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SUPPLEMENTAL TECHNICAL BULLETIN
ST – 2007 – 05

Title: THE PDQ™ PLATELET FUNCTION CENTRIFUGE AND AUTOMATED PLATELET COUNTS

Bulletin No: ST-2007-05

This Supplemental Technical Bulletin (ST) has been developed as a laboratory aid. This ST does not alter, revise or change the information provided in the Technical Bulletin included with each product. In accordance with Good Laboratory Practice and regulatory requirements, each laboratory must develop, validate and adopt its own written procedures.

THE PDQ™ PLATELET FUNCTION CENTRIFUGE AND AUTOMATED PLATELET COUNTS (WHY DON'T THE PLATELET COUNTS FROM PRP PREPARED BY ORDINARY CENTRIIFUGATION MATCH THOSE ON THE PRP PRODUCED BY THE PDQ? WHICH ONE IS RIGHT? WHAT DO I DO ABOUT IT? ANSWERS AT THE END OF THIS SUPPLEMENTAL TECHNICAL BULLETIN)

Platelet aggregation is measured as percent transmittance based on the difference in opacity between Platelet Rich Plasma and Platelet Poor Plasma. It is not based on platelet count.

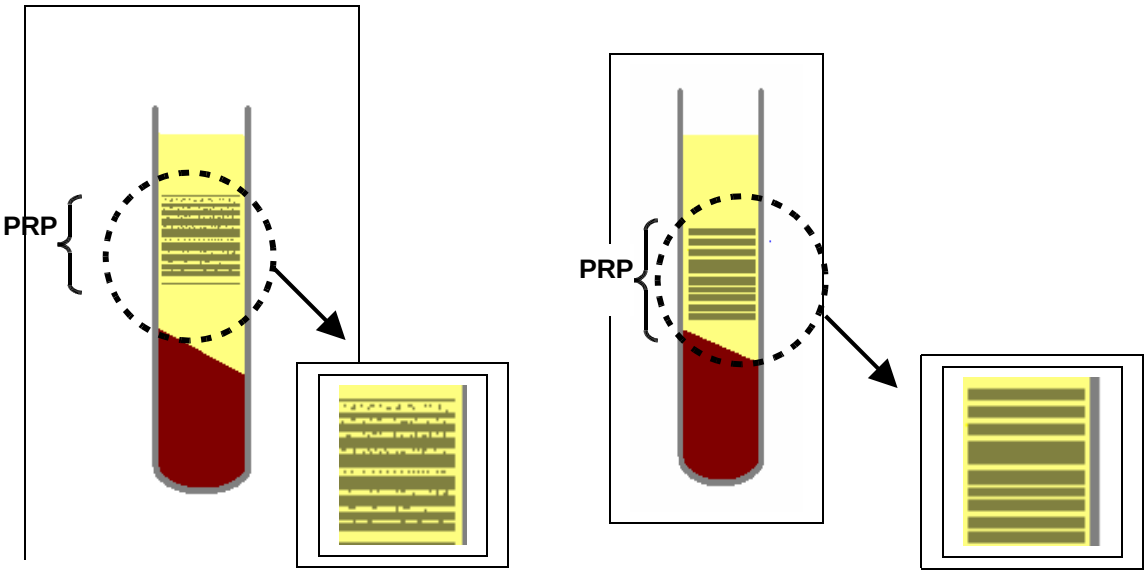
The PDQ Methods Manual states that routinely counting platelets on Platelet Rich Plasma (PRP) is not required.[1] This practice pre-dates modern instrumentation. Some contemporary light transmission (platelet) aggregometers (LTA) can accept samples with platelet counts of 25 - 50,000/cumm. (Platelet counts are required on specimens for whole blood aggregation) Newly available guidelines recommend that aggregation tests be performed on samples with platelets counts of 100,000/cumm or more.[2] PRP platelet counts below 100,000/cumm are uncommon.

The PDQ generated PRP (Fig 1b) has a more uniform distribution of platelets than do samples prepared by ordinary centrifugation (Fig 1a). (see Figures 1a and 1b) The stratification of platelets in PRP from ordinary centrifugation is a significant cause of platelet count variability.

Figure 1

1a
STRATIFICATION OF PRP PREPARED BY ORDINARY CENTRIFUGATION

1b
HOMOGENEITY OF PRP PREPARED USING THE PDQ



Cattaneo and other have demonstrated that adjusting or normalizing platelet counts with autologous Platelet Poor Plasma (PPP) in platelet aggregation samples may result in artificial low results. [1]

Like other laboratory analyses, platelet counts can be affected by pre-analytical and analytical processes. The PDQ has been performance tested using specimens collected with syringes and evacuated specimen collection tubes with 21 g winged needles. Results for platelet counts, platelet aggregation and coagulation tests were as expected. Our findings are consistent with those reported by Lippi and Mani. [4, 5]

In a 2007 survey of major manufacturers of automated hematology analyzers including Abbott Laboratories, Seimens Medical (Bayer Diagnostics) Beckman-Coulter and Sysmex Corporation, each

of the available analyzers has an intended use of counting blood cells in EDTA anticoagulated whole blood. Other uses, unless fully validated by individual laboratories are considered "off-label".[6] In comments to an FDA Exert Panel on platelet collection, Dr. J. AuBuckon reported the following:

"As is well known in the industry and to the agency, currently available automated instruments intended for counting platelets in whole blood samples of patients provide widely divergent platelet counts when applied to platelet rich plasma....A reference method for counting platelets has been published (AJCP, 2001;115:460-4), and an immunologically based method has been shown to compare accurately to this (BJH 2000; 108:228-35). Utilization of methods that have not been appropriately validated and calibrated leads to discrepancies....An alternative would be to publish a list of hematology instruments that have been approved for counting platelets in platelet rich plasma and require the use of one of these. This list of approved instruments is a short one and not widely known." [7]

Counting platelets in PPP or Platelet Free Plasma (PFP) has all the limitations described above as well as those specific and well known to confound very low platelet counts.

Van Pape reported that a stand alone centrifuge, properly set for time and g force specified in published guidelines failed to produce PPP with platelet counts less than 10,000/cumm. The 1500 X *g* for 15 minutes cycle produced PPP with platelet counts of 30,000/cumm. At 2500 x *g* for 15 minutes, the PPP had platelet counts of 11,000/cumm. [8]

Highly automated centrifuges such as the Beckman-Coulter Power Processor require ten minutes to produce PPP with platelet counts of 800/cumm compared to the PDQ's 120 second cycle. [9]

PRP and PPP samples produced by a dedicated platelet function centrifuge are more suitable than those produced by ordinary or automated centrifuges. Platelet counts are consistently and repeatedly in the desired range for each sample type.

The consistency of test results confirms the acceptability of the PDQ generated PRP. (see Figure 2)

Figure 2

Epinephrine aggregation on PDQ prepared PRP

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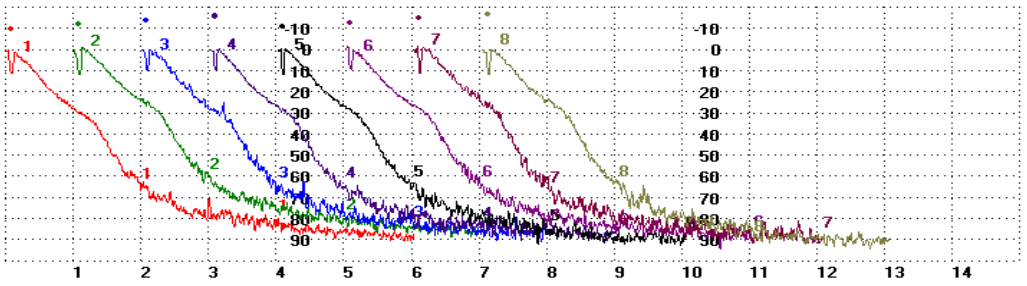

Ch	Sample ID	ID Number	Date	Test Type	Details	PA	PS	SA	SS	AUC*	LP	DA	MA	FA
1	ma	7225	9/27/2007	Epinephrine		31	18	57	25	386	0	0	89	87
	Comment:													
2	ma	7225	9/27/2007	Epinephrine		27	16	59	24	371	0	0	86	84
	Comment:													
3	ma	7225	9/27/2007	Epinephrine		31	18	56	25	382	0	0	87	85
	Comment:													
4	ma	7225	9/27/2007	Epinephrine		28	18	59	27	387	0	0	88	86
	Comment:													
5	ma	7225	9/27/2007	Epinephrine		29	18	61	25	394	0	0	90	88
	Comment:													
6	ma	7225	9/27/2007	Epinephrine		28	17	60	25	387	0	0	88	86
	Comment:													
7	ma	7225	9/27/2007	Epinephrine		89	28	0	0	388	0	0	89	87
	Comment:													
8	ma	7225	9/27/2007	Epinephrine		26	17	65	26	392	0	0	91	89
	Comment:													

 PLATELET AGGREGATION PROFILER® Model PAP-8E S/W Ver:1.0.8 Printed: 9/27/2007 1:45:27 PM

Final aggregation has a mean of 86% and ranges from 84 to 89%. The AUC has a mean of 387 and ranges from 371 to 394. This enhanced repeatability is the result of the PDQ's pre-set and non-variable parameters and the uniformity of the PRP generated by the PDQ.

The use of automated hematology analyzers to count platelets in PRP, though frequently performed, **is unnecessary and is not a validated determination**. Platelet counts on specimens other than EDTA anticoagulated whole blood are not accurate.

Platelet counts are neither a valid performance verification test for PDQ sample preparation, nor a reliable or comparable predictor of sample acceptability. There is no need to routinely perform platelet counts of LTA samples.

Platelet aggregation measurements are based on the difference in the amount of light passing through PRP and are reported as %T, % Aggregation, or Final Aggregation (%). 100% T is the same for samples with a platelet count of 200,000/cumm as those with a 500,000/cumm count. The appropriate performance verification for the PDQ is to compare platelet aggregation results on samples from known donors prepared by ordinary centrifugation and using the PDQ.

(ANSWERS: AUTOMATED CELL COUNTERS ARE NOT INTENDED TO COUNT CELLS IN PRP. IT IS LIKELY THAT NEITHER PLATELET COUNT IS RIGHT! FOLLOW MANUFACTURER'S INSTRUCTION FOR PERFORMANCE VERIFICATION TESTING)

Validation and correlation assistance is available from Bio/Data Corporation. Contact Customer Service for details.

REFERENCES:

1. Bio/Data Corporation. PDQ Methods Manual, Doc No 106909 . Bio/Data Corporation. Horsham, PA. September 2007.

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