DIAGNOSTICS

Calibration and QC Guidance for the Abbott Alinity hq Hematology Analyzer

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INTRODUCTION AND OBJECTIVE

Alinity hq is a state-of-the-art hematology analyzer that utilizes Advanced Multi-Angle Polarized Scatter Separation (MAPSS™) Technology, using 7 light detectors positioned at different angles and a fluorescence detector (FL1). Alinity hq employs a combination of photometry, optical flow cytometry and fluorescence analysis, together with advanced software algorithms to enumerate cells and report 29 different measurands.

Alinity hq is a fully optical hematology analyzer. A complete blood count (CBC) is reported with a 6-part white blood cell (WBC) differential that includes immature granulocytes (IG). In addition, a nucleated red blood cell (NRBC) count is performed with every CBC. Reticulocyte analysis includes the immature reticulocyte fraction (IRF) and mean hemoglobin content of the reticulocytes (MCHr) as well as percent reticulated platelets (%rP).

The purpose of this document is to familiarize users with the configuration and measurement principles of Alinity hq, as it applies to routine calibration and quality control procedures, and to provide guidance on the best practices of calibration and optimal use of quality control.

OVERVIEW OF CONFIGURATIONS, SAMPLE AND FLUIDICS PATHWAYS OF THE ALINITY h-SERIES

In the simplest arrangement, one Alinity hq unit is used as a standalone instrument. Multiple Alinity hq units can be connected and integrated with or without the addition of an Alinity hs (slide maker stainer) unit to create a multi-module system configuration. In this document, “module” refers to one Alinity hq or one Alinity hs unit.

Each Alinity hq module has two incubation blocks, designated as the left incubation block and the right incubation block (1). Each incubation block contains three incubation cups: WBC cup, RBC cup, and HGB cup (Figure 1). This design allows for higher throughput and more flexibility for sample handling. During routine use, Alinity hq automatically alternates between the two blocks as whole blood samples are processed. When running the precision application, the user can select either the right block, left block, or both blocks. It is highly recommended to use both incubation blocks during regular precision runs, as using one block serves mostly for troubleshooting.

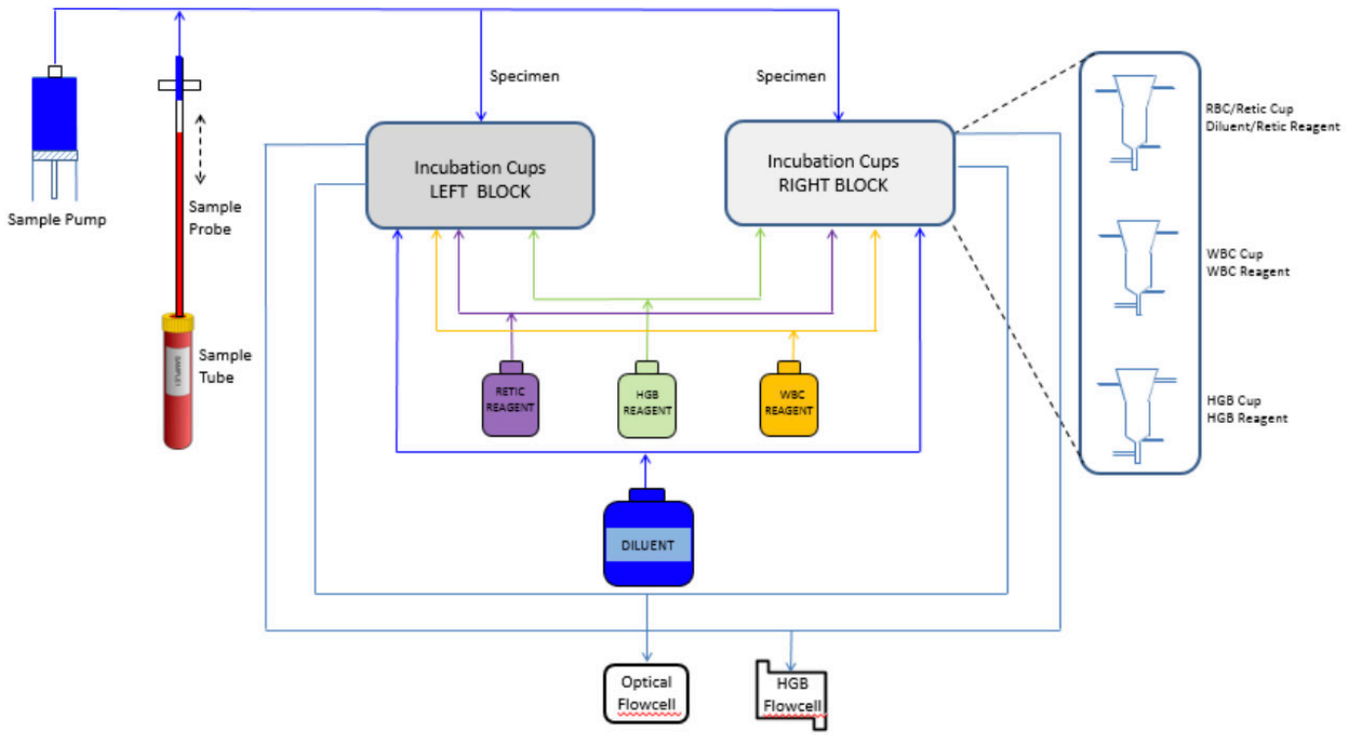

Figure 1. Schematic diagram of reagent and sample workflow

Alinity hq has one sample aspiration probe and aspiration pump. The aspirated sample is directed alternately to the incubation cups contained in the left and right incubation blocks. Separate reagent fluidic paths lead to the two incubation blocks (Figure 1). After mixing and incubating the sample with the appropriate reagent, the contents of the incubation cup are transferred to the flow cell. All samples are measured in the same flow cell, using the same light source and optical detectors.

In summary, some of the fluidic paths are shared between the left and right incubation blocks, though some are at least partially separate, justifying independent calibration and quality control (QC) of the incubation blocks. In a 2+1 configuration (two Alinity hq units and one Alinity hs unit), there are 4 incubation blocks that need to be calibrated.

In addition to calibration and QC, carryover should also be assessed separately on the two incubation blocks. The built-in carryover software module automatically performs the run and the calculation, and reports the results for the left and right incubation blocks (1).

ALINITY hq CALIBRATION

Frequency of Calibration

Calibration of an Alinity hq module should be scheduled in compliance with guidelines that are established by regulatory and accreditation agencies. Calibration should be verified on a regular basis according to the laboratory's procedures and local regulatory requirements.

Calibration or calibration verification is usually required:

- At complete changes of reagents (i.e. change in type of reagent from same vendor or change to a different vendor)
- When indicated by QC data
- After major maintenance or service
- When recommended by the manufacturer
- When required by the regulatory agencies that govern the laboratory (1, 2).

Users must carefully consider the need for calibration when trending is observed in control data. In addition to QC results, moving averages and historical data should also be taken into account. Calibration should only be considered after all other troubleshooting is completed.

Frequent, unnecessary calibration can mask an underlying problem with instrument performance.

Calibration with Commercial Calibrator

Calibration of Alinity hq can be accomplished by using a commercial whole blood calibrator or assayed fresh whole blood (1, 2).

A commercial whole blood calibrator is a whole blood-based material with assayed reference values. These values are traceable to a national or an international reference preparation or method. When using a commercial whole blood calibrator, follow the instructions in the product documentation for correct storage, handling, and mixing.

Follow instructions in the Operations Manual for performing calibration with a commercial calibrator.

Calibration with Assayed Whole Blood

Calibration with assayed whole blood is an alternative to calibration with a commercial calibrator. Assayed whole blood must be assigned values by using a reliably calibrated instrument or a reference methodology. Professional guidelines (2, 3) recommend that:

- At least 10 samples, run in duplicate, are used for cross-calibration
- Specimens used for calibration are less than 4 (maximum 8) hours old
- The determination of whole blood reference values must be completed within 2 hours of whole blood analysis on the system
- The mean of WBC, RBC, HGB, HCT and PLT values of the 10 specimens must fall within the normal range for the laboratory
- Samples must not have morphological or data invalidating flags
- All specimens must be collected in tubes that contain EDTA anticoagulant

Follow instruction in the Operations Manual for performing whole blood calibration.

Initial Calibration During Setup of the Analyzer

When the analyzer is initially set up, it is recommended to use the same lot of commercial calibrator for calibrating each incubation block separately, at the same time. In the case of a single-module configuration, that means two blocks, and in a 2+1 configuration, that means 4 incubation blocks. If the laboratory has more than one Alinity h-series system, all should be calibrated with the same calibrator, at the same time, to ensure equivalency between systems and modules. Calibration factors on each incubation block can be different, even within one module.

Recalibration During Routine Use

If calibration becomes necessary on one module (for example, after major maintenance or a service procedure), it should be performed on both incubation blocks simultaneously. In a multi-module configuration, the other module's calibration needs to be verified at the same time, using the same lot of calibrator. If calibration verification criteria fail, the other module needs to be re-calibrated. In addition, if the laboratory has more than one Alinity h-series system, calibration verification needs to be performed on each system.

It is not recommended to change the calibration on only one incubation block or one module at a time, as results generated by the various blocks and modules will eventually drift from each other.

ALINITY hq QUALITY CONTROL

General Rules

Quality control (QC) procedures for Alinity hq may include (2):

1. Use of commercial whole blood controls
2. Moving average monitoring
3. Retained patient specimens
4. A combination of the above

Each laboratory needs to determine the frequency of quality control runs. There should be a relationship between the frequency of control runs and the number of patient specimens processed (for example, if the lab works only one shift, running controls once a day may be sufficient, but in the case of 24/7 operation, one run per shift may be required) (2).

The Alinity h-series Operations Manual (1) recommends running controls in the following situations:

- After a reagent lot number change
- After maintenance, component replacement, or field service action
- After a software change
- After calibration
- In accordance with the laboratory's quality control program
- In accordance with regulatory requirements

When QC samples are run, each tube is aspirated twice and the instrument automatically tests the control on each incubation block (i.e. generates two data points for each measurand). QC results are displayed on Levey-Jennings graphs and results originating from the left and the right block can be visualized separately or combined (left block = blue line, right block= brown line) (1). The Westgard rules status (if set up) is also displayed on the Levey-Jennings (QC) screen. Status is displayed for each parameter and for both incubation blocks. Westgard rules can be individually enabled and disabled (1).

Commercial Whole Blood Controls

Stabilized assayed or un-assayed control material can be used. It is usually recommended to test a minimum of two different levels of control every 24 hours (2), although local regulatory and accreditation agency requirements as well as internal quality system requirements must also be considered. It is not appropriate to run three levels of control and consider QC results "pass" when QC results are within pre-established QC limits for only two of them. Low-level controls are less informative indicators of calibration status (2).

Moving Average Monitoring

Moving average programs automatically and continuously monitor system performance by tracking the results of various parameters in the patient populations that are being analyzed.

Moving average analysis for hematology is an automated means developed by Dr. Brian Bull (4, 5) to monitor performance by using the known stability of the red cell indices (MCV, MCH, MCHC).

This approach is extended on Alinity hq by applying algorithms that are similar to those of Dr. Bull's for moving average analysis of other RBC, WBC, PLT and Retic parameters.

The Alinity hq moving average programs calculate the means for each parameter in batches of 20 samples. The software includes a moving average statistical formula that smooths and trims data to minimize the weight of outliers (1). The mean that is calculated for each batch is compared to the target value and its action limits. If the mean for a batch is outside of the action limits, a notification is displayed. If the means for the previous two batches for a moving average program are outside the action limits, an alert is displayed.

It is recommended that the moving average programs be used initially with the default settings for acceptance limits and action limits, until the laboratory can establish its own values (1).

ESTABLISHING LABORATORY-SPECIFIC MEAN VALUES AND RANGES FOR COMMERCIAL CONTROLS

Assayed Controls

Assayed commercial controls have expected ranges for each parameter, provided by the manufacturer. The mean of such ranges may not be the exact mean value in a given laboratory. Each laboratory must determine its own mean values that are based on initial analysis of the material. This mean value should fall within the control limits supplied by the manufacturer, but need not exactly match the package insert mean (2, 3, 6).

The laboratory also must establish specific control limits for each measurand. The manufacturer-provided control ranges for commercial control materials are not the same as between-run SD ranges, and are too wide for daily QC of a single instrument (2, 3, 6). Manufacturer-supplied means and ranges usually encompass the variation observed across many labs (7). Changes in product attributes also need to be considered; for example, MCV and HCT in commercial products may show an increase over time. The laboratory must establish control limits that accommodate for these known changes in product attributes, assuming that calibration status has not changed (3).

For establishing QC mean and QC ranges, guidelines are provided in CLSI H26-A2 document Appendix E. Briefly:

1. Analyze the new lot of control material parallel with the current lot
2. Run each level twice a day for 3 to 5 days and calculate mean values
3. If the calculated mean is within the manufacturer's specified range, use it as the expected mean instead of the manufacturer's mean
4. Acceptable control ranges are determined by evaluating 3 to 6 months of control data at each level, by calculating average weighted %CV
5. The average weighted %CV is used with the newly established mean

To summarize, the use of data supplied from outside the laboratory is meant to be temporary until the laboratory accumulates data based on real performance. SD results obtained during the initial 3 to 5 days are overly optimistic, and should not be used for establishing QC limits. Using results spanning 3 to 6 months will include variables originating from storage, different lot numbers, different users, and will result in establishing realistic control limits.

During the initial time while the data are still being accumulated, the manufacturer's ranges can be used if they are narrow enough to detect medically significant error. If the manufacturer's ranges are too wide, the initial SD value may be used; however, with periodic re-calculation and adjustment. Another option is using historical SD values from the predecessor instrument.

During routine use of commercial controls, lot changes require running the new lot of control material in parallel with the current lot. The current lot is used to determine if the method is in control, and the values obtained on the new lot are used to determine the new mean value.

Un-assayed Control Material

If un-assayed control material is used, establishing the mean values and expected QC ranges becomes the responsibility of the laboratory. The same procedure is recommended to be followed as for assayed controls.

Alinity hq-specific Considerations

As described in the Calibration section, all blocks of each module and of each Alinity h-series system must be calibrated the same way to ensure that obtained results are equivalent. This also creates the condition of creating universal QC mean and ranges for all units/systems in the lab.

The procedure described above for determining QC mean and QC ranges should be performed on each module in parallel, and the mean should be calculated on all results. Similarly, establishing QC ranges should include results generated on each block, module and system during a 3 to 6 months of evaluation period, and the average weighted %CV should be calculated on all results.

APPLYING QC RULES

Laboratories should establish their own QC strategy that complies with regulatory requirements and fits the needs of the laboratory. The strategy has to define the following (2):

1. The number and measurand concentration for each QC sample
2. The frequency of QC sample testing
3. Statistical QC limits (such as multirule analysis)
4. Actions taken when results exceed acceptable limits

It is important to optimize the QC strategy based on real-life performance data. Instead of arbitrarily applying rules to a test, the laboratory should design or select its QC procedures based on the quality that is required for the intended use of the test, the precision and accuracy observed for the method, and known rejection characteristics of the control rules and numbers of control measurements (7-9). According to Dr. Westgard, decisions based solely on the +/- 2SD rule are simplistic and insufficient, as well as inefficient, and usually generate many false rejections. The traditional use of 1:2s "warning" rule is no longer recommended (7, 10). Conversely, the across-the-board implementation of Westgard multirules (11) on all tests is not the most efficient and appropriate way to manage the quality of the tests in the laboratory (10).

When analytical performance is measured on the Six Sigma scale, each test will have a distinct Sigma-metric. For high performing tests (≥ 5.5 sigma), a single control rule (such as 1:3s) can provide desired error detection (although regulatory agencies may require running two controls). For methods with lower sigma values, running three levels of controls, and using the 2of3:2s (reject when 2 out of 3 control measurements exceed the same mean plus 2s or mean minus 2s control limit), the 3:1s (reject when 3 consecutive control measurements exceed the same mean plus 1s or mean minus 1s control limit), or the 6:x, 9:x rule (reject when 6 or 9 consecutive control measurements fall on one side of the mean) should be considered. The 2 of 3:2s and 3:1s rules could be applied over multiple runs on a single control level, but it is also possible to use those within one run (when three levels of controls are used). The 6:x and 9:x rules are used over multiple runs. Multirule analysis is recommended for methods with moderate to low performance (9).

It is crucial to select control methods that are sensitive enough to reveal loss of performance that could compromise patient results, but not oversensitive to the point of signaling error when none exists (3). If the control method is not sensitive enough, erroneous results may not be detected. If the control method is oversensitive, it can lead to the rejection of valid results (false rejection), needless and repetitive calibration, unnecessary anxiety, work and cost for the laboratory.

SUMMARY

In addition to the general application of the rules of laboratory quality control and calibration, the hardware design and sample processing characteristics of Alinity h-series of analyzers require additional consideration.

The presence of the two incubation/mixing blocks within one module and the combination of multiple units into one system makes it necessary to treat them as parts of an integrated system, while performing calibration and quality control on individual elements.

This document provides necessary information for the users of Alinity hq on optimal set-up and use of quality control.

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Product is not commercially available in all countries including U.S.
Alinity hq instrument is a Class I laser product. For *In Vitro* Diagnostic use.

www.corelaboratory.abbott/alinity

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