

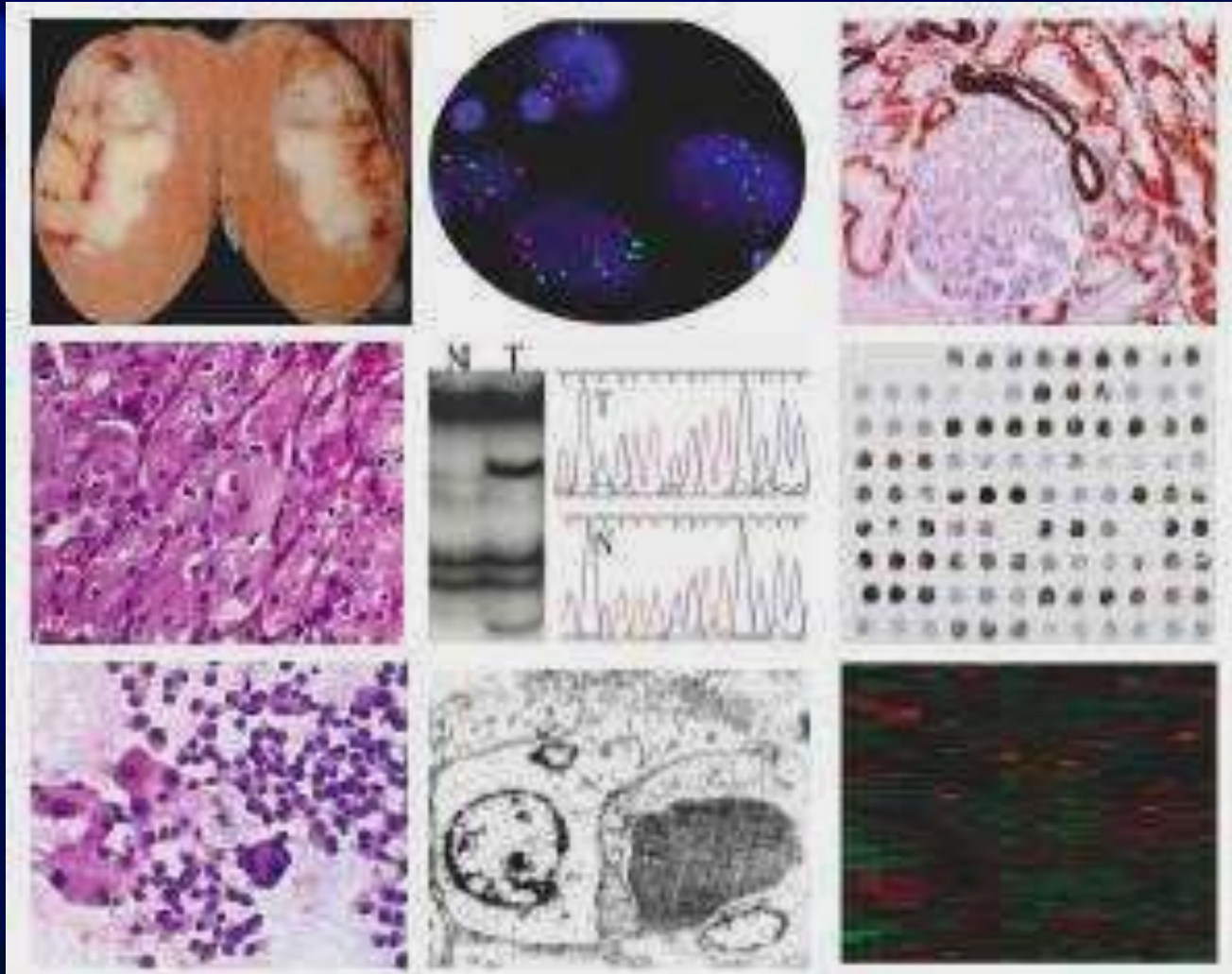
Quality Control and Monitoring of Molecular Tests

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Why do we need more quality assurance?



Clinical Laboratory Regulation in the U.S.

(In The Name Of Quality)

- Clinical Laboratory Improvement Act – 1967
- Clinical Laboratory Improvement Amendments – 1988 (CLIA88)
- 1992, revised CLIA88 (QC and personnel)
- 2003, further revised QC, personnel and proficiency testing grading
- All clinical labs must register with the Department of Health and Human Services
 - Centers for Medicare and Medicaid Services (CMS)

What is quality?

- Conformance to requirements of customers
- Satisfaction of customer needs and expectations

TQM and Cost

- Cost of conformance to customer requirements
 - Prevention costs (ex. training, calibration, maintenance)
 - Appraisal costs (inspection, QC)
- Cost of non-conformance to customer requirements
 - Internal failure (repeats)
 - External failure (complaints)

Quality and Cost



Deming “improve quality, decrease waste, decrease cost”

The goal of clinical laboratories is to:

- 1. produce timely and reliable measurements of analytes.***
- 2. assist in diagnosis, management and prevention of human disease.***

***R*eliable measurements** ---> **ACCURATE &
PRECISE**

***T*imely measurements** ---> **MEET SPECIFIED
TURN AROUND TIMES**

Insuring “reliable” laboratory results

Standard operating procedures

- develop
- use
- document

Systems

- monitor *overall* lab performance
- analytical performance
 - accuracy & precision
- respond to errors
- proactively improve operations

QUALITY ASSURANCE PROGRAM
- Quality control

Quality Assurance is the preventive medicine to Quality Control

*Quality Assurance makes sure you are
doing the right things, the right way*

*Quality Control makes sure the
results of what you've done are
what you expected*

QA?



Tissue retrieval

surgeon
pathologists

Tissue transport

technician

Tissue preparation

Tissue selection

technician

Molecular extraction

PCR
qPCR
RT-PCR
Western Blot
IHC
FISH

Specialist
technician

Interpretation

pathologists

Quality assurance programs evaluate

- **Pre-analytical phase**
- **Analytical phase**
- **Post-analytical phase**

Preanalytic Phase: Specimen Accession

- **Pre-analytical:** error caused by events prior to sample analysis.
- Condition of the specimen and requisition is reviewed upon receipt in the laboratory.
- No specimen is accepted without proper **labeling** and identification.
- If a specimen is unacceptable, the disposal or retention of the specimen is recorded.

Preanalytic Phase: Specimens for Molecular Testing

- Numerous types, sizes and volumes
- Cross-contamination must be avoided.
- Solid tissues can include fresh, frozen, or FFPE tissues (i.e. tumor cell content?)
- The quality of nucleic acid from fixed tissue depends on the fixing process and the fixative used.

Preanalytic Phase: Specimen Collection: Anticoagulants

Additive	Color	Testing
None	Red	Chemistry, serum, viral antibody studies
Sodium heparin (freeze dried)	Green	Immunology, virology studies
Sodium heparin	Brown	Cytogenetic studies, molecular studies
Tripotassium EDTA (7.5-15% solution)	Lavender	Virology, molecular biology
Acid citrate dextrose (ACD) solution	Yellow	Molecular biology

Analytical Phase

- SOPs
- Extraction and specimen storage
- Contamination control
- Laboratory design
- Laboratory practices (i.e aliquotting reagents, carry-over prevention)
- Controls
- Test Validation
- Equipment maintenance
- Personnel competency
- Proficiency testing

Analytic Phase: Specimen Holding and Storage: DNA

- Specimens
 - Blood, Bone marrow, Fluids
 - ≤ 1 day, 23°C; 3 days, 4°C
 - WBC, ≥ 1 year, -20°C or -70°C
 - Tissue
 - 23°C (not recommended)
 - ≤ 1 day, 4°C
 - ≥ 2 weeks, -20°C
 - ≥ 2 years, -70°C
- Isolated DNA
 - ≤ 26 weeks, 2-25°C
 - 1-3 years, 4°C (1 year for Southern blot)
 - ≤ 7 years, -20°C, -70°C (not frost-free)

Analytic Phase: Specimen Holding and Storage: RNA

- Specimens
 - Blood, Bone marrow, Fluids
 - <2 hours, 23°C or 4°C
 - 5 days, 23°C; 7 days 4°C in denaturant
 - 1-2 weeks, -70°C in denaturant
 - WBC, 2-4 weeks, -20°C; >6 months, -70°C
 - Tissue
 - <2 hours, 4°C
 - snap frozen, -70°C, ≥ 2 years
 - nitrogen vapor -140°C– -150°C, ≥ 2 years
- Isolated RNA
 - 2–25°C (not recommended)
 - <30 days, -20°C in DEPC-treated water
 - <30 days, -70°C in DEPC-treated water
 - >6 months, -70°C in ethanol

Analytic Phase: Laboratory Design

Polymerase Chain Reaction (PCR):

- Amplicon contamination
- Unidirectional workflow
- Positive air pressure in pre-PCR area
- Negative air pressure in post-PCR area
- Dedicated lab coats, pipettors, etc., in each area
- Isolate reagent preparation area
- Checkerboard controls
- NTC controls; bracketing

Analytical Phase: Laboratory Preparation for RNA Analysis

- Bench, equipment
 - separate laboratory area designated RNase free or RNF
 - wipe with RNase ZAP, RNase AWAY
- Disposables
 - certified RNase free
 - rinsed in 0.1% diethyl pyrocarbonate (DEPC)
- Reagents
 - certified RNase free
 - add 0.05–0.1% DEPC (except Tris)
 - test with RNase Alert (Ambion)
- Reactions
 - add Rnasin (Promega)

Analytical Phase: Test Performance

Federal regulations from the Food and Drug Administration (FDA) require validation of the performance of clinical test methods and reagents in accurately detecting or measuring analytes prior to use in human testing.

Analytical Phase: Test Validation

- Test validation is performed on **specimen types** that will be encountered in the routine use of the test.
- The number of specimens tested varies with the procedure and availability of test material.
- Results from the new test methodology are compared to results from established procedures or correlated to clinical diagnosis.

Analytical Phase: Test Validation

- FDA-approved molecular methods need to be verified
- If the FDA-approved test is modified, validation is required to show performance
- Procedure is documented in the laboratory according to Clinical and Laboratory Standards Institute (CLSI) guidelines.

Analytical Phase: Proficiency Testing

- **Proficiency testing:** refers to external specimens from a reference source supplied to independent laboratories.
- The College of American Pathologists (CAP) and other organizations supply specimens for molecular analysis.
- If proficiency specimens are not commercially available, laboratories can exchange blinded split specimens, or alternatively, blinded specimens measured or documented by independent means such as chart review can be tested within the laboratory.

Analytical Phase: Controls

- Controls are samples of known type or amount that are treated like and run with patient specimens.
- For qualitative tests: **positive**, **negative**, and in some cases, a **sensitivity** control
- For quantitative methods: **high-positive**, **low-positive**, and **negative** controls
- For amplification procedures, **amplification controls** are required to avoid false-negative results.

Analytical Phase: Controls

- Quantitative PCR methods that automatically analyze results require a **standard curve** or dilution series of the positive control.
- For methods requiring detection of a target-specific product or relative amounts of target, **internal controls** are run simultaneously, preferably in the same reaction mix as the test specimen.
 - *e.g.*, housekeeping genes, centromere probes, amplification controls

Analytical Phase: Other Quality Assurance

- Periodic review and documentation of test results is required.
- For molecular quantitative methods, define **dynamic range, sensitivity level, and accuracy**.
- Assay levels that distinguish positive from negative results (**cut-off values**) must also be well defined and verified at regular intervals.

Analytical Phase: Instrument Maintenance

- Manufacturers supply recommendations for **routine maintenance**.
- The laboratory maintains a **schedule, instructions, and documentation** of all routine maintenance.
- Technologists should know the limits of user-recommended repairs and when service calls are indicated.
- Regular calibration, or fitting an instrument or test system output with the actual concentration of a reference analyte, is required for detection systems.

Analytical Phase: Reagents

- Instructions on the preparation of reagents and the quantities used in each assay are included in the SOP.
- The sequences of primers and probes are documented.
- Primer binding sites and sizes of expected amplicons are documented.

Analyte Specific Reagent

- ASRs are probes, primers, antibodies, or other test components that detect a specific target.
- Most ASRs used in the molecular laboratory are class I, not subject to special controls by the Food and Drug Administration.
- Class II and III ASRs include those used by blood banks to screen for infectious diseases or those used in diagnosis of certain contagious diseases such as tuberculosis.

Postanalytical Phase: Documentation of Test Results

- Test results in the form of electropherograms, gel images, etc. should be of sufficiently high quality that results are unequivocal.
- Documentation of assay conditions, reagent lot numbers and quality and quantity of the isolated DNA or RNA is required.
- In situ results such as FISH are correlated with histological findings (stained sections) of tissue morphology.
- Raw data is retained with the final report and clinical interpretation of the test results.

Postanalytical Phase: Reporting Test Results

- The test report must convey the method or manufactured kit used, the locus, mutation or organism tested, the analytical interpretation of the raw data, and the clinical interpretation of the analytical result.
- The likelihood of false-positive or false-negative results should also be included on a report.

Postanalytical Phase: Reporting Test Results

Disclaimer

When Class I ASRs are used in an analytical method, the following disclaimer must be included in the test report:

“This test was developed and its performance characteristics determined by [laboratory name]. It has not been cleared or approved by the U.S. Food and Drug Administration.”

We add: “The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high complexity clinical laboratory testing.”

Personnel

MOL.40100 Personnel - Technical Operations

The person in charge of technical operations of the molecular pathology laboratory is qualified as one of the following.

1. Person who qualifies as a director
2. MB(ASCP), BS, BA or MLS(ASCP)/MT(ASCP) with at least 4 years experience (at least 1 in molecular pathology methods) under a qualified director

Personnel

MOL.40150 Technologist Qualifications

Persons performing the technical aspects of molecular pathology qualify as one of the following:

1. Experienced in field under direct supervision of a qualified director or supervisor & qualified to perform high complexity testing
2. MT(ASCP) certified or equivalent
3. BA or BS degree in biologic sciences with appropriate experience in molecular pathology

Personnel

MOL.40200 Training/CME

There is an adequate training program for new technologists, and a continuing medical laboratory education program.

MOL.10160 Alternative Performance Assessment

For tests for which CAP does not have PT, lab at least semiannually 1) participates in external PT, or 2) exercises an alternative performance assessment system for determining reliability of analytic testing.

Alternatives:

- split sample analysis with reference or other labs**
- split samples with an established in-house method**
- assayed material, regional pools, clinical validation by chart review, or other suitable & documented means**

Responsibility of lab director to define alternative performance assessment procedures

41042 (2)

Refrigerator/freezer temperatures are checked and recorded daily.

Applies to refrigerators/freezers containing reagents or patient/client specimens. "Daily" means every day (7 days/week, 52 weeks/year). Lab must define acceptable temp ranges for these units. If temperatures are found to be outside of acceptable range, document appropriate corrective action, which may include evaluation of contents for adverse effects.

05075 (1)

The laboratory's current CAP Activity Menu accurately reflects the testing performed.

Accurate Activity Menu required to properly assess Lab's compliance w/PT requirements. Activity Menu accuracy can be assessed by inquiry of responsible individuals & by examination of Lab's test requisitions, computer order screens, PMs, or patient reports.

20300 (1)

There is evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test.

Appropriate TATs vary by test type & clinical application. There are certain clinical situations in which rapid completion is essential.

20550 (1)

When appropriate, statistics on molecular pathology test results (e.g. percentages of normal and abnormal findings, allele frequencies) are maintained, and appropriate comparative studies performed.

Periodic evaluation of test result statistics (rates of positives & negatives) can be used to identify changes in test performance or shifts in populations being tested.

32050 (2)

There is a summary statement, signed by the laboratory director or designee, documenting review of validation studies and approval of the test for clinical use.

29865 (2)

There is documentation of at least annual review of all policies and procedures by the current director or designee.

Director must ensure that the collection of policies & technical protocols is complete, current, & has been thoroughly reviewed by a knowledgeable person.

Technical approaches must be scientifically valid and clinically relevant.

To minimize burden on lab & reviewer(s), it is suggested that a schedule be developed whereby ~1/12th of all procedures are reviewed monthly.

Paper/electronic signature review must be at level of each procedure, or as multiple signatures on a listing of named procedures.

A single signature on a Title Page or Index of all procedures is not sufficient documentation that each procedure has been carefully reviewed. Signature or initials on each page of a procedure is not required.

29750 (2)

A complete, current procedure manual is available at the workbench or in the work area.

Use of inserts provided by manufacturers is not acceptable in place of a PM. However, such inserts may be used as part of a procedure description, if the insert accurately and precisely describes the procedure as performed in lab. Any variation from this printed or electronic procedure must be detailed in PM. Appropriate reviews must occur.

A manufacturer's PM for an instrument/reagent system may be acceptable as a component of the overall departmental procedures. Any modification to or deviation from the PM must be documented.

Card files or similar systems that summarize key information are acceptable for use as quick reference at workbench provided that:

- a complete manual is available for reference
- card file or similar system corresponds to the complete manual & is subject to document control

31475 (2)

Validation studies document test accuracy, analytical sensitivity, analytical specificity and precision.

REFERENCES:

- MR Pincus. Interpreting Laboratory Results: Reference Values and Decision Making. In: Clinical Diagnosis and Management by Laboratory Methods. Chapter 4. 19th ed. JB Henry, ed. WB Saunders Company, Philadelphia. 1996.
- L Jennings, VM Van Deerlin, ML Gulley (2009) Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. *Arch Pathol Lab Med*: 133, (5), 743-755.

34065 (2)

New reagent lots and/or shipments are validated before or concurrent with use for patient testing.

For qual tests, minimum cross-checking includes retesting at least 1 known (+) & 1 known (-) pt sample from the old reagent lot or shipment against the new reagent lot, ensuring that the same results are obtained w/the new lot.

For quant assays, several pt samples should be run at different levels to check system calibration.

Good clinical laboratory science includes patient-based comparisons in many situations, since it is patient results that are "controlled."

A weakly (+) control should also be used in systems where patient results are reported in that fashion, *i.e.*, sensitivity control.

Use of QC material to check new reagent lots/shipments is acceptable, but lab should be aware that matrix interference may affect such material & mask a change in patient results.

Summary

- Proper specimen handling is required for accurate test results.
- Specimens should be held and stored under conditions that will preserve nucleic acids.
- Molecular test performance is monitored by the use of quality controls.
- Instruments should be maintained and calibrated for accurate detection and measurement of analytes.
- Reagents are prepared, stored, and used as recommended by manufacturers and/or laboratory protocol.
- Raw data should be documented and results clearly reported.

What if.....



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