

Doing It Right When Establishing New Molecular Tests in Your Hospital or Pathology Laboratory



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Disclosures

- Nothing to disclose



College of American Pathologists



American Society for
Clinical Pathology

Click to **LOOK INSIDE!**

Molecular
Pathology in
Clinical Practice



Association for Molecular Pathology

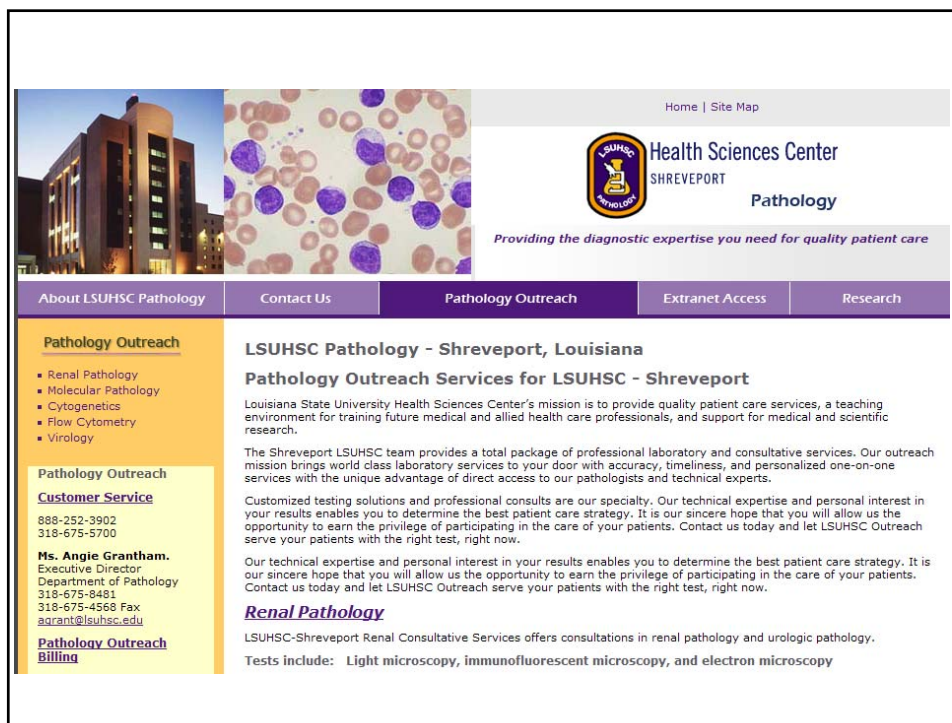


WASHINGTON G-2 REPORTS




Building a Test Menu

- Generalist vs. Specialist
 - Centralized molecular diagnostic lab
 - Develop molecular diagnostics tests in one of the current disciplines of Pathology or Genetics
- What will be your area of testing?
 - Infectious diseases
 - Hematopathology
 - Solid tumor
 - Molecular Genetics
 - Cytogenetics
 - Identity testing
 - HLA
- The service you provide will depend on your interest and the needs of your customers.



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Providing the diagnostic expertise you need for quality patient care

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Pathology Outreach
Customer Service
888-252-3902
318-675-5700

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Department of Pathology
318-675-8481
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agranam@lsuhsc.edu

Pathology Outreach
Billing

LSUHSC Pathology - Shreveport, Louisiana
Pathology Outreach Services for LSUHSC - Shreveport

Louisiana State University Health Sciences Center's mission is to provide quality patient care services, a teaching environment for training future medical and allied health care professionals, and support for medical and scientific research.

The Shreveport LSUHSC team provides a total package of professional laboratory and consultative services. Our outreach mission brings world class laboratory services to your door with accuracy, timeliness, and personalized one-on-one services with the unique advantage of direct access to our pathologists and technical experts.


Customized testing solutions and professional consults are our specialty. Our technical expertise and personal interest in your results enables you to determine the best patient care strategy. It is our sincere hope that you will allow us the opportunity to earn the privilege of participating in the care of your patients. Contact us today and let LSUHSC Outreach serve your patients with the right test, right now.

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Renal Pathology

LSUHSC-Shreveport Renal Consultative Services offers consultations in renal pathology and urologic pathology.


Tests include: Light microscopy, immunofluorescent microscopy, and electron microscopy



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- Ordering Procedures
- Specimen Collection
- Results and Reports
- Lab Contacts
- News and Information

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Molecular Pathology Tests - LSUHSC- Shreveport, Louisiana

Tests by Disease and Specialist


We test for virtually every inherited & oncologic disease. ([Test Downloads](#))

The tests have been organized by test-type, your patient's disease, and by your specialty.

[TEST SCHEDULES](#)
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Cellular	Bladder Cancer Breast Cancer	Allergist / Immunologist
Flow Cytometry	Coagulation Disorders	Bone Marrow Transplant
Immunohistochemistry	Colorectal Cancer	Cardiologists
	Genetic Syndrome	Endocrinologists
	Hematologic Disorders	Gastroenterologists
	Immunodeficiency	General Practice / Family Medicine
Sub Cellular	Infertility	Infectious Disease Specialists
Classic Cytogenetics	Leukemia	Oncologist / Hematologist
Digital Image Analysis	Lymphoma	Ob/Gyn
	Lung Cancer	Pathologist
Sub Chromosomal	Myelodysplasia	Pediatric / Geneticist
Molecular Cytogenetics	Melanoma	PCPs
FISH		

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Molecular Pathology News

Dr Nordberg's next speaking event

Thursday, April 29, 2010
Molecular Diagnostics for Community Hospital Laboratories

8:40 AM-9:20 AM

Doing It Right when Establishing New Molecular Tests in your Hospital or Pathology Laboratory

Mary Lowery-Nordberg, Ph.D., Director, Molecular Pathology and Cancer Cytogenetics, LSU Health Sciences Center, Shreveport, LA

The big story in 2010 is Congressional Health Reform. The Executive War College is your timely first opportunity to get the inside scoop on how legislation will affect clinical laboratory testing and anatomic pathology professional services.

You'll also experience more than 70 speakers, topics, and sessions that cover the A-to-Z of laboratory strategy, operations, and management. Check out the special sessions below!

[Laboratory and Pathology Mergers & Acquisitions!](#)

Special four-hour session on Tuesday, April 27

- Review of transactions, prices paid, valuations
- Roundtable of laboratory and pathology group sellers
- New legal, compliance, business issues surfacing in lab sales
- Best hospital strategies for profitable lab outreach

Copping Award Honors Excellence in Teaching

Mary Lowery-Nordberg, Ph.D. & T. Nathan Davis.

Complete program, schedule & times!

CLICK HERE to Download

LAUNCH PROCEDURES

WEAPON PREPARATION FOR LAUNCH (RN-N)

NOTE

Perform these checklists not later than 30 minutes prior to the HEW or to the IP.

1. Cabin Pressurization – COMBAT (if applicable) (CP)
2. Personal Locator Beacon Lanyards – Set as briefed (All)
3. Exterior Lights – As briefed (P)
4. Jettison Power & Jettison Control Circuit Breakers – In (RN)
5. Weapon Status Check: (N)

- a. FRMT-7 – Entered
- b. Weapon Targeting – Checked

When a weapon has been assigned to a launch point number and targeting has occurred, the status displays xx (xx = launch point number 1 to 99), in bright underlined video. Mission group number displays "a" (where a = A to F) in bright video.

- c. Weapon Navigation Quality – Checked

When the weapon is aligned or in alignment process the WEAPON STATUS should indicate AL in normal video. Weapon status should change to GO within 10 minutes of beginning alignment. With a status of GO, the weapon can be released. The Transfer Alignment (TA) quality should be 1 to 2, indicating the weapon is good for launch. A TAL maneuver should be accomplished if the WEAPON STATUS indicates AL in normal blinking video or the TA quality displays from 3 to 6 indicating a marginal quality; or 7 to 10 indicating unsatisfactory quality, and the TA quality is not displayed in bright underlined video. If weapon navigation is good, the Transfer Alignment Quality/Navigation Solution Quality in Window 5 of the FRMT-7 screen should be displayed in bright underlined video.

6. Pilot's Munitions Consent Panel LOCK/UNLOCK Switch – UNLOCK (P)

Locked status is maintained by not activating the LOCK/UNLOCK switch on the Weapons Control Panel.

Trends In Molecular Diagnostic Technologies

- Labor intense – **more automated**
- Low Throughput – **mid to high throughput**
- No Standardization - **CLSI Guidelines**
- Costly – **Less expensive**
- High complexity – **More turn key**
- Staffing – **Less expertise required**





Advancing Excellence

COMMISSION ON LABORATORY ACCREDITATION

Laboratory Accreditation Program

Molecular Pathology Checklist

Molecular Pathology

OUTLINE

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What's new in Molecular ?

Molecular Microbiology Checklist

- Covers all of molecular testing for infectious diseases – bacterial and viral.
- All FDA cleared (IVD); FDA modified & Lab Developed Tests (LDT) including those using ASR's and RUO's.
- Qualitative, Quantitative and Genotypic tests
- Separate sections for tests using
 - amplification methodology (PCR, signal amplification, hybridization, sequencing)
 - Non-amplification methods (DNA probes, PNA FISH)



Molecular Pathology Checklist (MOL) Required

- Clinical molecular diagnostics testing:
 - Oncology
 - Hematology
 - Inherited diseases
 - ~~Infectious diseases~~
 - HLA typing
 - Parentage
 - Forensics
- Test types
 - All laboratory-developed molecular tests (LDTs)
 - Lab-modified FDA-approved molecular tests
 - FDA-approved molecular tests



Effective 15 June 2009

- NEW CHECKLIST QUESTIONS
 - MOL.34932
 - Sequencing variants
 - MOL.48590
 - Concentration methods for quant. tests
- REVISED CHECKLIST QUESTIONS
 - Mol.34003
 - MOL.36842
 - MOL.39288
 - MOL.48588

 - DELETED
 - MOL.39714

Decision to Set Up a Test

- Is it in your area of interest?
 - Establish a presence in a new field
- Clinical Usefulness
- Volume
 - What volume is enough?
- Cost vs. reimbursement

Reasons For Setting Up Tests

- Factor V Leiden
 - Established as an extension of an existing coagulation test
- Cyclin D1/BCL1
 - Difficulties of distinguishing mantle cell lymphoma from chronic lymphocytic leukemia – initiated by Pathology
- B- and T-cell Gene Rearrangement
 - Clonality assessment
- A number of other tests were established based on current literature and clinical demand
 - JAK2, FLT3

Decision to Set Up a Test

- Personnel
 - Do you have enough people and do they have the proper training and experience?
- Is there instrumentation available?
 - Need to wait for capital budget?
 - Reagent rental/leasing options?
- Lab Space

Inspector Requirements

- Actively practicing molecular scientists preferred
 - Familiar with Checklist
 - Technical and interpretive skills
- CAP provides list of potential regional specialty inspectors in Inspector's packet

Follow the Specimen

Accessioning

DNA/RNA extraction

Microscopy

Probe labeling

Amplification/hybridization

Electrophoresis or
other signal detection

Reporting

Computer entry

Filing

PERSONNEL

- **MOL.40000Phase II**
Is the director of the molecular pathology laboratory a pathologist, board-certified physician in a specialty other than pathology, or doctoral scientist in a biologic science, with specialized training and/or appropriate experience in molecular pathology?
- *NOTE: In the case of forensic identity testing, the above or appropriate degree, training, or experience in forensic science is required.*
- **MOL.40100Phase II**
Is the person in charge of technical operations of the molecular pathology laboratory qualified as one of the following?
 - Person who qualifies as a director
 - CLSp(MB), BS, BA or MT(ASCP) with at least 4 years of experience (at least 1 of which is in molecular pathology methods) under a qualified director
- **MOL.40150Phase II**
Do persons performing the technical aspects of molecular pathology qualify as one of the following?
 - Experienced in the field under the direct supervision of a qualified director or supervisor, and, for laboratories subject to U.S. regulations, qualified to perform high complexity testing
 - MT(ASCP) certified or equivalent
 - BA or BS degree in biologic sciences with appropriate experience in molecular pathology methods
- **MOL.40200Phase I**
Is there an adequate training program for new technologists, and is there a continuing medical laboratory education program?

What to Look For

- Turn-around time
- Competency (technical skills, clinical judgement, communication skills) assessed periodically
 - Direct observation
 - PT results
 - Written exam
 - Look for documentation; sign-off sheets

Know What is in Kit Format

- What does a kit format mean?
 - Food and Drug Administration approved tests (FDA)
 - Analyte specific Reagents (ASR)
 - Research Use Only (RUO) and Investigational Use Only (IUO)

What is in Kit Format? FDA-Approved Tests

- What is an FDA-Approved Test?
 - “In vitro diagnostic (IVD) products are those reagents, instruments, and systems intended for use in diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body” [21 CFR 809.3]
www.fda.gov/cdrh/oivd/guidance/1587.html
 - Like other medical devices, IVDs are subject to premarket and postmarket controls.
- Where to go for a list.
 - FDA’s Office of In Vitro Diagnostic (OIVD) Evaluation and Safety
 - Association of Molecular Pathology (www.amp.org)

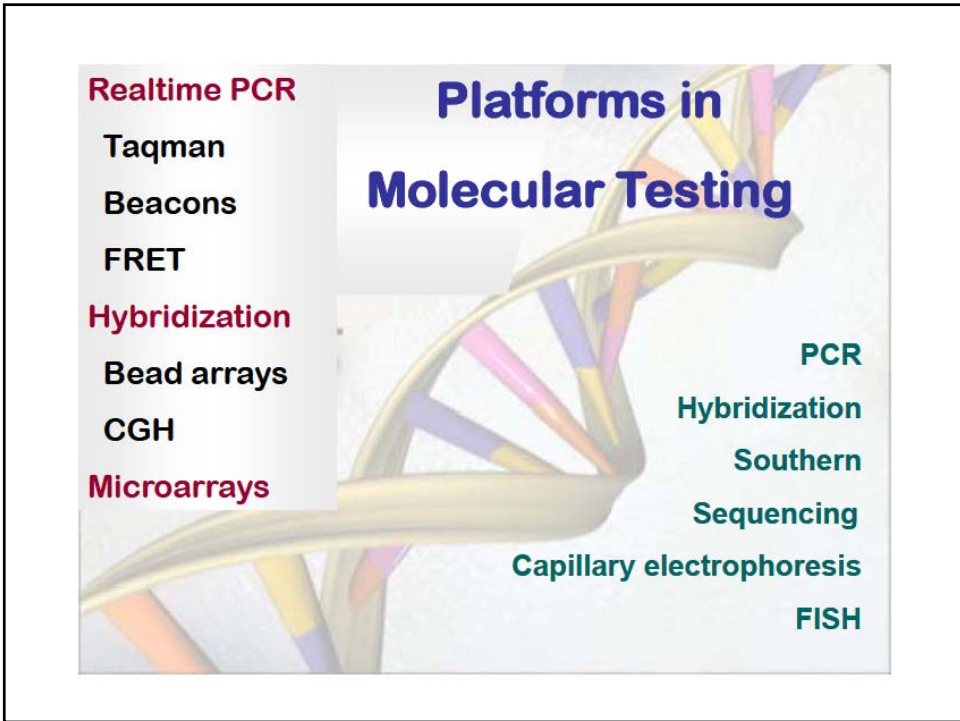
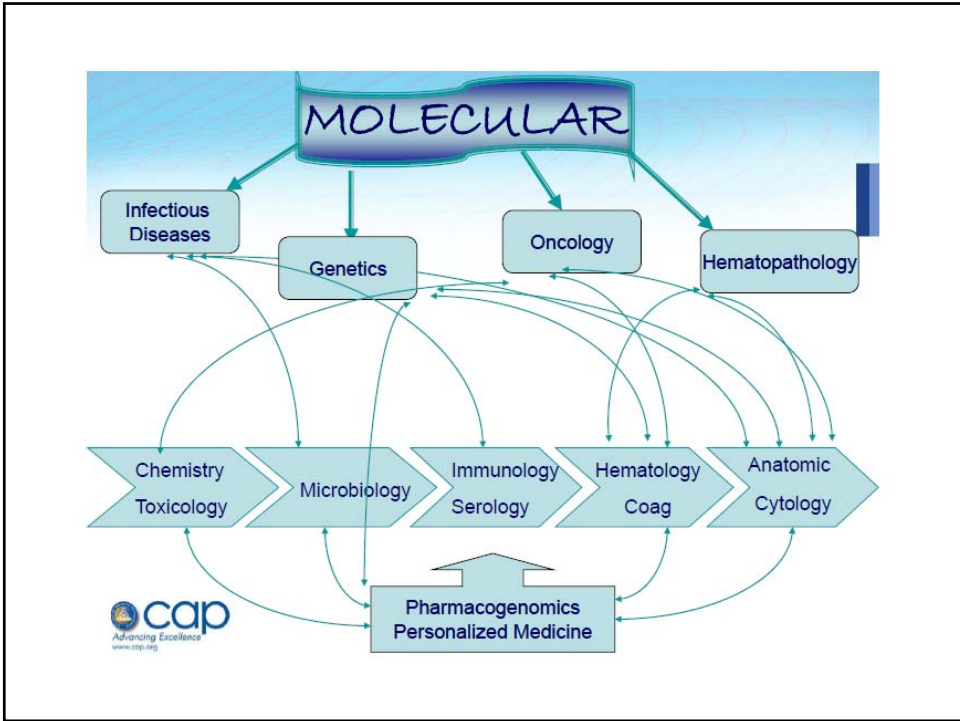
Know What is in Kit Format Analyte Specific reagents (ASR)

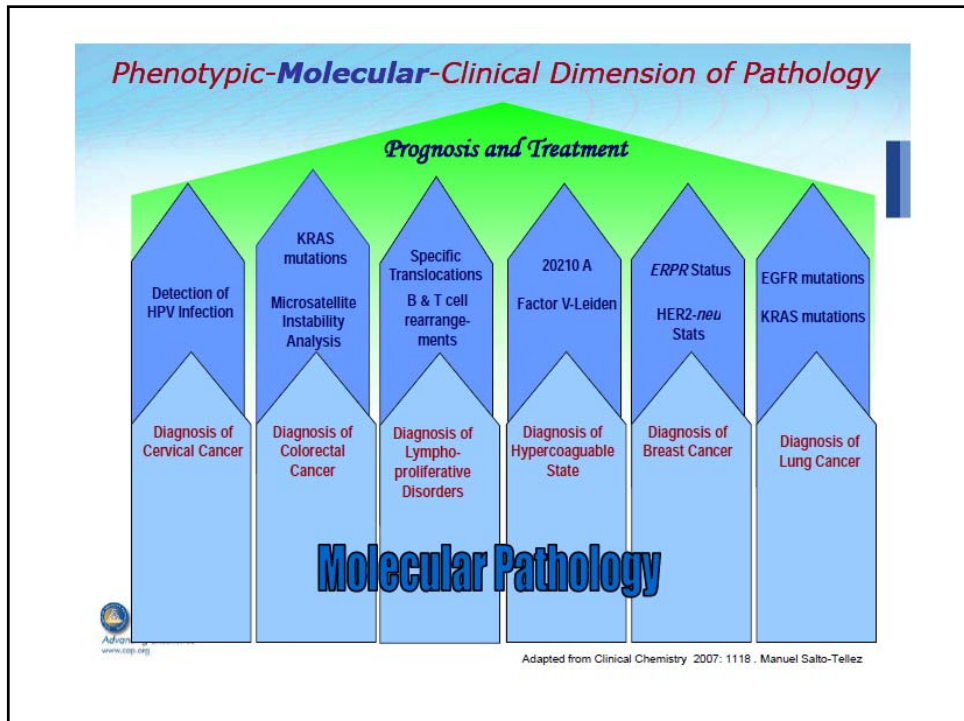
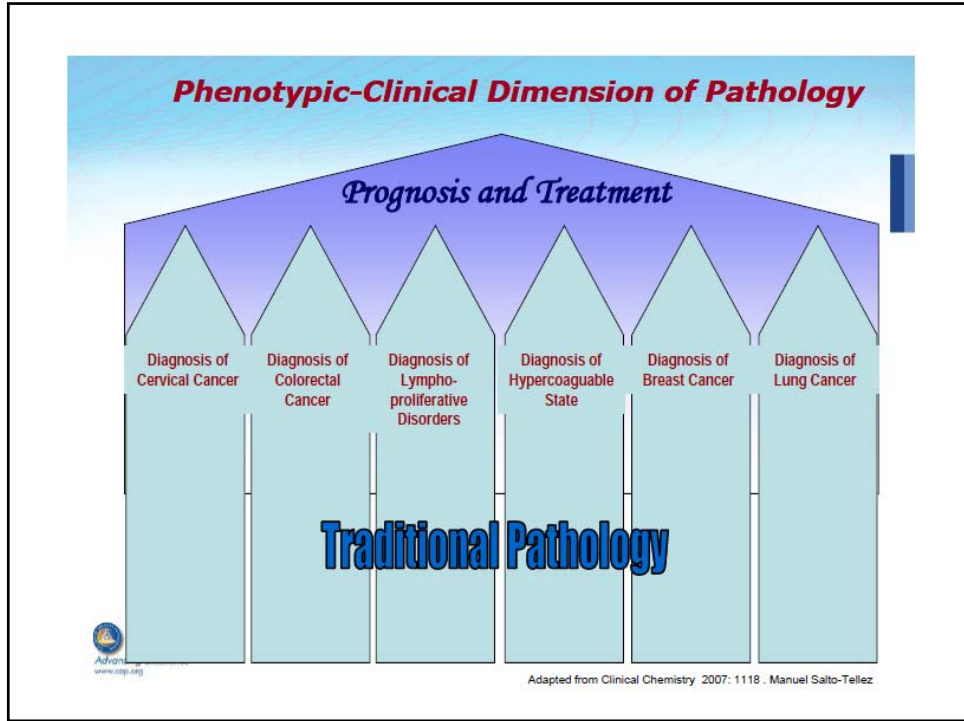
- What is an Analyte Specific Reagent (ASR)
 - ASRs are defined as “antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents, which through specific binding or chemical reactions with substances in a specimen, quantification of an individual chemical substance or ligand in biological specimens.” 21 CFR 864.4020(a).
 - ASRs are intended to be sold as building blocks for use in the design of a diagnostic test by the test developer.
 - ASRs are only sold to CLIA accredited labs and sold with out instructions for their use.
- Where to go for a list
 - A limited number of ASRs can be found in the Device Listing database (<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfRL/listing.cfm>).

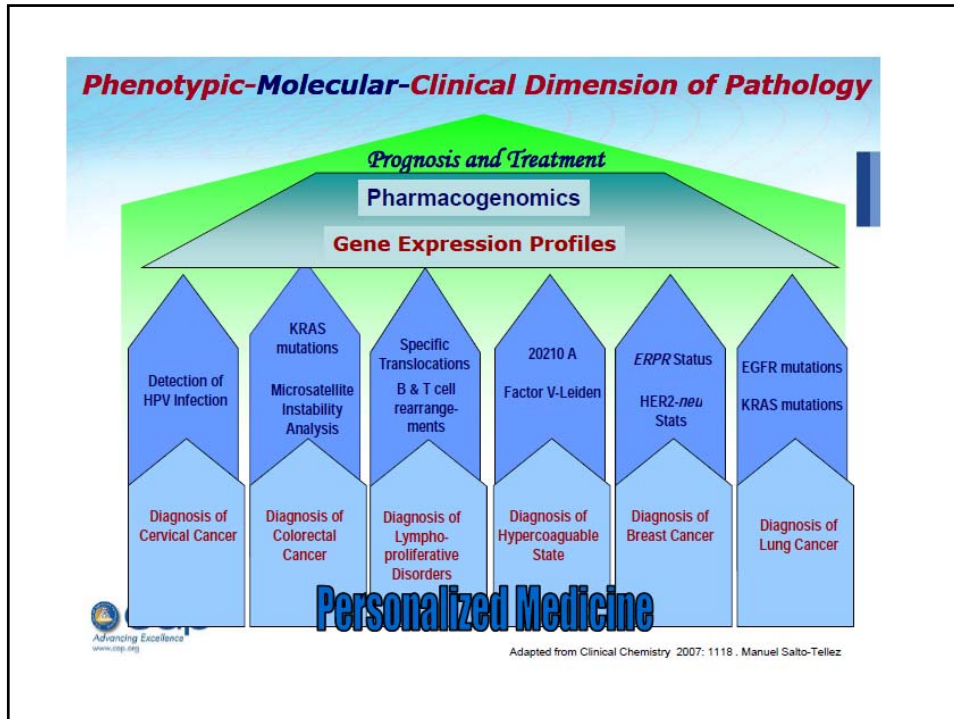
Guidance for Industry and FDA Staff Commercially Distributed Analyte Specific Reagents (ASRs) FDA Website

Know What is in Kit Format RUO Tests

- RUOs “purchased from commercial sources may be used in laboratory-developed tests only if the laboratory has made a reasonable effort to search for IVD- or ASR-class reagents. The results of that failed search should be documented by the laboratory director.”







Lab Developed Tests (LDT)

- LDT's are tests that are
 - developed totally within a CLIA certified laboratory; verified & validated for clinical use.
 - assembled in a CLIA certified laboratory with analytes (ASR's) obtained from a single or multiple sources; verified & validated for clinical use.
- LDTs are performed by the clinical laboratory in which the test was developed

RUO's should be verified & validated with the same rigor as LDT's



CAP's proposal is to include all LDTs in a three-tier 'risk based' oversight model with a graduated system of review, based on a test's potential risk to patients.

Risk-based classification from low → moderate → high involves

- strengthening CLIA accreditation standards on labs using low- and moderate-risk LDTs,
- requiring FDA review of all high-risk LDTs.

Changes would incorporate oversight of claims of clinical validity, and specify scientific and regulatory standards to be applied to all LDTs.

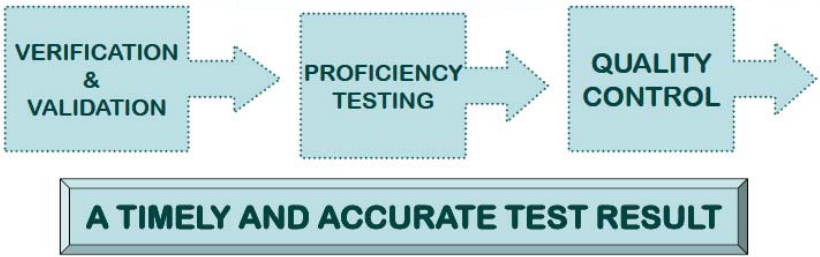
College of American Pathologists Laboratory-Developed Test Oversight Model			
Classification	Determining Factors	Oversight	Examples
Low Risk	The test result is often used in conjunction with other findings to establish diagnosis.	The laboratory internally performs and reviews validation prior to offering for clinical testing. The accreditor during the normally scheduled inspections will verify that the laboratory performed appropriate validation studies.	Cytokeratin Fragile X
	No claim that test result indicates prognosis or direction of therapy. The test presents low risk to patients.		
Moderate Risk	The test result is often used for predicting disease progression or identifying whether a patient is eligible for a specific therapy	The laboratory must submit validation studies to the accreditor for an external review prior to offering the test clinically.	KRAS HER2
	The laboratory may make claims about clinical accuracy or clinical utility. The test has higher risk to patients.		

College of American Pathologists Laboratory-Developed Test Oversight Model

Classification	Determining Factors	Oversight	Examples
High Risk	<p>The test result predicts risk, progression, patient eligibility for a specific therapy, AND;</p> <p>The test uses proprietary algorithms or computations such that the test result cannot be tied to the methods used, or inter-laboratory comparisons can not be performed.</p> <p>The test poses potentially significant risk to patients.</p>	The laboratory must submit a high-risk test to FDA for review prior to offering the test clinically.	Oncotype Dx®



Important considerations in Molecular Testing



Interpretation

Verification:

Rationale: Ensure that the analyte(s) meet their performance specifications

- a one-time process
- verify the analytical performance of a test that is approved by a regulatory body (FDA)
- Often done with some mathematical rigor



Verifying Performance Specifications

- Applies to unmodified, approved test systems
- Laboratory must
 - Demonstrate it can obtain performance specifications comparable to the manufacturer before reporting patient results (analytical)
 - Accuracy
 - Precision
 - Reportable range of test results
 - Verify appropriate reference intervals (normal values) for the laboratory's population (clinical)



Validation:

Rationale: Establish that the analyte fulfills its intended use in its intended environment

- Validate the performance characteristics of laboratory- developed tests
- Examine the components of a test that are assembled for the intended use without regulatory approval. – ASR; RUO
- Establish the analytical performance and clinical (diagnostic) performance of a test as it applies to its intended use
- **An extensive process AN ON-GOING PROCESS**



Validating Performance Specifications

- Applies to
 - Laboratory-developed tests
 - Approved tests that have been modified by the laboratory
- Before reporting patient results, the laboratory must establish performance characteristics for
 - Accuracy
 - Precision
 - Reference and Reportable ranges
 - Analytical sensitivity (LOD) (LOQ)
 - Analytic specificity
 - Interfering substances
 - Diagnostic (Clinical) Sensitivity



Pre-Validation Considerations for the design of Laboratory developed tests

- Stringent design/analysis of primers and probes
- Quality and quantity of extracted nucleic acid
- Appropriate platform for the test
- Commutable calibrators and controls
- Optimization of amplification and detection



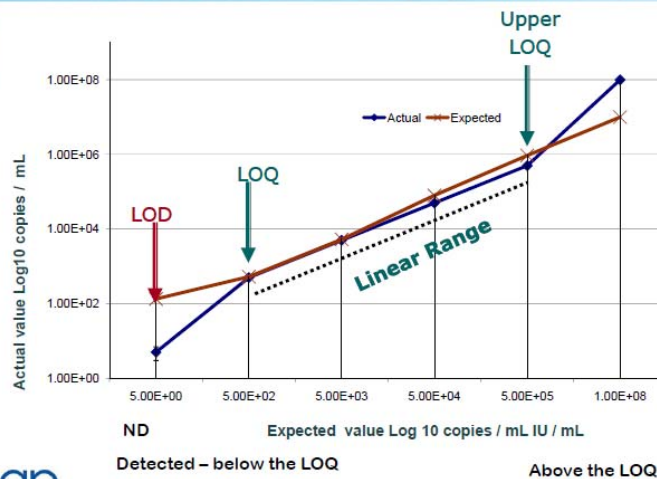
Analytical Sensitivity can be done by..

- Control material of known concentration or copy number (calibrators / standards)
- Dilutions of analyte (microorganism, gene) of known quantity
- Quantify amount of RNA or DNA extracted

Compare sensitivity to a predicate device



Relationship between LOD and LOQ



****NEW**** 06/15/2009

MIC.63275

Phase II

Are acceptability limits defined for all control procedures, control materials, and standards?

NOTE: Acceptability limits must be defined for all control procedures, control materials, and standards. These controls must be appropriate for the range of sensitivities tested and should, ideally, focus on result ranges that are near clinical decision points.



	FDA Cleared	Lab developed Modified, ASR
Accuracy	≥ 20 specimens	≥ 30 specimens
Precision	Positive & Negative controls in triplicate	High Positive and Low positive controls in triplicate Inter and Intra run
Analytical Sensitivity	5-10 specimens in triplicate at LOD and LOQ	Establish LOD and LOQ with 3-5 calibrators run in triplicate 3-5 times (different runs)
Analytical Specificity	Test related analytes	Evaluate interfering substances
Reportable Range	3-5 specimens in triplicate at different concentrations	Establish measuring range and linear range with 3-5 calibrators run in triplicate
Reference Range (Normal)	10 -20 specimens	50 – 100 specimens depending on analyte



Modified from Clinical Microbiology Newsletter 29:12 2007

Clinical (Diagnostic) Validation

Address the clinical significance and utility of the test.

- ✓ Diagnose a disease or disease state
 - ✓ Confirm the results of another laboratory test, or clinical diagnosis
 - ✓ Monitor and assess disease progression, prognosis or resolution
-
- Comparing the test to a gold standard
 - Another test (culture, EIA, chemistry test, FISH)
 - Clinical outcome / endpoint (response to therapy)
 - Use Positive and Negative Predictive values
 - Use ethnic variation and geographic distribution
 - Cite references to clinical studies in the literature



For some disorders, it may not be possible to identify large numbers of positives (i.e., patients with the disorder) to establish the diagnostic sensitivity of the assay.

In such instances, the laboratory should procure as many positive cases as is reasonably possible for method validation and in addition cite any publications that have investigated the diagnostic sensitivity of the assay.



Validation of a modified IVD

Alteration of any process or analyte in an approved test that may effect performance

- Pre-analytical modifications
 - change the collection tube
 - storage conditions
 - different specimen types
- Analytical modifications
 - change test platform or amplification parameters
 - extraction method (manual → automated)
 - increased linear range
- Post-analytical modifications
 - reporting units



Validation of a modified IVD

- Changes in
using the test for another purpose
Diagnostic vs Screening
Qualitative vs Quantitative



Verifying or Validating Performance Specifications

- When multiple instruments are used to perform the same test, the laboratory must compare the performance on both instruments at least twice a year
- If using more than one platform to test the same analyte, the lab should verify or establish performance specifications including agreement.



MOL.31015

Phase II

- Were validation studies with an adequate number and representative (reasonable) distribution of samples performed for each type of specimen expected for the assay (e.g., blood, fresh/frozen tissue, paraffin-embedded tissue, prenatal specimens)?

Verification and Validation studies should be performed on all types of specimens that the test is used for.



Assay Validation

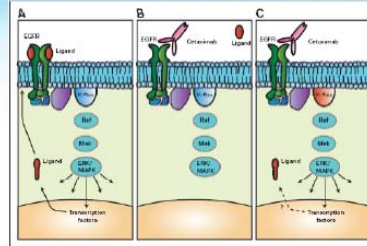
Example - Mutations in KRAS - therapeutic implications

1. Determine the number and type of specimens to be used
 - > 50 specimens for mutation analysis
 - > FFPE or frozen tissue with at least 70% tumor -
2. Select the test to be used for comparison
 - > Mutations in Exons 12 & 13 capillary electrophoresis vs pyrosequencing
3. Document
 - > Accuracy, Precision, Repeatability
4. Establish acceptance criteria for analytical sensitivity
 - > Lowest amount of DNA that can be analyzed
 - > Lowest acceptable amount (percentage) of Tumor cells / DNA that can give the expected result.
5. Evaluate analytical specificity
 - > Test other tumors known to be negative for mutations
 - > Use tumors with more than one mutation



The EGFR pathway and KRAS

The KRAS status of a tumor may be indicative of prognosis and predictive of response to drugs that block EGFR



(A) Binding of ligand to EGFR activates the receptor and triggers signaling through Ras/MAPK pathway leading to proliferation & survival.

(B) In the presence of Cetuximab, ligand binding is prevented, and there is deactivation of EGFR signaling in cells dependent on this pathway.

(C) KRAS mutations can lead to dysregulation of MAPK pathway and constitutive signaling in the absence of ligand binding.

www.ccp.org

Khambata-Ford, et al JOURNAL OF CLINICAL ONCOLOGY 25: 3230 2007.

Outline for Assay Validation

Example - Mutations in KRAS - therapeutic implications

6. Assess (Diagnostic) Clinical performance
 - > Retrospective correlation with clinical data
 - > Cite literature references
7. Resolve discrepancies
 - > Send-out to another facility or reference lab
8. Assess limitations
 - > Lowest detectable % of detected tumor to call Positive
 - > Detection of more than one mutation
9. Set the parameters for interpretation of results
 - > Exons 12 & 13. Mutations in codon 61
10. Summarize all of the above in a "Validation document"
 - > Expand on each section
 - > Statement on acceptance of validation for clinical use
 - > Review & signature by Director

Multiplex tests in Molecular Pathology

Considerations...

- Number of targets in a single reaction
- PCR efficiency of the targets
- Probes labeling
- Ability to differentiate targets
- Competition of targets
- Positive controls for multiplex assays

MOL.34229

Phase II

For QUALITATIVE tests, are positive, negative and sensitivity controls run for each assay, when available, appropriate, and practical?

Ideally, one should use a positive control for each analyte in each run.

In some circumstances - large multiplex panel one can rotate positive controls in a systematic fashion.

The sensitivity control is used to verify detection of low-level target sequences, e.g., pathogens or tumor markers.



CAP Laboratory Improvement Programs

(Arch Pathol Lab Med. 2009;133:743–755)

Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests

Lawrence Jennings, MD, PhD; Viviana M. Van Deerlin, MD, PhD; Margaret L. Gulley, MD;
for the College of American Pathologists Molecular Pathology Resource Committee

• **Context.**—The use of DNA- and RNA-based tests continues to grow for applications as varied as inherited disease, infectious disease, cancer, identity testing, human leukocyte antigen typing, and pharmacogenetics. Progress is driven in part by the huge growth in knowledge about the molecular basis of disease coupled with advancements in technologic capabilities. In addition to requirements for clinical utility, every molecular test also may have limitations that must be carefully considered before clinical implementation. Analytic and clinical performance characteristics, as well as test limitations are established and documented through the process of test validation.

• **Objective.**—To describe the established principles of test validation, along with relevant regulations in the United States, in order to provide a rational approach to introducing molecular tests into the clinical laboratory.

• **Data Sources.**—PubMed review of published literature, published guidelines, and online information from national and international professional organizations.

• **Conclusions.**—These resources and recommendations provide a framework for validating clinical tests.

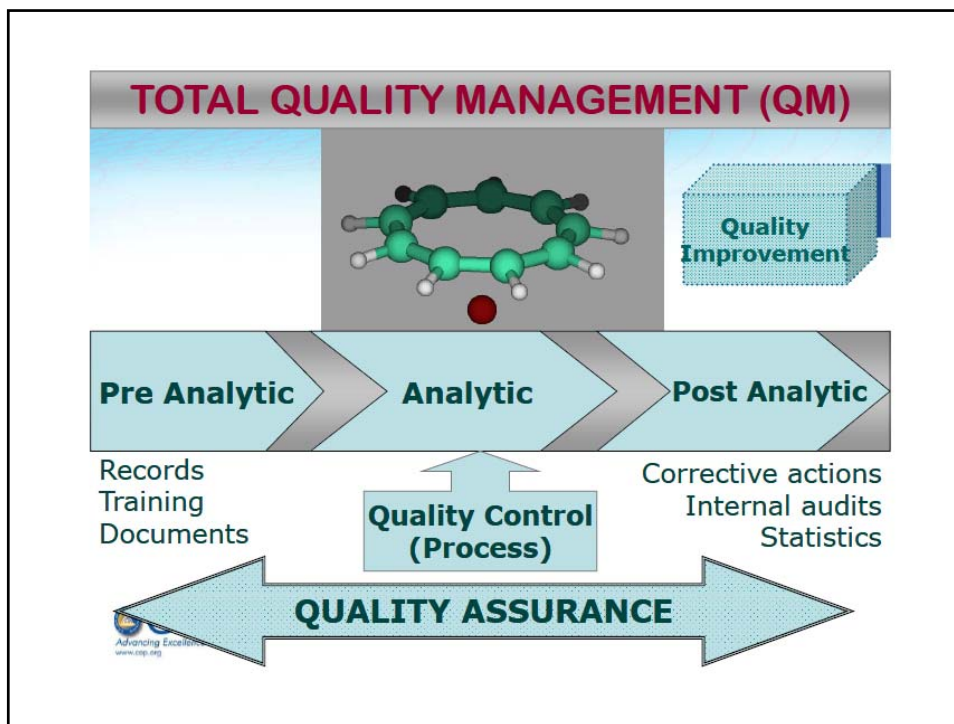
(Arch Pathol Lab Med. 2009;133:743–755)



Proficiency Testing in Molecular Pathology

- CAP Proficiency Surveys
- Alternate Proficiency
 - Inter-laboratory comparison
 - Send-out to another / reference lab
 - In-house proficiencies (Blind panel, split sample analysis)
 - Comparison with another method (gold standard)
- How many specimens ???
 - Assay dependent (expense?)
 - Volume dependent (# of tests ↑)
 - Types of specimens / matrix
- In general three events with 3-5 samples / year
- Evaluation criteria
 - < 80% passing grade in 2 consecutive PT's = PROBLEM !!





Challenges in Quality Management in Molecular Diagnostics

- Technology driven field
- Transition from research to clinical laboratories
- Rapid evolution and change of technologies & applications; emerging pathogens; new mutations and associations with disease
- Paucity of material for validation and standardization
- Non-uniformity of calibrators and controls

Challenges in Quality Management in Molecular Diagnostics

- Limited quality assurance / proficiency programs
- Clinical validation can be challenging
- Limited diagnostic testing guidelines
- Results for the same test use different methodologies that may not be in agreement
- Lack of concordance on units of reporting
- Interpretation can sometimes be difficult
- Some esoteric tests performed in a single lab



Challenges in Quality Assurance in Molecular Diagnostics

- Since most technologies involve amplification technical precision is mandatory
- Contamination of specimens, equipment reagents and aerosol contamination are legitimate problems
- Samples can be inhibitory to amplification
- Mutations in the nucleic acid can interfere with testing
- Discrepancies could be an issue





QA / QC is an ongoing process that affirms verification & validation.

It needs to be monitored on a regular basis for trends

You need to be in touch



Quality Monitors

- **Molecular tests are multi-step processes extraction → amplification → analysis**
- **Monitors provide information on the analytical performance of the test at all stages**
- **Assist in troubleshooting and help identify contamination events**

QC Monitors are selected to assess....

Pre-analytical process

- ❖ Specimen integrity (DNA, RNA)

Analytical process

- ❖ Sample prep, amplification, detection
- ❖ Calibrators & Controls

Post-analytical process

- ❖ Documentation, interpretation, reporting



QC at the PREANALYTICAL LEVEL



- Requisition forms
Ethnic information and consent for genetic tests
- Specimen collection
Test specific tubes & containers (PPT); swabs
Storage specs until transport (separate plasma)
- Specimen transport & storage
Temperature, Conditions
- Specimen Handling / Processing
Speed & temp of centrifugation



Preanalytical monitors

- Time of collection → Time of transport
- Time of collection → Time of processing
- Collection tube / container (EDTA..Heparin..
Tissue collection & transport
- Storage conditions

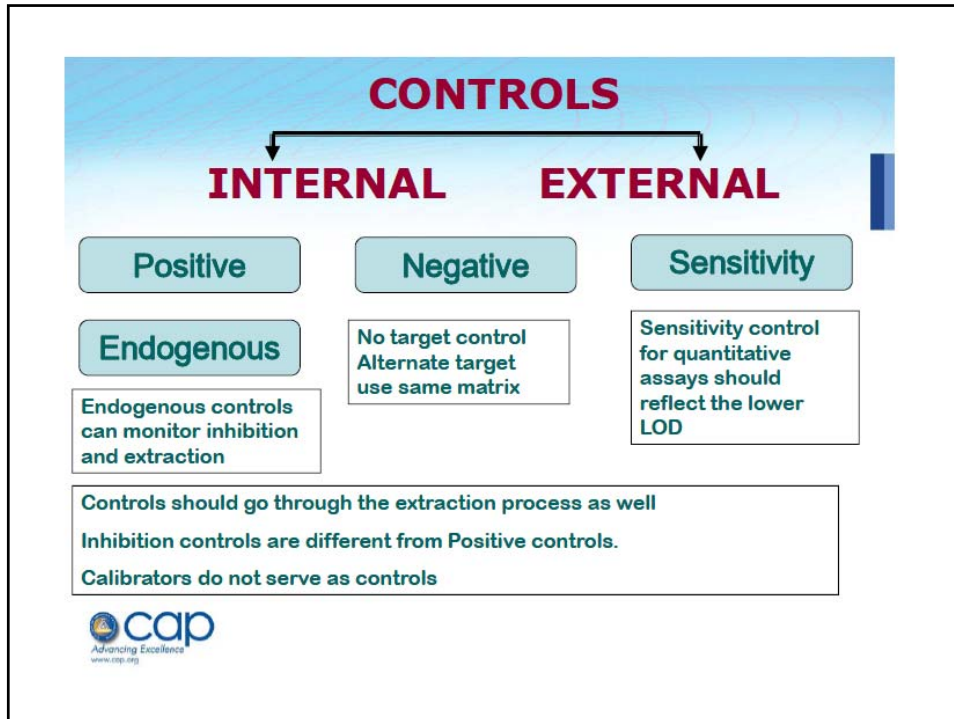


QC at the ANALYTICAL LEVEL



- Proficiency testing
- Use of Positive, Negative and Sensitivity controls
- Use internal, external, extraction & endogenous controls (inhibitors)
- Contamination control






Other analytic QC monitors

- Melting curve temperature- T_m range ($\pm 2^{\circ}\text{C}$)
- Blank OD values
- For realtime PCR monitor Ct values of controls (qual or quant)
- Lot – Lot check
- Failed runs /reactions

Instrument QC

Amplification machines; Laser driven instruments (realtime, sequencers, bead arrays)



QC at the POST-ANALYTICAL LEVEL

Interpretation of Results:

- Molecular tests can be subjective**
- Have more than one person review results**

Difficult problems:

- Discrepant analysis**
- Blips at the end of an amplification curve**

Reporting in different units of measure

- Copies/mL; IU/mL; genome eq/mL**



QC monitors Post Analytical

Turn around time

Notification of rapid tests

Records of quantitative tests when used for monitoring e.g. Viral Load

Record all new / rare mutations or variations in a notebook

Correlation between tests

Errors in reporting



MIC.63252

Phase I

When appropriate, are appropriate statistics (e.g., percentage of results that are positive for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*) maintained and monitored?

NOTE: An increase above the expected positive rate within a run or over multiple runs should prompt investigation for potential false positive results.

****NEW**** 06/15/2009

MIC.63256

Phase I

Is there evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test?

NOTE: There are certain clinical situation in which rapid completion is essential. An example is detection of HSV in CSF.



****NEW**** 06/15/2009

MOL.34932 Phase I

Does the laboratory follow professional guidelines for interpretation of sequence variation?

NOTE: The laboratory should have an algorithm for decision-making in interpretation of pathogenic variants, benign variants and variants of unknown clinical significance

MOL.48590

Phase I

Are concentration methods for quantitative tests verified?

NOTE: Methods used to concentrate specimens for analysis must be verified at specified, periodic intervals



****REVISED**** 06/15/2009

Is standard nomenclature used to designate genes and mutations?

NOTE: Whenever possible, human genes, loci and mutations should be designated according to standard nomenclature as defined in the references below. Where a common name is also in wide use in the medical literature, it may also be given in the report to improve clarity and prevent misunderstanding. One approach is to put the common name in the diagnosis section of the report, and the standard name in the methods section or in an interpretive comment.

REFERENCES

- 1) Wain HM, *et al.* Guidelines for Human Gene Nomenclature. *Genomics*. 2002;7:464-470
- 2) den Dunnen JR, *et al.* Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion. *Human Mutation*. 1999;15:7-12



QC SUMMARY

Continual Quality Control required

Documentation of all QC monitors on a regular basis – monthly

- **Graphing of control values**
- **In-house control in quantitative tests for reagent and tech variation**
- **Melting temperatures for variants**
- **Ct value for probe integrity**
- **Background OD for contamination**



Overall.....??

Does the test perform the way it was intended to?

Would you use your test to help your clinician make a decision for you or your family member ??



Assistance

<http://www.cap.org>

Email: accred@cap.org

800-323-4040, ext. 6065



Documents for reference

- CAP Molecular Checklist
 - www.cap.org
- CLSI (NCCLS) Approved Guidelines
Clinical Laboratory Standards Institute
 - www.clsi.org
- AMP – List of FDA approved tests
Association for Molecular Pathology
 - www.amp.org
- American College of Medical Genetics
 - www.acmg.net



CAP Laboratory Improvement Programs

(Arch Pathol Lab Med. 2009;133:743–755)

Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests

*Lawrence Jennings, MD, PhD; Viviana A. Van Deerlin, MD, PhD; Margaret L. Gallely, MD;
for the College of American Pathologists Molecular Pathology Resource Committee*

Context.—The use of DNA- and RNA-based tests continues to grow for applications as varied as inherited disease, infectious disease, cancer, identity testing, human leukocyte antigen typing, and pharmacogenetics. Progress is driven in part by the huge growth in knowledge about the molecular basis of disease coupled with advancements in technologic capabilities. In addition to requirements for clinical utility, every molecular test also may have limitations that must be carefully considered before clinical implementation. Analytic and clinical performance characteristics as well as test limitations are established and documented through the process of test validation.

Objective.—To describe the established principles of test validation, along with relevant regulations in the United States, in order to provide a national approach to introducing molecular tests into the clinical laboratory.

Data Sources.—PubMed review of published literature, published guidelines, and online information from national and international professional organizations.

Conclusions.—These resources and recommendations provide a framework for validating clinical tests.
(Arch Pathol Lab Med. 2009;133:743–755)



CLSI guidelines @ www.clsi.org

MM01-A2	Molecular Diagnostic Methods for Genetic Diseases
MM02-A2	Immunoglobulin and T-Cell Receptor Gene Rearrangement Assays
MM03-A2	Molecular Diagnostic Methods for Infectious Diseases
MM05-A	Nucleic Acid Amplification Assays for Hematopathology
MM06-A	Quantitative Molecular Methods for Infectious Diseases
MM07-A	Fluorescence In Situ Hybridization (FISH) Methods
MM09-A	Nucleic Acid Sequencing
MM10-A	Genotyping for Infectious Diseases
MM11-P	Molecular Methods for Bacterial Strain Typing
MM12-A	Diagnostic Nucleic Acid Microarrays
MM13-A	Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods
MM14-A	Proficiency Testing (External Quality Assessment) for Molecular Methods
MM16-P	Use of External RNA Controls in Gene Expression Assays

Common Phase II Deficiency

MOL.34106

Phase II

Are reagents and solutions properly labeled, as applicable and appropriate, with the following elements:

1. Content and quantity, concentration or titer,
2. Storage requirements,
3. Date prepared or reconstituted by lab,
4. expiration date?

Revised- MOL.34003

- Phase II
- If the laboratory uses more than one instrument/method to test for a given analyte, are the instruments/methods checked against each other at least twice a year for correlation of results?

Revised - MOL.39288

- Phase II
- Are photographic or digitized images retained for documentation of all FISH assays (at least one cell for assays with normal results and at least two cells for assays with abnormal results)?
- *NOTE: For assays where multiple chromosomal loci (>2) are targets in part of a single test, an image of at least one cell must be retained for documentation of each target. Images of at least two cells are required to document all abnormalities. Images of FISH assays must be retained for 10 years.*

Checklist Questions

MOL.36025

Phase II

If patient testing is performed using Class I analyte-specific reagents (ASRs) obtained or purchased from an outside vendor, does the patient report include the disclaimer required by federal regulations?

NOTE: Laboratories must include an FDA-required disclaimer on reports for tests using analyte-specific reagents, whether obtained commercially or produced in-house.

Checklist Addition, cont.

The mandatory language is "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." The CAP recommends additional language, such as "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 (CLIA-88) as qualified to perform high complexity clinical laboratory testing."

Checklist Addition

Quality Control

Phase II

- 38400 Acceptance limits all QC materials/standards
- 38550 QC verified before reporting results
- 38700 Corrective Action when QC exceeds limits
- 38850 QC tested in same manner as patient

CAP

Laboratory Accreditation Program

Common Phase II Deficiencies

MOL.33450

- **If aliquoting of specimens is performed, is there a written procedure to prevent any possible cross-contamination of the specimens?**
- *NOTE: Although in some cases it may be appropriate to aliquot a specimen, the laboratory must have a policy that no aliquot is ever returned to the original container.*

COMMENTARY:

The laboratory must have a policy that no aliquot is ever returned to the original container.

MOL.34065

- **Are new reagent lots and/or shipments checked against old reagent lots or with suitable reference material before or concurrently with being placed in service?**
- *NOTE: For qualitative tests, minimum cross-checking includes retesting at least one known positive and one known negative patient sample from the old reagent lot or shipment against the new reagent lot, ensuring that the same results are obtained with the new lot. For quantitative assays, several patient samples should be run at different levels to check the calibration of the system. Good clinical laboratory science includes patient-based comparisons in many situations, since it is patient results that are "controlled". A weakly positive control should also be used in systems where patient results are reported in that fashion. The use of QC material to check new reagent lots/shipments is acceptable, but the laboratory should be aware that matrix interference may affect such material and mask a change in patient results.*

MOL.40666
MOL.41132

Is there evidence of ongoing evaluation of instrument maintenance and function, temperature, etc., for all procedures as required?

Are there documented standard procedures for set-up and normal operation of instruments?

NOTE: All laboratory instruments and equipment must be maintained in a manner consistent with safe and reliable testing. The laboratory should have an organized system for monitoring and maintaining all instruments. The procedures and schedules for instrument maintenance must be as thorough and as frequent as specified by the manufacturer. Since some equipment has no standard frequency or extent of maintenance, each laboratory should establish a maintenance schedule that reasonably reflects the workload and specifications of its equipment. All servicing and repairs must be documented. The Inspector should identify the specific instruments involved in any deficiency during the summation conference.

MOL.31200

Are enzymatic amplification procedures (e.g., PCR) designed to minimize carry over (false positive results) using appropriate physical containment and procedural controls?

NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that the laboratory take special precautions.

MOL.34875

Are nucleic acid amplification procedures (e.g., PCR) designed to minimize carry over (false positive results) using appropriate physical containment and procedural controls?

- *NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that the laboratory take special precautions. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimize aerosolization. In a given run, specimens should be ordered in the following sequence: patient samples, positive controls, negative controls (including "no template" controls in which target DNA is omitted and therefore no product is expected). Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.*

MOL.10160

For tests for which CAP does not require PT, does the laboratory at least semiannually 1) participate in external PT, or 2) exercise an alternative performance assessment system for determining the reliability of analytic testing?

NOTE: Appropriate alternative performance assessment procedures may include: split sample analysis with reference or other laboratories, split samples with an established in-house method, assayed material, regional pools, clinical validation by chart review, or other suitable and documented means. For FISH testing, alternative assessment may be performed by method and specimen type, rather than for each tested abnormality (i.e., one program for all FISH cytogenetics tests performed on cell suspensions).

It is the responsibility of the laboratory director to define such alternative performance assessment procedures, as applicable, in accordance with good clinical and scientific laboratory practice. Participation in ungraded/educational proficiency testing programs also satisfies this checklist question.

Semiannual alternative assessment must be performed on tests for which PT is not available.

MOL.36025

If reports are based on the use of reagents labeled by the manufacturer as "analyte-specific reagents", is the federally-required clarifying statement part of the report?

NOTE: Laboratories must include an FDA-required disclaimer on reports for tests using analyte-specific reagents, whether obtained commercially or produced in-house. The mandatory language is "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."

MOL.36025

NOTE, continued: The CAP recommends additional language, such as "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 (CLIA-88) as qualified to perform high complexity clinical laboratory testing."

MOL.31350

Are appropriate internal or external validation studies referenced?

REFERENCE: Association for molecular pathology statement. Recommendations for in-house development and operation of molecular diagnostic tests. *Am J Clin Pathol.* 1999;111:449-463.

MOL.36000

Does the final report include an appropriate summary of the methods, probes and endonucleases used, the loci or mutations tested, the objective findings and a clinical interpretation in an easy-to-interpret format?

MOL.10150

Does the laboratory participate in an appropriate available CAP or CAP-approved alternative interlaboratory comparison program for the patient testing performed?

CAP

Laboratory Accreditation Program

Common Phase I Deficiencies

MOL.50150

**Is there adequate space for technical work
(bench space)?**

MOL.20550

**Are statistics on all molecular pathology
laboratory results (e.g., percentages of
normal and abnormal findings, allele
frequencies) maintained, and appropriate
comparative studies performed?**

MOL.20600

Are these statistics (e.g., percentages of normal and abnormal findings, allele frequencies) and comparative studies reviewed at regular intervals by the Laboratory Director or designee and appropriate corrective action taken, if indicated?

Commentary: This process may detect trends, systematic errors, or local population variations that may affect test results or interpretation.

MOL.39890

When possible, are individual wells (or a representative sample thereof) of thermocyclers checked for temperature accuracy before being placed in service and periodically thereafter?

NOTE: If it is not physically possible to check individual wells, a downstream measure of well-temperature accuracy (such as productivity of amplification) may be substituted to functionally meet this requirement.

Space Deficiencies

Is there adequate space for:

- **Instruments** **1.3%**
- **Administrative functions** **1.1%**
- **Shelf storage** **1.1%**
- **Clerical work** **1.0%**

MOL.33360

Are there written criteria for questioning or rejection of clinically inappropriate test requests?

NOTE: For every test offered, there should be documentation describing the appropriate (and inappropriate) clinical indications.

MOL.33360

COMMENTARY: Many of the disease genes subject to molecular genetic testing are extremely complex as to their size, mutational heterogeneity, penetrance, and expressivity. Especially with regard to presymptomatic testing, application of these tests to patients not carefully screened and counseled can be meaningless or damaging. For certain tests, only those patients with strict family history criteria are eligible. There are also many ethical considerations, such as the policy of not offering predictive genetic tests to children unless there is a viable clinical intervention to be initiated prior to adulthood.

MOL.33360

COMMENTARY, continued:

Because primary care physicians may not be conversant with these matters, it is sometimes left to the molecular diagnostic laboratory to provide consultation. The laboratory therefore should have established guidelines for the rejection or questioning of test requests felt to be inappropriate on clinical or ethical grounds. Reference to the policies promulgated by professional organizations and government agencies, and/or consultation with a medical ethicist, may be of help on a case-by-case basis.

MOL.40200

Is there an adequate training program for new technologists, and is there a continuing medical laboratory education program?

iPassport- Document Control



The screenshot displays the Genial Genetics website. The header features the Genial logo and the tagline "Genial Genetics Innovation for Genetics and Pathology Professionals". A navigation menu includes links for News, Products, Support, Forums, Partners, and Find Us. The main content area is titled "Laboratory Document Management" and describes the iPassport QMS system. A sidebar on the left lists various products, with "iPassport QMS" highlighted.

Genial Genetics
Innovation for Genetics and Pathology Professionals

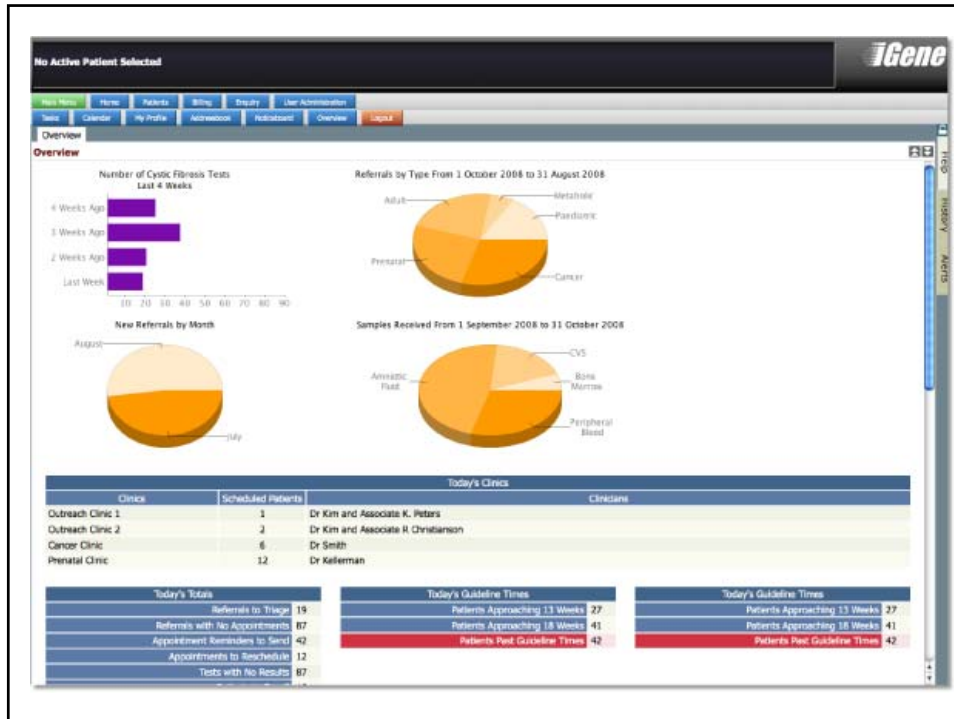
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(New) iGene Genetic Database
iPassport QMS
mWare HL7 Integration Engine
Shire Genetic Database
Cytogenetic Automation
Procell Reagents

Laboratory Document Management

iPassport provides complete Laboratory Document Management for SOPs, Policies, General Documentation and COSHH control. Using advanced pdf functionality, the system can easily create 'View Only' .pdf documents from Word™ and other .doc files. The system also allows users to control the versioning of other documents, such as Risk Assessment records or other laboratory documents of their choice. By recording and storing each version of the laboratory document, iPassport provides a complete audit trail which can be quickly demonstrated to an inspector.

Controlled document authorisation can only be carried out by higher-access users and the system provides the option of automatic authorisation (MFA) for this, enhancing the security of the laboratory.



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- Thank you for your attention!