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SUPPLEMENTAL TECHNICAL BULLETIN
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Title: von Willebrand Factor Assay (vWF), (Ristocetin Cofactor Assay) (RCoF) - Methods and Techniques – PAP-8E

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von Willebrand Factor Assay (vWF), (Ristocetin Cofactor Assay) (RCoF) - Methods and Techniques

The vWF assay is a system for the quantification of the von Willebrand Factor (% Activity) present in plasma. The system is usually described as using an “Assayed Reference Plasma” (Assayed for RCoF Activity), and a Lyophilized “fixed” Platelet Suspension. The Assayed Reference Plasma is used to construct a “Standard Reference Curve” using dilutions of the Reference Plasma. Controls and patient plasmas are then diluted and assayed using the same platelet suspension. The results of the test plasmas are (graphically) extrapolated against the constructed reference curve to project an estimated activity of the test plasma. The system of estimation and graphical extrapolation is completed in a Log / Log coordinate system. This coordinate system best represents the dilutions used and the expected activities measured.

The RCoF system was originally developed to “estimate” the vWF Activity and to distinguish between specimens in the normal range (50% to 150% Activity), and samples having activity below the normal range (< 45%). With development, this same system is being used to accurately project activities in the range of 5% to 60% Activity. Inherent to the methodology are the difficulties encountered in performing the construction of the standard reference curve and the assay of test specimens. These problems are directly related to the Coordinate System used, the use of reagents systems which have not been optimized for true quantitative behavior, and techniques.

The best results may be obtained by technicians who have a good knowledge of the system and its weaknesses, and have developed good techniques applicable to their assay environment.

Presented below are some additional details relating to technique and reagent preparation when performing the construction of the standard reference curve and completing patient assays.

LYOPHILIZED PLATELETS, C/N 101258, 1 X 10 mL, C/N 101595, 3 X 4 mL

1. Remove Platelet vial and TBS vial from refrigerator and allow equilibration to room temperature (RT) (20°C to 28°C)

2. Reconstitute with the required volume of TBS (10.0 mL for the 10 mL Vial or 4.0 mL for the 4 mL Vial.

   Invert gently to "wet" contents and allow the vial to stand at RT for 5 to 10 minutes. After standing, place on "rocker" and mix for at least 20 minutes, but no more than 30 minutes.

   Set aside at RT for use. Remember to re-suspend the contents prior to use each time material is taken from vial

   Storage of Platelet material at RT for up to 4 hours will not reduce the long term reconstituted stability of the material if correctly stored after use. **Do not freeze.**

3. Prepare the "Blank" for the PAP-8E (Dilution = 1 part of platelet suspension + 1 part of TBS)

   To a siliconized 7.25 X 55 mm tube (C/N 101521) add 125 µL of TBS. Add 125 µL of the platelet suspension. Make sure that the platelets are mixed and suspended before pipetting and dispensing into the tube with the TBS. (The dilution is a 1part of TBS + 1 Part of Platelets. This may be adjusted to accommodate your needs)

   Mix TBS and Platelets. Be careful not to generate bubbles during mixing. Mixing should be by inversion but not shaking.

   Cover tube and set aside till needed in the assay process. The platelet blank should always be stored at RT and inverted for mixing prior to use for setting the blank.

vW NORMAL REFERENCE PLASMA (vWNRP), C/N 101269, 3 X 0.5 mL

1. Remove vWNRP vial and purified water vial from the refrigerator and allow equilibration to room temperature (RT) (20°C to 28°C)

2. Reconstitute the vial of vWNRP with exactly 0.5 mL of purified water.

   Invert gently to "wet" contents and allow the vial to stand at RT for 5 to 10 minutes. Invert the vial 3 or more times to mix the contents. Allow to stand for an additional 10 to 15 minutes.

   Set aside at RT for use. If the material is not going to be used within 1 hour, refrigerate. The reconstituted plasma is stable for up to 8 hours when refrigerated at 2° to 8°C. **Do not freeze**. After refrigeration and equilibration to RT, invert prior to use.

vW Normal reference Plasma Dilutions:

1. Reconstitute and preserve plasma as detailed above.

2. Make vWNRP dilutions in 1-3mL polypropylene tube(s) as follows:

   NOTE: Mix dilutions completely but gently to avoid bubble generation or splashing.

% Dilution	Reagents (RT)	Stability	USE
100%	400uL TBS + 400uL vWNRP reconst.	up to 40 min	within 40 mins
50%	300uL TBS + 300uL 100% vWNRP Diln.	up to 50 min	within 30 mins; not sooner than 5 mins after prep.
25%	200uL TBS + 200uL 50% vWNRP Diln.	up to 50 min	within 30 mins; not sooner than 5 mins after prep.
12.5%	200uL TBS + 200uL 25% vWNRP Diln.	up to 30 min	within 20 mins; not sooner than 5 mins after prep

Reagent proportions may be adjusted to accommodate your needs.

NOTE: Each of the above dilutions has a slightly different stability. The 50% and 25% dilutions are the most stable. The 100% and 12.5% dilutions are the least stable. Plasma may vary as to stability and reactivity after storage and preparation. Technique must be adjusted to accommodate lot-to-lot variations. Stability for any plasma dilution is time and technique dependent, and "minimum time before use" precautions must be observed.

vW RISTOCETIN, 1 X 0.5 mL per vial @ 10 mg/mL per vial.

1. Reconstitute the vial of vW Ristocetin with exactly 0.5 mL of purified water.

   Invert gently to "wet" contents and allow the vial to stand at RT for 5 to 10 minutes. Invert the vial 3 or more times to mix contents and allow to stand for an additional 5 to 10 minutes.

   Set aside at RT for use. If material is not going to be used within 2 hours refrigerate. The reconstituted ristocetin is stable for up to 7 days when stored refrigerated at 2° to 8°C. After refrigeration and equilibration to RT, invert prior to use.

AGGRECETIN®, C/N 100970, 1 X 15 mg per vial ristocetin powder, and 1 X 2.0 mL diluent per vial

1. Reconstitute the vial of Aggrecetin with exactly 1.5 mL of supplied diluent.

   Invert gently to "wet" contents and allow the vial to stand at RT for 10 minutes. Invert 3 or more times to mix contents and allow to stand for an additional 5 to 10 minutes.

   Set aside at RT for use. If material is not going to be used within 2 hours refrigerate. The reconstituted ristocetin is stable for up to 7 days when stored refrigerated at 2° to 8°C. Reconstituted Aggrecetin is stable for up to 8 weeks when stored at < -20° C. Thaw frozen material at 37° C. After thawing, allow to equilibrate to RT and invert prior to use.

vW ABNORMAL CONTROL PLASMA (vWACP), C/N 101270; vW NORMAL CONTROL PLASMA (vWNCP), C/N 106426

1. Remove vW Abnormal Control Plasma (vWACP) vial and purified water vial from refrigerator and allow equilibration to room temperature (RT) (20°C to 28°C)

2. Reconstitute a vial of the appropriate plasma control with exactly 0.5 mL of purified water.

   Invert gently to "wet" contents and allow the vial to stand at RT for 5 to 10 minutes. Invert the vial 3 or more times to mix contents and allow to stand for an additional 10 to 15 minutes.

   Set aside at RT for use. If material is not going to be used within 1 hour, refrigerate. The reconstituted plasma is stable for up to 8 hours when stored refrigerated at 2° to 8°C**. Do not freeze**. After refrigeration and equilibration to RT, invert prior to use.

3. Reconstitute and preserve plasma as detailed above.

4. Prepare the 100% dilution to use as a test plasma:

   To 1-3ml polypropylene tube(s) add 400 µL of the reconstituted vW Control Plasma. Then add 400 µL of RT TBS. Mix completely without the generation of bubbles or splashing. The (100%) diluted plasma is stable for up to 40 minutes. This dilution must be used within this time frame. Allow to stand for at least 10 minutes before using to make additional dilutions. The dilution is 1part of TBS + 1 Part of vW Control Plasma. This may be adjusted to accommodate your needs.

TEST PLASMAS AND OTHER CONTROL PLASMAS

1. Reconstitute (If required) and preserve plasma as detailed above.

2. Prepare the 100% dilution to use as a test plasma:

   To a 1-3 ml size polypropylene tube(s) add 400 µL of the appropriate Plasma. Then add 400µL of RT TBS. Mix completely without the generation of bubbles or splashing. The (100%) diluted plasma is stable for up to 40 minutes. This dilution must be used within this time frame. Allow to stand for at least 10 minutes before using to make additional dilutions. The dilution is 1 part of TBS + 1 Part of Plasma. This may be adjusted to accommodate your needs.

OPERATIONAL CONSIDERATION FOR THE PAP-8E SYSTEM

1. Setup and prepare the instrument for operation. Operate the instrument as directed in the manual and as in the training provided. Following are some suggestions and considerations relating to the performance of an assay.

   Prepare tubes to be used. Add a stir bar (new) to each tube to be used in the test run.

   Add 25 µL of ristocetin to each tube. Take care not to splash reagent on the sides of the tube or form bubbles in the tube.

Add 200 µL of the (mixed) platelet suspension to each of the tubes with the ristocetin. Again, take care not to splash reagent on the sides of the tube or to form bubbles in the tube.

After the platelets are mixed with the Ristocetin, the mixture must be used within 5 minutes. Prolonged exposure of the ristocetin to pH above 7.0 will degrade the ristocetin activity.

Incubate the platelet ristocetin mixture for 1 minute @ 37°C without stirring. Move the tubes to either the incubation or test wells and incubate an additional 2 minutes with mixing. Total incubation time for each test should not exceed 5 minutes

While the incubation is being completed, the blanks for each test channel should be set and the system made ready for assay.

Place tubes in the test wells for 15 seconds to mix and equilibrate. Close well covers and begin the test procedure.

2. When ready to start testing, press the "START" button and observe that the instrument has started to measure and record the % Aggregation. % Aggregation before sample is added should stabilize to a constant at between ± 3% Aggregation. If this does not happen, the blank was not set correctly or the incubation cycle was faulty.

3. When the baseline is stable add 25 µL of sample to the reaction mixture. Press the "INJECT" button within 5 seconds of adding the sample. You should observe that the baseline % Aggregation will change when the sample is added but will reset to 0 % Aggregation after the "Inject " button is pressed. For some strong reactions, the baseline will not reach "0" but will start to aggregate. This is not unusual when dealing with highly abnormal specimens.

4. Pipetting techniques and suggestions:

   In the ristocetin cofactor assay, the addition of the sample should be as gentle as possible. The pipette tip should be just over the liquid surface and centered in the test tube. The sample should dispense completely into the base liquid and gradually mix with the action of the stir bar. The injection of the sample should not cause mixing to any major extent.

   During pipetting, it should be observed that a "full" volume is aspirated and that full volume has been dispensed. Pipetting should not create any bubbles in the reaction mixture or bubbles on the surface of the liquid in the tube. Be careful not to "carry over" any excess material on the outside of the tip. (Wipe tip if necessary) The best pipetting "technique" should always be used.

5. Although the test is not defined as an "exactly timed" assay, operations and testing from channel to channel should be as consistent as possible and each test should be setup and started within a similar and consistent timing sequence.

6. If a test does not come out correctly or as expected, that tube should be examined to determine if there were some problems with the technique or setup. Examples: No stir bar in tube; 2 or more stir bars in tube; liquid on side of tube; bubbles in tube; bubbles in liquid, tube is defective; and so on.