

## SUPPLEMENTAL TECHNICAL BULLETIN ST – 2006 – 06

**Title:** Ristocetin Induced Platelet Aggregation (RIPA)

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### 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.

In a normal test system, 25  $\mu$ L of the ristocetin dilution is added to 225  $\mu$ L 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 25  $\mu$ L of the "working" concentration is added to the 225  $\mu$ L 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	<b>MEAN</b>	28.7	66.9	37.7	83.7	48.0	88.9	
	<b>SD</b>	10.0	24.9	9.0	16.9	8.6	9.4	
	<b>CV</b>	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.