

Comparison of Antigen Stability Between Three Manufacturers of Reagent Red Cells

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BACKGROUND

Reagent red cell antigenic stability may vary between manufacturers due to differences in manufacturing processes, preservative solutions or diluents. Our study evaluated antigenic stability of reagent red cells from three manufacturers over time.

STUDY DESIGN

Thirty-four samples with known antibody specificities were tested in gel technology (Ortho Clinical Diagnostics) with reagent red cells from the following manufacturers: Ortho Clinical Diagnostics 0.8% Resolve Panel B, ImmucorGamma Panocell 16, and Medion Diagnostics Data-Cyte Plus distributed by Olympus America Inc. The antibody specificities represented the major clinically significant blood groups and are summarized in Table I.

Table I: Antibodies included in study

Blood Group System	Antibody Specificity	Number
Rh	Anti-D	4
	Anti-C	2
	Anti-E	11
	Anti-c	1
Kell	Anti-K	8
Duffy	Anti-Fy ^a	2
Kidd	Anti-Jk ^a	4
MNSs	Anti-M	2

The reagent red cells from ImmucorGamma and Medion were diluted to a final 0.8% concentration with MTS Diluent 2 the day of testing according to the guidelines provided by Ortho Clinical Diagnostics. Cells tested were homozygous for the corresponding antigen to the previously identified antibody.

The following three phases of testing were performed:

- Phase I; week the panel was received
- Phase II; week of the manufacturer's stated expiration date
- Phase III; two weeks after the manufacture's stated expiration date

The week of expiration varied between manufacturers as follows:

Table II: Reagent Red Cell Shelf-life

Manufacturer/Product	Manufacturer's Shelf-life	Phase III Testing
Ortho 0.8% Resolve Panel B	3 weeks after receipt	5 weeks after receipt
ImmucorGamma Panocell 16	4 weeks after receipt	6 weeks after receipt
Medion DataCyte Plus	5 weeks after receipt	7 weeks after receipt

Reaction strengths were graded and then scored using the Race and Sanger Scoring System. Antibodies that did not exhibit expected results were classified as IgG or IgM with 0.01M DTT (Sigma Chemicals) using the PeG (ImmucorGamma) method.

Race and Sanger Scoring System	
Strength of Reaction	Race and Sanger Score
4+	12
3+	10
2+	8
1+	5
w+-Neg	0

RESULTS

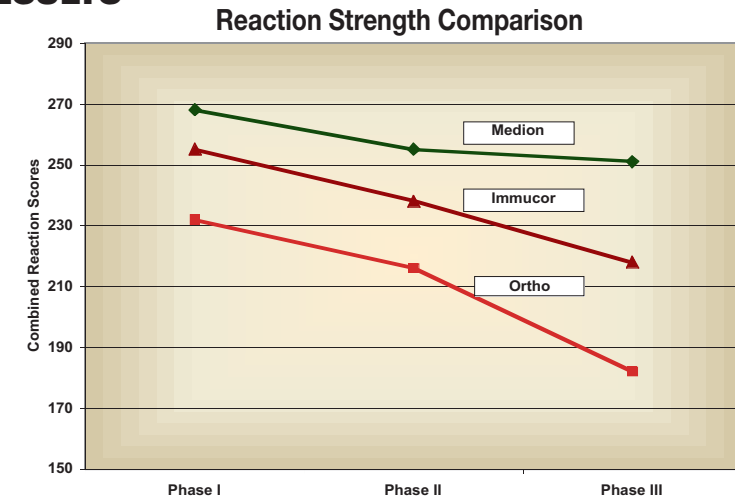


Table III: Reaction score summary of all antibodies

	Manuf.	Rxn Score	Rxn Score	% Decrease	Rxn Score	% Decrease
		Phase I	Phase II		Phase III	
Total	Medion	268	255	4.9%	251	6.8%
	Ortho	232	216	6.9%	182	27.5%
	Immucor	255	238	6.7%	218	17.0%

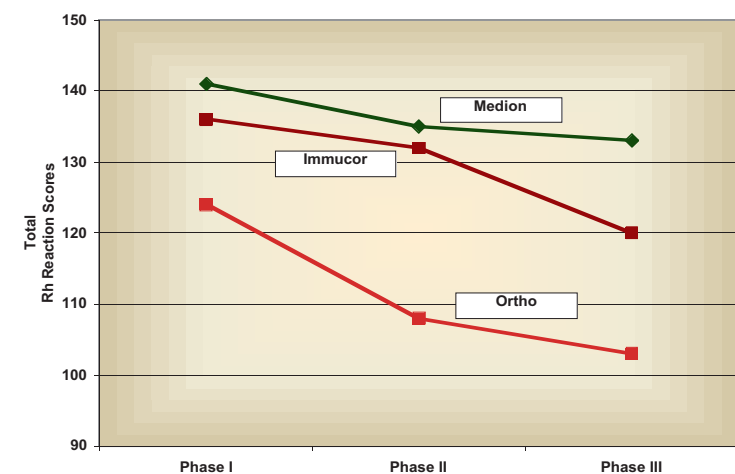


Table IV: Rh antibody detail

Reaction Score					
Antibodies	Manuf.	Phase I	Phase II	Phase III	
D	Medion	32	28	28	
	Ortho	35	28	28	
	Immucor	40	38	35	
C	Medion	20	18	18	
	Ortho	18	18	18	
	Immucor	18	18	18	
E	Medion	81	81	79	
	Ortho	63	54	49	
	Immucor	68	68	62	
C	Medion	8	8	8	
	Ortho	8	8	8	% Decrease
	Immucor	10	8	5	Phase II Phase III
Total Rh	Medion	141	135	133	4.3% 6.0%
	Ortho	124	108	103	12.9% 20.4%
	Immucor	136	132	120	2.9% 13.3%

Table V: Detail of non-Rh antibodies

Reaction Score					
Antibodies	Manuf.	Phase I	Phase II	Phase III	
K	Medion	72	68	66	
	Ortho	63	63	63	
	Immucor	73	64	56	
Fy ^a	Medion	16	16	16	
	Ortho	16	16	0	
	Immucor	16	16	16	
Jk ^a	Medion	21	18	18	
	Ortho	13	13	0	
	Immucor	10	10	10	
M	Medion	18	18	18	
	Ortho	16	16	16	% Decrease
	Immucor	20	16	16	Phase II Phase III
Total	Medion	127	120	118	5.5% 7.6%
	Ortho	108	108	79	0.0% 36.7%
	Immucor	119	106	98	10.9% 21.4%

The Medion DataCyte Plus panel had the smallest decrease in antibody score with a decrease of 19 points between Phase I and Phase III. The ImmucorGamma Panocell 16 had an overall decrease in antibody score of 37 points. The decrease in score is almost double that of Medion and occurred over a six-week testing period. The Ortho 0.8% Resolve Panel B had an overall decrease in antibody score between Phase I and Phase III of 50 points. This was the largest decrease in antibody score and occurred over a six-week testing period.

Table VI: Breakdown of antibodies identified with each manufacturer over time

# Antibodies Identified at Each Phase					
Antibody	N=	Manuf.	Phase I	Phase II	Phase III
D	4	Medion	3	3	3
		Ortho	4	4	4
		Immucor	4	4	4
C	2	Medion	2	2	2
		Ortho	2	2	2
		Immucor	2	2	2
E	11	Medion	10	10	10
		Ortho	9	9	8
		Immucor	10	10	10
c	1	Medion	1	1	1
		Ortho	1	1	1
		Immucor	1	1	1
K	8	Medion	8	8	8
		Ortho	8	8	8
		Immucor	8	8	8
Fy ^a	2	Medion	2	2	2
		Ortho	2	2	2
		Immucor	2	2	2
Jk ^a	4	Medion	3	3	3
		Ortho	2	2	2
		Immucor	4	4	4
M	2	Medion	2	2	2
		Ortho	2	2	2
		Immucor	2	2	2
Total	34	Medion	31	31	31
		Ortho	30	30	29
		Immucor	33	33	33

MEDION

Medion did not detect a weak anti-E and anti-Jka that could not be classified as either IgG or IgM as the controls were positive after being diluted 1:2. The anti-D not detected with the Medion cells was unable to be classified as either IgG or IgM. This sample was from a prenatal patient who had received RhIg at 28 weeks gestation and was being retested prior to delivery at 39 weeks.

ORTHO 0.8%

The Ortho 0.8% cells missed two IgG anti-E antibodies and one IgG anti-Jka antibody. The additional anti-E that lost reactivity when tested with the Ortho cells in Phase III was determined to be IgG. One of the anti-Jka samples that was not detected with Ortho cells, was also not detected with Medion cells. It could not be classified as IgG or IgM.

IMMUCOR

The anti-E not detected with Immucor cells, was also not detected by Medion cells, it was unable to be classified as IgM or IgG.

CONCLUSION

The Medion cells are more robust than Immucor or Ortho. Medion testing was performed over the longest period of time and had little loss of reactivity between phases. Ortho had the largest decrease in reactivity and did not detect four clinically significant antibodies during testing.

Variations in reagent red cell manufacturing processes, preservative solutions or diluents may have an effect on antigen expression over time. As seen by the decrease in score of the OCD cells, long-term storage in enhancing diluents may have a negative effect on the stability of some antigens.