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SUPPLEMENTAL TECHNICAL BULLETIN
ST – 2006 – 06

Title: Ristocetin Induced Platelet Aggregation (RIPA)

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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.

Ristocetin Induced Platelet Aggregation (RIPA)

RIPA is a test performed on human platelets using ristocetin reagent. This assay measures the response of patient platelets to a series of decreasing concentrations of ristocetin reagent. RIPA tests are recommended when the RiCoF activity assay is abnormal, or type 2BvWD is suspected (based on family history or laboratory evaluation).

A common variation of the RIPA test, sometimes called RIPA screen, uses a high and low concentration of ristocetin, typically 1.2 and 0.6 mg/mL.

Ristocetin will react with normal human platelets causing aggregation. This aggregation will be proportional to the concentration of ristocetin in the platelet rich plasma / ristocetin mix as the concentration of platelets remains constant.

A person (patient) with a von Willebrand like bleeding disorder will show reduced or no aggregation when ristocetin (of a specific concentration) is mixed with platelet rich plasma of the standard concentration. A normal person will show a standard defined aggregation profile of **Slope** and **Total Aggregation**.

Laboratory Considerations:

The aggregation profile produced with varying concentrations of ristocetin will be different with each lot of ristocetin for testing.

Each normal donor used for testing will also exhibit a varying profile.

Normal profiles should be developed using at least 4 concentrations of ristocetin for at least four (4) normal donors.

The 4-concentration profile must be used to develop the lower limit for the ristocetin concentration that generates a “normal” aggregation pattern.

Recommended ristocetin concentrations are: 1.3, 1.1, 0.9 and 0.7 mg/mL for the development of a normal profile for each normal donor with each different lot of ristocetin.

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In a normal test system, 50 µL of the ristocetin dilution is added to 450 µL of the platelet rich plasma, inducing aggregation. Aggregation is quantified by observing both the rate of aggregation (Slope) and the total aggregation induced (% aggregation). In this test system, "working" concentrations of 13, 11, 9 and 7 mg/mL must be prepared. When 50 µL of the "working" concentration is added to the 450 µL of PRP it results in a "final" concentration in the test system (1.3, 1.1, 0.9, and 0.7 mg/mL)

Concentrations below 0.7 mg/mL are not recommended in this screening test.

A two (2) concentration "screening" assay can now be derived from the above testing. An expected Normal Profile (Slope and % aggregation) may also be determined with this testing. Expected Slopes and % aggregation are specific for the instrumentation used for testing.

Normal donors will exhibit unique profiles for each lot of ristocetin.

For Example:

Testing of all normal donors may show that for a specific lot of ristocetin, all normal donors showed acceptable Slope and % aggregation at a concentration of 1.2 mg/mL. The same normal donors all showed unacceptable Slope and aggregation at a concentration of 0.7 mg/mL. Using this data, a two point screening (test) may be developed using the concentrations of 1.2 and 0.7 mg/mL to screen an unknown donor.

These two points are unique for a specific lot of ristocetin. Results with other lots of ristocetin may vary to a significant amount as developed by the 4-point dilution profile on the normal donor.

The following is provided to illustrate the variability of the ristocetin reagent raw material. Data provided was generated on a Platelet Aggregation Profiler®, Model PAP-4C from Bio/Data Corporation

		1.0 mg/mL		1.25 mg/mL		1.5 mg/mL		TESTED WITH "NORMAL" PLATELET DONORS
		Slope	Agg.	Slope	Agg.	Slope	Agg.	
For 17 lots tested 1994 - 2004	**MEAN**	**28.7**	66.9	**37.7**	83.7	**48.0**	88.9	
	SD	10.0	24.9	9.0	16.9	8.6	9.4	
	CV	34.94%	37.29%	23.95%	20.16%	17.80%	10.54%	

References:

NCCLS – Assays of von Willebrand Factor Antigen and Ristocetin Cofactor Activity: Approved Guideline; Guideline # H51-A, page 7.