QUALITY CONTROL/QUALITY ASSURANCE IN THE MOLECULAR MICROBIOLOGY LABORATORY

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Molecular QA/QC

- One of the largest stumbling blocks for traditional Clinical microbiology Laboratories that start performing molecular testing
- Molecular QA/QC more closely resembles Clinical Chemistry QA/QC
- New CAP Microbiology Checklists have been refined and full sections now devoted to Molecular Microbiology
- Many changes in QA/QC; many are new to traditional microbiologists

QA/QC Program

- Written clearly defined program that ensures quality throughout the preanalytical, analytical, and post-analytical phases of testing
- Capable of identifying systems problems and opportunities for system improvement
- The laboratory must be able to develop plans for corrective/preventative action based upon data from its QA/QC system

QA/QC Program

- Pre-Analytical Phase – addresses activities that occur prior to testing (e.g., test orders, patient identification, specimen collection transport, handling, and processing)
- Analytical Phase – Laboratory must meet specific requirements to perform testing
- Post-Analytical Phase – Involves assessing the accuracy and timely reporting of laboratory results while maintaining patient privacy

Pre-Analytical Phase

- Test Requests
- Specimen Collection and Handling
- Rejection of Specimens
- Informed Consent

Test Requests

- Develop well-designed manual or electronic test request forms
  - Allow for sufficient identification of patient and ordering physician, the test requested, and pertinent clinical information
  - Should include date and time of specimen collection and specimen type
  - Should accompany patient's specimen to the laboratory
- Ensure that clinical indications are appropriate for test(s) requested

Specimen Collection and Handling

- Specimen collection and handling can have a significant effect on final test results; integrity of target nucleic acid sequence must be maintained
- Establish clearly defines criteria for monitoring proper specimen collection, labeling, preservation, transportation, and storage of specimens
- Establish educational programs
- Develop firm (but fair) rejection policy

Guidelines for Specimens

- Clearly defined method(s) of collection of specimens from all sources
- Sample type and quantity of specimen
- Preparation of collection site
- Timing of collection during disease
- Specimen quality/adequacy
- Collection and transport devices
- Identification of specimens
- Appropriate transport conditions
- Storage time, temperatures, and conditions

Specimens for RNA Detection

- Specimens for RNA detection require special collection and handling conditions
- RNA may be degraded by RNases
- Use method that stabilizes RNA until specimen can be processed and stored appropriately

Analytical Phase

- Written Procedure Manual
- Nucleic Acid Extraction and Storage
- Contamination Control
  - Laboratory Design
  - Laboratory Practices
- Controls
- Test Performance
- Equipment Maintenance
- Personnel Competency
- Proficiency Testing
- Accreditation

Procedure Manual

- Written instructions that contain sufficient detail that qualified laboratory personnel can perform tests consistently and accurately
- Must include:
  - Principle of Test
  - Clinical Significance
  - Specimen Requirements
  - Reagents Needed
  - Procedural Steps
  - Quality Control
  - Calibration
  - Reference Ranges
  - Calculations
  - Result Reporting
  - Test Limitations
  - References
- Yearly review by Director or designee; review all new procedures and revisions prior to implementation
- Electronic or paper procedures acceptable

Nucleic Acid Isolation

- Adequate procedures for release and isolation of target nucleic acid
- Keep process simple and minimize number of manipulations
- Maintain integrity of target while inactivating or removing inhibitory or interfering substances
- Yield, purity, intactness can be measured
- Ideally, isolated nucleic acid should be tested immediately; if delay, store under appropriate conditions until testing can be completed

Analyzing DNA

- Concentration of DNA
  - 1 A_{260} Unit of dsDNA = 50 μg/ml H_2O
  - 1 A_{260} Unit of ssDNA = 33 μg/ml H_2O
- Purity of DNA
  - Pure DNA: $A_{260}/A_{280} \geq 1.8$
  - An $A_{260}/A_{280} < 1.8$ (Contaminated with proteins and aromatic reagents)
  - An $A_{260}/A_{280} > 2.0$ RNA contamination

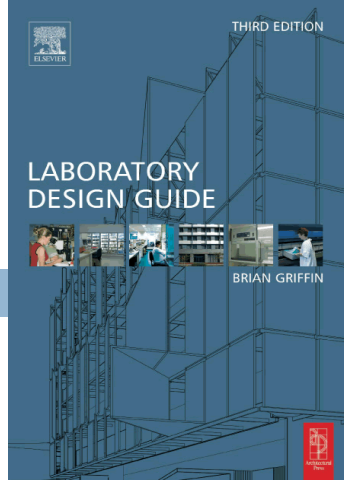
Analyzing RNA

- Concentration of RNA
  - 1 A_{260} Unit of ssRNA = 40 μg/ml H_2O
- Purity of RNA
  - Pure RNA: $A_{260}/A_{280} \geq 2.0$
  - An $A_{260}/A_{280} < 2.0$ (Contaminated with proteins and aromatic reagents)

Contamination Control

- PCR creates millions of new replication-competent molecules
- Amplified nucleic acid (amplicons) can contaminate gloves, skin, clothing, and the environment
- Requires appropriate laboratory design and good laboratory practices

Laboratory Design



- Molecular space should be divided into at least 3 separate work areas:
  - Area 1 – Reagent preparation area/room
  - Area 2 – An area/room for specimen processing/nucleic acid extraction
  - Area 3 – An area/room for amplification and detection
  - *Area 4 – For preparation of controls, calibrators, standards

Laboratory Practice

- Dedicated supplies and reagents for each work area
- Use plugged aerosol resistant pipette tips
- Use closed systems or methods to control product carryover (e.g., uracil-N-glycosylase (UNG) to inactivate amplified product)
- Use appropriate positive and negative controls

Laboratory Practice

- Follow unidirectional flow from pre-PCR to post-PCR
- Pulse-spin reagents, samples, controls; Add reagents to vials before samples
- Use gloves and protective clothing

  which are dedicated to each area
- Decontaminate pipettes and instruments and wipe work surfaces with 10% bleach; rinse with 70% ethanol
- Meticulous cleaning is a must

Laboratory Practice

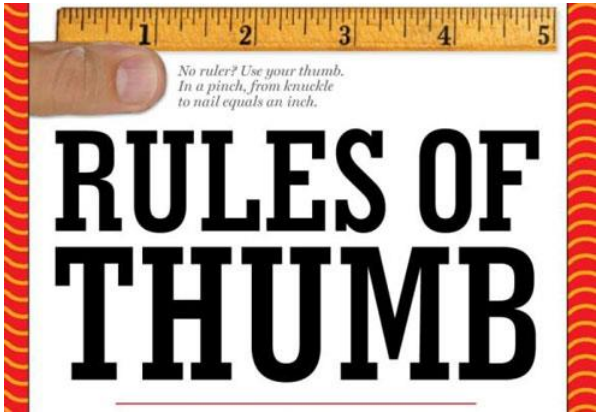
- Carefully open and close all tubes to minimize aerosolization of contents
- Keep all non-essential tubes closed during sample addition
- Order of preparation and loading of samples for PCR should be actual clinical specimens first, followed by positive and then negative controls
- Hoods and/or glove boxes (with UV light) are often necessary for nucleic acid isolation and set up of amplification reactions

General Rules of Thumb

- Traffic can only go from clean to dirty area
- Objects can only go from clean to dirty area
- Reagents and supplies needed in a clean area need to be prepared and stored in a clean area
- Equipment used in clean area cannot be moved and used in dirty area
- Personal safety equipment cannot move with you from dirty to clean area
- Meticulous cleaning is a must

General Rules of Thumb

- Protocol books, worksheets, sample tubes, test data and results cannot move from dirty area to clean area
- Can employ color coded lab coats, pipettes, safety glasses, racks, etc. to monitor traffic
- Have respect for RNases if working with RNA
- Meticulous cleaning is a must



Reagents & Solutions

- Prepare and divide into single-use aliquots
- Store in area that is separate from specimen preparation and post amplification
- Dedicated equipment and supplies should be used
- Reagents can be premixed into single-use, master mixes

Reagents

- Pulse-spin reagents, samples, controls; Add reagents to reaction vessel before samples or controls
- Use gloves and protective clothing

  which are dedicated to reagent preparation
- Decontaminate pipettes and wipe work surfaces with 10% bleach; rinse with 70% ethanol

Reagents

- Test new lots for purity, functionality, concentration, and contamination before use
- Compare performance of new reagents to ones currently in use
- Test new reagents against reference materials; monitor quality with use
- Keep meticulous QC records

Reagents

- Lot-to-lot testing
  - The extent of lot-to-lot testing depends upon whether the lab is using:
    - In-house developed assays
    - Analyte-specific reagents
    - In vitro diagnostic use
- Tolerance and acceptability limits for all control procedures, control materials, and standards

Assay Controls

- Negative Control(s)
- Positive Control
- Sensitivity Control
- Internal Control – To determine whether nucleic acid has been extracted and is amplifiable without inhibition
- External Control(s)
- Standard Curve - Quantitative Assays
- Calibrators - Quantitative Assays

Assay Controls

- Qualitative Assays
  - Positive, Negative, Sensitivity Controls
  - At least a positive and negative control for each analyte in each run
- Quantitative Assays
  - Positive and Negative Controls
  - Quantitative Controls at more than one concentration (level) used in every run
- Controls should have a matrix that closely matches specimens being tested

Commercial Sources of Controls, Calibrators, and Standards

- Acrometrix
- Advanced Biotechnologies
- American Type Culture Collection (ATCC)
- BBI Diagnostics
- Zeptometrix

Internal Control Needed?

- What is likelihood of encountering inhibitors in a particular specimen
- Need to control for inhibition should be decided during test validation
- Clinical implication of a false negative result
- Low target copy number or sequence variability in primers and probes may account for false-negative results
- The degree to which the clinical diagnosis depends upon the lab result

Equipment & Instruments

- Ensure equipment and instrument function
- Establish system for monitoring
- Detection of drift, instability, or malfunction before problem affects test results
- Regular preventive maintenance and cleaning
- If multiple instruments, functions must be check against each other every 6 months or after repair
- Keep meticulous maintenance, service and repair records
- Establish service contracts with vendor

Quality Assurance/Monitoring

- A continuous process
- Documents that a test which has already been validated is repeatedly giving the expected results as test is performed over time
- Confirms that test continues to perform according to laboratory's requirements and its intended use

Quality Monitoring Process

- Personnel competency assessment
- Quality control monitoring
- Quality improvement
- Internal and external proficiency testing
- Correlation with clinical findings
- Trend analysis
- Integral part of a laboratory’s quality assurance program

Quality Control Monitoring

- Have system in place to continuously monitor performance of positive and negative controls, standards, and calibrators
- Perform wipe testing – can reinforce need to disinfect the environment daily
- Monitor prevalence rates of specific viral diseases

Training & Competency

- Establish a complete program of employee training, verification, and competency
- Verify personnel competency at least semiannually during first year and at least annually thereafter
- Reverification necessary if change in method or instrumentation

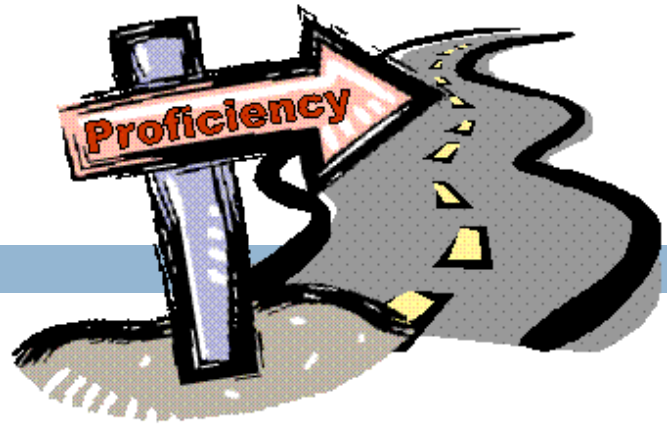
Assay Training

- Determine magnitude of training and skill level required
- Each operator must demonstrate proficiency with the method; may require certification
- Should include understanding of:
  - Sample collection, handling and storage
  - Reagent handling and storage
  - Proper test protocol
  - How to interpret and report results
  - QC/QA for the system

Competency Assessment

- Direct observation of routine patient test performance, including specimen handling, processing, testing, instrument maintenance, and function tests
- Monitoring test result recording and reporting
- Review worksheets, QC records, PT results, and PM records
- Wet testing with proficiency samples
- Assessment of problem-solving skills

Proficiency Testing



- CAP Proficiency Tests
- Alternative performance assessment
- Integrated into the workload
- Rotated among people at the bench
- Corrective action on failed PT surveys

Alternative Performance Assessment

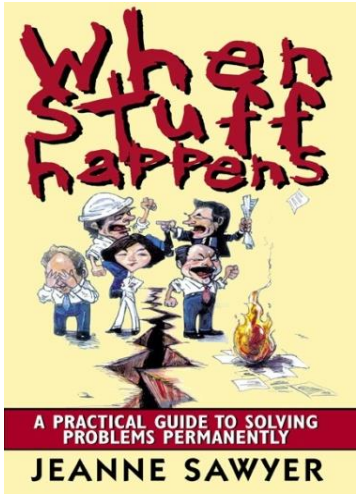
- Participate in alternate PT Programs (e.g. QCMD)
- Participate in ungraded/educational PT
- Split sample analysis with other laboratories
- Establish in-house PT Program
- Use regional pools (can be purchased)
- Clinical validation by chart review

Internal or Split-Sample PT

- Minimum of 3-5 samples per testing event
- At least 2-3 testing events at equal intervals per year
- Use full range of positive and negative specimens in panel
- Samples should represent clinical specimens

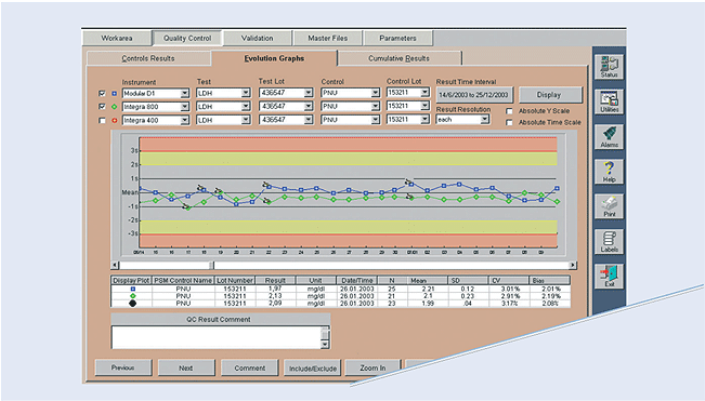
Corrective Actions

- Mistakes will occur; avoid assigning blame
- Clearly document what has happened
- Thoroughly investigate, take immediate corrective action, institute preventive measures
- Errors often result of larger process issues with reagents, personnel, equipment, workflow
- Ultimate goal: **DO NO HARM**



Statistics

- Maintain statistics on percentages of normal and abnormal results
- Maintain surveillance data
- Perform comparative studies on these statistics
- Director review at regular intervals and corrective action taken as needed



Instructive Case Log

- Instructive or unusual cases
- Maintained and reviewed

Post-Analytical Phase

- Laboratory Test Reports
- Timeliness of Reporting
- Correction of Errors
- Patient Confidentiality

Molecular Reports

- Patient Name, age, date of birth, gender
- Unique identifier number
- Name and location of laboratory
- Specimen source and condition
- Date specimen collected and test performed
- Summary of the method
- Objective findings and clinical interpretation
- Limits of testing

Recording & Reporting Results

- Laboratory test reports should be clear, concise, accurate, and fully interpretive
- Verify all results before the final report is released
- Screen for clerical and reporting errors
- Monitor and report specimen inadequacy
- Good practice to have reports reviewed by Supervisor or Director

Interpretation & Reporting of Results

- Significance of results must be evaluated with respect to agent, specimen site, and clinical situation
- Interpretive criteria must include provisions for equivocal results
- Reporting of results should include as much information as necessary for the clinician to properly interpret the results

Turnaround Times

- Provide test results in a timely manner to have greatest impact on patient care and management
- Have system to monitor TATs
- Are TATs appropriate for the intended purpose of the test?

Additional Items

- Correction of Errors – Laboratory should have a system in place for timely review and correction of clerical and analytical errors
- Confidentiality – Testing records and reports should be maintained in a manner that preserves patient privacy and confidentiality