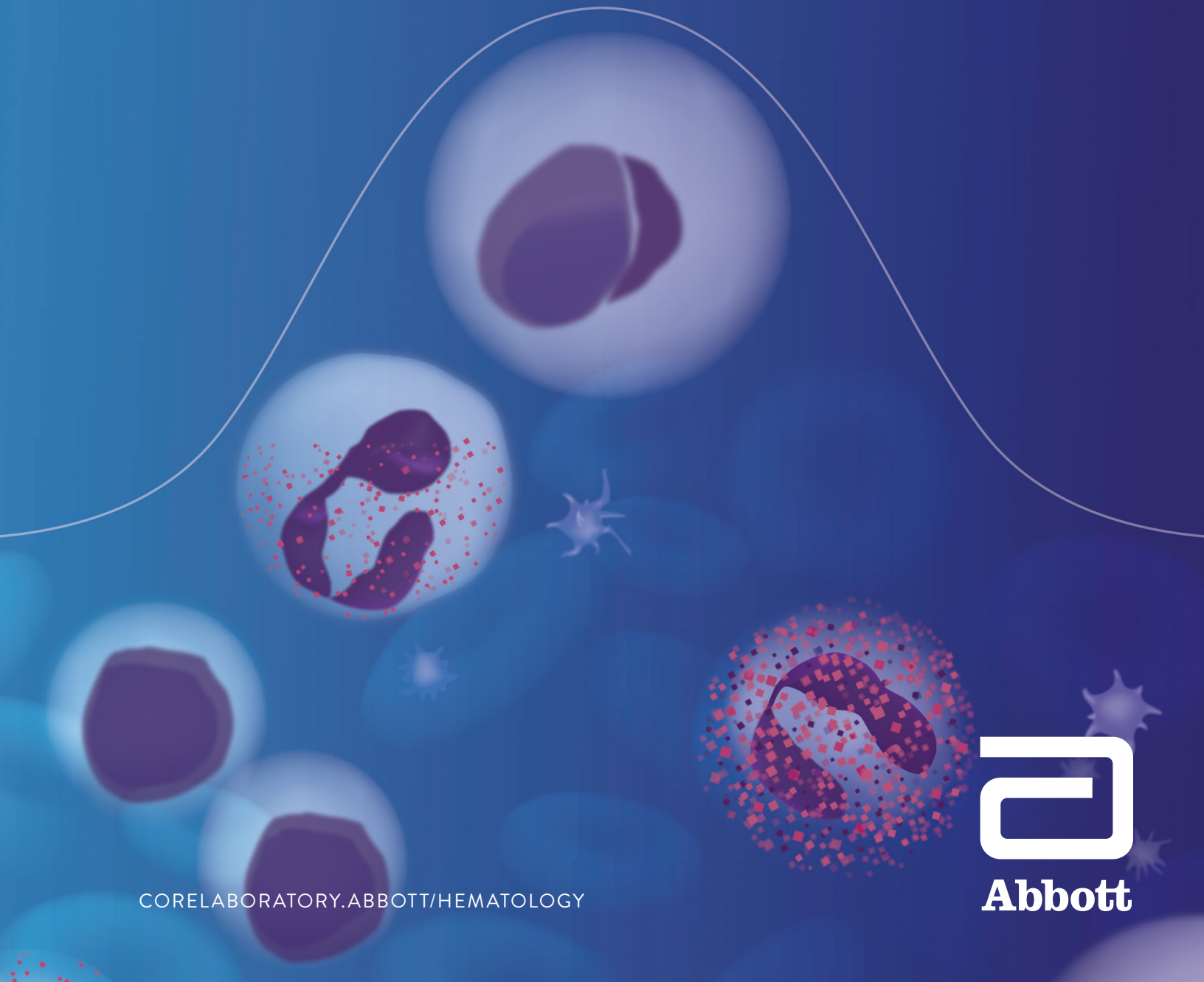
LEARNING GUIDE

QUALITY CONTROL IN THE HEMATOLOGY LABORATORY



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ABOUT THIS LEARNING GUIDE

Quality Control in the Hematology Laboratory is not a comprehensive guide to clinical laboratory quality control or statistics. It is designed to provide a basic understanding of the principles of quality control in the hematology laboratory and give some direction as to what to do when QC results may be other than expected.

Your most important guide is your instrument's Operations Manual. Important information also is contained in the package inserts/IFUs and assay sheets that come with your control and calibration materials. For the best performance, always follow all procedures and warnings contained in these documents.

Finally, your Abbott team is always available to help. For questions or concerns, consult your local Customer Support representative for answers and assistance.

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SECTION 1

INTRODUCTION TO BASIC QUALITY CONTROL IN THE HEMATOLOGY LABORATORY

LEARNING OBJECTIVES

After reading this guide, you will:

Understand basic quality control concepts and learn how to use basic quality control procedures to ensure the best results from your hematology analyzer. This is an important step on your journey towards achieving measurably better healthcare performance.

Quality Control (QC) need not be difficult or mysterious. Through this guide, we will address the basics in a way we hope you will find interesting and intuitive.

WHY PERFORM QUALITY CONTROL?

Quality Control serves one main purpose – to verify that your analyzer is performing as it should, which will help make sure the patient results you produce are correct.

The goal of QC is to ensure that the analytical bias and analytical variability of each measurement does not adversely affect medical decisions that are based on test results. The total analytical error must be lower than clinical needs. In general, the most medically important components of the Complete Blood Count (CBC) are white blood cells (WBC), hemoglobin (HGB), hematocrit (HCT) and platelets (PLT), although other parameters may also be important in specific patient situations.[1] QC protocols are required to address both systematic and random error detection related to precision and accuracy (bias).

Typically, laboratories use an internal (intra-laboratory) QC process designed to maximize error detection and subsequent corrective actions on a daily basis, and an external interlaboratory QC process that provides peer data comparison on a longer-term basis.

PEER GROUPS

A peer group is a group of users that uses the same instrument and the same lot number of control materials that you are using. Reporting your control results to a program such as *STATS*-Link® gives you access to the averaged (and anonymous) results of all the other users who have reported to the program.

There are several benefits to having the peer group result information:

- Your lab is compared to the other labs using the same analyzer and control material so that you can see where your controls are performing vs. all other users in your group.
- The *STATS*-Link® system evaluates your lab compared to the other labs and tells you how far away you are from the results they are reporting.

By reviewing this data, you are able to determine if you have any instrument, reagent or other issues which should be investigated.

In many countries, there are regulatory agencies or other organizations that also will expect you to perform quality control on a regular basis. The reason is to ensure that each laboratory is providing good patient care.

WHAT AM I ACTUALLY CONTROLLING?

When you test a patient sample on your instrument, you may think that you are using a single device. In reality, it is a system that is composed of a number of elements, all of which affect performance and the results that are produced. There are four components that need to be considered:

1. THE ANALYZER

This is the most obvious part. It contains the physical hardware – the pumps, the valves, the counting mechanisms, electronics and software – that creates the measurements. Current CBC hematology analyzers offer a mix of methodologies designed to enhance analytical performance. Today's analyzers use the traditional impedance or electronic resistivity method and a mix of optical light scatter, laser light scatter, and fluorescent flow cytometric methods to obtain results. Each method has its strengths and limitations related to cellular analysis of fresh whole blood specimens, as well as stabilized material used primarily for the internal QC process.

2. THE REAGENTS

These dilute the specimen, preserve the cells, convert hemoglobin into a measurable form and allow the analyzer to do its job.

Different cellular analysis technologies require development and use of specific reagents.These reagents may react differently with fresh whole blood and fixed control bloods. For example, a reagent intended to sphere fresh human Red Blood Cells (RBCs) for analysis will have a minimal sphering effect on stabilized control cells. The effect of this minimal sphering will produce a significantly lower Mean Cell Volume (MCV) value when compared to the MCV value for the same control cells run on an analyzer using non-sphering reagent.

The intended use for a lytic reagent is to destroy RBCs. During the analysis process, RBC destruction is required to:

- Accurately count WBCs
- Release HGB and convert it to a measurable form[1]

A lytic reagent will react differently with controls that have different degrees of cellular fixation. Any remaining or unlysed RBCs or fragments contribute to result variability that will affect the accuracy of the WBC and HGB concentrations.

3. THE CALIBRATOR

This material acts much like real blood. In fact, it is manufactured using real blood in a very long and complicated process. It is used to set the system to known values. Normally calibration or calibration verification is done infrequently. We use the control material on a frequent and regular schedule to make sure the calibration is stable and nothing has changed. Assayed whole blood may also be used to calibrate an analyzer.

4. THE CONTROLS

Commercial controls are assayed to obtain target means (values and ranges) for either a single or multiple hematology systems by using instruments that have been calibrated to reference methods. The quality control material you use also mimics, as closely as possible, the characteristics of real blood.

The range or mean range for each parameter is set wider than a single instrument's ± 2SD (standard deviation) range to accommodate known result variation for a given parameter over the stated use period for that control product, as well as to accommodate interlaboratory calibration differences.

Variations may be seen between the mean target or assay values for a selected parameter for different analyzers. This variation can be slight or quite significant. For example, assay count differences for WBC, RBC, and PLT should vary only slightly between optical, impedance, and focused impedance systems, while MCV assay values can vary based on the reagents and method used.

QUALITY CONTROL PRODUCTION

Stabilized human and mammalian blood cells are the cellular components most frequently used in the manufacture of control materials; however, other materials have been used to simulate cells. During the preparation process, base components for each of the three cell types (WBC, RBC, and PLT) are obtained by removing them from whole blood. WBCs may be further separated to obtain lymphocytes and granulocytes. The base components are then treated to "fix" or stabilize them. Over a period of several weeks, the cellular components may be subjected to additional processing and testing before they are finally blended with a suspending solution to yield a predetermined count range for each cell type at each control level. The common practice is to produce two to three levels of control. The prepared mixture is then packaged and assayed.

Typically, the total time required to produce a control product is from three to six months. The source of the cellular components and the degree of their fixation or stabilization are key factors related to the control's performance. When the source is living cells that have been lightly fixed, the product will closely mimic fresh whole blood; however, such a product will have a shorter use period and will show decreased stability for parameters such as MCV, red blood cell distribution width (RDW) and WBC. When the source is from living cells that have been durably fixed or even from non-blood cell particles, the product will be very stable but will most likely be difficult to lyse.

QC can assure all of the components of the system – analyzer operations such as dilution, mixing and counting, as well as the reagents – are working properly. It also tells you if your calibration is correct. By looking at the data produced by the control material, you will not only know when your system is operating correctly, you will know where to look for problems when it is not. We will explore what this means, and what to do about it, in future sections. But, first we need to understand two basic concepts – accuracy and precision.

SECTION 2

ACCURACY AND PRECISION

WHAT IS ACCURACY?

Accuracy is the ability to get the right answer.

Think of an archer shooting arrows at a target. The archer wants his arrow to hit the center of the target to get the best score. He loses points when he is not in the center. The farther from the center the fewer points he gets until, if he misses the target altogether, he gets no points. This is accuracy: the ability to hit the center of the target.

In the laboratory, accuracy is the ability to get the right answer, whether we are analyzing a patient's sample or a control sample. If the control sheet says that we should have a result of "x", if everything is operating properly we would expect to get this result, or very close to it. A little bit of variation is normal and expected.

WHAT IS PRECISION?

Precision is the ability to get the same answer, over and over.

Consider our archer again. He does not want to hit the center of the target just once, he wants to hit it every time. His archery is said to be precise if all of the arrows are close together.

In our case, we want all of the control results to be close together. As with accuracy, a little bit of variation (or, to get technical, imprecision) is normal. Your control limits will tell you how much is allowed.

UNDERSTANDING THE DIFFERENCE BETWEEN ACCURACY AND PRECISION

Now that we know what accuracy and precision are, we need to be sure we understand that they are not the same. Let's look at four examples:

ACCURATE AND PRECISE

If all of the arrows are in the center of the target and close together, the results are both accurate and precise. If your control results are near the target values, and they are all close to each other, your instrument system is also accurate and precise **(Figure 1a)**.

ACCURATE, BUT NOT PRECISE

If instead, the archer's shots are in the middle of the target but not very close to each other, he is accurate but not precise (we say that he is experiencing imprecision) **(Figure 1b)**.

PRECISE, BUT NOT ACCURATE

Our archer shoots again. This time all of the arrows are very close to each other, but nowhere near the center of the target. This is an example of inaccuracy, but good precision. Before shooting again, our archer may need to adjust his sights; we might say he needs to recalibrate **(Figure 1c)**.

NOT ACCURATE OR PRECISE

Finally, if someone who had never used a bow before were asked to shoot at the target, the results probably would not be anywhere near the center, or even near each other. This would be an example of both a lack of accuracy and precision **(Figure 1d)**.

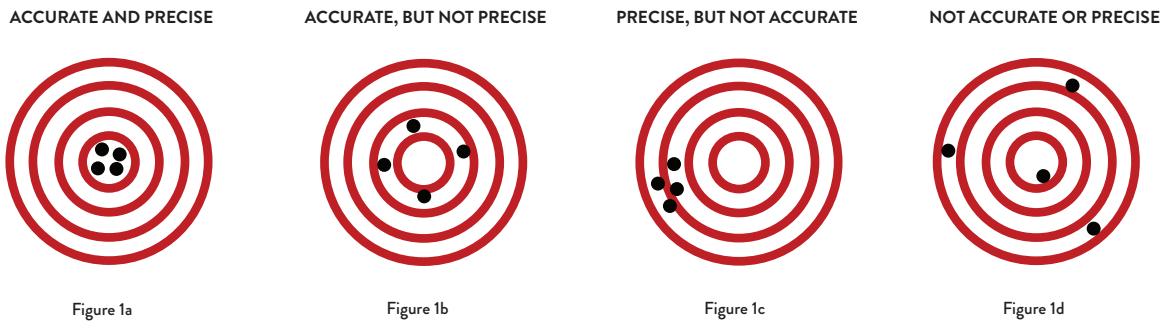

Figure 1. Illustration of the difference between accuracy and precision

ACCURACY AND PRECISION IN THE LABORATORY

Why is it important to understand these four situations? Obviously, it is not because we all want to become expert archers. Each of the scenarios above describes a different situation that can occur when you are performing QC. Understanding what causes each situation makes it easier to know the proper corrective action, should your controls be "out". Additionally, knowing these two basic concepts helps us understand the statistics that are normally used in the laboratory for monitoring performance. We will discuss each situation in more detail in the following section.

SECTION 3

STATISTICS MADE EASY

For our purposes, there are only three statistics that you need to know – mean, standard deviation and coefficient of variation. On a daily basis, only the mean and standard deviation will normally be required.

MEAN

The mean (expressed as $\bar{x}$) is simply the average of all of the values you generate when you run a control sample. For example, if you analyze the normal control 10 times and average the WBC counts (divide the total by the number of events) you will get the mean WBC for the normal control.

Run Number	WBC Results
1	5.8
2	6.2
3	6.1
4	5.9
5	6.3
6	5.8
7	6.0
8	6.0
9	5.9
10	6.1
Mean	**6.0**

Table 1. Calculation of the mean WBC for the normal control

STANDARD DEVIATION

Standard deviation (SD) is a way to talk about precision by looking at variation, or the dispersion of values around the mean. It sounds complicated, and there is a complicated-looking formula that is used to produce it. However, we will just stick to what it means:

- If you take any number of results and calculate the standard deviation, it will tell you, on average, how far the values are from the mean.
- Standard deviation is usually expressed as 1, 2 or 3 times its value (1SD, 2SD, 3SD). Most often you will expect control results to be within 2SD, but not always. As mentioned earlier, an occasional value outside of this limit is expected.
- In the example above where we used the normal control WBC values, the standard deviation is +0.166.

It looks like the results are pretty close to each other with less than 0.2 units of variation, and our precision is good. But, how do we know it is acceptable performance? We compare it to what is expected, using the control Assay Sheet and the Operations Manual. We will discuss this in more detail later.

COEFFICIENT OF VARIATION

For your everyday quality control, you will be most focused on the mean and standard deviation for each parameter.

However, there is another value we often use when reviewing results obtained at different levels, or when using different methods.

Coefficient of variation (CV) is another way to look at the precision of a group of results (see p. 7, "Accuracy and Precision"). The calculation (Standard Deviation / Mean) * 100 produces a result expressed as a percent (%CV), allowing comparisons that SD alone does not. For example, if you wanted to compare the precision of a hemoglobin measurement performed by two different methods, %CV would allow you to do this.

SECTION 4

GETTING STARTED WITH YOUR HEMATOLOGY ANALYZER

Congratulations! You have a brand new hematology system. The Service Engineer has set it up and it is ready to go. It is time to start testing patients, right? Well, not quite yet. First, we need to calibrate and run our controls.

CALIBRATION

Before starting, the system needs to be calibrated. This is to make sure that the calibration factors which ensure correct results are set to the proper values. As a part of the setup procedure of your new system, the Service Engineer or Application Specialist may have completed the calibration for you. If not, or if something has changed with the system, you will have to perform a calibration.

To calibrate your system, follow all directions in the Operations Manual, as well as the instructions included with the calibration material.

The accuracy of the results obtained from hematology analyzers is directly related to proper calibration. The College of American Pathologists (CAP) Checklist[2] states in the Complete Blood Count (CBC) Instruments, Calibration subsection:

Commercially available calibrator materials represent a convenient way to ensure that CBC analyzers yield accurate results. Because of differences in technology, such calibrators or target values are typically instrument-specific, and are cleared by the Food and Drug Administration for such use. These calibrators have more rigorous assignment of target values than ordinary commercial QC materials. Commercial control materials are not suitable for routine instrument calibration.

Abbott hematology analyzer calibrators that meet these recommendations are available for all Abbott CELL-DYN and Alinity hq hematology analyzers. Calibration and/or calibration verification is recommended:

- When a change in the reagent type from the same vendor occurs or a change to a different vendor occurs
- When verification is indicated by trends in quality control data
- After major maintenance and service procedures
- At least every six months or as required by the regulatory agencies that govern the laboratory.

For calibration, the general recommendation is to use the Abbott CELL-DYN or Alinity h-series Calibrator developed for use with the CELL-DYN or Alinity hq analyzer to be calibrated.

Hematology laboratories often have more than one hematology analyzer. Accurate calibration of each analyzer is critical. A clinically significant difference or calibration bias should not be evident between results obtained for the same specimen on each analyzer.

A practice that is typically used for cross-system calibration is:

- Calibrate a designated reference or primary instrument with a stabilized calibrator
- Cross-calibrate each additional instrument using 10 whole blood specimens that have been run on the reference or primary instrument

The calibration procedure must be done as rapidly as possible to minimize error due to potential changes in the whole blood as it ages. A sufficient quantity of whole blood must be available to ensure that the minimum required volume for aspiration, usually 0.5 to 1 mL, is met for all aspirations. Follow any additional instrument-specific instructions found in the appropriate Operations Manual.

CONTROLS

After calibration, you will need to run the controls. This will confirm that the calibration was performed correctly and the system is operating properly.

As discussed earlier, commercial controls are materials as physically and chemically similar to patient samples as possible. These control materials and retained patient specimens need to be tested similarly. Two types of QC materials will be discussed in this section: commercial controls and also retained patient specimens (secondary controls). Many laboratories use both types of control materials.

TYPES OF CONTROLS

1. Commercial Controls. Commercial controls are made from pooled whole blood that has been preserved to delay deterioration. The manufacturer provides an expiration date for each lot of control material, which applies only to unopened bottles. Opened containers have their own open stability dates. Stability is decreased if the manufacturer's recommendations for handling and storage are not followed. Also, as with reagents, evaporation and contamination are concerns.

When commercial control preparations are used, either two or three "levels" of control are run. One level has values in a typical patient reference range, while the other levels have abnormally low and high values. Each control preparation has been assayed by the manufacturer. Information on the range within which the laboratory's mean should fall is provided for each level. Each laboratory will then determine its own mean from repeated runs of the control materials. The laboratory's range of acceptable values should be established based on three to six months of historical data. Once these values are established, the controls are run at an appropriate frequency; any deviation from the acceptable range of values must be dealt with as outlined by Westgard analysis or laboratory policy.

2. Retained Patient Specimens (Secondary Controls). Ideally, control preparations should be very similar to actual patient samples. Obviously, commercial controls are not identical to patient samples and must be preserved in some fashion. Unlike commercial controls, the "true" values for parameters of patient samples (secondary controls) are not known. However, patient samples have the advantages of being identical to routinely tested samples and less expensive than commercial controls. Patient samples are also useful to distinguish between (1) instrument or operator errors and (2) problems with commercial controls. A patient specimen whose values

were determined when the instrument was "in control" may be used for a maximum of 24 hours after collection. The RBC, HGB, HCT, MCV, MCH, MCHC, RDW, PLT, and MPV are stable for up to 24 hours, while the total WBC should be within 5% of its initial value for up to 12 hours, and within 7% for up to 24 hours. The WBC differential parameters are stable to within 10% of their initial values for up to 12 hours. Stability studies indicate that samples exhibit increased stability when they are stored at room temperature rather than stored in the refrigerator. Target values must be established for each new patient control. The range of acceptable values is based on historical data. Establishing both the target values and historical limits requires the following assumptions:

1. Precision studies have been performed and all values fall within the manufacturer's specifications (as listed in the Operations Manual).
2. Instrument calibration has been verified using the appropriate reference method or commercial calibrator.
3. Routine maintenance has been performed (and documented) as per schedule.

For many years, hematology laboratories would retest one or more patient specimens some hours later, and a results comparison made to determine if the analyzer had shifted its calibration. When there was a results discrepancy, commercial QC materials were then run to determine if the change was reproduced. All too often, failure of the commercial material to replicate the difference noted in the patient specimen would lead to the conclusion that the labile patient specimen had "deteriorated."

As noted in the CAP Checklist:[2]

Use of retained patient specimens alone is inadequate for routine QC of the primary CBC instrument, and must be considered as a supplemental procedure, in combination with another QC system. Retained patient specimens, while conveniently available, present some difficulties in mathematically defining 'agreement' between CBC results separated in time, as these are not stabilized samples. This is in contrast to commercial control materials that have been treated to reduce time-dependent degradation.

Thus, laboratories that elect to incorporate retained patient specimens in their QC programs must have a statistically valid basis for results comparison.

CONTROL LEVELS

In most cases, you will want to analyze three levels of control. Abbott's hematology controls come packaged with materials to represent low, normal and high levels of hematologic values. Running all three levels helps ensure that patient results across the entire range will be correct.

ESTABLISHING TARGETS AND LIMITS

The control ranges provided with the control product insert are not intended to serve as the boundaries for acceptable performance of a single instrument. Instead, the ranges serve as a reference during "pre-use" or parallel study product performance verification. The target value adopted by individual laboratories should be established through analysis of the mean value obtained for each parameter after processing multiple replicates of the respective controls.[3,4,5] This lab-generated mean value should fall within the manufacturer-provided range, and serves as a check on control product integrity. In other words, Assay Sheets or electronically-provided target values and ranges from a manufacturer are simply broad starting guidelines, but not the values for use during the life of the control material in a particular laboratory.

Abbott recommends use of at least 10 control run replicates to establish this mean value.[3,4,5] Ideally, these 10 replicates should consist of duplicate analyses performed over five days. When the user is choosing to set limits (or ranges) around the target value, there are a number of choices. One is to use the SD derived from the initial 10 replicates of the control. However, this may prove to be too stringent during use, due to the small, but inevitable changes that can occur in some parameters over time. A good example of this is the MCV, which typically shows an increase of around 1-2 fL during the expected life of the control product. This is especially important if Westgard rules are used to monitor control results.

An alternative is to use the SD of historical data collected from previous batches of control product. There are two principal sources of these limits. The first is the overall SD of the control data during a period of three or more months. The mean %CV over the period can be used to estimate ± 2SD for the new lot of control material, using the following formula:

$$\frac{\text{Mean \%CV x current assay mean}}{100} \times 2 = \pm 2 \text{ SD for current control}$$

Note that this calculation must be performed for each analyte for each control level, since higher imprecision is generally expected for cytopenic samples, due to Poisson statistics.

Another alternative is the use of interlaboratory (peer group) QC programs. Here, the SD and %CV are calculated each month in a cumulative report that may be used for long term analysis. Laboratories must use their own imprecision statistics, and not those of the peer group, since the latter values reflect broader interlaboratory imprecision. These mean and SD limits should only be applied to specific analyzer and control product combinations. Once done, the control is then verified and ready for use in the laboratory's QC process.

FREQUENCY OF ANALYSIS

How often you run the controls will depend on your laboratory's operating schedule and policies, as well as applicable regulatory requirements. It is common, and good laboratory practice, to run the three levels of control at least once on each work shift. However, your schedule may vary and some labs run controls more often, others less.

LEVEY-JENNINGS CHARTS

We use charts, called Levey-Jennings (or L-J) charts, to graph the results of control performance. Each time a control is analyzed, the result is added to the chart. We can then look at the series of dots and detect patterns of performance.

When you look at an L-J chart, you will see a horizontal line across the middle. This represents the "mean" or expected result. Other lines, above and below the mean, represent the acceptable limits of the results. The limits are calculated from the standard deviation.

An L-J chart of an accurate and precise set of data might look as illustrated on **Figure 2**.

Occasionally, a result will be outside of the expected limits, and that's expected. As long as it does not happen too frequently, some variation is normal.

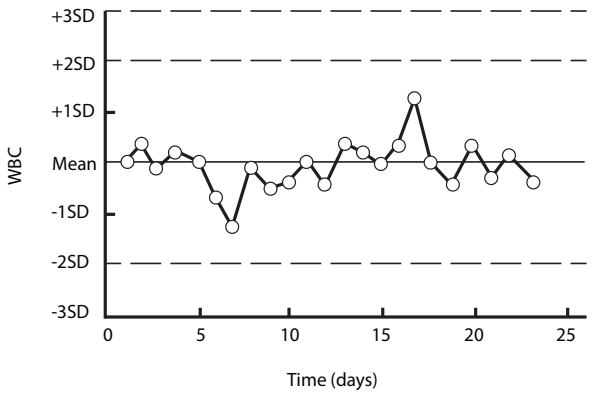

Figure 2. An accurate and precise set of data

ANALYZING THE CONTROL MATERIAL

To analyze the controls, follow the directions in the Operations Manual, as well as the instructions included with the control material.

A common problem in Hematology QC is the failure to adequately prepare the controls for sampling. The manufacturer's directions should be strictly followed. The two most critical steps are warming the controls to room temperature and adequate mixing. Failure to warm and mix causes inaccurate results, because the cells typically settle at the bottom of the tube.

MISUSE, MISHANDLING, AND STATISTICAL VARIATION

Control misuse or mishandling can result in poor performance, and results that are frequently out of range. Examples of mishandling or misuse are:

- Refrigeration storage location that could allow freezing
- Refrigeration storage outside the manufacturer's recommended storage range (storing calibrators and controls in the refrigerator door should be avoided due to the associated temperature changes during opening and closing)

- Inadequate mixing or resuspension
- Severe agitation or vortexing
- Failure to follow instructions for use
- Use of control on an unspecified analyzer
- Use of control on an analyzer that is not operating with specified reagents
- Use after the open stability date
- Use after the expiration date
- Use when the volume in the tube is below the required minimum tube volume

Even when the controls are stored and used appropriately, the impact of simple statistical variation must also be considered. For example, if the laboratory chooses to use ± 2SD limits around the mean of all their charted data, then, by inference, the values of any single parameter will fall outside these 2SD limits on 5% of occasions. When multiple parameters are monitored (typically 20 or more), then it becomes almost an expectation that one of the parameters will fall outside these limits on almost every occasion that a control is tested. This becomes even more relevant with multiple control levels. It is therefore important that more complex strategies for monitoring QC are applied than a single point failure at the 2SD level. The application of Westgard rules is particularly valuable.

Despite stringent manufacturing procedures, the use of biological cell-based products will occasionally give rise to unexpected anomalies, an example of which might be the occurrence of the so-called lymphocyte-basophil "flip." In this condition, the software algorithm finds the lymphocyte population close to the threshold of discrimination for the basophil population and as a consequence can erroneously misclassify a portion of the lymphocytes as basophils.

When you are finished, store the control material as directed on the label or product insert. Following these simple directions will pay off when it comes to generating proper QC results and getting the most life out of your control.

Each time you analyze your control material, points will be added to the control charts. Usually, you will analyze three levels of control – low, normal and high. If you look at the charts, you will see points for each parameter and each level. Both your current analyses, as well as previous results, are shown. By observing the results and the patterns, you will know when your QC results are good ("IN") and when something may need attention (QC is said to be "OUT").

SECTION 5

USING THE RESULTS OF QC ANALYSIS

Let's now begin to explore some of the situations you may encounter when analyzing control materials.

Results are compared to the acceptable limits of performance. You may have found the ranges on the documents in the control package or provided electronically, but as stated earlier, laboratories establish their own acceptable limits of quality control values. Those limits are used to determine if the control results are within range.

IN RANGE RESULTS

Control results falling within the acceptable limits are said to be "in range". When all three levels of control are within range, your system is "in control" and you may analyze patient specimens.

OUT OF RANGE RESULTS

Control results produced by the instrument that fall outside your laboratory ranges for the material are "out of range". Individual results may be "out" **(Figure 3)**, or you may detect a shift or trend in the control behavior.

What to do:

1. Remix and repeat the controls – if still out of range, go to step 2.
2. Open new vial of control – if still out of range, go to step 3.
3. Call Abbott Technical Customer Support for troubleshooting assistance.

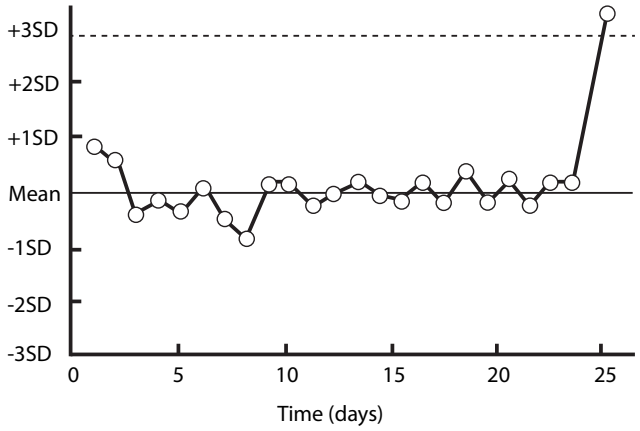

Figure 3. A set of data showing out of range result

SECTION 6

SHIFTS, TRENDS AND IMPRECISION

Control results also may exhibit other behaviors that are undesirable. This includes shifts, trends and imprecision.

SHIFTS

When our archer begins to shoot his arrows, they are all in the center of the target. However, his next five shots are at the top of the target. A shift has occurred.

When performing QC, we may be getting good results, all near the center value, and then, suddenly, all of the values are low (or high). This also is called a shift **(Figure 4)**.

Some variable has suddenly changed in the instrument, the reagents or the environment that has caused this shift. Review maintenance records for part changes on the instrument, lot number change for reagents, sudden temperature changes in the lab. What to do:

1. Remix and repeat the controls – if still out of range, go to step 2.
2. Open new vial of control – if still out of range, go to step 3.
3. Call Abbott Technical Customer Support for troubleshooting assistance.

TRENDS

Maybe our archer is having a bad day. His first arrow hits the top of the target. The next is closer to the middle, but the next after that is below the middle. Finally, his fourth shot is right at the bottom of the target. This movement from top to bottom (or bottom to top) over time is a trend **(Figure 4)**.

When performing QC, a gradual change in the control results is called a trend.

Trends are typically caused by a drift of the instrument. Some variables that may contribute to a trend would be:

- Preventative maintenance may need to be performed.
- Controls and reagents should always be used and stored according to the manufacturer's directions.
- Check the date and storage conditions for the controls and reagents.
- Controls and reagents should not be used past their expiration dates.
- The date of last calibration – is the six-month calibration verification date approaching?

What to do:

1. Remix and repeat the controls – if still out of range, go to step 2.
2. Open new vial of control – if still out of range, go to step 3.
3. Call Abbott Technical Customer Support for troubleshooting assistance.

IMPRECISION

You may remember that we discussed results showing a lack of precision (imprecision) at the beginning of this guide. Even when the results were within range, they varied widely. This is shown above on the right side of **Figure 4**.

Although a number of conditions may affect results in this way, imprecision is often caused by poor mixing of the control material. Always follow all instructions for the proper handling of the control materials. Not only will this improve performance, it also will save money that would otherwise be spent repeating control testing.

What to do:

1. Remix and repeat the controls – if still out of range, go to step 2.
2. Open new vial of control – if still out of range, go to step
3. Call Abbott Technical Customer Support for troubleshooting assistance.

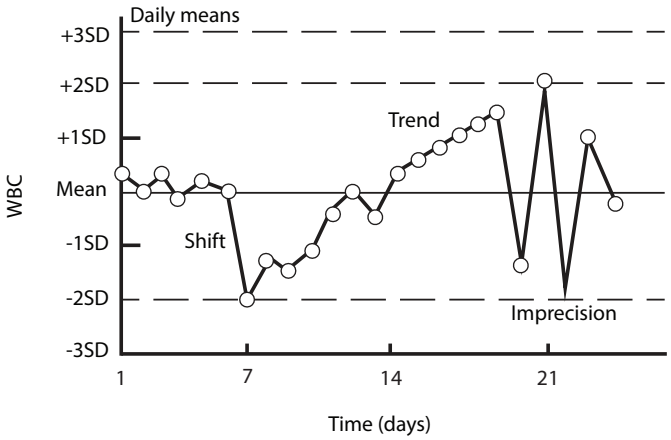

Figure 4. A set of data showing shift, trend, and imprecision

SECTION 7

QUALITY CONTROL RULES AND REGULATIONS

REGULATIONS GOVERNING USE OF STABILIZED CONTROLS

Regulations governing the laboratory and daily QC vary, based on requirements of the United States (U.S.) or international agencies. In the U.S., the CAP Checklist[2] states that laboratories should run two different levels of control materials in each 24-hour period. The same document also indicates: "Longitudinal process quality control (QC) procedures for individual instruments or inter-instrument comparisons may include:

1. Use of preserved or stabilized whole blood controls
2. 'Moving average' monitoring
3. Retained patient specimens or
4. Some combination of the above."

In the subsection related to Stabilized Controls the CAP Checklist[2] also notes the following:

NOTE: Stabilized control materials must be at 2 different analytical levels (i.e., 'normal' and 'high'). Three levels of control is a conceptual carryover from clinical chemistry, and does not apply to hematology particle counting. Dilute, 'low-level' (e.g., leukopenic and thrombocytopenic) 'oncology' controls are less informative indicators of calibration status, and are neither required nor recommended. For example, a 10% calibration bias will be numerically most apparent in a high-level control, less apparent in a normal-level control, and perhaps inapparent in a low-level control; it would be quite extraordinary for a low level control to indicate a calibration problem that is not revealed by the other controls. There should be some relationship between the frequency of control runs and the numbers of patient specimens processed. If the frequency of commercial control use is less than two control specimens per 24 hours, one or more of the additional approaches to QC must be employed to produce a total of at least two different data points per 24 hours.

USE OF WESTGARD MULTIRULES REGULATIONS GOVERNING USE OF STABILIZED CONTROLS

Several hematology analyzers include a multirule QC program that uses a combination of decision criteria, or control rules, to decide whether an analytical run is in-control or out-of-control. The most well-known of these is the Westgard Multirule QC procedure. According to Westgard: "The advantages of multirule QC procedures are that false rejections can be kept low while at the same time maintaining high error detection. This is done by selecting individual rules that have very low levels of false rejection, then building up the error detection by using rules together." More information regarding the use of Westgard Multirule QC can be obtained from www.westgard.com.

Abbott hematology analyzers allow the laboratory to select from 1 to 7 modified rules to apply to QC runs. To use on-board multirule QC, each laboratory should establish mean and SD values for each new lot of stabilized controls, select the desired rules, and run the controls.

Mean values can be established during the pre-use performance validation period described above in Establishing Targets and Limits. However, because multirules use SD, it is recommended that long term or cumulative limits be established over several lots.[3,4,5] Cumulative limits can be calculated using SD data from a peer comparison inter-laboratory report, such as the *STATS*-Link® ILQC Program.

QUALITY PROGRAMS

An additional source of supplementary external quality assurance is a manufacturer's QC peer review scheme. For users of Abbott CELL-DYN or Alinity h-series control products, Abbott provides an electronic QC peer review program. This service enables customers to submit their summarized monthly QC data electronically via the Internet for comparison with other users of the same instruments and stabilized QC products. In this scheme, reports of the individual laboratory's summary QC data are analyzed and displayed alongside the summarized statistics of the population of users who have also submitted data.

Customers wishing to enroll in this service should create an account and register analyzers at www.statslink.streck.com.

Another additional source of QC information is peer review comparison data.[6] Proficiency Testing (PT) and external quality assurance schemes (EQAS) are widely available, including the long-standing ones available from the CAP and by the U.K. National External Quality Assessment Scheme (UK NEQAS). Many other countries operate national or regional PT or EQAS schemes. Involvement in such programs is often a regulatory requirement or an expectation of voluntary accrediting agencies.

USE OF ON-BOARD STATISTICAL PROGRAMS

The availability of fully automated walk-away hematology systems, in combination with an increasing number of parameters, forces one to look at ways to monitor system performance. Most hematology analyzers available today include laboratory-selectable programs that use statistics to monitor system performance. These include Levey-Jennings graphical analysis of commercial QC materials and moving average algorithms.

X-B MOVING AVERAGE PROGRAM

The X-B (Bull's moving average) program has been successfully used for more than 30 years to monitor the RBC indices as a QC technique.[7,8,9,10] It relies on the biological consistency of the RBC and its HGB content over a fairly narrow range for the Wintrobe RBC indices of MCV, mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). Specifically, X-B uses a mathematical formula to evaluate the MCV, MCH, and MCHC for patient specimens as they are run on the analyzer. The formula compares the new result for each parameter to a batch mean, then uses a square root function to smooth and trim data to minimize the weight of a single result

falling far from the mean. When specimens are run randomly, X-B program acceptance criteria can be set to accept results for most patient specimens. A new batch mean for each parameter is calculated for each batch of 20 specimens. Published literature showed that target means established for laboratories around the world show only slight variations from the means established by Dr. Maxwell Wintrobe in the early 1930s for each of the RBC indices. For this reason, the X-B moving average program is widely accepted, and used as an additional QC process to monitor the development of any RBC parameter calibration bias. From an analytic perspective, moving average data are completely free of matrix effects that occur with commercial controls, and are financially free to the laboratory. Further, they can serve as more continuous performance monitors than daily commercial QC runs for high-volume laboratories.

The CAP Checklist[2] lists moving averages as an acceptable method for longitudinal process QC procedures for individual instruments or inter-instrument comparisons. In the Moving Averages subsection, the following is stated:

The technique of weighted moving averages (derived from multiple batch analysis of patient samples) is acceptably sensitive to drifts or shifts in analyzer calibration if a supplemental QC routine (stabilized control material or retained patient specimens) is employed. The latter is needed to detect random error and to avoid bias due to masking of drift by characteristics of the subpopulations within each individual batch. Laboratories analyzing fewer than 100 CBC specimens daily (long term average) should not use moving averages as the primary method for process control, as this would not generate sufficient data within a day to be of value. Depending on the particular instrument, there may be 'on-board' moving average analysis for RBC indices only. In such cases, additional QC techniques are required for WBC, PLT, and WBC differential parameters. However, some laboratories have found the mathematical logic of moving averages, modified average of normals, etc., applicable to other CBC parameters, and some instruments have these capabilities built into their software. Or, such calculations may be performed with an associated computer.

WBC DIFFERENTIAL MOVING AVERAGE PROGRAM

Abbott CELL-DYN Ruby, CELL-DYN Sapphire and Alinity hq analyzers with an optical five-part differential offer a WBC Differential Moving Average Program. The same mathematical formulation used by the X-B program is employed to monitor the lymphocyte and neutrophil population location means and CV statistics from patient specimens. Because this program uses data from patient specimens, all aspects of the differential analysis process, including the lytic reagent, software, hardware, fluidics, and specimen, are monitored. Any shift or change in the differential analysis process can be rapidly identified and addressed.

EXPANDED MOVING AVERAGE

The Abbott CELL-DYN Ruby, CELL-DYN Sapphire and Alinity hq analyzers have expanded the use of the moving average formula further to monitor results for WBC, RBC, PLT and reticulocytes, and to monitor population location mean and CV statistics. Significantly more different measurements can be monitored using the expanded moving average programs.

For easy viewing, the moving average program status panel on the main screen automatically updates the number of specimens in the current batch and displays the on/in, on/out 1, and on/out 2 status for each program as specimens are run.

A color change visually alerts the operator when data exceed established limits. Program logs provide summary data and Levey-Jennings plots for the last batches that can be quickly reviewed for shifts and trends on a routine basis or during troubleshooting.

GLOSSARY, APPENDIX AND REFERENCES

ADDITIONAL RESOURCES

As we explained at the start, our approach in this guide to QC has been basic. To learn more about laboratory QC, consider one of the following references:

- Basic QC Practices", 3rd Ed., James O. Westgard, Ph.D., Westgard QC, 2010 (www.westgard.com)
- "Hematology Control Assay Value Download Instructions", 9150722E. Abbott Laboratories, 2019. Available in Customer Portal at www.corelaboratory.abbott (last accessed 3.19.2020)
- "Establishing Quality Control Means and Standard Deviations for Hematology Instrumentation." Available at https://streckinc-wpengine.netdna-ssl.com/wp-content/uploads/2017/09/02_Establishing_Quality_Control_Target_Values_and_Standard_Deviations_for_Hematology_Instruments.pdf (last accessed 3.18.2020)
- "Q.C. Concepts." Available at https://www.streck.com/wp-content/uploads/sync/Services/STATS/STATS/02_Literature/06_Streck_STATS_QC_Concepts.pdf (last accessed 3.18.2020)
- Additional resources are available on Streck *STATS*-Link® website https://www.streck.com/services/stats/ (last accessed 3.18.2020)
- "Calibration and QC Guidance for the Abbott Alinity hq Hematology Analyzer", Abbott Laboratories White Paper, 2018. Available from your local Abbott representative or distributor
- "Quality Assurance In The Hematology Laboratory." Available at https://hematologyacademy.com/on-demandwebinar/quality-assurance-in-the-hematology-laboratory/ (last accessed 3.18.2020)
- "Alinity hq Sigma Metrics." Available at https://hematologyacademy.com/on-demandwebinar/alinity-hq-sigma-metrics/ (last accessed 3.18.2020)

GLOSSARY

Accuracy: a measure of how close a measured result is to the "true" value.

Analyte: any substance that is measured.

Assay sheet: an information sheet provided with commercial control materials that lists an acceptable range of mean values attributed to a specific control.

Bias: error introduced into sampling or testing by selecting or encouraging one outcome over another; tendency of an individual analytic measurement to be shifted in one direction from its true value; usually caused by systematic error.

Biohazardous materials: materials that contain biological agents that may be harmful to humans or the environment.

Calibrator: a material with known values used to adjust (calibrate) a laboratory instrument to ensure accurate measurement of results.

Coefficient of variation (CV): a statistical measure of population heterogeneity.

$$\%CV = (SD / Mean) * 100$$

Commercial control: a stabilized blood preparation with a range of assayed values for the parameters being analyzed.

Cumulative: describes data that has been collected over a period of time.

Distribution histogram: in hematology, a graph that compares one property of a cell (size) to the relative number of cells analyzed; used for RBCs, WBCs, and platelets.

Expiration date: the date after which a product should not be used.

External quality programs: programs that analyze the results of a laboratory's control samples and compare them to the results obtained by other similar laboratories; Proficiency Testing such as NEQAS or CAP surveys.

Gaussian distribution: a "normal" distribution with a bell-shaped curve, where 95% of the data points lie within 2 standard deviations of the mean.

Infectious: capable of transmitting microbial disease.

Interlaboratory QC program: a peer QC comparison program set up among several different laboratories that use similar instruments.

Internal QC program: a QC program that is used to analyze data generated within the laboratory, e.g., X-B analysis.

Levey-Jennings graphs: plots that show how data points distribute relative to the mean; outliers, trends and shifts are easily identified on these plots.

Mean: the average value of a set of measurements.

$$Mean = (a+b+c \ldots z)/n$$

Mean Corpuscular Hemoglobin Concentration (MCHC): a value that describes the average concentration of hemoglobin in red cells. It is calculated using MCHC = HGB/HCT x 100.

GLOSSARY (CONTINUED)

Mean Corpuscular Volume (MCV): a measure of the average red cell volume that acts as an indicator of cell size. It is calculated using MCV = HCT/RBC x 10 or MCV = HCT x 10/RBC.

Moving averages: a type of statistical analysis that generates an average based on current and previous batch means.

Open-vial stability: how long a product may be used after it has been opened.

Precision: a measure of how repeatable a result is.

Probability: the likelihood of an event or result occurring.

Procedural error: error due to mistakes in performing an assay.

Proficiency testing: a form of external QC where the laboratory analyzes unknown samples sent by a testing organization. The results are compared to those obtained by other participating laboratories and referees.

Random error: error introduced by chance.

Range: the set of values that a result must fall within to be acceptable.

Reliability: the ability to have both accuracy and precision in test results.

Secondary control: a retained patient specimen that is used as a control.

Shift: describes an abrupt change in several data points that lie above the mean (upward shift) or below the mean (downward shift).

Standard Deviation (SD): the amount of dispersion of data points about the mean.

Standard Deviation Index (SDI): the SD expressed as a percent of the mean value; indicates the degree of separation between the peer group mean and an individual lab's mean.

$$SD = \sqrt{\frac{\sum (x-\bar{x})^2}{n-1}}$$

$\bar{x}$ = mean
x = any individual result
$\sum$ = sum (total)
n = number of values

Systematic error: error introduced into a system that affects all results equally; instrument problems are often the cause of systematic error.

Trend: describes data points that gradually increase above the mean (upward trend) or gradually decrease below the mean (downward trend).

Westgard analysis: an approach for deciding when the results of testing controls suggest that a true change in instrument performance has occurred.

Westgard Rules: a set of guidelines used to determine whether a result should be accepted or rejected.

X-B analysis: a software feature on CELL-DYN and Alinity h-series analyzers that monitors instrument stability by comparing the average values of certain results in successive groups of patient samples. X-B analysis as applied to the RBC indices was developed for use in hematology by Dr. Brian Bull.[7]

REFERENCES

1. CLSI. Validation, Verification, and Quality Assurance of Automated Hematology Analyzers; Approved Standard - Second Edition. H26-A2. Wayne, PA: CLSI, 2010.
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3. CELL-DYN Sapphire System Operator's Manual 9140426D - October 2014.
4. CELL-DYN Ruby System Operator's Manual 9212934G - November 2017.
5. Alinity h-series Operations Manual 80000023-107 - September 3 2019.
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10. Streichman S, Gescheidt Y. Using Patient Blood Samples to Assess and Harmonize Laboratory Test Results. Lab Hematol. 1998;4:21-25.

www.corelaboratory.abbott/hematology

Alinity hq, CELL-DYN Ruby and CELL-DYN Sapphire instruments are Class I laser products. For in vitro diagnostic use only.
Refer to the Operator's Manual for operational precautions, limitations, and hazards.
Alinity h-series is available in select countries, not including the U.S.
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