Standards of Operation and Best Practices for Future Biomarker Evaluation

Assuring Quality with New Biomarkers

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Criteria for Assessment of Novel Biomarkers

1. Can the clinician measure the biomarker?

1. Measure

2. Does the biomarker add new information?

2. More

3. Will the biomarker help the clinician to manage patients?

3. Manage

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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)

Standards of Operation defined by CLIA
Sec. 493.1445 (of Federal Register) Standard Laboratory Director responsibilities.

- The laboratory director is responsible for the overall operation and administration of the laboratory, including the employment of personnel who are competent to perform test procedures, record and report test results promptly, accurately and proficiently, and for assuring compliance with the applicable regulations.
College of American Pathologists has ‘deemed status’ from CMS under CLIA
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
Other Items

MOL.40200 Training/CME

MOL.41042 (2) Refrigerator/freezer temperatures are checked and recorded daily.

MOL.05075 (1) The laboratory's current CAP Activity Menu accurately reflects the testing performed.

MOL.20300 (1) There is evidence that the laboratory monitors sample turnaround times that are appropriate.

MOL.20550 (1) Validation studies document test accuracy, analytical sensitivity, analytical specificity and precision.

MOL.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.
Three most important issues for Best Practice

- Validation / Documentation

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Best Practice Programs Define and Evaluate

- Pre-analytical phase
- Analytical phase
- Post-analytical phase
## Preanalytic Phase: Specimen Collection Defined

<table>
<thead>
<tr>
<th>Additive</th>
<th>Color</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Red</td>
<td>Chemistry, serum, viral antibody studies</td>
</tr>
<tr>
<td>Lithium heparin (freeze dried)</td>
<td>Green</td>
<td>Immunology, virology studies</td>
</tr>
<tr>
<td>Sodium heparin</td>
<td>Brown</td>
<td>Cytogenetic studies, molecular studies</td>
</tr>
<tr>
<td>Tripotassium EDTA (7.5-15% solution)</td>
<td>Lavender</td>
<td>Hematology, Virology, molecular biology</td>
</tr>
<tr>
<td>Acid citrate dextrose (ACD) solution</td>
<td>Yellow</td>
<td>Molecular biology</td>
</tr>
</tbody>
</table>
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. **All of which must be validated.**
Preanalytic Phase: Specimen Accession

- Pre-analytical = 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.
Analytical Phase

- SOPs
- Extraction and specimen storage
- Contamination control
- Laboratory design
- Laboratory practices (i.e. aliquotting reagents, carry-over prevention)
- Controls (Monitor Method Performance)
- 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** 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

• 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.
Analyte Specific Reagent (ASR)

• 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 are 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.
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.”

Some institutions 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.”
What It Takes for Best Practices

You need a plan!

Commitment  Space/Facilities  Equipment  Staff  Culture

- EB Practice
- Validation
- Consistency
- Sustained Quality

Thank You!

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