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Technical Report 6270

CHRONO-PAR P/N 396 RISTOCETIN AND MICROCENTRIFUGE ALIQUOT TUBE TESTING REPORT

1. Objectives

- a. The primary purpose of this study was to confirm the stability of the CHRONO-PAR P/N 396 Ristocetin reagent for platelet aggregometry testing following reconstitution and storage of reagent aliquots for 90 days at -20°C. Tests were performed immediately, using newly reconstituted Ristocetin, and at monthly intervals using frozen aliquots. Whole Blood Aggregation (WBA) testing was used.
- b. The secondary purpose of this study was to check the stability of various aliquot tubes of different sizes [0.3, 0.5 and 1.7 mL], plastics [polypropylene and polyethylene] and caps [snap cap or twist-on with O-ring seals]. Reagent volume loss was determined by comparing the weight of each tube prior to freezing to the weight of each tube immediately after thawing. Reagent was reconstituted July 1, 2011 with final aliquot thawed on October 1, 2011. Data reported is from the final thaw, 90 days after the reagent reconstitution and preparation of the aliquots.

The study was performed at Medical Research Technology Laboratory, University of Maryland, Baltimore, Maryland, USA.

2. Reagent Information

The CHRONO-PAR Ristocetin reagent is intended to be used for analysis of platelet function via agglutination studies in whole blood and platelet rich plasma. The Reagent used for this study was CHRONO-PAR, P/N 396 Ristocetin, Lot No. B-1186-8, Expiration Date of April, 2014.

3. Materials

Aliquot Tubes	Part No.
Free-Standing Polypropylene Tube with Natural Color + Caps with O-ring seal, 0.5mL	C19053/C19046 [Denville Scientific]
Free-Standing Polypropylene Tube with Amber Color + Caps with O-ring seal, 0.5 mL	C19053-A and C19046-A [Denville Scientific]
Microcentrifuge Polypropylene Clear Conical Tube with Snap cap, 1.7 mL	20170-333, www.vwr.com
ADDITIONAL TUBES: 0.3 mL Polypropylene and Polyethylene	--

Instrumentation Used: Chrono-log Model 560CA and 810CA

4. Reagent Stability

Testing was performed with a single lot of CHRONO-PAR P/N 396 Ristocetin, shipped overnight at ambient temperature and stored at 2-8°C immediately upon arrival. On day one (1) of testing, four (4) aliquot tubes were prepared for each tube type by adding 50 uL of Ristocetin into each tube and weighing each tube with a Mettler Scientific Lab Balance Scale. Three (3) of each tube type was then placed in -20°C freezer and one (1) of each placed on ice for immediate testing. One (1) aliquot for each tube type was hand thawed for testing at monthly intervals.

a. Assays:

A single drug-free normal donor was used throughout the study. Whole Blood Aggregometry (WBA) was tested with a Ristocetin final concentration of 1.0 mg/mL. At each testing day, citrated whole blood was analyzed in accordance with the Chrono-log Whole Blood Aggregation procedure on a Chrono-log Model 560CA/810CA. Maximum Aggregation Amplitude was evaluated. Tests were run following the manufacturer recommended procedures.

b. Ristocetin Stability Claim:

Lyophilized - Until the expiration date, when stored in the refrigerator at 2-8°C;
Reconstituted - 3 months, stored frozen at -20°C.

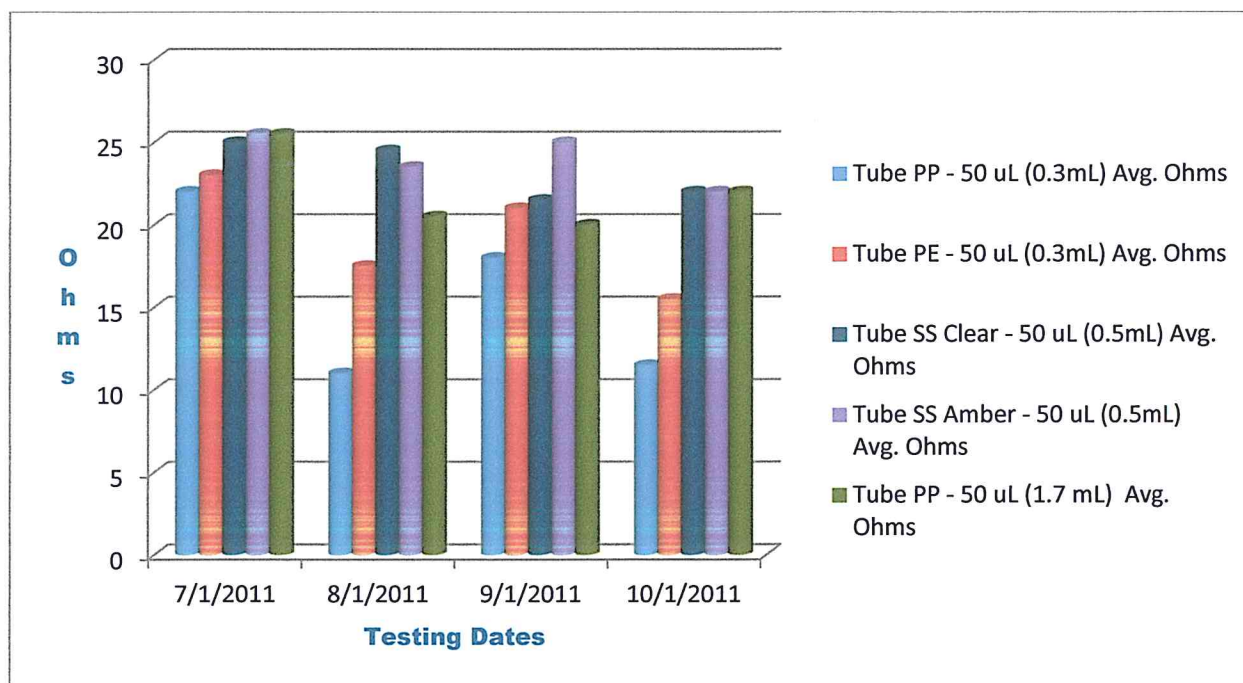
c. Aim:

- 1) To confirm the stability claim of the Chrono-log Ristocetin reagent throughout the recommended 3-month storage at -20°C, in various aliquot tubes.
- 2) To investigate the stability of Ristocetin when stored in various aliquot tubes of different sizes [0.3, 0.5 and 1.7 mL], plastics [polypropylene and polyethylene] and caps [snap cap or twist-on with O-ring seals]. This was done by measuring reagent volume loss by weight and by comparing the results of Whole Blood Aggregation testing at each time point.

- 3) To verify that the P/N 396 Ristocetin reagent is stable for 3 months and to determine which aliquot tube provided the most stable test results with minimum loss of reagent volume.

d. Results:

Chart 1 - Ristocetin-Induced Whole Blood Aggregation with Various Aliquot Tubes



Whole Blood Aggregation test results from various types and sizes of aliquot tubes containing 50uL of Ristocetin stored at -20°C. Maximum Aggregation Amplitude, calculated in Ohms, compared over the (4) time points with each tube type. Tube SS Amber had most consistent Aggregation test results.

PP = Polypropylene tubes with snap caps
PE = Polyethylene tubes with snap caps
SS = Free-Standing Polypropylene tubes with twist-on caps and O-rings

Table 1 - Reagent Loss Determined by Weight in Each Tube Type [mg]

Test Date	PP – 50 uL (0.3 mL)	PE – 50 uL (0.3 mL)	SS Clear – 50 uL (0.5 mL)	SS Amber – 50 uL (0.5 mL)	PP – 50 uL (1.7 mL)
7/1/2011	0.4310	0.4325	1.6260	1.9504	1.0555
10/1/2011	0.3880	0.4000	1.6150	1.9499	1.0499
Weight loss	- 0.0430	- 0.0325	- 0.0110	- 0.0005	- 0.0056

PP = Polypropylene tubes with snap caps
PE = Polyethylene tubes with snap caps
SS = Free-Standing Polypropylene tubes with twist-on caps and O-rings

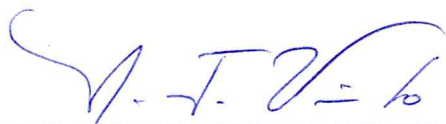
5. Results

The data from this study shows that:

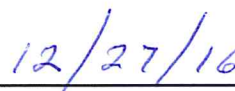
- The reconstituted CHRONO-PAR RISTOCETIN reagent is stable for 90 days when 50 uL frozen aliquots are stored at -20°C in the proper type tubes.
- A tube size of between 0.5 mL and 1.7 mL provides the greatest reagent stability based on Aggregation test results.
- A tube size of between 0.5 mL and 1.7 mL provides the greatest reagent stability based on reduced weight loss.
- Polypropylene tubes with tube size of between 0.5 mL and 1.7 mL gave the most consistent results for each time point tested.

6. Conclusion

Based on consistency of Aggregation testing over four (4) time points and on lower Reagent Loss determined by weight, the SS Clear (0.5 mL) and the PP (1.7 mL) tubes are acceptable. The SS Amber (0.5 mL) tube provided the most consistent Aggregation results and had the least amount of reagent loss, determined by weight, three (3) months after reconstitution. Therefore, the reconstituted CHRONO-PAR Ristocetin reagent is stable for 90 days when 50 uL frozen aliquots are stored at -20°C, in Polypropylene tubes with a tube size of between 0.5 and 1.7 mL.



Nicholas J. Veriabo
Executive Director



Date