Validating assayed QC materials

Q Currently, we use unassayed quality control (QC) for chemistry and establish our own reference ranges by running the new lot number over a period of 20 to 30 days prior to activating. If we were to use assayed QC, would we be able to run fewer samples, since we are only verifying the manufacturer’s ranges—not establishing our own—prior to activating?

A Different areas of the clinical laboratory analyze different numbers of controls to obtain preliminary control means. For example, many blood-gas laboratories analyze single vials of quality-control product on 10 different days, whereas many high-volume clinical-chemistry laboratories require analysis on 20 to 30 different days. In hematology, break-in periods of five to 10 days have been recommended.

In hematology, it is acceptable to assure the control values are near the midpoint of the package insert value and then use the manufacturer’s recommended range.

In some work presented a few years ago, we recommended that assays with little variation (CVs [coefficient of variations] of 1% to 2%) need minimal break-in periods. These include testing done on high-volume hematology analyzers, which usually use short-lived control products, blood-gas analyzers and electrolytes, and blood gases done on the i-STAT. In hematology, it is acceptable to assure the control values are near the midpoint of the package insert value and then use the manufacturer’s recommended range. For assays with CVs around 4% or 5%, 20 to 30 replicates suffice. For very noisy assays—those with CVs greater than 10%—more than 40 replicate measurements will be needed; additionally, these assays probably should be done in duplicate.

The advantage of generic (not provided by the instrument manufacturer) assayed controls is that the laboratorian knows roughly the concentration of the control’s documented constituents. This information is useful in control selection. Usually, the break-in period of the assayed controls should probably be the same as that of the unassayed control. In the case of controls provided by the instrument manufacturer, these controls tend to be more expensive and are sometimes reserved for demonstrating a need for instrument service or reagent exchange. As these controls may be better matched to the reagents, the break-in period may be abbreviated.

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Delta check and precision standards for sodium

Q What would you consider a significant delta regarding sodium on repeated analysis of a patient’s specimen? We have noticed that when we repeat low sodiums (128 mmol/L to 134 mmol/L), we will see an increase on the repeated analysis of anywhere from 4 mmol/L to 10 mmol/L. We are repeating these sodiums because we are seeing low anion gaps (1 to 5). QC and calibration are okay. Our specimens are plasma; we have not noted anything floating in the specimen that might cause the interference. At what point would you consider the delta significant on a repeated sodium on the same specimen?

A The determination of what to consider a significant change in an analyte has been widely studied. Unfortunately, there are almost as many recommendations as there are publications devoted to this particular topic. Your question brings up two different issues; delta checks and within-run precision. Delta checks, defined as “the comparison of laboratory test results obtained on a current specimen with those values obtained from the same patient on a previous specimen,” is one technique employed by laboratories to detect specimen mix-up errors. The expected variability between test results obtained on the two specimens depends on both the analyte and time interval between determinations. Delta check limits that have been recommended for sodium are 5% and 10 mmol/L.1

The easiest way to evaluate what the expected variation observed with repeated measurements would be to perform a replication experiment.

The question you ask, “What would one consider to be a significant change after repeating the same specimen,” refers to within-run precision rather than true delta checking. The easiest way to evaluate what the expected variation observed with repeated measurements would be to perform a replication experiment. A replication experiment is typically performed by obtaining test results on 20 samples of the same material and calculating the mean, standard deviation, and coefficient of variation. The purpose of this experiment is to observe the variation expected in a test result under similar operating conditions. Various factors, however, such as differences in matrices between samples, and the concentration of analyte within the sample may result in greater than normal imprecision. In our laboratory, within-run imprecision for sodium is less than 2 mmol/L between repeated measurements of this analyte.

Another way to look at this problem is to establish the maximum difference in repeated measurements that can be toler-
ated before the difference is considered to be clinically significant. This method takes into account the analytical and within-subject variation of an analyte and is calculated, for $P \leq 0.05$, as $2.77 \left( CV^2_A + CV^2_D \right)^{1/2}$, where $CV^2_A$ is the square of the analytical CV for sodium and $CV^2_D$ is the square of the normal intraindividual variability of sodium. Using an analytical CV of 0.75 mmol/L and an intraindividual variability of 0.86 mmol/L for sodium, a critical difference of up to 3.2% between repeated measures for sodium are acceptable.2

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References

**Pediatric reference ranges derived by different methodologies**

**Q** We are a mid-size hospital with low pediatric volume due to a children’s hospital near by. Can I use a reference range from literature if I do not have access to pediatric specimens to establish my own? I am nervous about using a reference range based on another methodology.

**A** Identifying pediatric reference intervals is a continuing problem for all laboratories. Numerous factors need to be considered including age, sex, diet, drugs, posture, stress, and time of day. The process of transferring and validating appropriate reference ranges or reference intervals, however, is the same for both adult and pediatric patients. This process has been spelled out in the document “How to Define and Determine Reference Intervals in the Clinical Library” available from the Clinical Laboratory Standards Institute (CLSI).1

It is acceptable to use a manufacturer’s reference interval or a reference interval from another lab using a similar analytical system. If the method is markedly different, this process cannot be used. This process, called transference, needs to be assessed for acceptability (validation). Three validation processes are delineated in the CLSI reference; I will only describe two in general terms. Please see the reference for further details.

**It is acceptable to use a manufacturer’s reference interval or a reference interval from another lab using a similar analytical system.**

The first validation process is a subjective assessment. The laboratorian must determine that the population tested for the transferred reference interval is similar to your testing population. Next, the pre-analytical and analytical procedures must be reviewed to determine if they are consistent with the processes in your laboratory.

In the second validation process, 20 specimens that represent your sampling population are identified and tested. The 95% reference interval is validated if no more than two of 20 test values exceed the proposed interval. If three or more values are identified, then the process should be repeated with an additional 20 specimens and checked for outliers. If there are three or more outliers on the second set, then further investigation into the analytical process and reference populations need to be initiated. If there is no explanation for the observed differences, then the laboratory may need to develop its own reference interval.

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References

**Correction for WBC in automated CSF count**

**Q** Our lab has recently begun to report cerebrospinal fluid (CSF) and body fluid cell counts from the Coulter (LH 750), and are using the procedure recommended by Beckman-Coulter. As the Coulter includes white blood cells (WBCs) in the red-blood-cell (RBC) count, my question concerns the potential for a false elevation of the fluid RBC count due to the inclusion of WBCs. It is not uncommon for fluids to have equal numbers of WBCs and RBCs or more WBCs than RBCs. At what point should the lab be correcting the Coulter RBC count on fluids for the presence of WBCs?

**A** Note: MLO generally does not seek answers from industry experts. We were, however, unable to find an expert not associated with industry to answer this question.

The Body Fluid Application was added to the Coulter LH 750 Hematology Analyzer in 2005. All Coulter hematology analyzers count RBCs in a dilution that contains RBCs, WBCs, and platelets. The RBCs are lysed in the WBC count dilution. The WBC represents the TNC (total nucleated cell count) in the analysis of body fluids.

As noted in the instructions for processing body fluids on the LH 700 Series analyzers, both the Coulter LH 750 and Coulter LH 780, the following lower limits of detection are indicated:

- Do not report the WBC if $\leq 0.20 \times 10^3$ cell/µL. Obtain WBC by an alternate method.
- Do not report the RBC if $\leq 0.010 \times 10^6$ cell/µL. Obtain RBC by an alternate method.
- These limits translate into a TNC $\leq 200/\mu L$ and RBC $\leq 10,000/\mu L$. Unless the WBC count exceeds the lower limit of detection for the RBC count, any interference from the WBC would not be reported in the RBC count.

In their three-site evaluation of the Coulter LH 750 Body Fluid Application, the authors noted:

“Of the 372 samples, 106 had RBC counts greater than 0.01 X $10^{12}/L$ and were used for method comparison. Low-level sensitivity excluded the majority of cerebrospinal (119) and a small number of peritoneal dialysate fluid samples (8), which require accurate enumeration at clinical decision points between one to

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100 cells/microliter. In the case of the synovial and serous fluids, however, most clinicians are interested in TNC counts above 0.2 X 10⁹/L and RBC counts are relevant only if they are significantly increased (≥0.05 X 10¹²/L).“¹”

Good laboratory practice suggests that each laboratory should evaluate the significance of interference between cell types.

In the evaluation of body fluids on the Sysmex XE-2100,² the authors noted that “RBC counts of body fluids other than cerebrospinal fluid are of limited diagnostic value.³,⁴ WBC counts <0.05 X 10³/µL and RBC counts <0.01 x 10⁶/µL should be confirmed with an alternative method.”

As provided in the reference material from Beckman Coulter, “… results should be interpreted in light of the total clinical presentation of the patient, including clinical history, data from additional tests, smear review, and other appropriate information.” In a review of the literature, the evaluators made no suggestion that correction was necessary. Good laboratory practice suggests that each laboratory should evaluate the significance of interference between cell types. –Maggie Worts, MT(ASCP)

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References

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