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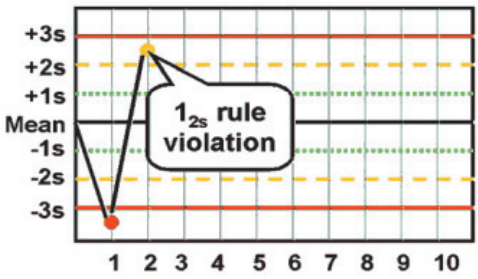
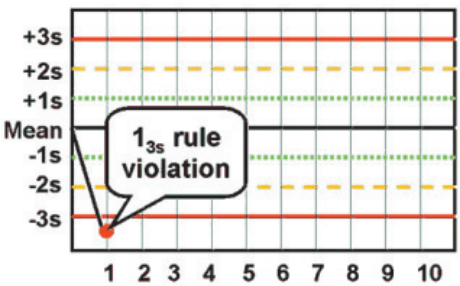
Quality control and standards in Clinical Laboratory





آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

LABRAD

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From the Editor's Desk

In the last decade, there has been a rapid expansion of healthcare services in Pakistan. Correspondingly, the consumer knowledge has also increased and they demand quality services in timely manner. Clinical laboratory is the center point of these services as over 70 per cent decisions are taken based on laboratory results.

The most important component throughout this whole process is quality assurance. With increasing awareness amongst patients along with increasing costs, a significant onus and urgency is placed on clinical laboratories to ensure that quality and error free work practices are maintained.

Over the years, the improvement in the quality of tests and methodology has been steady. Following the introduction of laboratory automation, quality assurance has been taken to a new level. Instruments have been interfaced with various laboratory automated systems and subsequently, information technology has drastically reduced human intervention during analytical procedure without compromising on the level of care.

Errors and challenges continue to occur but time has indicated that the path to improvement is not always a straight line; there are periods of success and periods of massive failures. Laboratory improvement has been a steadfast experience till now. One lesson to be learnt from this experience is that we cannot predict all errors; but through active quality process we can detect errors earlier and prevent them from happening.

This issue has been focused on articles pertaining to quality assurance in a clinical laboratory. The articles have been carefully selected so that our readers can build upon the culture of quality assurance and increase knowledge of the same by following the goal of continuous improvement.

Dr Natasha Ali
Haematology

An Overview of Proficiency Testing

Dr Hafsa Majid
Chemical Pathology

An important aspect of clinical laboratory quality management is 'Quality Assessment', and it can be conducted in several ways. Internal quality control compares laboratory performance to itself over time, assuming that the performance observed earlier represents correct or accurate test results. This assumption should be validated initially by method validation experiments and must be validated on a continuing basis by external quality assessment (EQA) or proficiency testing (PT) programs. It is also mandated by accreditation bodies that laboratories participate in PT programs for all types of analyses undertaken in that laboratory. In cases where PT programs are not available for a specific analytes alternate PT methods (split sample testing, sending specimen to reference laboratory for analysis) should be adopted for EQA.

The EQA or PT programs refer to the process of controlling the accuracy of an analytical method by interlaboratory comparisons. This comparison can be made to the performance of a peer group of laboratories or to the performance of a reference laboratory. The key requirements of such comparisons are that the samples are homogenous and stable, matrix is as similar as possible to patient specimen and the instrument, method, calculations and units used are appropriate and similar to comparing laboratories.

Benefits of PT Programmes

Proficiency testing is used to judge the quality of laboratory testing.The unique capability of an EQA program is to monitor the accuracy of methods/ instruments and make sure that the performance

of a laboratory's method is stable on the period between two surveys. Harmonization of results among laboratories and among methods is another important objective of EQA because clinical practice guidelines can be standardized if results are consistent among different laboratories. By participating in PT programs, a laboratory can maintain a level of competence comparable with other laboratories. Continued and significant deviation in PT should alert a clinical laboratory to a possible accuracy problem.

PT Providers

Multiple private organizations are providing PT surveys of different analytes. Common ones include College of American Pathologist, American Proficiency Institute, United Kingdom National External Quality Assessment Service, Centers for Disease Control and Prevention, BIO-Rad External Quality Assessment Services and National External Quality Assurance Program Pakistan. Two types of surveys are available; peer group comparison and accuracy based survey. In accuracy based survey actual value of PT sample measured by a reference method and individual laboratory's results are compared with true or target value of PT specimen. Proficiency testing provider is selected on basis of national and regulatory agencies requirement, previous surveys of that PT provider, surveys taken by other regional laboratories, cost of surveys, type of comparison and commutability of PT specimen. Frequency of PT testing is recommended by different regulatory authorities, at least twice a year and more frequent for routine analytes.

PT Report Interpretation

Peer group comparison is the common method used by PT providers, same PT specimen is sent to all laboratories participating in a survey and results are reported as mean, standard deviation (SD), standard deviation index (SDI), control limits for each analytes and graph of previous and current survey. A peer group is comprised of at least 10 or more laboratories performing same analyte on same instrument using same method and reporting in similar units. If no peer group is formed laboratory is evaluated against all methods mean. Limits for all analytes is different based regulatory authority's acceptability criteria and biological variation. A laboratory's results are acceptable if within limits. Standard deviation index (SDI) is another accuracy measure calculated by (Laboratory's mean – Peer group mean)/ Peer group SD and it should be <2. Acceptable PT results with high SDI should alert laboratory to system's bias. Last to look at in a PT survey is graph for bias. Ideally a graph should show PT results near and on both sides of mean. Graph showing results of previous and current surveys on one side of mean shows positive or negative bias in a system. For a survey to be acceptable at least 80% of PT results should be acceptable.

Proficiency testing results can be affected by preparation/ transport conditions of the PT specimens, matrix effects, clerical errors, selection of statistical methods of evaluation, and peer group definition. It is not appropriate to use PT as the only means for evaluating the quality of a laboratory as it will not detect all problems in the laboratory, particularly those that address the pre and post analytical processes. In conclusion PT programs can be an efficient tool in assessing a laboratory's analytical systems/methods. However complete utility of any PT program requires complete understanding of the PT report issued.

Sources of Pre-analytical Variations

Dr Khushbakht Arbab
Chemical Pathology

Laboratory testing can be divided into three phases that is pre-analytical phase which begins with the patient and ends with preparation of a sample for testing, analytical phase which includes steps involved in the actual performance of a laboratory test and post analytical phase which begins with the reporting of results to the health care provider and ends with actions being taken by the health care provider that are based on test results. A common assumption is that errors are most likely to occur in the analytical phase, the component of laboratory testing considered the most complex. Perhaps

as a consequence of the focus on technological improvements, it is actually the pre-analytical phase in which most errors occur. The healthcare providers, laboratory staff and the patients themselves should be aware of the common causes of pre-analytical errors. These errors can be further divided into pre-collection, blood collection and post collection causes (Table 1). To prevent pre-analytical errors, the procedures for collection, handling, and processing prior to analysis, as well as the physiologic patient variables that may directly affect the test result, must be clearly understood.

Specimen composition is influenced by any of the patient variables listed in Table 1. Some are controllable, some are not. The laboratory personnel must be aware of these influences and minimize the effects when possible. For example, in the basal state, a patient is at rest and fasting. Collection of a blood specimen from a patient in that state minimizes the effect of diet, exercise, and other controllable factors. A broad category of variables related to phlebotomy technique and procedures can introduce pre-analytic error. A few studies have demonstrated that pre-analytical errors are less common when dedicated laboratory personnel collect blood samples as opposed to nursing or other health care personnel. Unacceptable specimens due to misidentification, insufficient volume to perform the assay, incorrect whole blood to anticoagulant ratio, or specimen quality issues (specimens that are hemolyzed, clotted, contaminated, or collected in the wrong container) account for the majority of pre-analytic errors. Hemolysis, lipemia, and icterus have variable effects on assays. How a specimen is handled from patient to laboratory is another area of potential error and mostly outside control of the testing laboratory. Careful handling of the specimen during transport and processing is imperative in maintaining the quality of a meticulously collected specimen. The means of transport, exposure to heat and cold, vibration, position of specimen tubes and overall time to delivery can significantly affect test results.

The pre-analytical phase is complex and with so many people involved in the blood collection process, it is not surprising that errors can occur. However, good practices, teamwork, and compliance with the established procedures and instructions can lead to a substantial reduction in pre-analytical errors.

Table 1: Preanalytical Causes of Errors Divided into Pre-collection, Blood collection and Post collection Phases

Pre-collection Phase Variables	Blood Collection Phase Variables	Post Collection Phase Variables

• Wrong/ inappropriate test request
• Test request written illegible
• Patient contributions:
  ○ Diurnal consideration
  ○ Fasting requirement for certain tests
  ○ Exercise
  ○ Stress
  ○ Age
  ○ Medications
  ○ Smoking
  ○ Dehydration
• Wrong labeling/ wrong barcode
	• Posture
	• Time of collection
	• Faulty phlebotomy technique
	• Tourniquet time (not >1 minute)
	• Skin preparation
	• Hemolysis
	• Clots in sample
	• Under filling of tubes
	• Cross contamination between tubes
	• Skin contamination
	• Inadequate mixing
	• Quantity not sufficient for analysis
	• Wrong vacutainer tube
	• Wrong order of draw
	• Air introduced in the sample
	• Venous sampling when arterial blood is required or venous mixing
	• Presence of intravenous fluids on the arm
		• Exposure to sunlight
		• Exposure to inappropriate temperature
		• Delay in transport
		• Hemolysis
		• Breakage or leakage
		• Delay in processing
		• Evaporation
		• Faulty centrifugation
		• Poor specimen storage condition
		• Incorrect aliquots
		• Wrong labeling

Critical Values in Surgical Pathology

Dr Nausheen Azam and Dr Arsalan Ahmed
Histopathology

Occasional diagnoses in surgical pathology, analogous to critical values (CVs) in clinical pathology, could require urgent contact of the clinician to facilitate rapid intervention. However, there are no established guidelines as to what type of diagnosis in surgical pathology should qualify as a critical value (CV).

The concept of critical value (CV) was first introduced by Lundberg in 1972 as a "pathophysiologic derangement at such variance with normal as to be life threatening if therapy is not instituted immediately." Since the introduction of the CV concept, the practice of notifying clinicians of CVs has become the standard of practice in clinical pathology, with well established guidelines for which specific laboratory results require that the laboratory personnel immediately contact the clinician responsible for the patient. The Clinical Laboratory Improvement Amendments of 1988, states that "the laboratory must develop and follow written procedures for reporting life threatening laboratory results or panic values." The College of American Pathologists (CAP) checklist also includes a requirement asking if there is a policy/ procedure regarding the timely communication and documentation of "significant" or "unexpected" surgical pathology findings.

However there are no well defined guidelines to address the concept of CVs in surgical pathology. Therefore in the absence of established guidelines and since surgical pathology results are not measured in numbers, common sense and personal experience of the pathologist determines when to urgently contact the clinician.

Table 1: Examples of Critical Diagnoss in Anatomic Pathology

Cases that have immediate clinical consequences
Crescents in > 50% of glomeruli in a kidney biopsy
Leukocytoclastic vasculitis
Uterine contents without villi or trophoblast
Fat in an endometrial curettage
Mesothelial cells in a heart biopsy
Fat in colonic endoscopic ploypectomies
Transplant rejection
Maligancy in superior vena cava syndrome
Neoplasms causing paralysis
Unexpected or discrepant findings
Significant disagreement between frozen section and final diagnosis
Significant disagreement between immediate intepretation and final FNA diagnosis
Unexpected malignancy
Significant disagreement and/or change between primary pathologist and outside pathologist consultation (at either the orginal or consulting institution)
Infections
Bacteria or fungi in cerebrospinal fluid cytology in immunocompromised or immunocompetent patients
Pneumocystis, fungi, or viral cytopathic changes in bronchoalveolar lavage,bronchial washing or brushing cytology specimens in immuncompromised or immunocompetent patients
Fungi in FNA of immuncompromised patients
Bacteria in heart valve or bone marrow
Herpes in Papanicolaou tests of near-term pregant patients
Any invasive organism in surgical pathology specimens of immuncompromised patients

Abbreviation: FNA, Fine needle aspiration

The Association of Directors of Anatomic and Surgical Pathology (ADASP) has supported the concept of critical diagnoses (CVs) in surgical pathology, recognizing that critical diagnosis guidelines would be of great help to surgical pathologists and, ultimately, facilitate the clinicians. As a result, several retrospective reviews and multi-institutional surveys led to the creation of a list of possible critical diagnoses in surgical pathology and cytology (Table 1).

ADASP recognizes that a generic anatomic pathology critical diagnosis guideline such as the one mentioned above should be used only as a template and it should be left to the individual hospital to customize the list following consultation with their relevant clinical services. While establishing the CVs guidelines it is also important to avoid overuse and remove non-critical diagnosis type cases from the list.

In conclusion, it is important to establish a surgical pathology CV guideline, because there are some diagnoses in surgical pathology which are life threatening and require fast remedial action for improved patient outcome, and secondly the presence of these guidelines would represent a practice improvement and patient safety initiative.

References:
Lundberg GD. When to panic over an abnormal value. MLO Med Lab Obs 1972;4:47-54
Critical diagnoses (critical values) in anatomic pathology. Human Pathology (2006)37, 982-984

Five Q-Framework for Implementing Total Quality Management in Laboratory

Dr Noreen Sherazi
Chemical Pathology

There has been a continuous challenge in the health care system to provide better diagnosis, while maintaining standard quality credentials. Total Quality Management (TQM) include all divisions of the organization, namely, laboratory operations, information management, documents and record maintenance, materials and purchase, customer care, safety etc. Quality Management System requirements cover management's commitment to quality, its focus on customer, resource management, employee competence, process management, quality planning, design, purchasing, monitoring and measurement of its processes, calibration of measuring equipment, processes to resolve customer complaints, corrective/preventive actions and continuous quality improvement program.

The traditional framework for quality management in a healthcare laboratory emphasizes the establishment of following processes i.e. (Figure 1)
(1) Quality Laboratory Processes (QLPs)
(2) Quality Control (QC)
(3) Quality Assessment (QA)
(4) Quality Planning (QP) and
(5) Quality Improvement (QI)
QLPs include analytical processes and the general

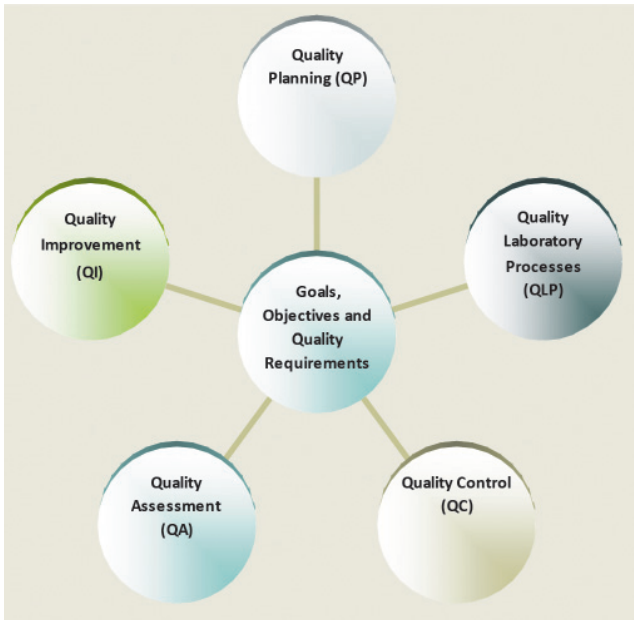

Figure 1: TQM Framework for Managing Quality in Healthcare Laboratory

policies, practices, and procedures that define how all aspects of the work are done. QC emphasizes statistical control procedures, but also includes non-statistical check procedures, such as linearity checks, reagent and standard checks, and temperature

monitors. QA, as currently applied, is concerned primarily with broader measures and monitors of laboratory performance, such as turnaround time, specimen identification, patient identification and test utility. Note that QA is the proper term for these activities, as opposed to quality assurance, which has been incorrectly used to describe these activities. Measuring performance does not by itself improve performance and often does not detect problems in time to prevent harmful effects. QA requires either that causes of problems be identified through Ql and eliminated through quality planning (QP) or that QC detect the problems early enough to prevent their consequences.

To provide a fully developed framework for quality management, the QI and QP components must be established. QI provides a structured problem-solving process to help identify the root cause of a problem and a remedy for that problem. QP is necessary to standardize the remedy, establish measures for performance monitoring, ensure that the performance achieved satisfies quality requirements and document the new QLP. The new process then is implemented through QLP, measured and monitored through QC and QA, improved through QI, and replanned through QP. The "five-Q framework" also defines how quality is managed objectively with the "scientific method," or the PDCA cycle (plan, do, check, act). QP provides the planning step, QLP establishes standard processes for the way things are done, QC and QA provide measures for checks on how well things are done, and QI provides a mechanism through which, to act on those measures.

Table 1: Essentials of TQM

Organization
Customer Focus
Facilities and Safety
Personnel
Purchasing and Inventory
Equipment
Process Management
Documents and Records
Information Management
Nonconforming Event Management
Assessments
Continual Improvement

Reference: CLSI guidelines QMS: A Model for Laboratory Services GP26-A4

Clinical and Laboratory Standards Institute (CLSI) document describes a quality management system (QMS) as a "set of key quality elements that must be in place for an organization's work operations to function in a manner to meet the organization's stated quality objectives." These Quality System Essentials (QMEs) are listed in Table 1. These depict the necessary infrastructure required by a laboratory to provide quality laboratory services which is implemented and monitored by five Q-framework to achieve the quality goals.

Delta Check in Clinical Laboratory

Hafsa Majid
Chemical Pathology

Decisions about diagnosis, prognosis and treatment are based on the results and interpretations of laboratory tests, and irreversible harm may be caused by erroneous test results. So early error identification have considerable implications for patient care and safety. Delta check is a very useful quality improvement measures that can help the laboratory identify possible patient-specific errors. It is a quality control method that compares the current test result with a previous result for the same test obtained over a short period of time (preferably within 96 hours) from the same patient and detects whether two values exceeds predetermined biological limits. A delta check failure or alert is caused by a discrepancy in patient results. It occurs when the difference between the patient's current laboratory result and previous result exceeds a pre-defined limit with a pre-defined length of time.

Delta check methods ensure the detection of pre-analytical errors (e.g. specimen mix-up errors, improper specimen acquisition, specimens altered by dilution with intravenous fluid, EDTA contamination, possible misidentification of a specimen and

transport error), clerical errors, biological variation (e.g. changes due to due to rhythmic, physiological or therapeutic changes) and random errors (e.g. sudden instrument or method related errors). If no error is identified in total testing process differences in results is due to a true change in patient's disease status. These errors cannot be detected using commonly used quality control methods; thereby delta check improves the reliability of clinical tests.

There are four delta check methods: delta difference, delta percent change, rate difference, and rate percent change. However, guidelines regarding decision criteria for selecting delta check methods have not yet been provided. Delta check methods statistically can be defined as:

- Delta Difference = Current Result – Previous Result
- Delta Percentage Change = $\frac{\text{Current- Previous Result}}{\text{Previous Result}} \times 100\%$
- Rate Difference = Delta Difference / Delta Time
- Rate Percentage Change= Delta Percentage Change/Delta Time

Studies have found that the majority of delta check failures (>75 per cent) can be attributed to true changes in the patient's medical condition and predictive value for detecting true specimen errors is between 0.4 and six per cent. Some analytes are more useful for delta checks than others. Ideally, analytes for delta checks should have little day-to-day variation, low reference change value and low index of individuality. Some common analytes on which delta check can be applied are listed in Table 1.

Table 1: Biochemical Analytes for Delta check Application

Appropriate analytes	Inappropriate analytes
Electrolyte (Na+, K+ & Cl-)	Glucose
Albumin	Phosphorus
Total Protein	Aspartate transaminase
Urea	Alanine transaminase
Creatinine	Creatinine Phosphokinase
Alkaline Phosphatase	Lactate Dehydrogenase

Modern automated analyzers now have the option of delta check flags in them. However to make this function practically possible delta check limits have to be derived for each analyte. In conclusion the delta check based quality control is very useful in identifying errors missed by laboratory quality control procedures and these programs should be performed in parallel with routine quality control practices.

Indicators of Quality Improvement in Surgical Pathology

Dr Muhammad Usman Tariq and Dr Arsalan Ahmed
Histopathology

A quality assurance program is meant to identify problems and recognize opportunities for improvement. The first step is the development of quality indicators which cover the most important aspects of patient care. Only few quality indicators have been identified by the surgical pathology laboratories worldwide. Few important variables of analytic and postanalytic phase are described in this article.

Diagnostic Accuracy (Intradepartmental Peer Review)

Quality in surgical pathology depends on the correctness of report which is assured by peer review which provides the opportunity to collect comprehensive information and straightforward identification of quality issues. Retrospective peer review often targets diagnostic biopsies with increased potential for adverse outcomes, recent cancer diagnoses, specific specimen types with known diagnostic difficulties and frequent mimics. The common examples include daily slide conference and reviews requested by the clinician in case of report ambiguity and lack of correlation with clinical findings. Other activities such as tumor boards, audits of cases sent to outside institution for routine review and audits focused on specific specimen types.

The responsibility remains with the primary sign-out pathologist who issues a revised/amended report if the diagnosis is after the review is changed.

Diagnostic Accuracy (Interinstitutional Peer Review):

External review can be initiated on the request of patient, clinician or pathologist but most importantly on institutional request because of patient's transfer. Studies have shown that these reviews revealed discordance in diagnosis including false –positive, false-negative, change in tumor type/grade, resection margin status and stage change. In addition, application of additional and/or repeat immunohistochemical stains results in significant change in diagnosis. In future, molecular diagnostics is further going to alter the diagnosis when reviewed in different institutions. For institutions treating new patients, the Association of Directors of Anatomic and Surgical Pathology (ADASP) recommends mandatory review of biopsy of cases for which major therapeutic intervention is planned.

Table 1: Monitors used to Assess Report Adequacy

	Data Element
Specimen of any type	Patient identification Pertinent clinical history Specimen site Statement of specimen adequacy (when appropriate) Adequate macroscopic description Clear diagnostic terminology
Primary cancer specimens	Tumor size Histologic type Histologic grade (if appropriate) Extent or depth of invasion (if appropriate) Number of lymph nodes examined Number of nodes with metastasis (if any) Status of surgical margins (if appropriate)
Image-guided biopsies	A comment on specimen adequacy A comment on whether the biopsy findings correlate with the imaging findings (eg. whether the findings explain the targeted abnormality) The presence of calcification in a biopsy performed for that reason

Correlation of Intraoperative and Final Diagnosis (Frozen Sections)

It is an integral component of quality improvement and it should be conducted on all the cases submitted for frozen section. It requires review of the slides prepared for frozen section and the slides prepared after formalin fixation. Reasons for discordant results (in order of decreasing frequency) include misinterpretation, specimen sampling, block sampling, technical inadequacy, inadequate clinical data and labeling errors.

Report Adequacy

Adequacy or completeness of the report is extremely important in cancer cases as the treatment relies on the complete information. College of American Pathologists and Royal College of Pathologists have devised comprehensive checklists and protocols for different cancers. Laboratories all over the world are adopting the synoptic reporting which presents all the essential information (data elements) required in an efficient manner. Moreover, monitoring of the reports for missing information has now become easier.

Turnaround Time (TAT)

TAT is as important as diagnostic accuracy and adequacy of a report and therefore, it is a critical component of patient care because of its impact on patient management. It is influenced by several factors related to the working of a hospital, anatomic pathology laboratory, with technical, clerical and human interpretive processes. Turnaround time data can help to identify the problems, their analysis and possible modification of the process in order to improve the overall service and eventually patient outcome.

Table 2. ADASP Benchmarks for Surgical Pathology Turnaround Times

Type of Specimen	Report Finalization
Frozen sections	20 minutes
Rush biopsies	2 days
Biopsies (small)	3 days
Surgical specimens	3 days
Additional Time for Special Procedures	
Overnight fixation	1 day
Decalcification	1 day
Regrosses	1-2 days
Recuts	1 day
Immunohistochemistry	1-2 days
Electron microscopy	2-3 days
Intradepartmental consultation	1 day

Reference: Quality Management in Anatomic Pathology. Chapter 6: Quality improvement plan components and monitors. 2005 College of American Pathologists (CAP)

Types of Analytical Errors

Shabnam Dildar
Chemical Pathology

Laboratory errors are normally classified into two categories; random errors (RE) and systematic errors (SE). Random errors are type of analytical error which arises from random fluctuations in measurement (Figure 1). Systematic error is defined as a component of error which, in the course of a number of analyses of the same analyte, remains constant or varies in a predictable way (Figure 2). Total analytical error =SE + RE. Statistically total error is defined as Bias + 1.65 x Imprecision. Under ideal circumstances, total analytical error equals to zero, but this cannot be achieved in daily practice.

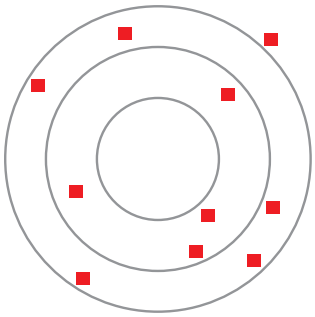

Figure 1: Representation of the values' dispersion in RE

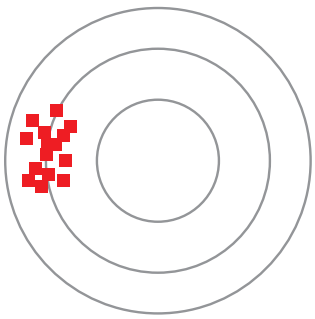

Figure 2: Representation of the values' dispersion in SE

Random Errors: cannot be absolutely identified (can occur due to differences in techniques between technologists, specimen characteristics, etc.) Some common causes of RE are:

- Instability of instrument
- Variation in the temperature
- Variation in reagents and calibrators (poor calibration curve stability)
- Variation in handling techniques such as pipetting, mixing, and timing
- Variability in operators

Random error affect the precision of all measurements, higher the precision of a measurement instrument, the smaller the variability (standard deviation) of the fluctuations in its readings. Large RE increases the dispersion of the results around a true value.

Systematic Error: Systematic errors can be attributed to certain reasons and therefore can be eliminated much easier than RE. They cannot be diminished by increasing the number of measurements. Some common causes of SE are:

- Incorrectly made standards, controls or reagents
- Instrumentation defects
- Variation in pipettes and volumetric glassware
- Variation in cuvettes
- Electronic and optical variation in instruments

Systematic errors are subdivided into the following two types:

Constant systematic error: When the error is consistently low or high by the same amount, regardless of concentration it is called constant systematic error. Factors that contribute to constant systematic error are independent of analyte concentration.

Proportional systematic error: When the error is consistently low or high by an amount proportional to the concentration of analyte is called proportional systematic error.

Table 1 presents an organization of experiments to be performed for specific error determination arranged in such a way that easy experiments can be done first and final performed only if errors estimated by these preliminary experiments are acceptable.

Table 1: Experiments for Estimating Specific Types of Analytical Errors.

Type of Analytic Error	Evaluation Experiments	
	Preliminary Study	Final Study
RE	Replication With In Run (using pure material, patient specimens)	Replication run to run (using patient specimens)
Constant Error	Interference	Comparison with comparative method
Proportional Error	Recovery	Comparison with comparative method

Validation of Modern Flow Cytometry Instrument

Dr Muhammad Shariq Shaikh, Dr. Arsalan Ahmed and Sarwat Kashif
Haematology and Histopathology

Flow cytometry is a dynamic technology which allows the multi-parameter evaluation of heterogeneous cell populations. Over the years, clinical flow cytometry has evolved as a distinct haematopathology diagnostic facility. Considering the requirement put forth by regulatory agencies and the complexity of multi-parameter analysis by flow cytometry, standardization and validation of flow cytometry instrument, reagents and procedures are essential to ensure interpretation of meaningful technical results. In this article, validation of flow cytometry instrument will be the primary focus. Validation of flow cytometry instrument includes instrument setup, daily calibration of both light scatter and fluorescence measurements and cross-instrument performance using relevant clinical specimens.

Instrument Setup

Manufacturer recommended procedures must strictly be followed to assure proper optical alignment for adequate light scattering, fluorescence sensitivity and resolution. Additionally when multiple fluorochromes are used, fluorescence compensation is essential to correct for spectral overlap.

Calibration of Light Scatter and Fluorescence Measurements

Optical alignment for optimal sensitivity and resolution of both forward (FSc) and side (SSc) scatter can be assessed by running uniformly sized beads that fall within the light scatter ranges observed with most clinical samples on a daily basis. The mean FSc and SSc channel numbers and percent coefficient of variation (CV) should be recorded. The acceptable ranges for each parameter can be established by running the beads 20 times over a five day period at the same photo multiplier tubes (PMT) setting. Levy–Jennings graphs are then used for validation by plotting the values obtained daily and an action plan is established for what to do when any parameter falls outside of the expected range. Owing to the difference in behavior of cells and beads, setting up of instrument using biological material (for example lymphocytes) is also recommended before running clinical samples. Similarly for establishing values for monitoring fluorescence sensitivity and resolution, recording of either the channel number and C.V of calibration beads or alternatively, the high voltage and gain to position the beads in the same channel each time.

Utilization of multiple fluorochromes is associated with spill-over of one fluorescence signal into another. For this, fluorescence compensation using a combination of biological control and hard dyed beads provided by manufacturer is preferable. For fluorescence linearity, a mixture of 4-5 known multi-level fluorescence beads should be run 20 times over a five days period to establish mean fluorescence intensity (MFI) ranges. Monthly fluorescence linearity should be stable unless the laser or PMT is unstable.

Cross-Instrument Performance

It should be done by laboratories performing the same clinical immunophenotyping protocols on more than one instrument. Five different representative clinical samples must be tested every six months. Corrective actions must be taken when cross-comparison results fail to meet performance specifications.

In conclusion, validation of instruments, reagents and procedures is the responsibility of diagnostic facility to ensure good laboratory practice and is integral to provide good patient care.
At Aga Khan University clinical laboratories, immunophenotyping by flow cytometry is offered for the diagnosis of leukaemia, lymphoma, minimal residual disease, ZAP-70 expression, lymphocyte subsets and CD34 count.

Reference:
Owens MA, Vall HG, Hurley AA, Wormsley SB. Validation and quality control of immunophenotyping in clinical flow cytometry. J Immunol Methods. 2000 21;243(1-2):33-50.

Method Validation for Quantitative Tests: CAP Recommendations

Dr Noreen Sherazi
Chemical Pathology

According to Food and Drug Administration, validation is ‘‘Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.’’ College of American Pathologist (CAP) regulations require that performance for any new method be ‘‘verified’’ prior to reporting patient’s test results. Precision and accuracy are specifically identified, along with analytical sensitivity, analytical specificity, reportable range, reference values, and any other applicable characteristic. The responsibility for method verification or validation resides with each laboratory and is accountable to see that adequate data has been collected and that this data shows that the new methods provide acceptable performance in the laboratory. Following protocol should be followed in validating a new test and it should be documented.

Verification of Precision

Precision implies repeatability, which means, analyze repeatedly to determine variation. To verify precision, samples should be processed twice a day in quadruplicate for five days generating 20 replicates. This is called inter-assay variation. For intra-assay variation, one sample was run 20 times. Imprecision is quantified by calculating the mean, standard deviation (SD), and coefficient of variation (CV %) of data collected from an analytical run: CV = SD/Mean x 100.Precision can be specified as: (i) repeatability (within run), (ii) intermediate precision (long term) and reproducibility (interlaboratory). If the precision is less than the total allowable error for that analyte then method is precise.

Verification of Accuracy

Agreement between test result and ‘‘true’’ result is done in mainly two ways:

(i) Comparison of results between new method and ‘‘reference’’ method (Method comparison)
(ii) Results using new method on certified reference materials or controls (Recovery)

The first approach is most commonly used. For this run 20 samples within testing range (CLSI document EP15-A2) by both new and comparative methods, and check whether the average bias between the two methods is within allowable limits or not. Recovery is done when there is no comparative method available and it should also be within total allowable error for the accuracy to be verified.

Verification of Reportable Range (AMR & CRR)

Reportable range is the span of test result values over which the lab can establish or verify the accuracy of the measurement response.

Analytical Measurement Range (AMR)

Range of analyte values that a method can directly measure on the specimen, without any dilution, or other pretreatment, not part of the usual assay process. AMR must be verified before a method is introduced, and checked at least every six months (and after recalibration or major maintenance) while in use. AMR verification must include three levels—low, midpoint, high. One can use commercial linearity materials, proficiency testing (PT) samples, controls or patient samples with known results, standards or calibrators. It can also be done by calibration verification, if three samples that span the measurement range are used. In absence of commercial materials, one will need to create one’s own materials. High and low samples can be mixed to create a mid-point sample. If it is found to be higher than measurement limit, one can dilute with low level sample to create a level near limit.

Clinically Reportable Range (CRR)

It is the range of analyte values that are reported as a quantitative result, allowing for specimen dilution or other pretreatment used to extend the actual AMR. CRR is a clinical decision by the laboratory director/ section heads, and does not require experiments or re-validation; however, dilution or concentration protocols must be specified in methods.

Verification or Establishment of Reference Intervals

It is not required for a laboratory to establish its own reference limits, but satisfy that limits it uses are appropriate for the patients. According to CLSI document C 28-A2, it is very useful to be able to transfer a reference interval from one laboratory to another by some process of validation which is less costly and more convenient. One can adopt reference limits from any of the following sources: manufacturer suggested, reference laboratory, published articles, neighboring laboratory or previous reference limits in the same laboratory. For verifying reference intervals, we should select 20 representative healthy individuals, and the test will considered validated if, ≤ 2 of them is outside the manufacturer's proposed limit. If >2 outside, can repeat with another 20, and accept if ≤ 2 is outside.

Barcoding for Reducing Pre-analytical Errors in a Clinical Laboratory

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Barcode is a system of using varying width bars as a way to provide identification information. It is an optical machine-readable representation of data, which shows certain data on certain products. in laboratory. Its purpose is to ensure sample identification and accession. Bar codes consist of a series of small parallel lines of varying width that are used to represent numbers or letters and numbers and are readable by automated equipment.

There are many types of barcodes the most common ones being one dimensional and two dimensional. In single dimensional barcode the vertical lines and their spacing constitute the code but whole length of the vertical lines are not essential for the codes. It is said that the code is repeated in vertical directions. So a symbol with printing defects, such as spots or voids, can still be read. On the other hand, two dimensional barcode stores information along the height as well as the length of symbol. As a result of that construction, these barcodes have a greater storage than is possible with the one dimensional barcode. A two dimensional barcode is not comprised of bars or lines, but rather of black-and-white cells arranged in a matrix pattern (often laid out in a square). This square design is often easier to scan than lineal barcodes because it fits better on curved surfaces such as test tubes or patient wristbands.

Bar-coding the specimen label greatly minimizes clerical errors and decreases errors in patient specimen handling as well as increase productivity. For clinical laboratories, timely and accurate specimen labeling is expected to ensure correct patient identification from collection to results reporting. Electronic identification such as two dimensional barcodes can certainly include two or more person-specific identifiers to comply with this requirement. Barcodes clearly provide patient tracking and sample management. They speed up record retrieval and are more secure for patients in terms of anonymity, accuracy, and elimination of human error. With barcodes, transcription errors and hard-to-read labels can be removed from the workplace. Besides laboratory specimens barcodes can be used on reagents/ kits, quality controls, calibrators and blood products in a clinical laboratory. With a high quality barcode label, a working scanner, and a trained operator, data collection can happen at lightening speeds with 100 per cent data accuracy.

Levey Jenning Chart and a Guide to Use Westgard Rules

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Levey Jenning chart (LJ chart) is a graph on which the quality control data is plotted on. It is named after S. Levey and E. R. Jennings who in 1950 suggested the use of Shewhart's individuals control chart in the clinical laboratory. It is a visual indication whether a laboratory test is working well or not. The data obtained from the daily analysis of quality control pools can be plotted to create a visual analysis. The expected analyte concentration, the established target value (mean), and the desired number of standard deviations are drawn on the y-axis, and the days of the month are indicated on x-axis. Lines run across the graph at the mean, as well as one, two and sometimes three standard deviations either side of the mean. This makes it easy to see how far off the result was.

Rules, such as the Westgard rules can be applied to see whether the results from the samples when the control was done can be released, or if they need to be rerun. In 1981, Dr. James Westgard and his associates developed a multi-rule procedure for interpreting control data. Since then, a number of sophisticated quality control schemes or analogues based on this multi-rule logic have evolved. Westgard rules are quality control rules to help analyze whether or not an analytical run is in-control or out-of-control. Any values violating Westgard rules will be either rerun or rejected depending on the rule violated. It uses a combination of decision criteria, usually five different control rules to judge the acceptability of an analytical run. Westgard rules specify the LJ chart. It makes use of a series of control rules for interpreting control data and also reduces the false rejection and improves the error detection. The formulation of Westgard rules were based on statistical methods. They are used to define specific performance limits for a particular assay and can be used to detect both random and systematic errors. For convenience, a short hand notation to abbreviate different decision criteria or control rules, e.g., 12s to indicate one control measurement exceeding 2s control limits is used.

Explanation of Individual Rules and Troubleshooting

$\mathbf{1}_{2s}$: One control measurement exceeding two standard deviations (SD) of control limits either above or below the mean. This rule is used a warning rule to trigger careful inspection of the control data. (Figure 1) False alarms are minimized by using the 12s rule as a warning rule, then confirming any problems by application of more specific rules that have a low probability of false rejection.

$\mathbf{1}_{3s}$: This rule is commonly used with a LJ chart when the control limits are set as the mean +3 SD of control limits. A run is rejected when a single control measurement exceeds the mean ± 3 SD. This is because either a random error or a very large systematic error has occurred, as less than one per cent of all test values exceed ± 3SD. Troubleshooting must be performed before further testing can be done.

$\mathbf{2}_{2s}$: Westgard rule 22s states that if two consecutive control measurements across runs exceed the same mean -2 SD or exceed the same mean +2SD, or, within a run, if two consecutive control values are outside the same two SD, the run must be rejected. If this circumstance occurs, a systematic error is likely. Troubleshooting must be performed before testing can continue. Had only one of the controls been greater than +2SD, the run would have been accepted as "in control," but would have been rejected on the next QC run if the same control was again out +2SD.

$\mathbf{R}_{4s}$: This rule rejects a run if two control measurements in a group exceed the mean with a four SD difference between the two consecutive controls.

4_{1s} : This rule rejects a run with the forth consecutive control measurement exceeding one SD on the same side of the mean. 41s is when four consecutive control measurements exceed the same mean plus one SD or the same mean minus one SD control limit.

$\mathbf{10}_{x}$: This rule rejects a control run when there are 10 consecutive controls on the same side of the mean.

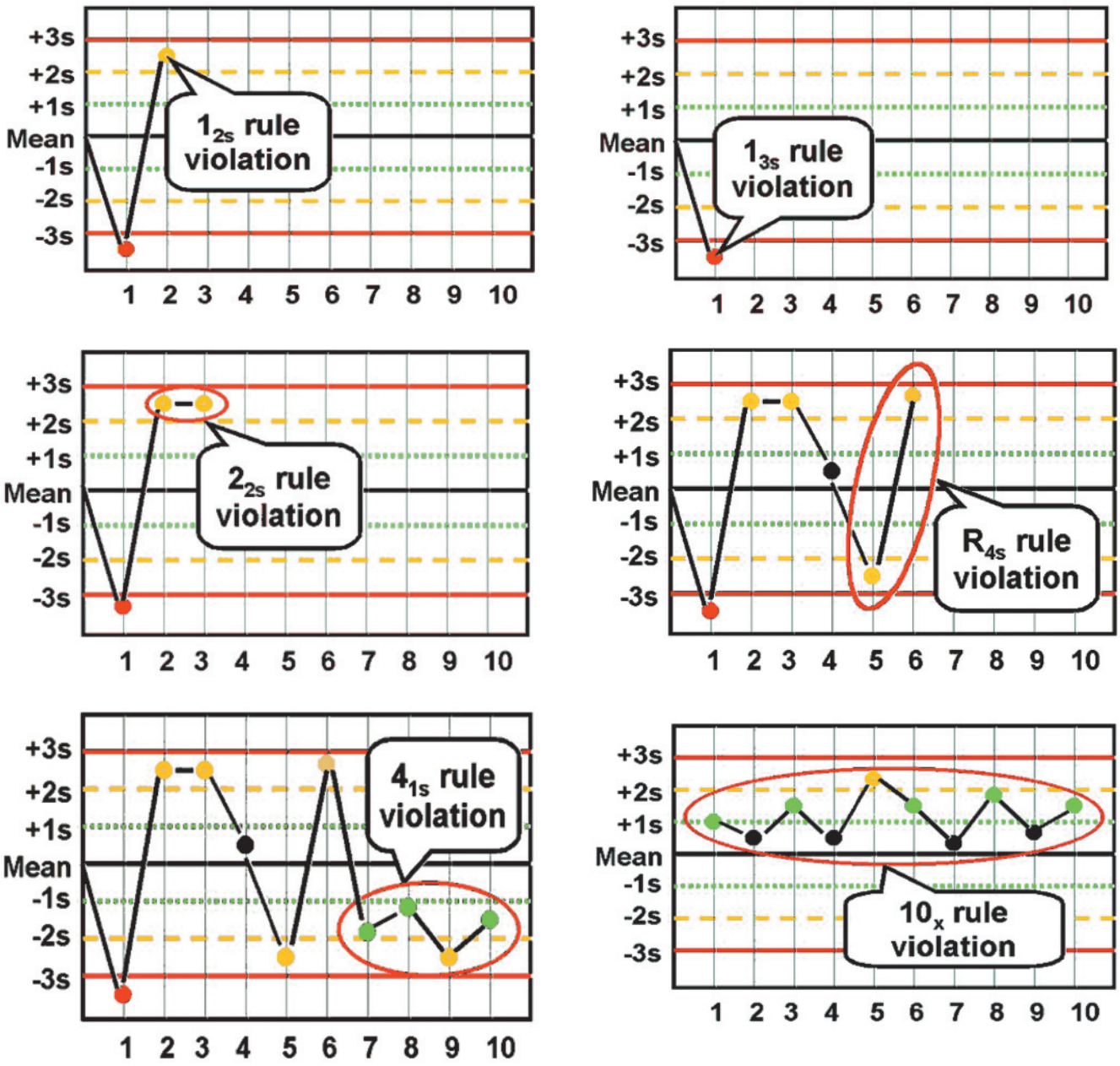

Figure 1: Graphs showing interpretation of Westgard rules on LJ charts (courtesy westgard.com)

True alarms or error detection are maximized by selecting a combination of the Westgard rules most sensitive to detection of random and systematic errors and then rejecting a run if any one of these rules is violated (parallel testing). The key in how to apply control rules with multiple materials and multiple runs is to identify which quality control results represent consecutive measurements. For example if one measurement is made on each of two different control materials in an analytical run, control rules can be applied as follows: the two control results 'within a run' can be inspected by applying a 13s rule to each material, as well as the 22s and R4s rules 'across materials'. Likewise the 10x rule can be applied to both control measurement in a run for the last five runs, or to the measurements on just one material for the last ten runs. Westgard rules are programmed in to automated analyzers to determine when an analytical run should be rejected. These rules need to be applied carefully so that true errors are detected while false rejections are minimized. The rules applied to high volume chemistry and hematology instruments should produce low false rejection rates.

Quality Control of VITEK-2 System

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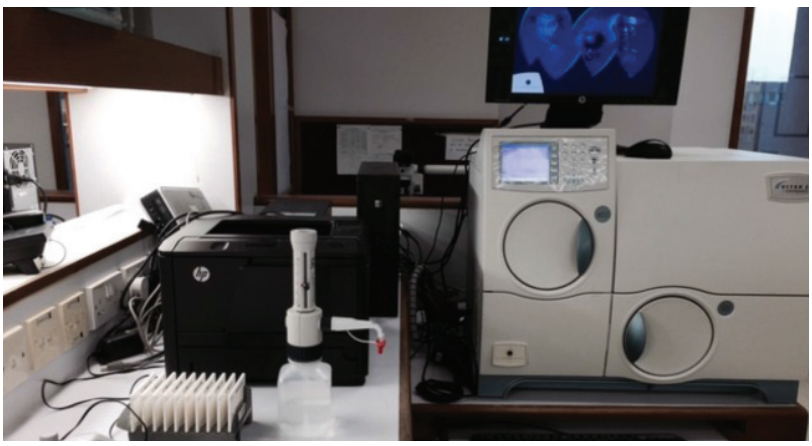
VITEK-2 system is an automated system for antimicrobial susceptibility testing. As with any automated system, it is very important to maintain quality control (QC) as microbial contaminants can enter a bio-manufacturing product system and impact the product outcome. QC is also performed to check that the instrument is functioning properly and all the values are within the reference ranges.

Principle

VITEK-2 system use advanced colorimetry, an identification technology that enables identification of routine clinical isolates. Advanced colorimetry provides a low rate of misidentified species and susceptibility testing can be performed accurately in a shorter period of time. The VITEK-2 Antimicrobial Susceptibility Test (AST) card is an automated version of determining the minimum inhibitory concentration (MIC). Each AST card contains 64 wells including a control well and wells containing premeasured portions of a specific antibiotic tested against a standardized suspension of bacterial or yeast isolates for 7-12 hours.

At the completion of the incubation cycle, a report is generated that contains the MIC value along with the interpretive category result for each antibiotic contained on the card.

The rapid results allow clinicians to discontinue empiric therapy and prescribe targeted therapy, resulting in improved patient outcomes and enhanced antibiotic stewardship.



VITEK-2 System

Quality Control

The consistency of results as analysed by the Vitek expert system indicates that the isolate fits an expected pattern for a defined phenotype. Results can be rapidly and confidently released with little or no intervention by laboratory personnel. Daily protocols are followed to control environmental factors and to maintain sterility for assuring quality through day to day operations. Important steps taken to maintain quality control of the VITEK-2 System include:

- QC of reference strains being done and the values are compared with standards. The results must be within recommended ranges as per guidelines for susceptibility results to be valid.
- VITEK-2 dispenser is cleaned with distilled water to remove any deposits and autoclaved weekly to maintain sterility.
- The tips used are also autoclaved before preparing suspensions
- The tubes used for making suspensions are sterilized by ethylene oxide
- Sterility check is performed daily to rule out the contamination of the dispenser and Vitek saline to ensure that the saline and dispenser are not contaminated with any foreign particle/bacteria
- For each isolate to be tested, purity plate is setup to ensure that the isolate is pure at the completion of the process. If the purity plates are not pure then the results are not valid and should be repeated.

Annual service conducted by manufacturer and on monthly basis PPM (planned preventive maintenance) by the vendor.

Proficiency Testing as a Quality Improvement Tool in Clinical Microbiology Laboratories

Dr Kauser Jabeen
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Infectious diseases are a great threat globally to human health. The morbidity and mortality related to infections is aggravated further in settings with limited resources, lack of government commitment and poor infrastructure. Clinical microbiology laboratories are pivotal for the diagnosis of infection and detection of antimicrobial resistance. It is therefore extremely critical that laboratories should ensure generation of accurate and precise results. In order to produce quality assured results it is essential that laboratories should have quality management systems in place. Proficiency testing (PT) is an external quality assessment tool that is used to assess laboratory performance and quality of results. It is recommended that clinical laboratories should participate in the PT programs. Alternate assessment of a split sample approach should be followed for a test for which an approved PT program is not available. The laboratory could also perform blinded testing of simulated specimens or use photographs or photomicrographs for this purpose.

The components and requirement of PT include enrollment in approved PT program, regular participation, meet criteria set by the PT program. It is essentially important that the PT samples are processed using similar protocols and methodology as a regular patient specimen is tested. Repeat testing should be avoided and all technologists involved in reporting should be provided with an equal opportunity to process and report PT sample. The PT result should be reported within the recommended time frame. After receiving the result the results should be compared with inter-laboratory comparison. In case of an incorrect result, the process should be reviewed to identify the cause of error. Corrective actions should be taken and ongoing monitoring continued.

Participation in a PT program could also be helpful as it could be used as a bench mark quality indicator of a laboratory. It could also be used as a marketing tool to instill confidence in the customer that this laboratory produces quality results. It could be used a toll to assess staff's competency and as an education tool for teaching and training of staff and junior doctors.

In conclusion regular participation in PT testing program is essential for a clinical laboratory as it is a valuable quality improvement tool. In Pakistan this participation is not a regulatory requirement, however many quality assured laboratories, such as the Aga Khan University laboratory regularly participate in the College of American Pathologists PT program.

Reference:
Stang LH, Anderson NL. Use of proficiency testing as a tool to improve quality in microbiology laboratories. Clin Microbiol Newletter 2013; 35: 145-52.

Establishing Quality Control Values for Haematology Parameters

Dr Muhammad Shariq Shaikh
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Quality control (QC) in the medical laboratory is a statistical process used to monitor and evaluate the analytical process that produces patient results. A quality control product is a patient-like material ideally made from human body fluids and contains one or more analyte in known concentration. In order to assess the validity of results on patient specimens, use of QC material is a requirement put forth by regulatory agencies. It is essential for laboratories to fulfill or surpass the regulatory standards set by regulatory agencies.

For most haematology analyzers, the target QC value and its limits for each analyte are provided by the vendor. These values are calculated by repeated testing of QC material and are provided in an assay sheet with each new lot of controls. However, owing to variation in different laboratories, instrument calibration, maintenance, reagents and operator technique, these values may not be the exact target value in a given laboratory.

Both The Joint Commission on Accreditation of Health Care Facilities (JCAHO) and College of American Pathologists (CAP) recommend calculation of own target values by each laboratory. This approach may not be a cost-effective option for the laboratories catering small number of patient samples. Utilization of manufacturer provided QC values however, is permitted as a guide for establishing own QC values and for very low volume tests where the range is narrow enough to detect clinically significant errors.

Calculation of own target values is a rather easy task. The new control should be analyzed a minimum of 20 times across three to five days. The average of these 20 values should be within the range stated on manufacturer provided assay sheet. A two standard deviation range should be calculated from this new target value for setting up the upper and lower limits. These new values should then be incorporated into the system and utilized throughout the dating of the product.

Mean corpuscular volume (MCV) is a haematology parameter that requires special consideration. It has tendency to rise by two units over the life of the control material. To accommodate this, it is acceptable to raise the new calculated target value by half this change (by one unit). This will result in values starting below the mean, rise through the mean and finish above the mean.

One drawback of establishing own QC values is an increased utilization of expensive commercial controls. However, in view of recommendations of regulatory bodies, each laboratory should establish its own quality control values to ensure reliability of test results thus avoiding medically significant errors.

References:
The Joint Commission - www.jointcommission.org
College of American Pathologists - www.cap.org

hospitals.aku.edu/Karachi/clinical-laboratories