported a positive crossmatch. B-cell crossmatches were all consensus positive due to the anti-DR7 antibody and the DR7 phenotype of CC31.

Methods	No Labs	T-cell			Result	B-cell			Result
		#	#	%T-cell		#	#	%B-cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	0	11	100	Negative	9	1	90	Positive
Wash	13	0	13	100	Negative	18	1	95	Positive
AHG	28	3	25	89	Negative	NT			Insufficient
Flow	31	13	18	42	Inconclusive	26	0	100	Positive
ELISA	0				Insufficient				

Transplant? Yes: 2 No: 25 More Information needed: 8

CS72 Vs. CC32**Crossmatch Consensus Results – CS72/CC32**

T-cell crossmatches were all consensus negative. B-cell crossmatches were consensus negative for all techniques except flow where 5/26 labs (19%) reported positive results.

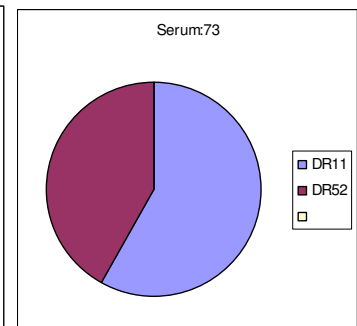
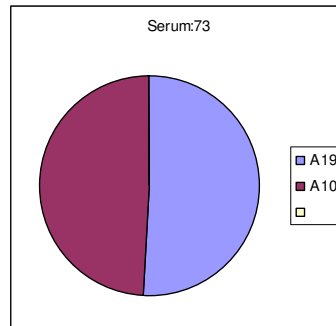
Methods	No Labs	T-cell			Result	B-cell			Result
		#	#	%T-cell		#	B-cell #	%B-cell	
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	0	11	100	Negative	0	10	100	Negative
Wash	13	0	13	100	Negative	2	17	89	Negative
AHG	28	0	28	100	Negative	NT			
Flow	31	2	29	94	Negative	5	21	19	Inconclusive
ELISA	0				Insufficient				Insufficient

Transplant? Yes: 25 No: 3 More information needed: 6

SERUM CS73

Antibody Analysis

Anti – Class 1: A10, (A19), (A28)
Class 2: (DQ1), (DQ4)



PRA Results

T-cell/ Class 1 PRA results ranged from 3-93%. Labs using serological methods reported A34 as specificity. Labs using enhanced methods reported A10, A28 and A19.

The labs reported the following results:

	No Labs	Consensus	% PRA Range	Specificity
No-wash T	4	Positive	3 - 30	A34
Wash T	7	Positive	3 - 14	A34
AHG-T	19	Positive	3 - 64	A26, 33, 34
Flow Class 1	33	Positive	22 - 93	A10, 28, 19
ELISA Class 1	20	Positive	20 - 83	A10, 28, 19

B-cell screening PRA values ranged from 0 to 79% depending on the technique used. Some labs reported DR8, DQ1 and DQ4 as specificities. No consensus for B-cell/ Class2 was met by any method

The breakdown, by technique is as follows:

	No Labs	Consensus	% PRA Range	Specificity
No-wash B	1	Inconclusive	0 -4	-
Wash B	10	Inconclusive	0 21	DQ4
B-cell AHG	0	Insufficient		
Flow Class 2	31	Inconclusive	6 -79	DR8, DQ1, DQ4
ELISA Class 2	19	Inconclusive	3 -66	DR8, DQ4

Crossmatching Results:

CS73 Vs. CC31

Both T-cell and B-cell crossmatches were consensus negative by all methods reported.

Crossmatch Consensus Results – CS73/CC31

Methods	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	1	10	91	Negative	1	9	90	Negative
Wash	13	0	13	100	Negative	0	19	100	Negative
AHG	28	0	28	100	Negative	NT			Insufficient
Flow	31	1	30	97	Negative	1	25	96	Negative
ELISA	0				Insufficient				

Transplant? Yes: 30 No: 0 More information needed: 6

CS73 Vs. CC32

Crossmatch Consensus Results – CS73/CC32

T-cell crossmatches by serological methods were all consensus negative. Flow T-cell crossmatches did not reach consensus since 14/31 labs (45%) reported positive results. B-cell cross matches also did not reach consensus by any methods. The breakdown is below.

Methods	No Labs	T-cell #	T-cell #	%T-cell	Result	B-cell #	B-cell #	%B-cell	Result
	T/B	Pos	Neg	Cons		Pos	Neg	Cons	
No-wash	11	0	11	100	Negative	2	8	20	Inconclusive
Wash	13	0	13	100	Negative	8	11	42	Inconclusive
AHG	28	1	27	96	Negative	NT			
Flow	31	14	17	45	Inconclusive	17	9	65	Inconclusive
ELISA	0				Insufficient				

CS73 vs. CC32

Transplant? Yes: 29 No: 0 More information needed: 6

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. One serum (CS69) appears to contain an IgM antibody due to the patterns reported by the labs. The next cell/ serum send out will be in November 2005.