BIO/DATA CORPORATION

155 GIBRALTAR ROAD, P.O. BOX 347, HORSHAM, PA. 19044-0347 USA
WORLDWIDE: (215) 441-4000 USA (800) 257-3282 FAX: (215) 443-8820
www.biodatacorp.com email: bdc@biodatacorp.com

SUPPLEMENTAL TECHNICAL BULLETIN
ST – 2006 – 01

Title: Application and Use of the Ristocetin Cofactor Assay

Bulletin No: ST-2006-01

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.

APPLICATION AND USE OF THE RISTOCETIN COFACTOR ASSAY

Background:

As originally developed the Ristocetin Cofactor Assay was a screening test to distinguish abnormal activity of the vW Factor (molecule) from normal activity. At this time the "normal range" was defined as between 60% and 150% Activity.

To use this assay, samples of a known normal (reference) activity were diluted and reacted with a (defined) concentration of Ristocetin Sulphate solution. The reaction produces data that included a value for "SLOPE" and % AGGREGATION. The SLOPE values from the dilutions (usually 3 dilutions at 100%, 50 % and 25%) were then plotted in an LOG/LOG coordinate system with the SLOPE values comprising one axis and the % Activity Values (from the dilutions) comprising the other axis. After plotting the data, a "linear" best-fit line was drawn between the data points to create a "Standard Reference Curve" (SRC),

In this initial development, the Ristocetin Sulphate, was classed an "antibiotic" and its properties as a reactant in the assay system were not defined. Purity and properties as a reactant in the assay system were never defined or determined.

This SRC was then used to extrapolate the Assay Value of unknown plasmas as they were assayed using the same Ristocetin Sulphate solution at the dilution as defined for the 100% point used to generate the SRC.

By practice and intent this system was considered a "linear" reaction for the utility of distinguishing between normal and abnormal activity of a patient test specimen.

As originally developed, this system of estimation was considered precise and reproducible if the Coefficients of Variation (CV) were ± 15% or less. The overall precision and reproducibility of the assay system was defined as between 12.5% and 15% (CV)

As the assay system evolved its utility for the diagnosis of von Willebrand Syndrome became more important. As this tool became more important, the users have modified the assay system to

generate more accurate and precise results and to attempt to “quantitate” the activity beyond the definitions of “normal” and “abnormal” and to use results form the assays for therapeutic intervention in the disorder.

There are many developed variants of the original assay system. These variants include the concentration of the dilutions, number of dilutions of the reference plasma used in constructing the SRC. Also variants include custom or standard concentrations of the reactants used in the assay system.

As the users of the assay system developed variants to the assay system, they attempted to “standardize” materials and methods to achieve better resolution and reproducibility.

The first important development in the improvement to the assay system was the development of a “standard” stable (By Lyophilization) preparation of the platelet suspension (essential to the assay system) that is commercially available. Bio/Data Corporation was one of the initial companies to market a standard platelet suspension preparation. Other sources developed and currently there are many sources for this material. But each source has its own processing methods and the reactivity of the platelet preparation (though “Standardized”) will vary from source to source. These variants of standard preparations are also applicable to the other commercially available components of the assay system such as the Ristocetin Sulphate, Standard Reference Preparations and “system” buffers and materials.

As standard components were becoming available there also were developed better and more specific instrumentation and methodology used to perform the assay. Again, Bio/Data Corporation was one of the initial companies to market instrumentation for performing the assay. With the introduction of specific instrumentation, some standard methodologies were also developed.

Currently there are a variety of selections for equipment and reagents for this assay system and although the Intended Use (estimation of vW Factor Activity) of these systems is similar, the application of the commercially available instruments and reagents will depend on the use and expectations of the user.

The purpose of this supplemental information is to aid the user in the selection of specific applications of the Bio/Data Corporation’s products to best achieve the intended purpose of the user.

Users will have different requirements as to specific use, accuracy and resolution of the Bio/Data Corporation’s system(s) and require more information to correctly select materials and equipment for their application(s).

About the Ristocetin Cofactor Assay Products currently (January 2006) available from Bio/Data Corporation

Product Availability:	C/N	Configuration
Ristocetin, Aggrecetin® (Bulk)	101241	1 X 100 mg, (No Diluent Supplied)
Aggrecetin (1.5 mg/mL)	100968	1 X 15 mg, 1.0 mL Diluent Supplied
Aggrecetin (1.0 to 1.5 mg/mL)	100970	1 X 15 mg, 2.0 mL Diluent Supplied
vW Abnormal Control Plasma	101270	3 X 0.5 ml
vW Normal Control Plasma	106426	3 X 0.5 ml
vW Normal Reference Plasma	101269	3 X 0.5 mL

vW Factor Assay® (Ristocetin Cofactor Assay), 10 Determinations

Lyophilized Platelets	1 X 4.0 mL
vW Normal Reference Plasma	1 X 0.5 mL
vW Abnormal Control Plasma	1 X 0.5 mL
Ristocetin	1 X 0.5 mL
TRIS Buffered Saline (0.06 M, pH 7.5)	1 X 10 mL

vW Factor Assay® (Ristocetin Cofactor Assay), 20 Determinations

Lyophilized Platelets	2 X 4.0 mL
vW Normal Reference Plasma	2 X 0.5 mL
vW Abnormal Control Plasma	2 X 0.5 mL
Ristocetin	2 X 0.5 mL
TRIS Buffered Saline (0.06 M, pH 7.5)	2 X 10 mL

TRIS Buffered Saline, 0.06 M, pH 7.5 1 X 473 mL

Instrumentation: C/N

Platelet Aggregation Profiler®, Model PAP-4	101460
Platelet Aggregation Profiler®, Model PAP-4D	104000
Platelet Aggregation Profiler®, Model PAP-8E	106075 (USA), 105077 (Export)

All the above products are for use in assay systems that are designated for the Intended Use of "the quantitation of von Willebrand factor" as a test application. When a specific instrument or reagent is a component of the test system for a specific application, the user must exercise specific considerations in the selection of appropriate components. This intended use covers a wide range specific of applications, varying from simple screening to a precise measurement of vW Factor Activity. The User should consult with Bio/Data Corporation's Customer Care Department or other knowledgeable sources for specific application to the users needs.

Additionally, the above components are designed to accommodate many specific applications of the generalized assay method, but the user must decide as to the acceptability of a component as it will be used in their specific application.

Variations to the generalized methodology:

1.0 Screening: Distinguishing between Normal And Abnormal

2.0 Activity Measurement in specific ranges

2.1 Measurement in High Normal Ranges or above Normal Ranges (100% or greater activity)

2.2 Measurement in the Normal Range (60% to 90% activity)

2.3 Measurement in the moderate below normal range below 60% Activity (25% to 60% activity)

2.4 Measurement in the extreme below normal range below 25% Activity (5% to 25% activity)

2.5 Measurements below 5% activity should not be performed using the Ristocetin Cofactor Assay System

Expected Accuracy and Precision in a generalized assay system without specific application considerations and reagent selections. The designated ranges apply both to test samples as well as Reference Plasmas used to construct a standard 3-point (100; 50; 25) curve:

1.0 Average Value ± 20% (100% Average = 80 to 120 range; 150 % Average = 120 to 180 range)
2.0 Average Value ± 15%
2.1 Average Value ± 20% (90% Average = 77 to 104 range)
2.2 Average Value ± 12.5% (70% Average = 61 to 79 range)
2.3 Average Value ± 10% (45% Average = 41 to 50 range)
2.4 Average Value ± 20% (20% Average = 20 to 30 range)
2.5 Average Value ± 70% (15% Average = 4 to 26 range)

Review of the above would indicate that a generalized 3-point assay system is provides only suitable results for Screening (Item 1) and testing in the low to mid normal ranges (Items 2.2 and 2.3). If the user's application is not any of these three categories, they should seek assistance to define the requirements for their specific application.

The Products of Bio/Data Corporation and Specific Applications and Techniques:

All of the Ristocetin Cofactor Assay Components and Instrumentation are designed to complete assay on test specimens that have values in the acceptable normal range (60 to 150%) as well as values in the Abnormal Range (20 to 59%) and will perform well in the classic generalized 3-point reference curve system. Also as design criteria, the flexibility to complete special application of the assay technique that goes beyond the classic described methodologies.