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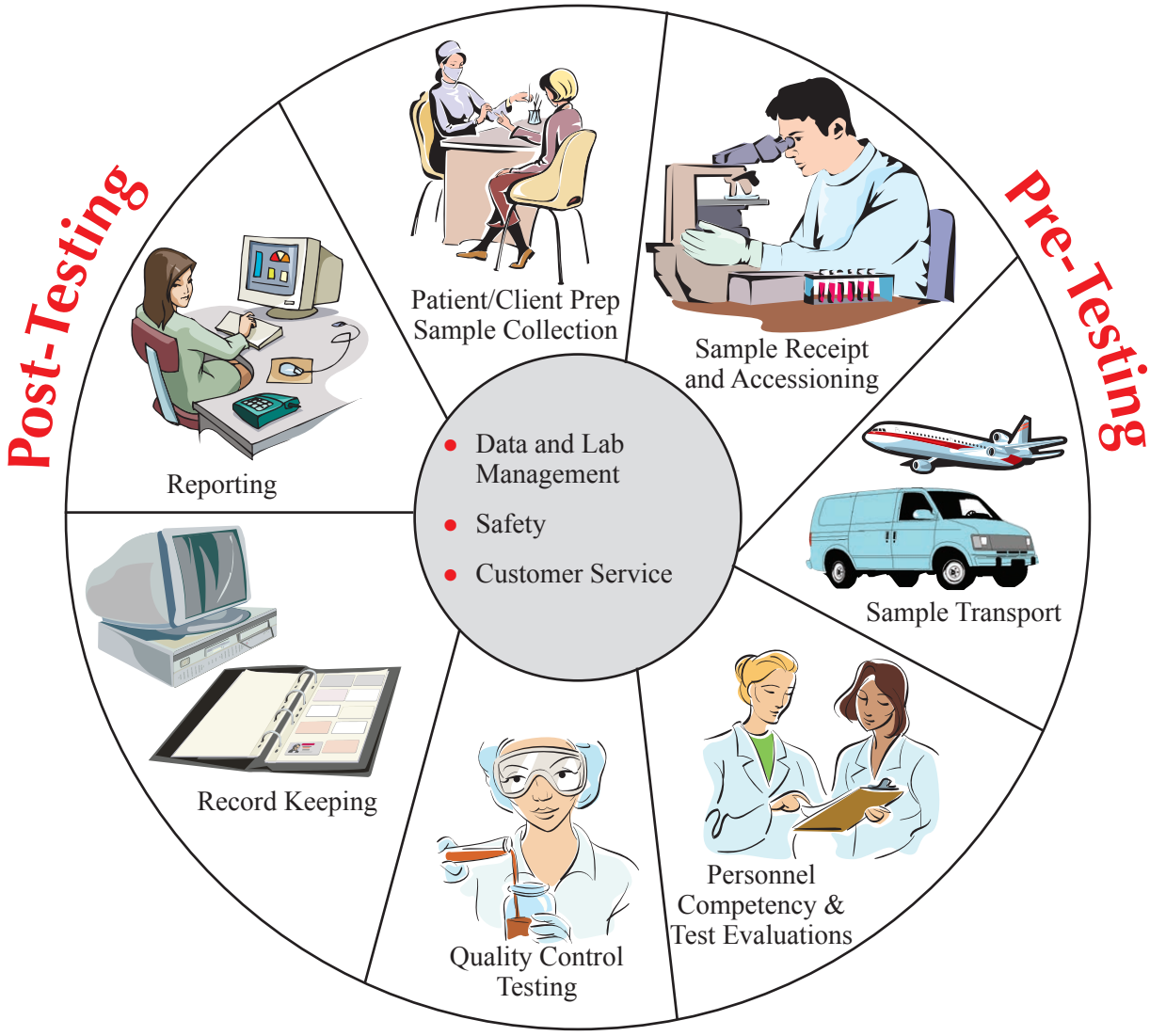
Quality Assurance in Laboratory



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Levey Jennings Chart and Guide to use Westgard Rules 3

Quality Assurance in Frozen Section Studies 4

Performance Improvement Monitors in Histology 5

Quality Assurance in Immunohistochemistry: The Way We Do It 7

Quality Assurance in Laboratory Biosafety 9

Quality Control of Culture Media Used in Clinical Microbiology 11

Quality Assurance (QA) in Qualitative Tests in the Clinical Laboratory 13

Radiology Pathology Correlation 16

Quality Requirements for Reporting Sickle Haemoglobin 19

Meeting Report of International Federation of Clinical Chemistry (IFCC) Conferences- 2017 23

From the Editor's Desk

This is the last issue of 2017 and we enter the New Year with a very well suited thematic issue on "Quality Assurance in Laboratory". Accredited laboratories follow a highly stringent compliance criterion of regulatory laboratory standards, quality assurance systems, validation protocol for diagnostic tests along with support of highly effective laboratory information system to guarantee quality of standards and reliability of analytical data.

Successful quality assurance programmes provide consistent test results with minimal chances of error and being a College of American Pathologists (CAP) certified laboratory we are constantly striving to provide this to our end users. With this background, the current issue contains articles on quality assurance being practiced in all sections of the laboratory. The topics include: guide to use Levy Jennings chart, quality assurance in frozen sections, quality assurance in laboratory biosafety and quality requirements for reporting sickling haemoglobin to name a few. We also have the pathology and radiology correlation article which has become a favorite by the readers.

We hope our readers enjoy the material as much as we enjoyed putting it together…

Happy New Year 2018!

Levey Jennings Chart and Guide to use Westgard Rules

Dr Syed Bilal Hashmi
Clinical Chemistry

Quality control (QC) results are used to validate whether the instrument is functioning within pre-defined specifications, deducing that patient test results are correct and reliable. Once the test system is validated, patient results can then be used for diagnosis, prognosis, or treatment planning. The data obtained from the daily analysis of QC pools can be plotted to create a visual analysis also known as the Levey-Jennings or LJ chart. 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 drawn 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.

In 1981, Dr. James Westgard and his associates developed a multi-rule procedure for interpreting control data. In short the Westgard rules specify the LJ chart. These rules are QC 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. There are six basic rules in the Westgard scheme. These rules are used individually or in combination to evaluate the quality of analytical runs. 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 also used to define specific performance limits for a particular assay and can be used to detect both random and systematic errors.

Rule	Interpretation	Type of Error
12s	One control measurement exceeds ± 2SD limits	Warning
13s	One control measurement exceeds ± 3SD limits	Random
22s	Two consecutive control measurements exceed ± 2SD limit	Systematic
R4s	If there is at least a 4SD difference between control values within a single run	Random
41s	4th consecutive control measurement exceeding 1SD on the same side of the mean	Systematic
10x	When there are 10 consecutive controls on the same side of mean	Systematic

Quality Assurance in Frozen Section Studies

Dr Arsalan Ahmed and Summayia Sohail
Histopathology

Correlation of Intraoperative and Final Diagnosis

Monitoring the correlation of frozen section diagnosis and permanent section diagnosis is an integral component of a quality assurance/quality improvement program in histopathology. It provides a very important measure of performance with respect to frozen section diagnostic accuracy. It is recommended that permanent section slides should be analyzed with the accompanying frozen section slides to establish if any discrepancy exists (CAP checklist item ANP.10075). Each frozen section disagreement (major) should be treated as an event that requires investigation and action, and discrepancies should be reconciled in the final pathology report (CAP checklist item ANP.10100). Local protocols should outline the process for treatment of a major discordance.

The Association of Directors of Anatomic and Surgical Pathology (ADASP) have recommended the following correlation categories.

- Concordance
- Deferral-Appropriate
- Deferral-Inappropriate
- Disagreement-Minor
- Disagreement-Major

Correlation Results

- Concordance – represents cases where frozen section and permanent section diagnosis are in agreement
- Deferral rate - The number of cases where frozen section diagnosis was deferred until final diagnosis was reached on permanent section
- Minor Disagreement/Discordance – represents a small change in diagnosis but there is minimal, if any, clinical relevance
- Major Disagreement/Discordance – represents a significant difference between the original frozen section diagnosis and the one rendered upon final diagnosis where potentially there is a serious impact on the patient's treatment or outcome.

Reasons for discordance and their reported relative frequencies include:

- Misinterpretation 25% to 45%
- Specimen sampling 30% to 45%
- Block Sampling 30% to 38%
- Technical inadequacy 30% to 38%
- Inadequate clinical data 6% to 8%
- Labeling errors 0.5% to 3%

A number of variables must be considered when collecting and interpretating quality assurance data on frozen section performance as follows:

- Differences in case mix potentially can account for difference in diagnostic accuracy of frozen section. It is recognized that certain frozen section activities (e.g. Sentinel lymph node, assessment of surgical margins) have a high discordance rate.

- Performance data will depend upon whether case discordance (# discordance/ # frozen section cases.) is calculated, or whether the number of blocks (# discordance/ # of all individual frozen sections) is utilized in the denominator.

- Calculations of discordance can be based on broad comparison of benign versus malignant or more specific diagnostic terminology.

ADASP recommends an acceptable accuracy threshold of three percent for intraoperative consultation. In every month the data is recorded as a quality indicator and the frequency of discordance compare with its bench mark and presented in sectional meeting and DQMC (Departmental Quality and Mangagement Committee).

Turnaround Time

Frozen section TAT has a critical impact on operative management. The turnaround time target is 20

minutes or less for single frozen section specimen, measured from the time that the pathologist receives a frozen section specimen to the time that a diagnosis is reported to the surgeon .More than 20 minutes is acceptable in cases where more than one block is processed or more than one specimen is received for the same patient. Frozen section record is kept in the quality indicator. The laboratory should be able to meet the 20 minute time limit for at least 90 percent of cases.

Monthly Evaluation of Frozen TAT

The monthly evaluation of frozen TAT is monitored and recorded. The list is presented and discussed in the departmental sectional meeting.This data is also shown in DQMC meeting.

Performance Improvement Monitors in Histology

Dr Arsalan Ahmed and Summayia Sohail
Histopathology

Turnaround Time

Turnaround time is the basic performance improvement monitor throughout anatomic pathology. The basic approach that can be used to track TAT in histology, is to use specific time "stamp" through the laboratory information system. This approach requires an entry at the time, tissue blocks are submitted or a special request is made, and an entry when the slides are released from the laboratory. With this approach, the actual TAT in hours can be specifically determined and trended.

Quality of Histologic Sections

The quality of histologic sections would be assessed by the pathologist receiving histologic preparations. The supervisor and technologists must work together to ensure that pathologist input is meaningful and consistent. A quality assessment form is designed and the pathologists are instructed to indicate a specific problem using coded comments and individual case numbers. This form assists the histology laboratory in documenting corrective action, in compiling summary reports by defects and in detecting trends.

Lost Specimen

A lost specimen is defined as "the loss of surgical pathology specimen that has occurred after the case has been accessioned in the laboratory and that prevents an adequate pathologist examination of that specimen. The acceptable threshold for these events was defined by ADASP as one in 3000 cases.

A tissue lost may occur at the time of grossing, at the time of processing due to failure of completely close cassettes, or loss of tissue blocks. Every effort should be made to find or account for "lost" surgical pathology specimens. The staff member discovering the discrepancy will immediately inform to the supervisor of Histology who in turn should notify the pathologist and the incident is considered as a non-conforming event and is documented on a lab non- conforming event form.

Floaters

Floaters are the small piece of tissue that arises from a source other than the case under examination. Floaters mainly arise due to cross contamination during grossing, embedding, Microtomy or staining. Identification of floaters by the pathologist can be tracked using the form or marked on a slide and number of occurrences per total tissue blocks or slides can be aggregated as a useful performance improvement monitors.

The average rate of occurrence on prospective slide review for participants in Q-probe studies were 0.6 percent of slides, 0.8 percent of blocks and 1.2 percent of specimen. Following steps can be taken to reduce its frequency.

- All instruments used in grossing e.g. forceps,

scalpels and scissors should be cleaned after every case.
- Floatation bath at microtome stations should be frequently cleaned, particularly following any poorly processed tissue that explodes on the water bath.
- Embedding forceps must be carefully cleaned off paraffin after every case.

Histology laboratories must provide high quality services by enforcing a technical quality control system. This can only be achieved by providing accurate, relevant, precise and comprehensive data, which is applied to medical management of patients. Any misdiagnosis or errors on reports can lead to massive impacts on the quality of service and health consequences to patients. Thus, laboratories must follow quality rules and standards that are already established. More so, the goal of quality management in laboratories should be to ensure continuous improvement which would guarantee quality service and quality laboratory results to consumers.

Assay Standardization and Challenges Faced by Laboratories

Dr Hafsa Majid
Clinical Chemistry

Accreditation bodies around the globe encourage assays harmonization to not only improve quality of care but also improve efficiency, patient satisfaction, and overall clinical operations. As numbers of clinical laboratories are expanding it is becoming more important that test performed by different laboratories are standardized. Patients receiving care across a healthcare network expect uniform laboratory services throughout and any differences in results reported from two sites or laboratories can create confusion for patients and clinicians. For example, serum creatinine performed at two different labs on a specific sample should give similar results (within analytes specific allowable error). In instances where assays are not standardized due to non-availability of primary reference materials, guidelines recommend that the follow up should be done with a single method and baseline be re-determined, when assay or methodology is changed.

Method standardization directly affects result accuracy which in turn affects patient's outcome. Ideally all methods should be completely comparable, but that is not noted in practice and intermethod differences can affect the clinical decision making. In long term monitoring a patient can change or switch hospital or the laboratory which has a different method of analysis for that specific analyte. Clinicians need testing standardization to interpret results properly. Seeing different results can be very confusing to clinicians and can potentially results in misinterpretation of laboratory results. For example when multiple laboratories in a country offer different cTn assays (I versus T and new high-sensitivity versus contemporary), misdiagnosis is a distinct possibility. Patient diagnosed based on cTn-T assay cannot be monitored with cTn-I assay.

Pathologists also face difficulties in interpreting reports of samples sent to reference laboratories for comparison when difference in methodology exists. Two non-standardized assays can produce different results. In such instances deriving qualitative or concordance for comparison will be helpful. Taking fecal calprotectin as an example for non-standardized assay; a patient with inflammatory bowel disease will have raised fecal calprotectin performed by two different methods with good concordance but the bias between results of the two methods can be high.

The main aim of clinical laboratories is to provide information that is useful to help medical decision-making, allowing optimal patient health care. The standardization of laboratory results assuring interchangeability of results would significantly contribute to improvements in health care, since lab results performed in different locations or times could be universally applied.

Quality Assurance in Immunohistochemistry: The Way We Do It

Dr Sabeehuddin Siddique and Dr Khurram Minhas
Histopathology

Introduction

Immunohistochemistry (IHC) is a method for localizing specific antigens in tissue or cells based on antigen-antibody recognition. It has become an indispensible tool in today's practice of surgical pathology. The Histopathology section at Aga Khan University Hospital Karachi has pioneered the use of immunohistochemical stains in laboratory practice in Pakistan and with the passage of time the number of procured antibodies is increasing. On the other hand, the accrediting agencies for clinical laboratories, such as College of American Pathologists have laid down stringent policies and guidelines for standardization, quality assurance, quality control and improvement of immunohistochemistry techniques.

Steps in Immunohistochemical Testing

The factors affecting the quality of results in immunohistochemistry include events spanning from the identification of the specimen to interpretation of the stain and then its assimilation into the final report. But before delving deeper into the details of quality assurance process in immunohistochemistry, and the way we achieve it in our laboratory, it is imperative to have a basic idea about how the process of immunohistochemical staining works. This entire process can be broadly divided into pre-analytic, analytic and post-analytic phases.

Pre-analytic steps: In order to reduce the ischemic time until fixation, the specimen should be immediately immersed in a standardized fixative (such as formalin) as soon as it is surgically removed. Therefore, upon being received in the laboratory and after accessioning, all the specimens are specifically checked if they have been adequately placed in formalin. Specimens received in other medium are transferred into formalin to prevent further autolytic changes. Use of non-formalin fixatives is strongly discouraged, and there are only a handful of exceptions to its use including Bouin's solution for testicular biopsies and Zenker's solution for lymph nodes.

During grossing, the specimen is visually examined for suspicious areas which need to be sampled for microscopic examination. In order to achieve optimal and reproducible immunohistochemical staining, thin tissue sections (usually less than 3mm in thickness; and 2 x 2 cm in size) are submitted for processing and cutting. During these processes, rigorous checks are kept on the system to detect any fault that may render the tissue block unsuitable for microscopic examination and subsequent immunohistochemical testing.

Analytic steps: Staining is the analytical part of the immunohistochemical process. It encompasses antigen retrieval (to recover tissue antigens that may have been altered by improper fixation); blocking of endogenous enzymes; application of the primary antibody (that may or may not be followed by application of secondary antibody); use of chromogen to visualize antigen/antibody complex; and visualization system, ending with counterstaining. In our laboratory, all these processes are performed on state of the art DAKO® autostainers using DAKO EnVision® Kits.

Post-analytic steps: In post analytic phase, the pathologist interprets the stains in context with positive and negative tissue controls using bright field microscopy. The observed results are analyzed in conjunction with the light microscopic features of the tumor and are then incorporated into the final histopathology report.

Routine Quality Control; Use of Daily Controls and Interpretations of Immunostains

The single best evidence that the antigen has been adequately demonstrated in a particular tissue is to look at the internal control. Internal control refers to antigens present in the normal tissue structures of the patient tissue being tested. Unfortunately, an internal control may not always be present. To overcome this issue, with each batch of immunohistochemical stains that we run in our laboratory a set of positive and negative tissue controls for a particular antibody

are also applied. The stains, along with the respective positive and negative tissue controls, are reviewed by an experienced histopathologist before being distributed. Any discrepancy observed is logged, notified to other pathologists and corrective measures are taken. In fact, the process of daily quality control does not end here. After the distribution of stains, each signing out consultant interprets the immunohistochemical staining in conjunction with the provided controls and in context with tumor morphology. Table 1 highlights the key parameters that may adversely affect the immunohistochemical staining quality.

Table 1: Key parameters that may have an adverse effect on staining quality

Monitor	Potential Adverse Effect
Temperature of processors, embedding centers and slide drying apparatus	Antigen degradation due to excessive heat
pH of antigen retrieval solution	Decreased immunoreactivity due to incorrect pH
Temperature of antigen retrieval solution/ chamber	Failure to reach the optimal temperature
Checklist for automated instrument set-up	Selection of incorrect protocol or failure to apply correct reagents results in absence of desired immunoreactivity
Reagent expiration dates	Use of reagents beyond the manufacturer's expiration date may result in inconsistent staining

Evaluation and Validation of new Antibody

Whenever a new antibody is introduced in our center, it is diligently evaluated, optimized and validated before being used for diagnostic purpose. The goal of this process is to establish the optimal titration, detection system and antigen retrieval protocol. Once the antibody has been optimized, it is tested on laboratory tissue in order to determine the sensitivity and specificity. Simple it may sound; this process can be quite cumbersome, especially if the optimal dilution titer is not achieved even on repeated attempts.

Validation of new Lot

Each new lot of antibody must be validated before it is used in clinical assays. For an established primary antibody that is performing well, the laboratory need only stain a small series of tissues, including the routine positive and negative controls. For new lots of detection system reagents, a similar side-by side comparison of the old and new lots should be performed and recorded using series of monoclonal and polyclonal antibodies that optimally test the specificity and sensitivity of the detection system.

External Quality Assessments; Participation in Cap Survey Program

Besides having a stringent internal quality assessment tools, Aga Khan Hospital Clinical laboratories has also been participating in the external quality assessment programs offered by the College of American Pathologists. One such program is The MK program of the American College of Pathologists in which unstained tissue sections are provided to our laboratory along with a clinical history and specifications as to which stains should be performed. After performing the relevant immunihistochemical stains, a Consultant histopathologist records the stain results and a favoured diagnostic interpretation based on history, the histologic appearance and the immunophenotype. These results are shared with the college, which then sends a summary report that includes the interpretation of the referees as well as statistics on the response of participants, stratified by antibody preparation used. These materials provide an excellent mechanism of external quality assurance and a source of published benchmarks for reproducibility.

Quality Assurance Monitors

Besides continuous monitoring of the immunohistochemical staining processes, it is crucial that the laboratory keeps track and record of various quality indicators pertaining to this process. Our laboratory is no exception to this rule. We not only periodically review the data inferred from these records but are bound to share this information with the College of American Pathologists as a part of our accreditation process. A few noteworthy of such parameters are listed below.

1. **Repeat Slides:** An inadequate immunohistochemical stain may be the result of less than optimal tissue selection and/or processing, antibody failure or technical failure. It is important for the laboratory to document all requests for repeat stains, the reason for the request, the corrective action performed and the final outcome. Interaction of the pathologist and technologist on a

case-by-case basis is essential for successful troubleshooting.

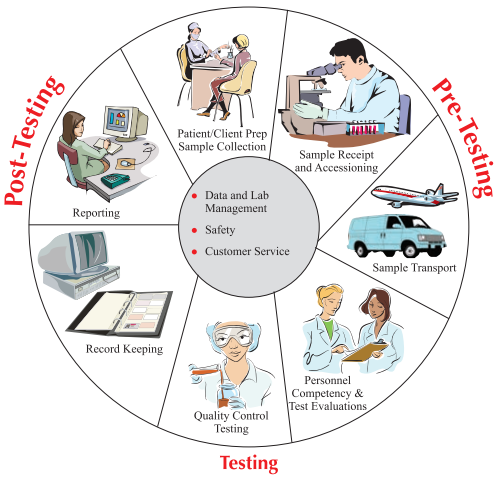
2. **Turnaround Time:** Immunohistochemistry may add significantly to the overall turnaround time of surgical pathology or cytopathology case. As these may be high profile cases, objective monitoring of turnaround time, either periodically through a retrospective audit or prospectively through the routine use of time stamps, is a useful quality assurance measure.
3. **Audits of Pathology Reports:** A retrospective review of reports from cases utilizing immunohistochemistry stains may provide very useful information on the efficacy and efficiency of ordering practices by the pathologists as a group and individually. More importantly, a review by a pathologist not involved in the case can provide useful feedback regarding the clarity and completeness of reporting.

Besides the aforementioned quality assurance, management and regulation processes in immunohistochemistry followed in our laboratory, additional requirements have been laid down by the College of American Pathologists, for reporting the results of predictive marker studies for breast cancer (including ER, PR and Her2-neu. We strictly adhere to these policies in order to produce the optimal staining results, as it has major implications in the management of disease.

Quality Assurance in Laboratory Biosafety

Syeda Kiran Zaidi
Clinical Microbiology

Laboratory safety is a vital part of total quality assurance program of our laboratory. “Quality assurance” is all the policies and systematic activities implemented within a quality system and, “Biosafety” is application of knowledge, techniques and equipment to prevent personal, laboratory and environmental exposure to potential infectious agents or biohazards. Together, we can say Biosafety is all the actions taken to improve the quality of work and personal safety.



Quality Assurance Cycle - Medical Laboratory

Image courtesy: Google Images

Risk Assessment

The backbone of the practice of biosafety is risk assessment. While there are many tools available to assist in the assessment of risk for a given procedure or experiment, the most important component is professional judgment. Risk assessments should be performed by the individuals most familiar with the specific characteristics of the organisms being considered for use, the equipment and procedures to be employed, and the containment equipment and facilities available. Most healthcare workers are familiar with biosafety levels we are working in.

Policies and Standards

Every institute must have a safety policy which should include:

1. A reporting hierarchy for safety officers designated to each area for monitoring safety indicators
2. Reporting any incident with a breach in biosafety
3. Regular audits of the facility to inspect for safety of the working environment

Image courtesy: Google Images

Policies provide a recommendation to uphold the standards set by the institute as per EPA (Environmental Protection Agency) Regulations, Federal, Provincial and Local Regulations, Joint Commission International and College of American Pathologist Standards are also included. Laboratory safety standards must include

- A chemical hygiene plan which covers all the hazardous materials used in the section. The Material safety data sheet (MSDS) should list and give details of all chemicals used in each department. Each employee should be well-versed in responding to biological and chemical spills, and use of spill kits. Eye wash stations are checked daily and on monthly basis to ensure proper working
- Blood borne pathogen standard which includes universal precaution (Discussed below) and vaccination and post-exposure prophylaxis.
- Personal hygiene especially hand washing and keeping work garments free of biohazard.
- Disposal of used linen and hospital garments for both patients and staff
- Disposal of sharps and biohazardous wastes in appropriate containers and incineration or deep burial

Any safety issues should be discussed in meetings held on regular basis. Moreover, monthly indicators are also sent to the chief safety officer for review. Knowledge of safe practices can be enhanced through planned lectures and drills. Each employee working in the laboratory must be aware of firefighting, location and usage of fire extinguishers, fire alarms, and fire rescue team numbers. There should be designated assembly areas in case of fire or earthquake for the workers of all departments to facilitate headcount.

Infection Control

Infectious diseases are a major risk to patients, visitors and staff. Infectious diseases can be spread through direct contact or the air.

- Blood borne pathogens include Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV). Exposure to a Blood-borne pathogen is a risk for faculty and staff of health-care facilities. Health Care Workers in hospital setting are at high risk of acquiring Hepatitis B viral infections. So immunizations are offered to all employees at risk of these. A full course of Hepatitis B vaccination consists of three (03) injections at zero, one and six months. All these three doses are compulsory for the safety of employees.

Universal Precautions:

1. Follow Standard (Universal) Precautions: Blood and body fluid from all patients must be handled as if they were infected with a Blood borne pathogen.
2. Wash hands: after removing personal protective attire, before leaving the restroom, after contact with blood/body fluids or after handling contaminated items, before and after eating

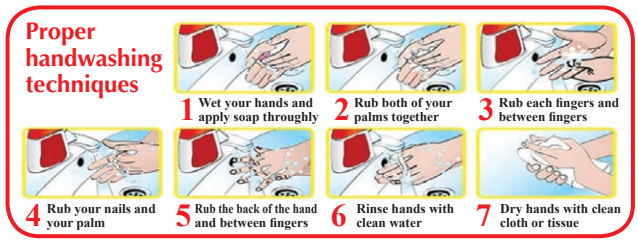


Image courtesy: Google Images

- Proper method for washing hands: Using regular soap, running water, rub hands 10-15 seconds of friction. After rinsing, dry hands and then turn off the faucet with the paper towel.

3. Personal protective equipment: Wear gloves when expecting to
   - Touch items or surfaces contaminated with blood or other body fluids.
   - Handle blood or other body fluids specimens.
   - Come in contact with patient's open skin lesions.
   - Obtaining a blood sample.

Always wash hands after removing gloves. Wear a face shield or a combination of a facemask and protective eyewear when at risk of splash or spray to the face. Apron or other barrier gown can protect personal clothing when it is likely to become soiled due to splashes; if penetrated by blood or body fluid, remove it as soon as possible.

4. Needle stick injuries (NSI)/sharps injury/ mucosal exposures to blood and body fluids are hazardous to your health. If injured, put the area under running water until it stops bleeding. Wash the site with soap and water, dry and cover with bandage. Trace patient's HIV, HBV, HCV status and notify the safety officer.

Quality Control of Culture Media Used in Clinical Microbiology

Sobia Noshad Baig
Clinical Microbiology

The reporting of culturable microorganisms, bacteria or fungus, depends upon the quality and sterility of the cultural media. The quality and quantity of media directly affects the observations and results. Different parameters of media such as poor growth support, physical characteristics and batch contamination can result in false reporting. The less quantity of media agar or supplements also results in poor growth. There are different methods of checking all these parameters systematically.

Culture media plays an essential role in the clinical microbiology laboratory. The isolation of microorganism is the prerequisite for identification and sensitivity of pathogenic and nonpathogenic organisms. Most laboratories prepare their own culture media for routine diagnosis and for research purpose. For ensuring the culture media's quality, quality control strains and materials are set simultaneously to assess the complete range of purpose for which the media is used. The batch of media agar and broth prepared is thus considered fit for use only after passing through rigorous quality checks.

Raw Material

The quality of the culture media depends directly upon the quality of the raw materials used for preparation.

Only distilled water is used for the preparation of cultural media. The water pH should be monitored daily, and should be slightly acidic to neutral but no lower than 5.5, as highly acidic pH may inhibit the growth of microorganisms.The petri dishes used for pouring of culture media should be ethylene oxide (EtO) sterilized or gamma irradiated. Only borosilicate glassware should be used because soda glass can leach alkali into the media and change its characteristics. There are various supplements used in preparation of culture media including supplements for enrichment, e.g. blood, NAD, hemin and certain vitamins, and other inhibitory additives, like antibacterial and antifungal agents etc. Traditionally, animal blood should be used e.g. sheep or horse, in most blood containing media, but human blood has also been used with acceptable results. Blood is collected aseptically and inoculated in agar media after cooling to 50oC so that it doesn't denature. For additives purchased as prepared supplements, the certificate of analysis and sterility and temperature at the time of addition are important considerations.

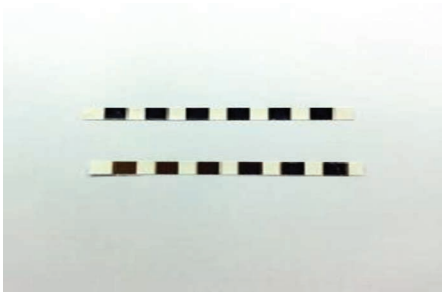
Sterilization

Sterilization plays an important role in the quality of the culture media. Generally, for media with heat stable components, autoclave is used for sterilizing the culture media. However, the time of autoclaving and the quantity of culture media sterilized should be closely regulated. The standard autoclaving cycle is maintained at temperature 121°C for 15 minutes and 15 psi pressure. Overheating of bacterial media can cause nutrient destruction, so temperature and pressure timings are very important.

The volume of the bacterial media in sterilization batch should be kept small, ideally two liters. Sterilization process indicators should be checked regularly. Temperature and pressure should also be plotted while the autoclave is running so that it can be monitored live. Records of biological indicators (e.g. spore strip or bromthymol purple broth with Geobacillus stearothermophilus spores) and chemical and steam penetration indicator (e.g. Bowie Dick

test) to check the efficiency of the process must be maintained.

EZ TEST (Biological indicator):
Bromthymol purple broth with
Geobacillus stearothermophilus spores.
VIOLET (Sterilized), YELLOW (Unsterilized)

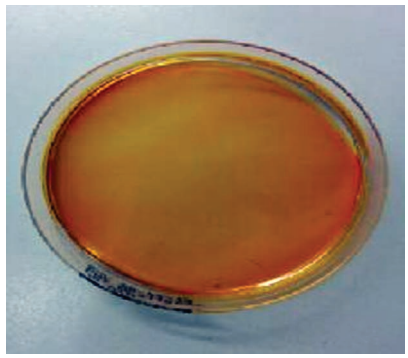


BOWIE DICK STRIPS DARK BROWN – lower strip (Poor steam penetration), BLACK- upper strip (Good Steam penetration)

Physical appearance
The gross physical appearance of bacterial media often shows the quality of media pouring. Prepared bacterial media should be screened for physical characteristics such as excessive bubbles or pits, unequal filling of plates (non-uniform levels), thickness of medium in the plate, which must be 4.0 ± 0.2 mm, cracked and frozen or crystallized medium in plate.

All the above mentioned characters can be checked visually by naked eye.

Excessive bubbles in media.

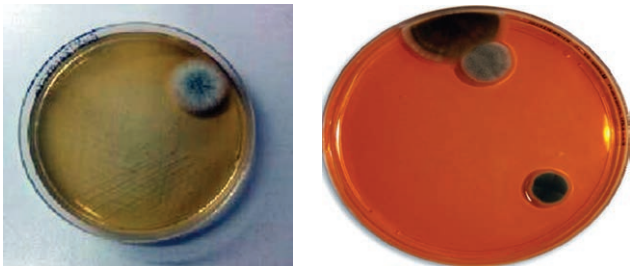


Cracked media.

The pH of the medium is also an extremely important physical characteristic, which must be checked. It can be measured after autoclaving of media by using the standard pH meter after proper calibration with standard buffers.

Microbiological parameters
Growth support is the most important parameter while conducting quality control of media. Standard inoculating procedures should be used, it includes positive control and negative control. National Committee for Clinical Laboratory Standards (NCCLS) has laid down certain guidelines for the control organisms to be used for every medium, the desired inoculum concentration and their expected growth results. After inoculation, the plates are incubated at 37°C for 24 hours and their growth and colony characteristics are observed. The results can be reported by mentioning presence or absence of growth and the growth characteristics. The results should be examined both qualitatively and quantitatively. While testing new lots, both previous batch and new batch should be simultaneously inoculated.

Contamination
This is a crucial parameter for the determination of the quality of media. The batch must be checked for contamination before passing for laboratory use. It is also suggested that the whole batch of the prepared media be checked for contamination by keeping the plates at least for three days at room temperature in case of a small batch (<100 plates). Alternatively, 5-10% of plates from the test batch can be taken and placed into the incubator set at 37°C for 24 hours. After required incubation, the plates are checked for any growth. If there is any growth, the sterility of the batch is rechecked, taking double the number of plates from the same batch. If contamination is confirmed in more than 10% of the incubated plates from the batch, the media must be discarded, as per recommendations.



Contaminated culture plates

Conclusion

The quality control (QC) of bacterial media used in clinical microbiology laboratory remains critical for accurate and acceptable isolation of pathogens from infected patients. Reference strains must be used as to assess whether the media is behaving as expected for both bacterial isolation, identification and sensitivity testing, before releasing the batch for use in the laboratory. Testing media using standard protocol can save time and resources and ensure reliable reporting.

Quality Assurance (QA) in Qualitative Tests in the Clinical Laboratory

Najma Shaheen
Clinical Microbiology

Quality Assurance is a way to achieve quality service. In the clinical diagnostic laboratory it ensures that laboratory processes are controlled at every stage and the results generated by the laboratory are accurate, reliable, timely and reproducible.

Infectious diseases serology is an important diagnostic modality because it provides analysis of disease without any invasive intervention with a limited span of time. These tests are qualitative semi-quantitative and quantitative. For better and accurate result interpretations, vigilant test procedure is required. However, the laboratories especially in resource limited countries have questionable reliability of infectious serology results due to noncompliance with the quality procedure and practices. Use of unreliable diagnostic kits without performing proper quality assurance is one of the major limiting factors for reproducing good quality report.

Components of Quality Assurance

Pre-analytical:

1. Sample collection:
   Sample must be collected in assigned appropriate tube/container and should be rejected if received in wrong container or wrong labeling.
2. Sample transportation:
   Recommended condition for sample transportation must be followed like maintaining temperature and adding preservative if there is any delay in transportation.
3. Sample processing:
   Before performing the quality of specimen must be assessed visually (Hemolysed, lipemic or turbid sample).
   The test sample must be processed according to kit manufacturer's instruction.

Analytical:

1. Method validation
   Before starting to use any diagnostic kit, the following important points must be taken into consideration by the users.

- Use those kits whose manufacturer is well known and reliable in diagnostic products.
- Diagnostic kit should be approved by some reliable international quality assurance agencies. (FDA or CE marked)
- Do not use kits which are intended for research purposes only.
- Ensure thorough literature review and market search before selecting any kit. Laboratories can select those manufacturers whose kit is in use by majority of peers if you are a participant of a Proficiency testing program (e.g. Proficiency testing by College of American Pathologist -CAP).

Once selection has been done and kits received in the laboratory, following quality assurance (QA)

parameters must be performed as a validation and verification process. Validation process must be repeated if there is a change in methodology or manufacturer.

a. **Sample selection**: To validate a diagnostic kit known positive and negative samples are required. Proficiency test samples (CAP surveys) can be used for this purpose. Well characterized positive clinical samples, having relevant clinical history of that disease and rechecked with authentic methodology by some other laboratory with better quality assurance process can also be used.

b. **Number of samples required**: 10-20 positive and 10-20 negative samples or well characterized samples is the minimum requirement for verification process of FDA approved tests. For non-FDA approved or modified FDA approved tests, the sample size is much larger, comprising 100-150 data points.

c. **Accuracy**: this parameter tells us that how much our method is accurate to generate the result, if we are running a sample of known concentration. It is calculated by:
   Accuracy=No. of correct results/ total no. of results x 100

d. **Precision**: it means how precise our method is, and can we get the same result upon repeating. It is achieved by testing same sample numerous times at different days by different individual under the same operating conditions. It is performed to determine intra-run assay and inter-run assay variation. Precision can be determined by:
   Precision= No. of repeated results in agreement/ total no. of results x 100

e. **Specificity**: it is the ability of method to detect only the analyte of interest in the presence of other factors "Specificity" can be defined as the ability of the method to specifically separate the particular analyte in the presence of other components.
   Specificity= No. of true negative results/ (No. of true negative results + No. of false positive results) x 100

f. **Sensitivity**: it is the ability of method to detect smallest quantity of analyte. Selectivity refers to the ability of the method to discriminate a particular analyte in a complex mixture without interference from other components
   Sensitivity: No. of true positive results / (No. of true positive results + No. of false negative results) x 100

g. **Positive predictive value**: it is performed to check the probability that a patient with a positive test result has the disease or the presence of an analyte in a specimen
   PPV=No. of true positive results / (No. of true positive results + No. of false positive results) x 100

h. **Negative predictive value**: it is performed to check the probability that a patient with a negative test result does not have the disease or the absence of an analyte in a specimen.
   NPV=No. of true negative results / (No. of true negative results + No. of false negative results) x 100

2. Quality Control-(Performance and documentation)
Kit's commercial controls and internal controls must be checked daily along with the patients' samples.
Results should be documented in designated forms and must be reviewed periodically (daily by bench in-charge and monthly by laboratory manager/consultant).

   In case of erroneous quality control results, patient sample results must be held back and the cause evaluated. Repeat the tests by alternative kit (same manufacturer and same lot or same manufacturer with alternate lot if available). If problem persists, promptly coordinate with senior laboratory management.

3. Equipment maintenance:
Equipment maintenance is scheduled as daily, weekly, biannually or annually. This schedule must be defined and documented for all instruments and coordinate with the defined protocols and the designated person who will perform that maintenance activity.
Equipment maintenance include daily cleaning of instrument, calibration (if required) before initiating to run the clinical sample. If there is any error or nonfunctional instrument or component, coordinate with biomedical department or inform laboratory manager if instrument is in warranty period.

4. Lot-to-Lot verification :
   Whenever new lot of any kit is received it must be verified before performing on patient's sample.

Post-analytical:

1. Review of results:
   All results are reviewed by consultant before reporting to the patient
2. Audits:
   Internal and external audits are performed at different interval to ensure compliance with national and international guidelines.
3. Reference ranges: In case of quantitative tests, reference ranges must be provided on patient's final report so that it can be helpful for the treating physicians to interpret the result cautiously.

Overcoming Challenges in Quality Assurance: Antimicrobial Susceptibility Testing by an Automated System

Asima Shahid Sabzwari
Clinical Microbiology

Quality Assurance ensures systematic monitoring and evaluation of the various aspects of a project, service, or facility to ensure that standards of quality are being met. Maintaining Quality Standards in Antimicrobial susceptibility testing is an important tool to ensure that the results are being correctly delivered to the patients.

For maintaining Quality Assurance of a certain automated testing system, there are certain elements which must be kept in mind when drawing up an Individualized Quality Control Plan (IQCP). These key elements are Specimen, Reagent, Environment, Testing Personnel, and Test System. All issues to be monitored can easily be distributed under these parameters and can thus be monitored accordingly, with preventive actions in place, anticipating any errors. If anything new comes up, a tailored preventive action can be added to the IQCP under the above categories and monitored. Each anticipated problem can be given a risk grade according to the seriousness of the problem. The main elements of IQCP include Risk Assessment, Quality Control Plan and Quality Assessment. An IQCP for the entire process must address the failures and errors in Pre Analytic, Analytic and Post Analytic phases of testing.

Risk Assessment

Risk Assessment is based on five elements Specimen, Test system, reagent, environment and testing personnel, and laboratory must ensures that all these five parameters are covered in the Risk Assessment plan.

- Specimen quantity should be acceptable according to the manufacturer's instructions otherwise inadequate quantity of specimen can cause false positive results and can cause kit reagents to perform improperly. Reagent integrity and expiration date needs to be checked before patient testing. Each lot should be verified before setting up susceptibilities, and reagents and kits should be stored in scientific grade refrigerator. QC is performed weekly to monitor effect of transient temperature fluctuations on the integrity of each analyte and performance of each equipment.
- Instrument diagnostics for temperature and optics fail which lead to frequent terminations and error. Dust from uncovered surfaces may interfere with the optics causing power failures and affects instrument. To prevent this, instrument should be kept in areas with less dust fallout, optics cleaned after frequent intervals and regular instrument maintenance is required.
- Training of staff is also necessary because if staff is not trained for setting up susceptibilities, incorrect results will be reported. Competency assessment and sign off by trainer of all staff must be completed before carrying out procedure on clinical specimens. Adherence to Patient Safety

Goal #1 deserves special mention here; matching the Name and Laboratory / Medical Registration Number is of foremost importance as there is zero tolerance for this error.

Quality Control Plan

The Quality control plan should have certain parameters that include:

- Error Detection: if an error occurs before during or after testing it should be detected immediately
- It should specify the number, type and frequency of Quality control material to be tested
- QC acceptable ranges should be defined
- QCs performed should be according to the manufacturer's instructions, no less
- Indicate that your laboratory director has reviewed, signed and dated the QC plan document

Quality Assessment

Quality assessment can be monitored by considering following aspects:

- There should be a record of Temperature logs for room temperature, refrigerator and freezer to ensure that temperature is being monitored on daily basis. Instrument ambient temperature can be affected if there is failure of engineering controls over air conditioning so this should be monitored by engineering department servicing lab. Since the test results are accepted as long as the QC remains within the acceptable range, ensuring the quality and integrity of the QC material is supreme for the analytic phase.
- If Quality control fails, results on patient samples are unacceptable or unreportable if no obvious cause is found. This can happen if Quality control ATCC strains are not pure, too old or too young QC cultures are used (correct age is 24-hour old colonies), incorrect control strains are used for the test, or inappropriate media is used for revival of QC strains. To avoid such type of errors, ATCC strains are revived weekly according to a standard ATCC maintenance plan, and all strains are saved at -80 degrees Celsius in a separate freezer box to ensure conservation of their genotype/ phenotype.
- Quality control log should also be maintained and reviewed regularly to ensure that corrective action has been taken for any unacceptable values
- Manufacturer's instructions should be reviewed with each new lot or shipment and if there is any change it should be updated in the policy
- Review of Personnel competency records should also be done

Quality Assurance can be maintained by following all the standards stated above. Our experience with the IQCP for automated AST (Vitek 2 system) has so far been very useful in ensuring correct reporting of results. Any inconsistencies or errors are analysed and if necessary, the IQCP is revised to include new occurrences or reduce the risk level of previously addressed issues. We have been following all these standards and performing quality control on weekly instead of daily basis since almost 2 years and are able to report automated susceptibilities with confidence.

Radiology Pathology Correlation

Dr Nasir Ud Din and Dr Dawar Khan
Histopathology and Radiology

A 14 year old boy presented with progressive pain in right leg, which was more at nights, and relieved by taking non-steroidal anti-inflammatory drugs (NSAIDs). A plain x-ray was performed followed by computerized tomography (CT) scan. The CT scan showed a radiolucent nidus surrounded by sclerotic bone (Figure 1A,B). Resection of the nidus with surrounding bone was done.

Histopathologically, the nidus was composed of multiple fragments of haphazardly interanastomosing trabeculae of variably mineralized woven bone with osteoblastic rimming. The intertrabecular stroma is fibrovascular (Figure 2A,B). The surrounding bone was thick sclerotic. A diagnosis of osteoid osteoma was made based on radiological and histological features correlation.

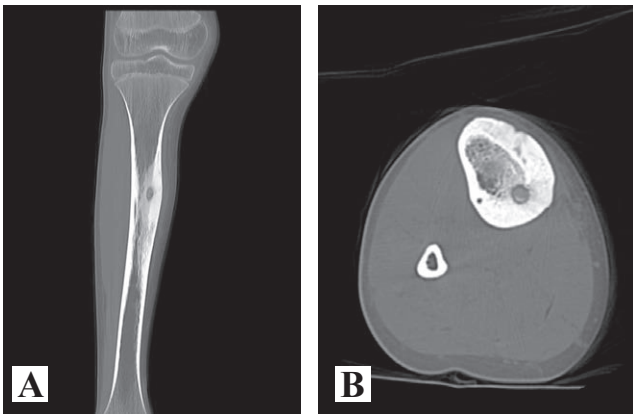


Figure 1A, B. CT coronal and axial images shows focal cortical thickening along a small lytic area and a nidus in proximal tibia.

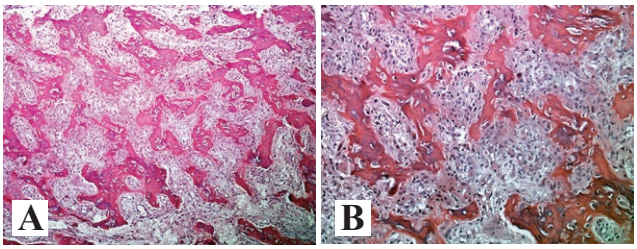


Figure 2A, B. Histological examination of osteoid osteoma nidus shows interconnecting trabeculae of woven bone with fibrovascular stroma

Osteoid osteoma is a benign bone forming lesion most frequently seen in children and young adults and has a peak incidence in the second decade of life. There is a male predilection. The tumor most commonly involves long bones of distal extremities, particularly femur neck.

Clinically, the most common presentation is pain of several months' duration seen is 80 percent of patients. The pain is intermittent at first, then becomes relentless and is more severe at night. Aspirin and other NSAIDs are very effective in alleviating the pain for several hours at a time. The pain is believed to be elicited by high levels of prostaglandin E2 and prostacyclin found within the nidus.

Radiological differential diagnosis of osteoid osteoma is osteoblastoma, brodie's abscess, cortical stress fracture, and bone island. Histologically, the closest differential diagnosis is osteoblastoma. The characteristic pattern of nocturnal pain in osteoid osteoma is not seen in osteoblastoma and these tumors preferentially involve the axial skeleton. The diagnosis of the two tumors is made by correlating overall clinical, radiological, and histopathologic features. In borderline cases, an arbitrary size cutoff of 1.5 cm has historically been used.

Identifying Problems before Proficiency Testing Fails

Dr Lena Jafri
Clinical Chemistry

Proficiency testing (PT) is the external component of quality control since it involves peer review. It primarily measures accuracy because each laboratory's results are compared to the interlaboratory or peer group mean, which is assumed to be the true value. The graphs in PT evaluation reports can be helpful in identifying problems even before PT results become unacceptable or before incorrect patient results are released.

Utilizing SDI for diagnostic interpretation of PT survey report

Proficiency test results are reported as standard deviation indexes (SDIs). This index represents the number of standard deviations each result is from the peer group mean. The SDI is calculated by the following formula: SDI = (your result - interlaboratory mean)/ interlaboratory SD. The

best way to detect problems is to examine these SDI results critically in PT evaluation reports. The following rules are useful in evaluating SDI results:

- Do two or more of the five SDI results exceed +/- 1? If not, a significant error is unlikely and evaluation is complete. If so, further questions need to be asked.
- Atleast one result exceeds +/- two SDI: review results to rule out possible problems, identify possible errors from non-analytical sources for results with very high SDIs.
- The average of the five SDI results is more than +/- 1.5: indicates significant bias (systematic error) , calibration data should be reviewed to determine if a shift has occurred. Bias can usually be eliminated by recalibration.
- One PT result is more than +/- three SDI : there is a high probability of random error.
- The range of SDI between the largest and smallest PT result exceed 4 SDI: random error is a possibility and the procedure should be evaluated for potential sources of imprecision.

Allowed Deviation and Patterns in PT Evaluation Graphs

The PT evaluation report also includes graphical summary using the relative distance of your results from the target. This distance is called the allowed deviation. Allowed deviation is calculated by subtracting the target value from your laboratory result and dividing this difference by the PT allowable error for that analyte. The ratio is then multiplied by 100 to bring the value on a percent scale ranging from -100 to +100. If results are beyond -100 to 100 'x' appears on the report indicating that results exceed the graphical limit. Monitoring PT graphs and rules using allowed deviation has also been useful in identifying problems before PT fails.

Patterns in PT Graphs for a Single Mailing/ Single Survey:

- One result in a mailing exceeds +/- 75 percent of the allowed deviation: review results to rule out possible problems, identify possible problems from non analytical sources such as clerical errors for results that exceed +/- 100% of the allowed deviation.
- All results are on one side of the target values with atleast one difference exceeding +/- 50 percent of the allowed deviation: shows bias indicating a possible calibration drift .
- Large positive and negative differences; combined lengths of longest positive and negative bars is >140 out of total range of 200: depicts random error

Patterns in PT Graphs over Multiple Mailings:

- Persistent results on one side of the target values: shows persistent bias, even if small, recalibration should have occurred within this time frame.
- Results flip from one side of target to the other (Figure 1): shows impact of system or process changes, longer bars are of more concern. Follow suggested action for systematic errors.

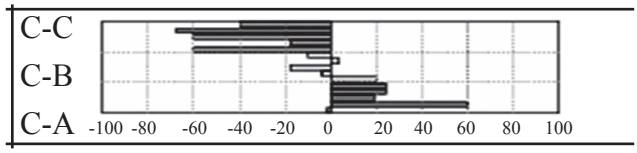


Figure 1: Results flip from negative to positive bias.

- Over time, length of bars increase (Figure 2): a sudden shift may show impact of system or process changes, most likely random error, may reveal new source of either systematic or random error.

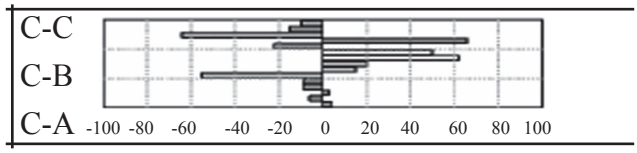


Figure 2: Lengths of bars increase over time on both sides.

- Over time length of bars increases primarily on one side (Figure 3): depict persistent bias, take corrective action for systematic error.

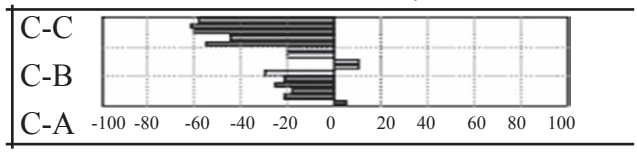


Figure 3: Lengths of bars increase over time on one side of target mean.

- Over time, length of bars decreases: shows impact of system or process changes, particularly as a result of corrective action.

Sometimes by reviewing results from multiple PT mailings or surveys, performance trends that could lead to PT failures are easily recognized and rectified. For example graphical plots by College of American Pathologists PT evaluation reports can identify trends that would be missed without reviewing multiple PT events together. As discussed above reviewing PT results carefully over time can identify persistent bias, trends, and shifts, change in system and/or process, systematic error, evidence of corrective action, training opportunities and staff competencies.

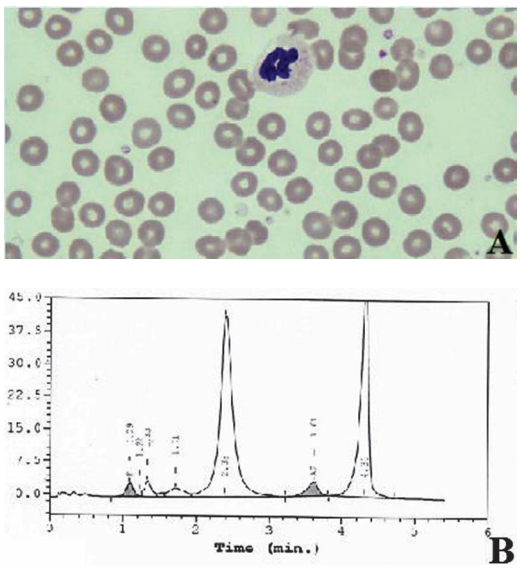
Quality Requirements for Reporting Sickle Haemoglobin

Dr Nazish Sana and Dr Muhammad Shariq Shaikh
Haematology & Transfusion Medicine

The haemoglobin molecule within red blood cells is essential for transporting oxygen to the tissues along with carbon dioxide removal and buffering action to maintain pH within red cell. Adult haemoglobin is a 64.4 kDa hetero tetramer comprising of iron-containing porphyrin called haem and two pairs of globin polypeptide chains that includes one pair of alpha and one pair of non-alpha chains. In adults, 96-98 percent haemoglobin A (α2β2) and 2–3.5 percent of haemoglobin A2 (α2δ2) are present. Sickle haemoglobin (Hb S) is a variant haemoglobin that results from valine for glutamic acid substitution at position 6 of β globin chain. Homozygosity for haemoglobin S (βSβS) causes a serious condition referred to as 'sickle cell anemia'. Heterozygosity for haemoglobin S (ββS), referred to as sickle cell trait, is usually asymptomatic. The βS gene may also be co-inherited with another β chain globin variant e.g. sickle beta thalassaemia. On deoxygenation and dehydration, HbS solubility is reduced and irreversible polymerization occurs, causing vascular obstruction. This phenomenon in vivo has deleterious effects such as hand–foot syndrome, acute chest syndrome, cerebral haemorrhage or infarction, priapism, acute pain crisis, splenic crisis, recurrent infections and others.

Various diagnostic methods for identification of HbS include haemoglobin electrophoresis at alkaline and acidic pH, isoelectric focusing and high performance liquid chromatography (HPLC). HPLC is one of the widely utilized methods for HbS detection. On HPLC, HbS elutes at retention time of 4.5 minutes (4.30–4.70 minutes) however, a number of other variant haemoglobins also co-elute at the same retention time such as Hb Q-Thailand (Mahidol), HbA2′, Hb Manitoba, HbE- Saskatoon, Hb Montgomery and others. Therefore, it is essential to identify HbS accurately in order to differentiate it from other haemoglobins for proper patient diagnosis and future counseling. Laboratory accrediting bodies such as College of American Pathologists (CAP) recommends that if a patient sample appears to have Hb S in the primary screening; the laboratory should perform a second procedure to confirm the presence of Hb S. In order to fulfill this quality requirement, section of haematology and transfusion medicine at Aga Khan University performs sickling test on every patient sample that have variant haemoglobin in S-window on HPLC. In this test, patient's sample is combined with Sodium Metbisulfite as a reducing agent which causes red cell deoxygenation. If HbS is present, red cells lose their smooth round shape and become sickle shaped. The presence of sickle shaped cells is confirmed by examining the slide under microscope along with review of both positive and negative control samples. Very rarely when sickling test is also negative, we advise patient to confirm presence of sickle cell disease by molecular testing.

In this way, identification of HbS is confirmed by two different methods and accurate report is provided to patients. Thus, patient safety and appropriate future management in ensured.



Patient with Sickle cell trait: A, Leishman stain at 40x showing absence of sickle cells. B, Chromatogram showing variant haemoglobin in S-window. C, Sodium metbisulfite preparation showing sickling RBCs

Quality Assurance of Bronchoscopically Collected Samples

Tazeen Fatima
Resident Clinical Microbiology

Bronchoscopes are medical devices widely used for diagnostic and therapeutic procedures in a different variety of patient populations. Flexible bronchoscopes are critical equipment as they are introduced in sterile body cavities and should be decontaminated after each procedure to eliminate all forms of microbiological life, including bacterial spores. Appropriate reprocessing of flexible bronchoscopes is a multistep procedure involving washing followed by sterilization or high-level disinfection with further rinsing and drying before storage. Proper storage is obligatory. However adequate decontamination of bronchoscopes is laborious and often ignored to save resources, time and workload which has serious implications on patients' health and can result in bronchoscopy-related healthcare associated infections, outbreaks and psuedooutbreaks.

Mycobacterium Tuberculosis, non-tuberculous mycobacteria (NTM), and Pseudomonas aeruginosa are the most common pathogens associated with transmission during bronchoscopy.

Contamination Sources	
Inadequate cleaning, disinfection, surface wiping and channel flushing, use of inadequate detergent/disinfectant (expired, low potency, resistance of microorganisms)	
Use of damaged and leaking scopes and re-use without decontamination	 
Improper storage (the scopes must hang down straight), and inadequate infection control practices and hand hygiene	

All images courtesy Google images

Potential Sources of Error in Report		
Preanylytical	**Analytical**	**Postanalytical**
1. Exogenous infectious material introduced in patient could lead to bronchoscopically transmitted infections (increased morbidity and healthcare cost!) 2. Collection of poorly representative and contaminated sample of BAL (Garbage in garbage out!)	1. Heavily contaminated samples (Possibility of missing out true pathogens!) 2. Extensive laborious and strenuous laboratory work (ensuring presence of true pathogens, identifying pseudo outbreaks and investigating true outbreaks) 3. Increased cost and logistic burden on labs	1. Delay in reporting/ communication to physician from laboratory 2. Commencement of unwarranted treatment to patient in case of over reporting

Bronchoscopically transmitted infections have grave consequences on patients' health and associated with enormous healthcare costs as positive cases are often investigated with repeat cultures and advanced radiological investigations to rule out false positivity. Those patients are bound to receive unnecessary antimicrobials until and unless contamination has been ruled out and also bear adverse effects of unnecessary treatment. Contaminated bronchoscopy respiratory samples are a huge burden from laboratory diagnostic point of view as it requires extensive laboratory work, increased uses of resources, manpower and time consumption.

Bronchoscopy related infectious complications reported are rare but the incidence is probably underestimated, with many episodes unrecognized and uninformed. Failure of adequate cleaning and disinfection procedures is the major cause and can be avoided by proper disinfection/ sterilization, adequate reprocessing of bronchoscopes and stringent infection control practices.

Antibody and Reagent QC for Flow Cytometry Assays

Dr Muhammad Shariq Shaikh and Dr Arsalan Ahmed
Haematology and Histopathology

Flow cytometry is a dynamic technology which together with specific antibody and fluorochrome reagents allows to preview at the surface, cytoplasm and DNA content of thousands of cells suspended in fluid. It is particularly useful to determine lineage and potential biologic behavior of targeted cells. The key feature of this technology is the use of monoclonal antibodies in order to ensure epitope specificity. Today, these antibodies are available from several commercial suppliers for utilization in diagnostic assays. These antibodies must go through stringent QC during manufacturing, distribution, evaluation and utilization in testing patient samples.

Besides general handling of reagents such as storage conditions, expiration dates, and "in-use" status, several other factors unique to flow cytometry reagents are critical in efficient antigen-antibody reactions. These factors include: the quality of the sample, the viability of cells, the pH of the environment, and the amount of antigen and antibody available for reaction.

The foremost requirement is validation of reactivity of each fluorescent-conjugated antibody before its intended use. The optimal concentration at which antigen-positive cells can be best distinguished from nonspecific binding on antigen-negative cells should be determined. For instance, over-expression is an increased number of antigens per cell and is observed in certain hematological malignancies. In contrast, weak or dim reactivity may result from decreased concentration of antibodies leading to misinterpretation of results. To resolve this, titration experiments are performed with variable dilutions. The data is then analyzed for signal to noise ratio, percentages of stained and unstained cells and intensity of the stain.

Additionally, to verify acceptable performance, all new lots of reagents used in laboratory testing must be verified against old lots. This includes all the reagents whether prepared in the laboratory or purchased from suppliers such as buffers and lysing reagents. A positive patient sample can be run concomitantly with the new and old lot of reagent. Depending on the use of reagents, acceptability criteria should be established and achieved results should be documented.

Method Validation: College of American Pathologists (CAP) requirements of Method Validation of Quantitative Tests in a Clinical Laboratory

Dr. Sibtain Ahmed
Chemical Pathology

What is method validation? According to Clinical & Laboratory Standards Institute (CLSI), validation is ‘‘The process of testing a measurement procedure to assess its performance and to determine whether that performance is acceptable.’’ The six elements of method validation are: precision, accuracy, reportable range verification, reference intervals establishment or verification, sensitivity* and specificity* (*for non FDA approved or laboratory modified assays). Following table shows the protocol to be followed in validating a new test and it should be documented.

Elements	How it is Done
Precision Precision indicates repeatability, which means, analyze repeatedly to determine variation.	To verify inter-assay variation, samples should be processed twice a day in quadruplicate for 5 days producing 20 readings. For intra-assay variation, one sample is 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
Accuracy Closeness of the agreement between test result and ‘‘true’’ result.	Two Approaches : 1. Comparison of results between new method and ‘‘reference’’ method. 2. Results using new method on certified reference materials or controls. 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. Results are acceptable if the average bias between the two methods is within allowable limits.
Reportable Range Analytical Measurement Range (AMR) Clinically Reportable Range (CRR)	AMR (Range of analyte values that a method can directly measure on the specimen, without any dilution, or other pretreatment) 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. CRR (range of analyte values that are reported as a quantitative result, allowing for specimen dilution or other pretreatment) 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
Reference Intervals	It is not mandatory but preferable for a laboratory to establish its own reference limit. 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. However reference interval verification is essential for standardized routine analytes. 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.

Meeting Report of International Federation of Clinical Chemistry (IFCC) Conferences- 2017

Reported by Dr Sibtain Ahmed and Dr Hafsa Majid
Clinical Chemistry

Figure 1: Participants from Pakistan at ICPLM & IFCC-Worldlab 2017

This year International Federation of Clinical Chemistry (IFCC) organized two events at Durban South Africa: XIVth International Congress of Paediatric Laboratory Medicine (ICPLM) and International Congress of Clinical Chemistry and Laboratory Medicine, Worldlab. The ICPLM was organized by the Task Force on Paediatric Laboratory Medicine (TF-PLM) in cooperation with the African Federation of Clinical Chemistry (AFCC), on 20-21st October 2017. International Congress of Clinical Chemistry and Laboratory Medicine, Worldlab was organized in partnership with South African Association for Clinical Biochemistry (SAACB) and AFCC, from October 22nd to 25th 2017.

One of the goals of the IFCC is to identify and nurture talented young scientists and increase participation of pathologists from developing countries. The IFCC achieves this goal by giving travel grants to its member societies from different countries. This year Dr Hafsa Majid and Dr Sibtain Ahmed were awarded these scholarships from Pakistan. Dr Sibtain Ahmed also received 2nd best prize for poster presentation on abstract regarding neonatal TSH reference interval in Pakistan. In both conferences high quality scientific programs were arranged. The plenary sessions covered wide range of topics ranging from innovations in techniques, neonatal and newborn screening, evidence based laboratory medicine, laboratory accreditation, incidence and spectrum of inherited metabolic diseases in South Africa, application of big data, standardization, reference intervals establishment and risk management.

The conference attracted people from many countries and continents and provided an eclectic mix of topics relevant to Clinical Chemistry including practical overview of latest advances in the field of laboratory medicine. To conclude, this was an overwhelming and rewarding experience.

hospitals.aku.edu/Karachi/clinical-laboratories