



**Final Summation on Separation Technology Protocol PRO 13-012
PlasmaPrep 12™ Centrifuge**

Protocol Intent:

This protocol was designed to evaluate the Separation Technology, Inc. PlasmaPrep 12 centrifuge in generating platelet poor plasma defined as a platelet count below 10,000/cu.mm in the residual PRP.

Protocol Design:

Four normal blood donors were evaluated using BD 3.2% citrate tubes of sizes 1.8mL, 4.5mL and 10mL. Samples were also obtained and tested using K2EDTA.

Samples were spun for 30 seconds, 2 minutes, 3 minutes, 5 minutes and 10 minutes. As a benchmark, the lab did a comparison spin in 3.2% citrate tubes in a Jouan centrifuge for 15 minutes at 2500G.

An Abbott Diagnostics Cell-Dyn 1700 was used for the platelet measurements.

Results:

As can be seen from the data sheets, all donors used had starting platelet counts between 270-408,000/cu.mm.

After only a 30 second spin, as expected, the 1.8mL samples exhibited the most significant drop in platelet count - dropping an average of 63% versus 10% and below for the 5 and 10mL volume tubes.

By the completion of the 2 minute spin, all tubes exhibited greater than 95% platelet removal and by the 3 minute spin more than 99% of all platelets were removed. By a 5 minute spin, there was greater than 99% removal but little was gained by this maneuver.

Testing for adequate coagulation function in the platelet free plasma was done using PT and aPTT measurements on the CliniSys Stago Coagulation analyzer.

There was good uniformity of results especially after the 2 minute or above centrifugation time where almost no differences were noted in PT or aPTT results. As anticipated, there was a significant difference in the results between the 3.2% citrate tubes and the K2EDTA tubes. This is due to the much higher concentration of EDTA as a calcium binding agent rather than 3.2% citrate. As the data indicated, all the 3.2% citrate results after 2 minute spins were the same and the K2EDTA results were the same.

Conclusion:

The PlasmaPrep 12™ performed very well. The optimum time for centrifugation to obtain a greater than 97% platelet free plasma was only two minutes. The marginal advantage for using a 3 minute spin to gain 1-2% less

platelets is not justified given that for stat plasma generation the advantage of the 1 minute more than offsets the marginally cleaner PPP.

The PT and aPTT results validate that the 2 minute spin is more than adequate and fulfills the need for any lab to obtain stat platelet poor plasma for clinical testing in an efficient and timely manner.

Since all coagulation testing is inherently time dependent and the pre analytical component remains a significant cause of delay, the proven advantage of this protocol versus the traditional 15 minute spin to obtain platelet poor plasma is clearly identified and validated.

Sincerely,

A handwritten signature in black ink that reads "Alexander Duncan" followed by a stylized flourish.

Alexander Duncan, MD
President & CEO
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PlasmaPrep 12 Rotor Test 6/12/14
Rotor Fully Loaded

Platelet Counts (K/uL)									
Donor	D1			D2			Average		
Tube Size - mL	1.80	5.00	10.00	1.80	5.00	10.00	1.80	5.00	10.00
Background Count	0	0	0	0	0	0	0	0	0
Base Count	242	287	354	265	333	345	254	310	350
30 Sec - PP12	213	306	371	106	284	483	160	295	427
2 Min - PP12	0	0	71	5	3	70	3	2	71
3 Min - PP12	22	0	19	72	5	14	47	3	17
5 Min - PP12	1	0	32	1	0	0	1	0	16
15 Min - Jouan	10	71	63	15	38	67	13	55	65

PT Seconds									
Donor	D1			D2			Average		
Tube Size - mL	1.80	5.00	10.00	1.80	5.00	10.00	1.80	5.00	10.00
30 Sec - PP12	12.6	20.0	19.6	12.8	19.7	19.8	12.7	19.9	19.7
2 Min - PP12	13.0	19.7	19.9	30.0	19.3	19.4	21.5	19.5	19.7
3 Min - PP12	12.8	19.8	19.5	12.8	20.0	19.8	12.8	19.9	19.7
5 Min - PP12	13.3	19.7	19.8	12.8	19.8	18.9	13.1	19.8	19.4
15 Min - Jouan	13.6	34.8	19.6	13.0	19.8	19.3	13.3	27.3	19.5

aPTT Seconds									
Donor	D1			D2			Average		
Tube Size - mL	1.80	5.00	10.00	1.80	5.00	10.00	1.80	5.00	10.00
30 Sec - PP12	25.0	45.4	44.4	25.0	45.9	45.6	25.0	45.7	45.0
2 Min - PP12	25.0	42.0	42.6	25.0	42.1	43.2	25.0	42.1	42.9
3 Min - PP12	25.0	42.8	41.2	25.0	43.8	43.8	25.0	43.3	42.5
5 Min - PP12	25.0	41.8	43.4	25.0	42.0	42.9	25.0	41.9	43.2
15 Min - Jouan	25.0	40.7	42.2	n/a	42.0	42.3	25.0	41.4	42.3

PlasmaPrep 12 Rotor Test 9/11/14

3 Minutes, Rotor Half Loaded

5 Minutes, Rotor Fully Loaded

Platelet Counts (K/uL)														
Donor	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	Average	Percent Removal
Tube Size - mL	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00		
Background Count	0	0	0	0	0	0	0	0	0	0	0	0	0	
Base Count	253	229	342	339	110	245	204	262	260	345	365	343	275	
3 Min - PP12	2	3	4	0	2	3	8	13	17	5	10	19	7	97%
5 Min - PP12	2	0	0	0	38	2	6	7	61	0	1	0	10	96%
15 Min - Jouan	87	49	59	111	27	38	39	38	72	87	81	105	66	76%

PT Times - Seconds - K2ETDA														
Donor	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	Average	
Tube Size - mL	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00		
Base aPTT	15.2	17.1	17.5	16.9	21.8	18.1	20.5	18.0	19.1	19.3	19.0	18.8	18.4	
3 Min - PP12	15.6	15.7	16.5	16.8	18.9	18.3	19.5	18.2	19.2	19.0	18.8	17.8	17.9	
5 Min - PP12	16.1	15.5	16.3	17.1	21.0	18.5	19.6	18.4	18.9	18.9	18.6	18.2	18.1	
15 Min - Jouan	15.2	17.1	17.5	16.9	21.8	18.1	20.5	18.0	19.1	19.3	19.0	18.8	18.4	

APTT TIMES - Seconds - K2ETDA														
Donor	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	Average	
Tube Size - mL	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00		
Base aPTT	68.3	69.7	45.5	45.7	68.7	52.4	62.6	51.3	40.8	40.6	40.8	41.0	52.3	
3 Min - PP12	63.1	71.9	44.3	44.8	59.9	55.0	58.6	53.4	40.7	40.1	40.5	40.0	51.0	
5 Min - PP12	73.5	72.5	44.2	47.0	67.8	53.3	60.3	54.2	40.5	40.5	39.2	39.5	52.7	
15 Min - Jouan	68.3	69.7	45.5	45.7	68.7	52.4	62.6	51.3	40.8	40.6	40.8	41.0	52.3	