Establishing a Quality System for Molecular Diagnostic Testing

Quality Control

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Quality Control

A system for verifying and maintaining a desired level of quality in an existing product or service through careful planning, use of proper equipment, continued inspection, and corrective action as required.

The most essential aspect of any laboratory test is that it provides correct results. Perfection is sought, but hard to achieve. For this reason, diagnostic scientists utilize a framework of quality control. At its very least, quality control alerts us to potential problems with a particular test being utilized in the laboratory. At its best, quality control provides us with specific information for particular failed testing events, and can even suggest reasons for the failure. Quality control saves money and time. Perceived through the most important lens, quality control improves the quality of medical care and protects peoples' lives.

The advent of nucleic acid based testing has been a significant addition to the field of diagnostic science. Such testing, often termed, "molecular", includes the utilization of the coding sequence of nucleic acid as a source of derived specificity. Molecular testing has been particularly impactful to the field of infectious disease, where organisms can be readily differentiated by DNA or RNA sequences that are unique to their species (or even sub-species). This means that molecular tests can be designed such that they theoretically have singular specificity for only their intended target. Additionally, many molecular tests operate on the principle of "target amplification" which denotes that detection of analytes is performed through amplification of the nucleic acid sequence target. Examples of such methods include polymerase chain reaction (PCR), transcription mediated amplification (TMA) and strand displacement amplification (SDA). This method of detection differs from "signal amplification" which has been the keystone of diagnostic science and includes chemiluminescence and enzyme or catalytic based reactions, two of the more common methods of generating a signal. Target amplification has resulted in exceptional sensitivity, with certain tests capable of detecting less than a single organism. It sounds as though we have described the perfect laboratory diagnostic: tests that can detect singular copies (or less) of pathogenic organisms and that do so with perfect specificity. Unfortunately, they are not.

The sensitivity of target amplification can also be an Achilles Heel. Even the minutest amount of contamination with a target sequence, or one quite similar to it, can provide false positive results. Moreover, the enzymes that perform target amplification can themselves be temperamental, in highly balanced chemical environs which are inhibited often by simple and common molecules and can result in failed amplifications and false negative results.

As with all diagnostic testing, molecular diagnostics are saved by quality control procedures, without which the risk of reporting incorrect patient results would be unacceptable. Herein, we will delineate the most important tenets of quality control for molecular tests as indicated by regulation, and best practice. There are many different accrediting bodies that regulate quality control, each with their own specific indications. Additionally, many individual States have their own specific regulations that overlay federal regulations. However, it is fair to say that all of these accreditation agencies adhere to the same basic principles codified in federal regulations. And all seek to attain the same results: high quality laboratory testing.

The required aspects of quality control for molecular tests in the United States of America include:

- **Validation or Verification**
- **Procedures**
- **Personnel Competency**
- **Maintenance (Equipment, Reagents)**
- **Controls, Calibrators, Standards**
- **Proficiency Testing**

These topics are not discussed below in order of importance. Many argue that Proficiency Testing is the cornerstone of quality control, for what it accomplishes and the manner in which it functions. Others may argue "Controls" because these really assess the performance of individual test runs, and provide a front line defense from the release of bad laboratory test results. But all are required by federal regulation and by all current accrediting bodies.

Validation and Verification

No two terms are perhaps more discussed amongst laboratory professionals, because few of us have any idea what the difference is. It is an interesting confusion, because the Center for Medicare and Medicaid Services (CMS) regulations never use the word "validation" in print, yet it is the most commonly utilized term. Regardless, both terms are now fully entrenched in the field of diagnostic science, and they are different in meaning.

Laboratory tests generally fall into two classes: those which are cleared by the Food and Drug Administration (FDA) and Laboratory Developed Tests (LDTs). I suspect we have or will hear a tremendous more on the differences between these two classes in the coming years, as the FDA and the CMS seek to determine how LDTs are regulated. Regardless, these two classes of tests are treated differently when they are assessed for use in the diagnostic laboratory environment.

FDA cleared assays are vetted through extensive internal or clinical trial processes that include the collation of a large amount of performance data. When a test is cleared, and subsequently marketed and sold, it must include a document ("package insert") which includes the most relevant data from such trials, including specificity and sensitivity. For molecular tests, there is also an assessment of "inclusivity" and "exclusivity" which are assessments of the ability of the test to react with many strains of a particular species (inclusivity); and not to react with species of organisms that are not the intended target (exclusivity). Regulation requires that such FDA cleared assays must be verified prior to use in a diagnostic laboratory. In doing so, one is verifying the performance claims of the product's package insert at their diagnostic laboratory.

In the process of verification, one is determining whether a particular product, works as expected, using the equipment, personnel and geography of one's own laboratory. Geography can bring a host of physical principles into play that can affect the performance of molecular tests and their associated equipment including humidity and temperature factors which can affect test results. It is very important to note that a test performed in a laboratory is only FDA cleared when the test is run according to the strict direction of the package insert. ANY deviation from such instructions immediately causes a test to be classified as a Laboratory Developed Test (LDT). Moreover, all LDT are classified as "High Complexity" according to the FDA, which means that only licensed staff can perform such tests, in most States.

The CLIA regulations are clear with regard to what assessments must be made for a verification study. Molecular tests can be both qualitative and quantitative and this is covered below. The requirements for a verification study include:

Accuracy

This includes an assessment of how well the test correctly determines the disposition of a specimen. Specimens can be purchased calibrators, quality control materials, or patient specimens for which a result is already determined. The number of such specimens is not indicated in regulation. Most laboratorians consider the number twenty to be a minimum number of specimens evaluated. Viewed from the scientific or statistical perspective, more is always better in the course of generating a data set in which there is high confidence.

Precision

While Accuracy assesses how "on target" a test is, relative to a gold standard, Precision assesses how repeatable a test result is. It is often assessed by testing a singular specimen (of adequate volume) to be evaluated on the test platform on multiple instances, preferably by different operators on different days of the week. This accounts for any variability in test performance or environment. The use of commercially available independent quality control materials are often used for assessing test precision.

Reportable Range

For qualitative molecular tests, this is essentially negligible, as test results are Boolean (positive or negative). However, it is still important to define reportable range in this part of the verification report. Such results were evaluated in the accuracy phase when positive and negative specimens were assessed. For quantitative tests, this is a more intensive operation. It includes the assessment of enough specimens

with adequate loads to show that a test can accurately assess results across a required range. For example, a typical viral load test that claims to have a reportable quantification range of 50 to 10,000,000 would have to be assessed to show that all specimens of various loads across that range, inclusive of the boundaries, are quantifiable.

Reference Range

This is the value that the test result would provide for a "normal" or non-afflicted individual. In qualitative molecular tests for infectious disease, this value is "negative". For quantitative molecular tests, this might likely be "target not detected" or zero.

When a test system being assessed for use is non-FDA cleared, or if it is a modification of an FDA cleared test, the test system must be validated for medical use. Validation is a process very similar to verification, requiring everything in verification, described above, but also including analytical sensitivity and specificity.

Analytical Sensitivity

This is the process whereby the limits of detection are assessed for a particular test. Whether qualitative or quantitative, the minimal concentration of analyte(s) that a particular test can detect must be determined. For qualitative tests, this is best performed using quality control or calibrator materials (purchased from a vendor) that have been accurately quantified. Dilutions of such materials across a broad range inclusive of a hypothesized threshold of detection would be adequate. The lowest dilution detectable would contain the assessed analytical sensitivity. In molecular tests such as PCR, there is a theoretical limit of one target nucleic acid molecule, but inefficiencies in extraction and amplification often make that number much higher.

Analytical Specificity

Molecular tests are of considerable value for their ability to sensitively detect targets and to discriminate on the basis of nucleic acid sequence. While theoretically this builds high specificity into a test, it is not guaranteed. For that reason, such tests must be further assessed. This is best achieved in two ways:

a) Challenge the test with specific organisms that may be found at the same physiologic environment as a typical tested specimen; and

b) Challenge the test with specimens of such physiologic regions known not to contain the target.

Achieving a), above, involves generating a list of known organisms, either closely related to, or found in physiologic concordance with, the target of the test in question. Those organisms are obtained/purchased, cultured, and then diluted into the appropriate testing matrix. Quality Control materials may also be of value here, if available. Diluted specimens are subject to the test in question and results are tabulated. A test specific for a particular target should not react with close relatives, or other organisms in the same physiologic site. b), above, is also very helpful in assessing specificity, and in this author's opinion, is perhaps more meaningful, scientifically. It includes obtaining known-negative specimens from the physiologic sites relevant to the test, and assessing them. Such specimens should contain the realistic amount and ecologic variety of the flora of the tested site, and thus should challenge the test in question with the most relevant materials.

Additional important notes

Many molecular methods include discrete steps of nucleic acid extraction and amplification. It is imperative that both aspects of the test are challenged by validation and verification. An amplification platform cannot be preceded with an extraction method unless that method has been included in the verification or validation. It is also of note that in many molecular LDT, analyte specific reagents for a test would include nucleic acid primers and/or probes ("oligos" as they are often called) that are manufactured in batches upon request by an external agency. It is crucial that quality control measures be established to continuously validate the functionality of batches of such materials. Note also that the verification or validation of a particular specimen type does not guarantee functionality of other specimen types. Assessing the accuracy of a test for detecting an analyte in stool does not validate or verify a tests ability to detect the same analyte in urine, for example.[1]

Procedures

Prior to the initiation of a diagnostic test, whether FDA cleared, or an LDT, the laboratory must construct an appropriate procedure document describing the test and how it is performed, among other important components relevant to the performance of the test. Federal regulations are specific regarding what a procedure manual must include. Such regulations indicate that textbooks cannot be utilized as procedure manuals, and that a test manufacturer's instruction manual (package insert) can be used as only a portion of a formal procedure. In such cases where a package insert is utilized, all aspects of a test that are specific to a particular laboratory setting must be documented in addition to the manufacturer's document (see bullet points below). Prior to the initiation of a particular test, the procedure must be reviewed and signed by the Laboratory Director and any personnel performing the test. Any changes in a procedure must also include a review of the document and a signature indicating such review. It is best practice for procedures to be reviewed at least annually by technical supervisors and laboratory directors, so that any changes in procedure can be integrated into the document.

Federal Regulations dictate that all procedure documents include:

- Requirements for specimen collection, labeling, storage, preservation, transportation, and processing; criteria for specimen acceptability and rejection
- Step-by-step performance of the procedure, including any necessary test calculations and interpretation of results.
- Preparation of any physical or chemical materials used in the test
- Calibration procedures
- The reportable range for test results for the test system often, but not always "positive" or "negative" for molecular tests, but take careful note of quantitative tests such as viral load tests
- Control procedures, including material used, testing frequency, and acceptance criteria
- Corrective action to take when control specimens (or calibrators) fail to meet the laboratory's criteria for acceptability
- Limitations of the test method, including inhibiting or interfering substances
- Reference intervals (results expected for a healthy, normal condition)
- Life-threatening test results, or "panic" or alert values.
- Relevant literature references
- The laboratory's procedures for entering results into a laboratory information management system (LIMS) and or into the patient record and for reporting patient results to clinicians who have requested the test and are responsible for working with the results
- Description of the course of action to take if a test system becomes inoperable

When a particular procedure is no longer in use, it should be noted and verified by signature and date of cessation of the procedure. Keep in mind that such procedures are subject to audit by accrediting bodies even after a procedure or test is no longer offered by a laboratory. It is best to keep such procedures for a minimum of 5 years after cessation.[2]

Personnel Competency

The ability to properly perform a test is an obvious necessity for assuring quality in the diagnostic setting. Molecular tests are of particular note in the realm of personnel competency because such tests span a considerable range in complexity. LDT molecular tests can be amongst the most complex of laboratory tests due to the large number of reagents used, handling conditions of such reagents, the particularly tiny volumes that are manipulated (often on the microliter scale) and the sophisticated nature of the equipment used to perform such tests. The last decade however has seen significant development in the automated molecular test market, some of which require minimal knowledge of molecular biology or of the components that power the tests. Certain molecular testing devices are even currently deemed "moderately complex" by the FDA and are as simple as adding a specimen to a cartridge and loading the cartridge into an instrument. Most clinical and public health laboratory training programs have begun to incorporate molecular biology training to lay the foundation of competence in this area of testing. Fortunately, the same guidelines for quality control in the area of personnel competency for "traditional" laboratory testing applies to molecular testing.

Federal regulations (and best practices) include six components in assessing competency:

1. ***Direct Observation***
2. ***Monitoring***
3. ***Reviewing***
4. ***Observe Instrument Performance***
5. ***Assess Personnel Competency***
6. ***Assess Personnel Problem Solving***

Federal regulations (and best practices) include these six components in assessing competency:

1. Direct observation of routine patient test performance, specimen handling, processing and testing;
2. Monitoring the testing personnel's ability to record and report test results;
3. Reviewing the testing personnel's test results and any worksheets, quality control records, proficiency testing results, and preventive maintenance records that are generated;

4. Directly observing the performance of instrument maintenance and function checks;
5. Assessing the ability of the testing personnel to perform the test by providing them with previously analyzed specimens, internal blind testing samples or external proficiency testing samples;
6. Assessing the testing personnel's ability to react to and solve problems with the test.

These components do not have to all be performed on the same date however all are to be documented, and dated.

Federal regulations require that all testing personnel be assessed for competency on an annual basis and that new laboratory personnel be assessed more often (twice in the first year, every six months of patient testing). It is important to note that all personnel who perform a test at any time must be assessed. This includes supervisorial personnel that might "fill in" for an absent technician or scientist, even if they only perform the test once in a given two year period. It is not uncommon for laboratories to be reminded of this upon audit, having forgotten that the technical supervisorial personnel, who may have performed a test only once or twice in a given period of time, were never themselves assessed.

Maintenance

The growth of molecular testing has created a concomitant increase in the use of equipment in laboratory testing. This includes the number of freezers and refrigerators in use due to the rather labile nature of molecular testing reagents. Equipment used in molecular testing is often complex, and may include multiple different types of devices. PCR and other amplification based tests cannot always be performed in singular amplification and detection devices, but may include separate nucleic acid extraction equipment which itself often requires an increased use of centrifuges and supporting equipment such as sonicators. Automated platforms that perform nearly all of the functions required for testing of specimens are now commonplace in larger laboratories. Such platforms invariably possess highly specific requirements for daily, weekly, monthly and annual maintenance.

Federal regulations are unambiguous regarding the maintenance of equipment / instruments used in diagnostic testing:

1. Follow the manufacturer's requirements
2. Document all maintenance
3. Ensure that equipment and instruments are functioning properly prior to use

In cases where the manufacturers do not provide maintenance requirements, the laboratory is responsible for determining that equipment is functioning within expected parameters, and documenting such parameters. This may mean that the laboratory must develop a test or evaluation process on its own to determine whether a particular piece of equipment is in functional order. The laboratory must define, perform and document that process. The use of commercially available independent quality control material after maintenance, but before patient samples are tested can help to meet this requirement.

It is crucial to note there are often processes associated with equipment maintenance that are NOT indicated in manufacturers' instructions. One example is an electronic interface connection to a Laboratory Information Management System, or Laboratory Information System (LIMS/LIS). Such interfaces between testing equipment and results databases have become very common, particularly in high volume laboratories where manual data entry is exhaustive and error prone. Electronic interfaces require maintenance and evaluation and every interface in which I have been a part of instituting has been initiated by a validation procedure. Routine assessment that such interfaces are delivering accurate results from testing equipment to their computer / server databases are essential aspects of quality control. Documentation of such evaluations and routine assessments are critical.

As a reminder, documentation is the most important process of all—for anything—that one does in diagnostic science. It is a common adage amongst good laboratorians and the auditors that inspect them:

"If it is not documented, it did not happen."

–42CFR 493.1254

Controls and Calibrators

It is often easy to forget that diagnostic testing is science and not manufacturing. Central to diagnostics, a laboratory test is a tool for answering a scientific question: is a patient experiencing a certain condition? The functionality of a test in answering that question can only be known through the use of controls. In some ways, the use of controls is the most powerful aspect of quality control. Controls, when used on the day of testing, and in the same "batch" or "run" of a test, can provide clear information regarding whether a particular test result is performing as expected. Validations and verifications accomplish this when a test is first introduced to a laboratory, but they do not assess the test existentially, at the particular time of its use. Controls therefore can immediately inform the diagnostician whether to accept or reject a result. Federal regulations require the use of controls and this is an area where regulations are very specific about how molecular amplification tests are controlled:

"Each molecular amplification procedure [shall] include two control materials and, if reaction inhibition is a significant source of false negative results, a control material capable of detecting the inhibition."[3]

Additionally, the regulations are unambiguous with regard to qualitative and quantitative molecular tests:

> *"At least once each day patient specimens are assayed or examined perform the following for -*
>
> *(i) Each quantitative procedure [must] include two control materials of different concentrations [calibrators];*
>
> *(ii) Each qualitative procedure, include a negative and positive control material";*[3]

Detailed descriptions of the requirements are described in these laws:

External vs. Internal Controls

Both 'external' and 'internal' controls exist for molecular tests. External controls are tested (often along with patient specimens) just as patient specimens are tested. They are often provided by a test manufacturer but can also be obtained from third parties. In some instances, laboratories will utilize patient specimens that are known to be either positive or negative for a particular analyte. While seemingly convenient, this mode of external control is not ideal. Ideal external control specimens would be highly characterized, often by a third party who can provide a "gold standard" level of confirmation to such patient specimens. External controls from reputable manufacturers are always vetted to standards much higher than provided to patient specimens by a typical diagnostic lab. The acquisition of external controls from third party manufacturers often includes certifications or documentation that establishes their performance. Moreover, such materials can be purchased in high volume with extended shelf life so that they can be used repeatedly for several test runs, even permitting monitoring across reagent lots. Unlike using an existing patient specimen, manufactured controls can be used without prior extensive evaluation or characterization. Often independent controls are targeted to be "low" positive, or closer to the assay level of detection which can be difficult to find with patient specimens. Additionally, acquisition of control materials from a manufacturer other than that of a particular test system may have particular merit since it provides a more objective assessment of a test system compared to materials in the system's run kits. Another benefit of using independent external controls is that they are often produced with whole intact pathogens providing a quality control material that is similar to patient specimens. This is in contrast to external controls provided by assay manufacturers which generally contain only nucleic acids or synthetic nucleic acid constructs or plasmids. Although assay manufacturers often require the use of their controls for each testing run, independent quality control materials can provide an important supplement. With intact pathogens similar to patient specimen, the use of these controls can help to monitor the entire testing procedure including lysis and the nucleic acid extraction phase.

Certain manufacturers of control materials provide additional attributes with their control material products. Some examples include single use packaging to minimize chances of amplicon contamination, multi-analyte products that offer increased convenience regarding inventory management and assay specific ranges for quantitative tests such as viral load tests. Software packages are also available that automate documentation for compliance and may include functionality such as the acquisition of data from quality control testing, provide real time alerts and reports, such as Levy-Jennings plots, for quantitative data (i.e. viral load testing or Ct values from qPCR methods). QC data management software gives laboratories the ability to track their controls' performance within a peer group that is using the same or related assays and the same lot of control material. It is also worth noting that well defined control materials obtained from a third party can play a significant role not only in the day to day assessments of test performance, but also in the verification and validation process of tests which may require that "gold standard" specimens be used to evaluate certain traits of a test, such as precision, sensitivity or specificity. When these third party controls are multi-analyte, they provide the lab with the opportunity to improve workflow and inventory management

When third party controls are multi-analyte, they provide the lab with the opportunity to improve workflow and inventory management by reducing the number of pooled patient samples or individual control materials maintained for routine testing.

by reducing the number of pooled patient samples or individual control materials maintained for routine testing. In addition, many third party, independent control materials have extended shelf lives allowing their users to avoid unnecessarily frequent cross-over studies.

Internal controls provide a means for identifying inhibition in molecular tests. Many molecular tests require the use of complex enzymatic systems for the amplification of target, analyte-specific sequences. These enzymes are sensitive to a variety of inhibitors which block their ability to provide amplification. The list of potential culprits is long and includes common molecules such as detergents, certain salts and alcohols. For this reason, when a negative result (aka "not detected") occurs in the process of the performance of an amplification based molecular test, it cannot immediately be known whether the result is based on the absence of an analyte, or on the inhibition of the amplification system. Internal controls aid in discerning this dilemma. Such controls are most often sets of reagents that reside within the chemical components of the molecular test system and are an essential component of the reaction for each sample processed including each patient specimens, and indeed even each external control tested.

Internal controls include a set of nucleic acid targets and reagents that will react and provide amplification of a particular nucleic acid sequence regardless of the presence or absence of analyte being tested. This set of separate nucleic acids will often provide amplification in a separate "color" or "detection channel" of the analyte, and their positive amplification is required for acceptance of the test result. This is because internal controls should amplify, and they will amplify if the chemical testing system is intact and functional. Should an internal control fail to amplify it is indicative of the presence of an inhibiting factor and immediately provides the necessary intelligence to call the test "indeterminate" rather than "negative", which may have significant clinical consequences.

Calibrators

Many molecular tests, particularly those for the detection of infectious agents provide Boolean, "detected or not detected" results and are not devised to provide data regarding the quantity present. However, a certain class of test, more popular since the mid-1990s and the rise of HIV treatment includes those that test "loads" or quantitative assessments of the agent present. Such tests provide numerical results which are used to guide pharmacological treatments and to provide prognostic information to clinicians. Quantitative tests are also subject to regulatory requirements for control, however in addition to assessment by both positive and negative control; such assays also require the use of controls of varying concentration of analyte. Calibrators are used by testing devices for the process of associating their measured signals with known amounts of target agent (analyte). When the devices accurately associate signal with known values of analyte, they can accurately "call" or assess the quantitative value of a given specimen. It is common for assay manufacturers to require their calibrators for regulatory cleared quantitative tests, but it is also possible to obtain such quantitative panels from third parties. Third party products, also called independent materials, often include more detailed information on source of the material and whether any genetic variability may be present in such material. As indicated for external controls above, third parties are currently supplying control and calibrator materials with considerable characterization and with additional attributes that allow for more detailed and thorough analysis of tests, prospectively. For example, unlike most assay calibrators, third party products may contain intact, inactivated pathogens, in contrast to assay provided materials which are generally only nucleic acids or synthetic constructs. Thus, even if an assay requires specific calibrators, the use of third party products can offer several benefits.[4]

Individualized Quality Control Procedures (IQCP)

On January 1, 2016, federal law was modified to include the provision that laboratories may utilize "Individualized Quality Control Procedures". IQCP provides laboratories with greater latitude in establishing control policies and procedures (for non-waived test systems only). In devising their own quality control procedures, the laboratory may not implement any system that is less "stringent" than those provided by the manufacturer's package insert. Any modifications to manufacturer's guidelines or creation of one's own quality control procedures must be justified by provision of data or risk analysis that shows that the plan does not put patients at risk. IQCP is perhaps most appropriately applied to molecular tests that do not fit well with the previously established federal regulations. Cartridge based, moderately complex, sample-to-answer tests which are becoming more commonplace often include very expensive per test costs,

or have the ability to test limited numbers of specimens at one time. As such, the use of external controls may be practical on a less frequent schedule, but each laboratory must assess the risk to patient results if implemented.

Proficiency Testing

One of the reasons that I have always enjoyed working in a diagnostic laboratory is that unlike many other types of business operations—we are routinely evaluated objectively, by a third party. There is little ambiguity to whether we are performing our work correctly or not. This is largely due to the audit/inspection process, which occurs biannually, but also in large part it is due to proficiency testing. When people ask how the laboratory is doing I usually tell them that it is doing fine, and that if they don't believe me, they can go and read our Proficiency Testing Binder and see for themselves.

Proficiency testing is a highly effective means of assuring quality in the laboratory workplace. It is the process whereby a third party provides to the laboratory a "subscription" of specimens for a laboratory test. The disposition of these specimens is unknown to the laboratory, and they are distributed up to three times per year, typically in groups of five. Regulations require that such specimens are tested in exactly the same manner as patient specimens are tested (if possible put them into the same "run" with such specimens). Results are tabulated and provided back to the party that provided them. Result concordance with intended results is assessed and graded, and grades are provided to the testing laboratory and also sent to the accrediting agency of that laboratory (CLIA, CAP, etc.). Laboratories that have robust quality assurance and quality control procedures are well positioned for achieving good results in their proficiency testing programs.

Regulations indicate that all diagnostic laboratories must enroll in proficiency testing for all "regulated" analytes. The list of regulated analytes is indicated in the Code of Federal Regulations (CFR) sections 493.821 through 493.865. If a laboratory performs a test that is not included in the list of regulated analytes, it is responsible for establishing a system for evaluating a test system, internally. If there are no third parties that provide a proficiency test subscription for a particular analyte / test that is performed, it is the responsibility of the laboratory to establish some system of routine evaluation for that test. Other pertinent aspects of regulation:

- Proficiency testing specimens are to be tested in exactly the same manner as normal patient specimens
- Only to be performed once (unless your procedure for a certain test requires duplication)
- Testing specimens are never to be sent to another laboratory for testing, under any circumstances; even if your laboratory normally sends such specimens out for testing to a reference laboratory
- Results are to be provided by the indicated deadlines
- Proficiency testing must be performed by laboratory staff that normally performs the test in question

Successful performance in a PT event is a score of 80% or greater. However any performance on a PT event that is less than 100% requires investigation of why a perfect score was not achieved. Such investigation must be documented, and must include a detailed assessment of whether any patients have been put into jeopardy as a result of the test in question. Unsuccessful participation in two consecutive PT events or in two of three PT events for a particular analyte requires that the laboratory cease performance of the test and initiate a major investigation into the performance of the test. Moreover, the investigation must include an assessment of whether patients were possibly harmed by inadequate test performance, including notification of all clients who have utilized the test.

Molecular testing is not specifically described in the CFR nor is molecular testing indicated in any of the subsections describing regulated tests. In the case of molecular testing for infectious disease however, it is prudent to consider that "microbiology" and "virology" are regulated test categories. As such, performance of molecular tests for virologic and bacterial analytes would best be accompanied with proficiency testing as per the normal regulations: three events per year, a minimum of 5 specimens per event. For many exotic molecular tests, it is certainly possible that no third party proficiency testing agency can be located. In those cases, it is best practice to establish a program for evaluating such tests internally. This can in many cases be accomplished by a member of the laboratory (other than the relevant testing personnel for a particular test) who creates a panel of "unknowns" for the analyte in question, perhaps using previously tested specimens, or stored validation specimens. Such a panel can then be provided to the relevant testing personnel who can perform an internal proficiency test on the panel, treating imperfectly performed events (less than 100%) in the same manner as those performed for regulated PT.[5, 6]

Regulations indicate that all diagnostic laboratories must enroll in proficiency testing for all "regulated" analytes.

References

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[3]Standard: Calibration and calibration verification procedures, Title 42 Code of Federal Regulations, pt.493.1255 (2010).

[4]Standard: Control procedures, Title 42 Code of Federal Regulations, pt.493.1256 (2010).

[5]Condition: Enrollment and testing of samples, Title 42 Code of Federal Regulations, pt.493.801 (2010).

[6]Approval of proficiency testing programs, Title 42 Code of Federal Regulations, pt.493.901 (2010).

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