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Texas Association for Clinical Laboratory Science

TACLS News

CLIA Test Performance Specifications Requirements

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This is the second of a two part series on Quality Control.

Last month, I discussed the new CLIA rules in terms of quality control and equivalent quality testing. The rules leave it up to the laboratory to establish the number, type, and frequency of testing control materials, but also state that the lab must verify the manufacturer's published performance specifications or establish new ones itself. The results of the laboratory's control procedures, proficiency testing, and assessment activities are used to verify test performance. This article will discuss the procedures that must be carried out to determine these performance specifications.

Unmodified/Approved Moderate Complexity Tests

According to the final CLIA requirements, all laboratories who do more than waived testing that introduces an unmodified, FDA-cleared or approved test system must, before reporting patient test results, demonstrate that it can obtain performance specifications comparable to those established by the manufacturer in the following areas

- **Accuracy**
- **Precision**, and
- **Reportable range** of test results for the test system

The term "FDA-cleared or approved test system" is defined in the November 9, 1997 revisions to the Food, Drug and Cosmetic Act (Pub. L. 105–115), to mean a test system cleared or approved by the FDA through either the premarket notification (510(k)) or premarket approval (PMA) process for in-vitro diagnostic use. This includes test systems exempt from FDA premarket clearance or approval.

It is up to the laboratory director to decide the extent to which these performance specifications are verified, based on the method, testing conditions, and personnel performing the test. Laboratories performing unmodified moderate complexity tests cleared or approved by the FDA are not required to retroactively verify the manufacturer's performance specifications.

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Standard methods to establish accuracy, precision and test range parameters are relatively well established procedures used when a new method is introduced into the laboratory. Below is a review of how to verify each parameter.

Accuracy Verification

In last month's article, accuracy was defined as the agreement of a measurement with the true value. The accuracy of a method is a check for systematic errors which could be either constant or proportional. Studies performed to verify accuracy include recovery studies and patient correlation studies.

Recovery studies can be performed by adding specific amounts of an analyte standard to a serum sample and then performing an analysis on that sample. This procedure will check for proportional error, looking for potential competitive interferences, with the ideal recovery = 100% and acceptable recovery range between 95-105%. The calculation for this study is:

$$\frac{\text{Analyte recovered}}{\text{Analyte added}} \times 100 = \%\ \text{recovery}$$

Minimally, the recovery of an analyte should be checked at the low, middle and high ranges of the procedure. Below is an example of recovery procedure:

Sample Preparation

Sample 1: 2.0 mL serum + 0.1 mL H_2O

Sample 2: 2.0 mL serum + 0.1 mL 20 mg/dL Analyte Standard.

Sample 3: 2.0 mL serum + 0.1 mL 50 mg/dL Analyte Standard.

Calculation of Recovery

1. Concentration added =
standard concentration x $\frac{\text{mL standard}}{\text{mL std + mL serum}}$

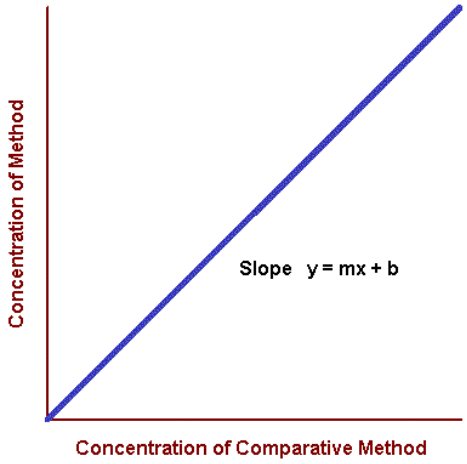
2. Concentration recovered =
diluted test concentration- baseline concentration

3. Recovery = $\frac{\text{concentration recovered}}{\text{concentration added}} \times 100\%$

For patient correlations, a minimum of 40 (100 preferred) patient samples over the range of medical interest are analyzed and the results compared. If possible, these patient samples should be analyzed 5 per day over at least 20 days. A linear regression analysis is performed on the data, assessing slope, y-intercept, standard error of estimate, and correlation coefficient. The data is plotted and checked for outliers. Acceptable ranges are:

- M (slope) between 0.95 - 1.05
- b (intercept) very low (ideal = 0)
- r (Correlation-coefficient) > 0.95

The slope of the procedure offers an estimate of the proportional error, while the intercept provides an estimate of the constant error. The standard deviation of the procedure will give an estimate of the random error between the 2 methods. The correlation coefficient will give an indication of the association of the 2 methods, but will not identify if the method is accurate.



Precision Verification

Precision is defined as the agreement between replicate measurements on the same material. Studies performed to verify precision include within-run precision and day-to-day precision. To verify with-in run precision, ten replicate analyses on five to ten samples which vary in level and matrix should be performed on the same day and within the same run of analyses. Between-day-precision is verified by performing measurements on 2-3 levels of control material for 20 days. If possible, 4 measurements should be performed per day for 20 days. Calculations included in this measurement are the mean, standard deviation, and coefficient of variation. This measurement is an indication of random error.

Reportable Range

Finally, to determine the range of reportable results, dilution studies must be performed to check the linearity of the sample matrix and to confirm the analytic range. This study should be performed like a "recovery" study, where a specific amount of analyte is added to a serum sample at specific intervals. These studies should be performed for the full range of the test procedure and should result in a recovery between 90-110% using the following calculation:

$$\frac{\text{Analyte found}}{\text{Theoretical amount}} \times 100 = \%\ \text{recovery}$$

Additionally, the procedure should be rechecked for accuracy and reproducibility of calibration at the medical decision levels of the analyte.

Unmodified/Approved Tests

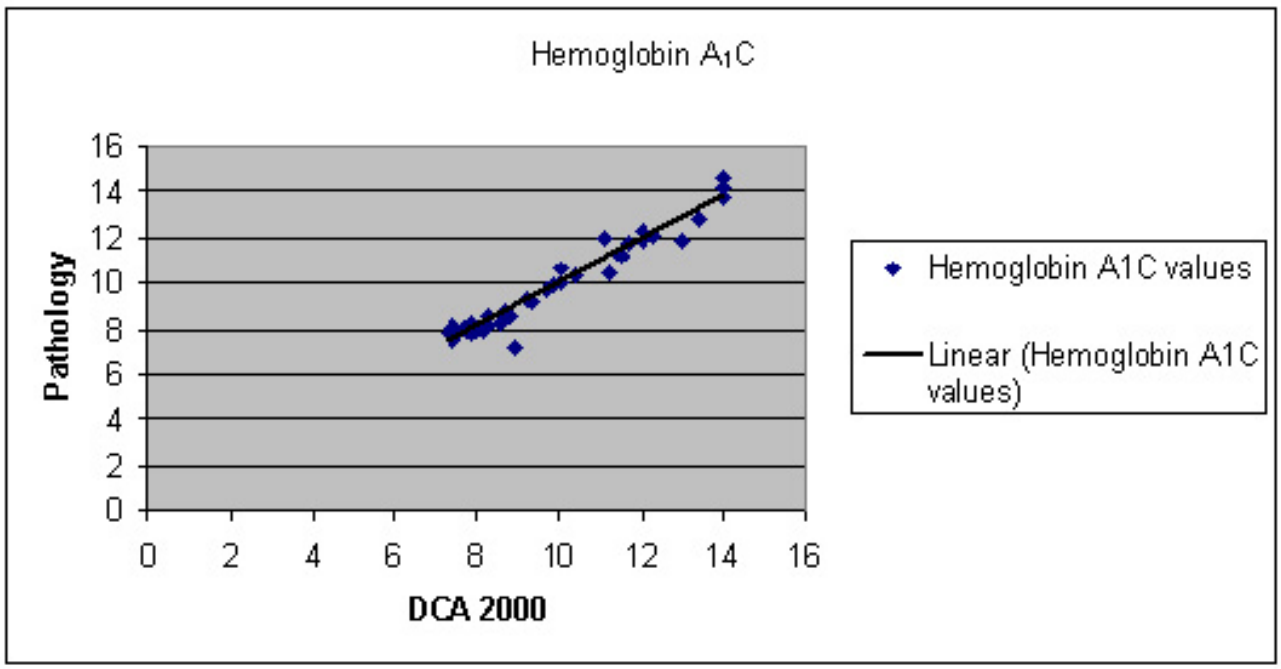
Laboratories that use a test system in which performance specifications are not provided by the manufacturer, modifies an FDA-cleared or approved test system or introduces test system not subject to FDA clearance or approval (including standardized methods and methods developed in-house) must establish **additional** performance characteristics, beyond accuracy, precision, and reportable range of results for the test system before reporting patient test results. These specifications include: analytical sensitivity and specificity (including interfering substances), reference intervals (normal ranges); and other performance characteristics required for test performance.

Analytical Sensitivity and Specificity

Analytical sensitivity is the lowest amount measured or detected by the procedure and can be determined by performing a calibration curve and including measurements of the sample and reagent blank to compare the magnitude of these measurements with the lowest analyte concentration.

Analytical specificity is when a false positive result may be due to the test measuring a related or other chemical rather than the analyte desired for testing. This can best be tested by performing interference studies. A specific amount of a potentially interfering substance is added to a serum sample and then an analysis on that sample is performed. This procedure will check for systematic (constant) errors caused by common interfering substances such as bilirubin or hemolysis.

CLIA Test Performance Specifications Requirements



Ideally, the interference from these substances will be 0. Minimally, the interference of a substance should be checked at the low, middle and high level. Below is an example of the test for Mg interference on a calcium procedure:

Sample Preparation
Sample 1: 1.0 mL serum + 0.1 mL H_2O
Sample 2: 1.0 mL serum + 0.1 mL 10 mg/dL Mg Standard
Sample 3: 1.0 mL serum + 0.1 mL 20 mg/dL Mg Standard

Calculation of Interference

1. Concentration added =
standard concentration x $\frac{\text{mL standard}}{\text{mL std + mL serum}}$

2. Interference =
concentration (diluted test) - concentration (baseline)

Reference Intervals

Reference ranges must be established for the area of the country in which the laboratory is located and must be appropriate for the laboratory's patient population. Therefore, reference intervals provided by instrument manufacturers are not adequate and each laboratory must determine their own ranges.

Additional Requirements for all Laboratory Tests

Calibration Requirement

Calibration must be performed at least once every 6 months using a minimum of 3 values to verify the laboratory's reportable range or whenever calibration verification procedures are unacceptable. However, the requirement for laboratories to perform calibration verification using calibration materials appropriate for the methodology and, if possible, traceable to a reference method or reference material of known value was removed to allow laboratories flexibility in choosing materials for calibration verification.

QC Record Retention Requirements

Confusion about the record retention requirements lead to a reinterpretation in the final CLIA rules. Requirements formerly may have been misinterpreted as permitting the laboratory to discard method performance

specification records after a 2-year period even though the method may have continued to be used beyond this timeframe. Under the final interpretation, records of the laboratory's establishment and verification of method performance specifications must be retained for the period of time the test system is in use by the laboratory, but not less than 2 years.

Hopefully, this article gives some guidelines to laboratory professionals on the CLIA requirement for the determination or verification of test performance characteristics. The article is not meant to be all-inclusive. Before performing any of these procedures, a laboratory textbook should be consulted to ensure proper methods are employed.

Suggested References

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A Message from the President

Christina Thompson, Ed.D., TACLS President

The ASCLS Annual Meeting is in Los Angeles from July 27 through July 31. If you have never gone to the national meeting, you should make every attempt to attend one and this meeting promises to be informative and exciting. In addition to the exhibits, presentations, research and case studies, the membership will discuss position papers and current issues affecting our profession. One of the presentations will be on a proposal from the career ladder task force. Rather than moving into management or out of the laboratory, this proposal will provide several levels for advancement as a clinical practitioner. The proposal provides specific tasks for each level and also introduces the advanced practice scientist to the profession. Additional issues include the medical laboratory personnel shortage, the proposed entry level Master's and proposed federal legislation. You can find information about the annual meeting and current issues affecting the profession on the ASCLS web page, www.ascls.org. I hope to see you in Los Angeles.

Austin Community College Hosts Phlebotomy Workshop

Dave Falleur., TACLS Editor

On Saturday, July 17, Austin Community College's Riverside Campus was the site for a full-day workshop on phlebotomy presented by Terry Kotrla and Dennis Ernst. Approximately 40 participants attended the program which was approved for PACE credit. The participants included nurses, phlebotomists, ACC students, and laboratory professionals.

Dennis J. Ernst, MT(ASCP) has been involved in phlebotomy for over 20 years as a medical technologist, educator and legal consultant. Dennis is the the Director of the Center for Phlebotomy Education in Ramsey, Indiana. He is one of the most prolific authors on phlebotomy issues and techniques with publications in *Advance*, *Health Care*, *MLO*, and *ASCLS Today*. He has a popular website (www.phlebotomy.com) and also has published a book *Phlebotomy for Nurses and Nursing Personnel* and a series of videotapes. For additional information go to his website, www.phlebotomy.com.

Terry Kotrla, MS, MT(ASCP)BB is a professor at Austin Community College and Phlebotomy Technician Program Director. She teaches blood banking, immunology, and phlebotomy, and also works as a PRN blood banker at Seton Medical Center in Austin.

The first session, *Protecting Yourself From Phlebotomy-Related Lawsuits* was presented byDennis Ernst who discussed the common errors which lead to disabling injuries, paralysis and death. They are (1) Nerve damage, (2) Arterial nicks, (3) Subcutaneous Hemorrhage, (4) Lymph Node Involvement, (5) Vertigo, and (6) Death. These errors fall in to three categories: Technical (for example inserting the needle at the wrong angle), Judgemental (i.e. unacceptable site selection), and Administrative (i.e. insufficient training). Many of the significant injuries to patients occur when phlebotomy is attempted by accessing the brachial vein. Dennis discussed the new NCCLS guidelines for phlebotomy and some of the changes that have been incorporated into these guidelines. The NCCLS document is *Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture*, 5th edition, and can be obtained from the National Committee on Clinical Laboratory Standards, www.nccls.org.

After a refreshment break provided by Judy Hough from Smiths Medical, Dennis presented the second session, *Identifying and Eliminating Preanalytical Errors*. In this session he identified errors before, during, and after collection, which can significantly alter laboratory results. The most serious errors that occur prior to collection are misidentification of the patient and improper timing of collection. Many errors can occur during the collection process and some common examples are excessive tourniquet time (>1 minute), pumping the fist, site preparation (i.e. contamination of blood cultures), insufficient volume, and hemolysis. Dennis discussed the recent change in the "order of draw" that has been

recommended by the NCCLS. The recommended order is

1. Sterile tube for cultures
2. Sodium citrate tube
3. Serum tube with or without clot activator
4. Heparin tube
5. EDTA tube
6. Oxalate-Fluoride tube

There was considerable discussion about this recommendation and the need for a "discard" tube for coagulation testing. The NCCLS recommends that discard tubes are not necessary for PTs and aPTTs, but should be used for factor assays and when blood is collected with a butterfly infusion set.

Errors that occur after collection include labeling errors, delays in processing, and improper storage and handling. Dennis discussed different strategies that laboratories can use to minimize these errors.

Lunch was provided by . Exhibits were set up by and participants had a chance to see the latest technology in phlebotomy equipment.

Lunch was provided by Steve Wilson of Becton-Dickinson. After lunch, Terry Kotrla spoke on *Age Specific Care for Phlebotomy*. In her presentation Terry discussed the needs of patients in various age groups. JCAHO requires that all healthcare staff annually meet competency expectations in performing Age Specific Care. "Managers are being challenged to make sure their phlebotomists are not only proficient at phlebotomy, but proficient with all age groups." Laboratory personnel performing phlebotomy should have a basic knowledge of human growth and development, age specific interpersonal skills, and technical expertise. Terry discussed the psycho-social needs of eight different age groups: (1) Neonate/Infant, (2) Toddlers, (3) Pre-School, (4) School Age, (5) Adolescent, (6) Young Adult, (7) Adults, and (8) Late Adults. Following this discussion Terry described observational and testing techniques that can be used to assess competence.

After a refreshment break provided by Chuck Zamutt of Greiner Bio-One, Terry presented the final program, *Needle Safety– When a Good Thing Goes Bad*. Terry discussed the newest safety devices, as well as the pitfalls that can lead to needle stick injuries. She provided a listing of websites for information about the mandatory Needlestick Safety and Prevention Act and revised Bloodborne Pathogens Standard. These regulations are not optional and employees should be part of the decision process for selecting safety equipment. Laboratory management needs to document training and document problems associated with various collection devices.

Mark your calendar now

ASCLS/AACC Annual Meeting, Los Angeles, July 27-31

Texas Clinical Laboratory Educators Meeting, Dallas, August 13

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