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SUPPLEMENTAL TECHNICAL BULLETIN
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Title: PAP- 8E – Blank (Baseline) Setting

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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.

PAP- 8E – Blank (Baseline) Setting

In platelet aggregation, the operation of setting the "blank" or baseline (0% Aggregation) is an extremely important step to achieve correct representation of the rate of aggregation (Slope) and the total aggregation. If the blank is not set correctly, or is not representative of 0% Aggregation, then the data presented (Slope and % Aggregation) may not be representative of the reaction you are measuring.

Common signs that there is a blanking problem are as follows:

1. The Baseline, upon addition of the sample, shows deflections of more than ± 10% from 0% Aggregation baseline.
2. The Maximum Aggregation is greater than an expected (up to) 110% Aggregation. There are certain Aggregation tests where it is common to achieve % Aggregations greater than 100%; sometimes up to 120%. This is due to the nature of optical aggregation and is not reflective of a compromised sample, reagent, or a problem with the instrumentation.
3. Error messages that indicate there is a problem with either the sample or the blank.
4. Failure to achieve an expected Slope or %Aggregation, or failure to show an aggregation reaction at all.
5. Large variations in test-to-test variation.

When setting the blank, make certain that the blank solution has the same source/composition, minus the platelets, as the test sample. Be sure to seat the blank completely in the test well and do not move or rotate the tube while the blank is being set. Some common errors that may occur during blanking process are:

1. The Platelet Poor Plasma (PPP) used for the blank is not completely free of significant quantities of platelets (<10,000 mm^3).
   Not enough PPP in tube for blanking – use at least 250µl.
2. While setting the blank, the tube was moved or raised before the blanking operation was complete.
3. The blank was not handled or stored properly prior to its use in the blanking operation. It is recommended that that the PPP (tube) be covered and stored at ambient temperature prior to use. Do not incubate or store at the 37°C test temperature.

4. Each specimen tested must have its own “unique” blank and that blank must only be used for the specimen being tested.
5. Samples and blanks that are highly icteric or lipemic may generate incorrect results or may not achieve an acceptable “blank setting” operation.
6. Samples and blanks that are hemolyzed, including very small amounts of hemolysis that are not visually observed, may generate incorrect results or may not achieve an acceptable “blank setting” operation.
7. In the ristocetin cofactor assay (Blank = 1 part TBS + 1 part lyophilized platelets) the following considerations must be observed:
    a. The blank must be covered and stored “off instrument” at ambient temperature. The blank must be at the same ambient temperature when setting all channels within a test or standardization run.
    b. Prior to setting the blank on each channel, the operator must make sure that the platelets in the blank are correctly suspended.
    c. The lot of platelets used for the preparation of the blank must be the same lot that is used for testing.

All blanking procedures are based on using standard aggregation and ristocetin cofactor testing and their blanking requirements. If your application is not standard, then you should establish laboratory specific methods and procedures for the preparation and utilization of the blank.

The blank as used on the PAP-8E system is very specific to the sample being tested and should not be generalized to using non-specific materials.

In standard platelet aggregation testing, it is very important that the blank be free of significant number of platelets. If there are significant numbers of platelets in the blank, it will cause the reported slopes (PS, SS) and maximum aggregation to (MA) to increase. If large numbers of platelets are in the blank, slopes may exceed expected ranges and aggregation may exceed 100%. If the processing of the platelet poor plasma (PPP) is such that it leaves large numbers of platelets, then the processing must be changed. If that is not possible, it may be acceptable to dilute the PPP with 0.85% (W/W) saline to achieve more acceptable properties. It is preferable to process the PPP to the acceptable platelet limits, but if dilution (with up 20% saline) is to be tried, the process must be validated by the laboratory performing the processing and testing before this technique is implemented.