

SUPPLEMENTAL TECHNICAL BULLETIN ST – 2006 – 03

Title: Ristocetin Cofactor Assay – Reference Curve Dilutions

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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.

Standard 3 point (100%, 50%, 25%)

1. Reconstitute reference plasma according to Manufacture's instructions.

Bio/Data Corporation C/N 101269: vW Normal Reference Plasma (NRP)

Allow vial to warm to room temperature before reconstitution

Add 0.5 mL purified water to the vial. Wait 5 minutes then invert vial to assure that all contents of the vial are re-hydrated. Allow the vial to stand an additional 10 minutes before using for making the dilutions. Prior to use invert the vial an additional time to assure complete mixing and re-hydration.

Prepare three tubes. Use either siliconized glass tubes or polypropylene plastic tubes.

Label one tube 100%. To this tube add 200 μ L of the reconstituted NRP then add 200 μ L of TRIS Buffered Saline. Mix and allow to stand for 10 minutes.

Label one tube 50%. To this tube add 200 μ L of the 100% dilution prepared above. Then add 200 μ L of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

Label one tube 25%. To this tube add 200 μ L of the 50% dilution prepared above. Then add 200 μ L of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

The three point reference curve dilutions are now ready for use in assay. The dilutions are stable for 45 minutes after preparation.

If the reference value of the NRP is not between 90% and 110% Activity, You may compensate and adjust the 100% dilution to a reference value of 100% by adjusting the TBS/Plasma ratio when the 100% dilution is prepared.

Example: If the reference value of the NRP is 110%, then to the tube labeled 100%, you would add 182 μ L of the NRP and 218 μ L of TBS. The remainder of the dilution instructions will be followed as provided.

Example: If the reference value of the NRP is 90%, then to the tube labeled 100%, you would add 218 μL of the NRP and 182 μL of TBS. The remainder of the dilution instructions will be followed as provided.

Standard 4 point (100%, 50%, 25%, 12.5)

1. Reconstitute reference plasma according to Manufacture's instructions.

Bio/Data Corporation C/N 101269: vW Normal Reference Plasma (NRP)

Allow vial to warm to room temperature before reconstitution

Add 0.5 mL purified water to the vial. Wait 5 minutes then invert vial to assure that all contents of the vial are re-hydrated. Allow the vial to stand an additional 10 minutes before using for making the dilutions. Prior to use invert the vial an additional time to assure complete mixing and re-hydration.

Prepare four tubes. Use either siliconized glass tubes or polypropylene plastic tubes.

Label one tube 100%. To this tube add 200 μL of the reconstituted NRP then add 200 μL of TRIS Buffered Saline. Mix and allow to stand for 10 minutes.

Label one tube 50%. To this tube add 200 μL of the 100% dilution prepared above. Then add 200 μL of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

Label one tube 25%. To this tube add 200 μL of the 50% dilution prepared above. Then add 200 μL of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

Label one tube 12.5%. To this tube add 200 μL of the 25% dilution prepared above. Then add 200 μL of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

The four point reference curve dilutions are now ready for use in assay. The dilutions are stable for 45 minutes after preparation.

If the reference value of the NRP is not between 90% and 110% Activity, You may compensate and adjust the 100% dilution to a reference value of 100% by adjusting the TBS/Plasma ratio when the 100% dilution is prepared.

Example: If the reference value of the NRP is 110%, then to the tube labeled 100%, you would add 182 μL of the NRP and 218 μL of TBS. The remainder of the dilution instructions will be followed as provided.

Example: If the reference value of the NRP is 90%, then to the tube labeled 100%, you would add 218 μL of the NRP and 182 μL of TBS. The remainder of the dilution instructions will be followed as provided.

Alternate 4 point (100%, 80%, 40%, 20%)

1. Reconstitute reference plasma according to Manufacture's instructions.

Bio/Data Corporation C/N 101269: vW Normal Reference Plasma (NRP)

Allow vial to warm to room temperature before reconstitution

Add 0.5 mL purified water to the vial. Wait 5 minutes then invert vial to assure that all contents of the vial are re-hydrated. Allow the vial to stand an additional 10 minutes before using for making the dilutions. Prior to use invert the vial an additional time to assure complete mixing and re-hydration.

Prepare four tubes. Use either siliconized glass tubes or polypropylene plastic tubes.

Label one tube 100%. To this tube add 300 μ L of the reconstituted NRP then add 300 μ L of TRIS Buffered Saline. Mix and allow to stand for 10 minutes.

Label one tube 80%. To this tube add 320 μ L of the 100% dilution prepared above. Then add 80 μ L of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

Label one tube 40%. To this tube add 200 μ L of the 80% dilution prepared above. Then add 200 μ L of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

Label one tube 20%. To this tube add 200 μ L of the 40% dilution prepared above. Then add 200 μ L of TRIS Buffered Saline. Mix and allow to stand for 5 minutes.

The four point reference curve dilutions are now ready for use in assay. The dilutions are stable for 45 minutes after preparation.

If the reference value of the NRP is not between 90% and 110% Activity, You may compensate and adjust the 100% dilution to a reference value of 100% by adjusting the TBS/Plasma ratio when the 100% dilution is prepared.

Example: If the reference value of the NRP is 110%, then to the tube labeled 100%, you would add 182 μ L of the NRP and 218 μ L of TBS. The remainder of the dilution instructions will be followed as provided.

Example: If the reference value of the NRP is 90%, then to the tube labeled 100%, you would add 218 μ L of the NRP and 182 μ L of TBS. The remainder of the dilution instructions will be followed as provided.