

## SUPPLEMENTAL TECHNICAL BULLETIN ST – 2006 – 14

**Title: Platelet Washing Protocol – HIT, HIPA**

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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.

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**PLATELET WASHING PROTOCOL Use for:  
HEPARIN INDUCED THROMBOCYTOPENIA (HIT) AND HEPARIN INDUCED PLATELET AGGREGATION (HIPA)**

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The American Society of Hematology recommends the use of washed platelets in platelet aggregation test (PAT) procedures for HIT and HIPA. Using washed platelets increases the sensitivity and specificity of PAT for heparin antibodies.<sup>1,2,3</sup>

There are many “platelet washing” procedures in the literature.<sup>4</sup> They vary in application, buffer used, washing cycle and complexity. Laboratories should select the washing procedure and buffer best suited for their test procedure.

One commonly used platelet washing procedure uses a Tyrode’s Buffer.<sup>5</sup> This procedure is simple and effective. It can be used on the PAP-4 and PAP-8E.

**PLATELET WASHING PROCEDURE:**

1. Draw an anticoagulated (3.2% Sodium Citrate) whole blood specimen following the laboratory’s approved procedure.

Refer to specimen collection procedures in the PAP-4 and PAP-8E Operation Manuals, Technical Supplements and the Platelet Aggregation Pre-analytics wall chart.<sup>6</sup>

**DO NOT COLLECT THE SPECIMEN IN SPECIMEN COLLECTION TUBES CONTAINING HEPARIN, OR FROM AN IV LINE OR PORT.**

2. Centrifuge (low G) the primary specimen collection tube (150 x G for 10 minutes)

**DO NOT UNCAP THE SPECIMEN COLLECTION TUBE DURING CENTRIFUGATION.**

3. Carefully aspirate the Platelet Rich Plasma (PRP) layer<sup>6</sup> with a plastic transfer pipette, and place the PRP sample into a plastic aliquot tube. Cap or seal the aliquot tube. (note PRP volume)
4. Centrifuge the PRP sample at 400 x G for 15 minutes to form a concentrated platelet pellet.

5. Carefully aspirate the remaining plasma (supernatant) from the concentrated platelet pellet, using a plastic transfer pipette.
6. Re-suspend platelet pellet in a volume of Modified Tyrode's Buffer (or your selected buffer) to achieve optimal platelet count for your test procedure.
7. Based on the washed platelets dilution, adjust the "blank" to achieve acceptable aggregation and slope. The laboratory should develop protocols to define the proper blanking mix for its application.

Start with a ratio of 1 part of washed platelet to 5 parts of buffer. A higher concentration of platelets will increase the slope and % Aggregation. A lower platelet concentration will reduce the slope and % Aggregation.

**THE MATERIAL USED TO SET THE BASELINE/BLANK MUST BE ADJUSTED TO A SUITABLE BUFFER / PLATELET COMBINATION.**

8. "Washed" platelets may now be used in test procedures as described in the Operation Manuals.

**WASHED PLATELETS SHOULD BE ASSAYED WITHIN 45 MINUTES OF PROCESSING.**

**STABILITY OF WASHED PLATELETS WILL VARY WITH THE BUFFER SELECTED.**

Formulation and Preparation Tyrode's Buffer – pH 7.35

Adjust pH to 7.35 using NaOH and HCl								
MATERIAL	Catalog Number	UNIT QUANTITY	(F) Liters	TOTAL REQUIRED	LOT #	VENDOR	BY	CK
Sodium Chloride		8.0 g/L	X	=				
Potassium Chloride		0.20 g/L	X	=				
NaHCO <sub>3</sub>		1.0 g/L	X	=				
NaH <sub>2</sub> PO <sub>4</sub>		0.05 g/L	X	=				
MgCl <sub>2</sub> ·H <sub>2</sub> O		0.203 g/L	X	=				
Glucose		1.0 g/L	X	=				
HEPES		2.38 g/L	X	=				
Purified Water		1.0 L/L	X	=				
5.0 N NaOH		as required	X	=				
5.0 N HCl		as required	X	=				

**REFERENCES:**

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2. Warkenin, TE. Heparin Induced Thrombocytopenia Diagnosis and Management. Circulation: 110:e454 – e458. 2004.
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4. Walenga, JM, Jeske, WP et al. Activation of Platelets by Heparin Induced Thrombocytopenia Antibodies in the Serotonin Release Assay is not Dependent on the Presence of Heparin. J Throm Haemost. Vol 13, No 10. 2168 – 2175. Oct 2005.
5. Tyrode, MV. Pharmacodyne: 17: 205 – 209. 1910.
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7. de La Vega, LP, et al. Association of Heparin Dependent Antibodies and Adverse Outcomes in Hemodialysis Patients : A Population Based Study. Mayo Clinic Proceedings. 2005;80: 995 - 1000

Operation Manual, Platelet Aggregation Profiler®, Model PAP-4, P/N 103400

Operation Manual, Platelet Aggregation Profiler®, Model PAP-8E, P/N 106221

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