Molecular Laboratory Design, QA/QC Considerations

Rachel Lee, Ph.D.
Texas Department of State Health Services

NBS Molecular Training Workshop
March 12, 2015

Quality Management Components

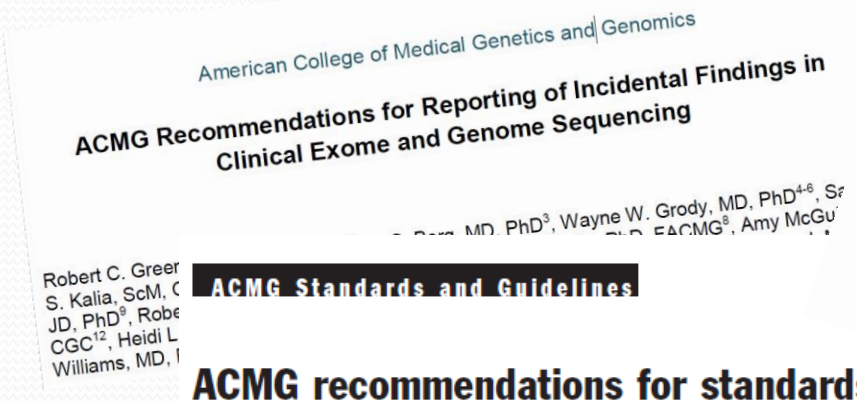
- Organization
- Personnel
- Documents and Records
- Advisory Services
- Laboratory Equipment
- Purchasing, Inventory, and Evaluation of Vendor Qualification
- Process Management
- Information Management
- Nonconforming Event Management
- Assessments
- Continual Improvement
- Facilities, Environment, and Safety
- Use of Referral Laboratories

Laboratory Regulatory and Accreditation Guidelines

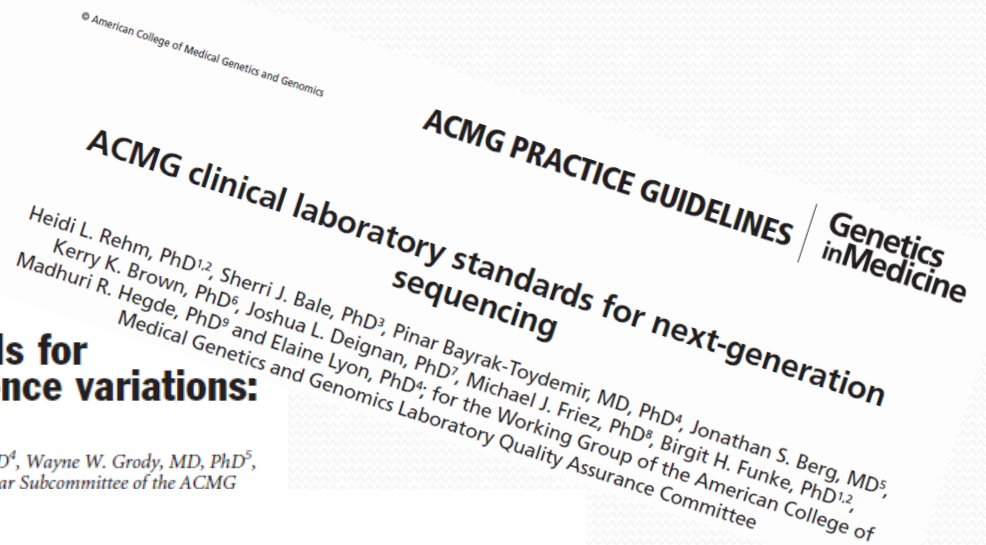
- US Food and Drug Administration (FDA):
  - Approves kits and reagents for use in clinical testing
  - Proposed oversight for Laboratory Developed Test
- Clinical Laboratory Improvement Amendments (CLIA):
  - Regulations passed by Congress 1988 to establish quality standards for all laboratory testing to ensure the accuracy, reliability and timeliness of patient test results regardless of where the test was performed
- College of American Pathologists (CAP):
  - Molecular Pathology checklist
- State Specific Regulations
  - NY Clinical Laboratory Evaluation Program (CLEP)

Professional Guidelines

- American College of Medical Genetics (ACMG)


American College of Medical Genetics and Genomics

ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing


© American College of Medical Genetics and Genomics

ACMG PRACTICE GUIDELINES | Genetics inMedicine

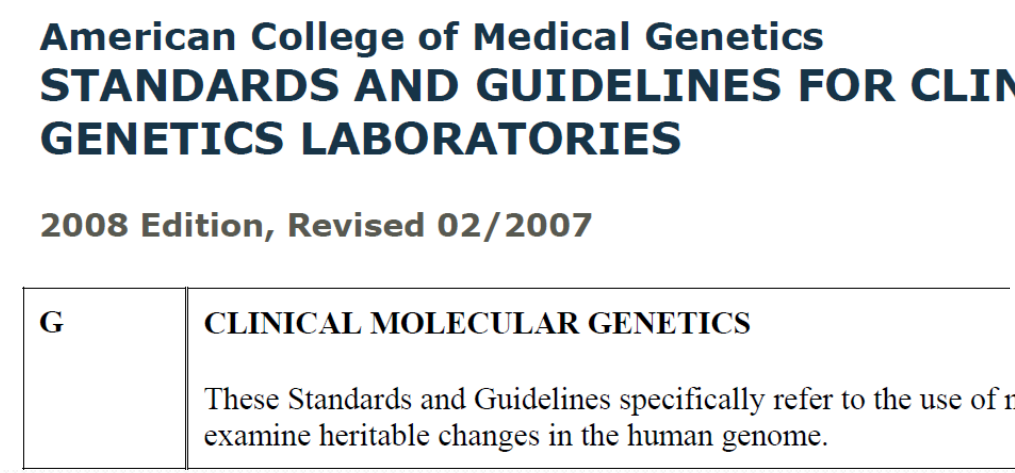
ACMG clinical laboratory standards for next-generation sequencing

Heidi L. Rehm, PhD[1,2], Sherri J. Bale, PhD[3], Pinar Bayrak-Toydemir, MD, PhD[4], Jonathan S. Berg, MD[5], Kerry K. Brown, PhD[6], Joshua L. Deignan, PhD[7], Michael J. Friez, PhD[8], Birgit H. Funke, PhD[1,2], Madhuri R. Hegde, PhD[9] and Elaine Lyon, PhD[4]; for the Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee


ACMG Standards and Guidelines

ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007

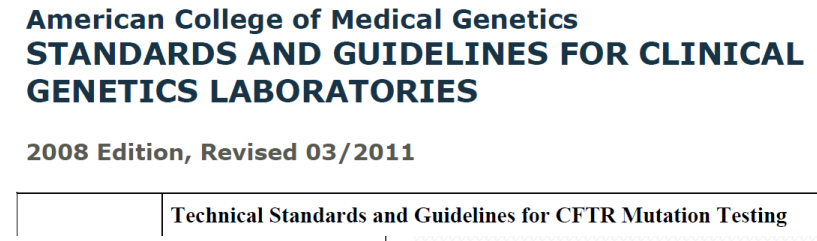
C. Sue Richards, PhD[1], Sherri Bale, PhD[2], Daniel B. Bellissimo, PhD[3], Soma Das, PhD[4], Wayne W. Grody, MD, PhD[5], Madhuri R. Hegde, PhD[6], Elaine Lyon, PhD[7], Brian E. Ward, PhD[8], and the Molecular Subcommittee of the ACMG


American College of Medical Genetics

STANDARDS AND GUIDELINES FOR CLINICAL GENETICS LABORATORIES

2008 Edition, Revised 02/2007

G	CLINICAL MOLECULAR GENETICS These Standards and Guidelines specifically refer to the use of molecular techniques to examine heritable changes in the human genome.


American College of Medical Genetics

STANDARDS AND GUIDELINES FOR CLINICAL GENETICS LABORATORIES

2008 Edition, Revised 03/2011

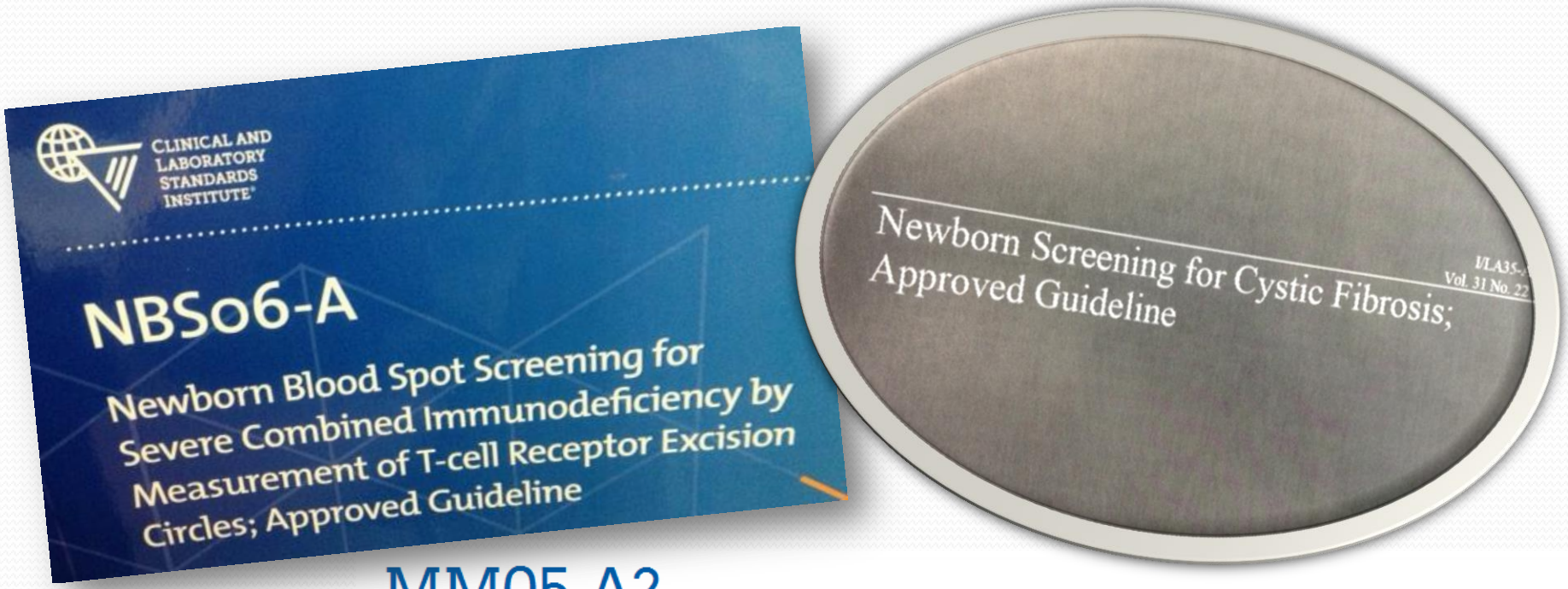
	Technical Standards and Guidelines for CFTR Mutation Testing

Professional Guidelines

- Clinical and Laboratory Standards Institute (CLSI)

Professional Guidelines

- Clinical and Laboratory Standards Institute (CLSI)



MM05-A2

Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline—Second Edition

Preview Sample Pages

This guideline addresses the performance and application of assays for gene rearrangement and translocations by both polymerase chain reaction (PCR) and reverse-transcriptase PCR techniques, and includes information on specimen collection, sample preparation, test reporting, test validation, and quality assurance.

Contamination

- Introduction of unwanted nucleic acids into specimen
  - the sensitivity of PCR techniques makes them vulnerable to contamination

- Repeated amplification of the same target sequence leads to accumulation of amplification products in the laboratory environment
  - A typical PCR generates as many as 10^9 copies of target sequence
  - Aerosols from pipettes will contain as many as 10^6 amplification products
  - Buildup of aerosolized amplification products will contaminate laboratory reagents, equipment, and ventilation systems

Potential Sources of Contamination

- Cross contamination between specimens
- Amplification product contamination
- Laboratory surfaces
- Ventilation ducts
- Reagents/supplies
- Hair, skin, saliva, and clothes of lab personnel

What happens if lack of contamination control

- Incorrect results
- Require extensive cleanup
- Loss of creditability
- Impact on financial and performance

How to Control Contamination

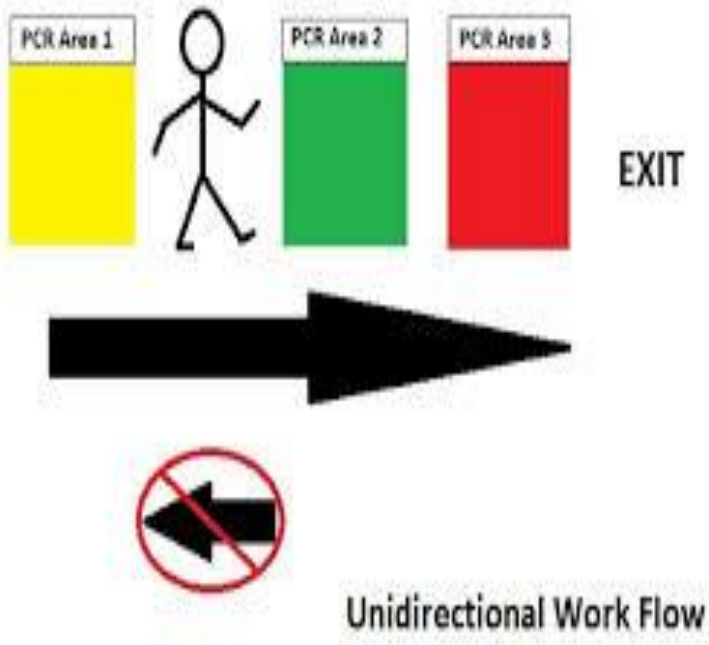
- Laboratory design
- Laboratory practices
- Chemical and enzymatic controls

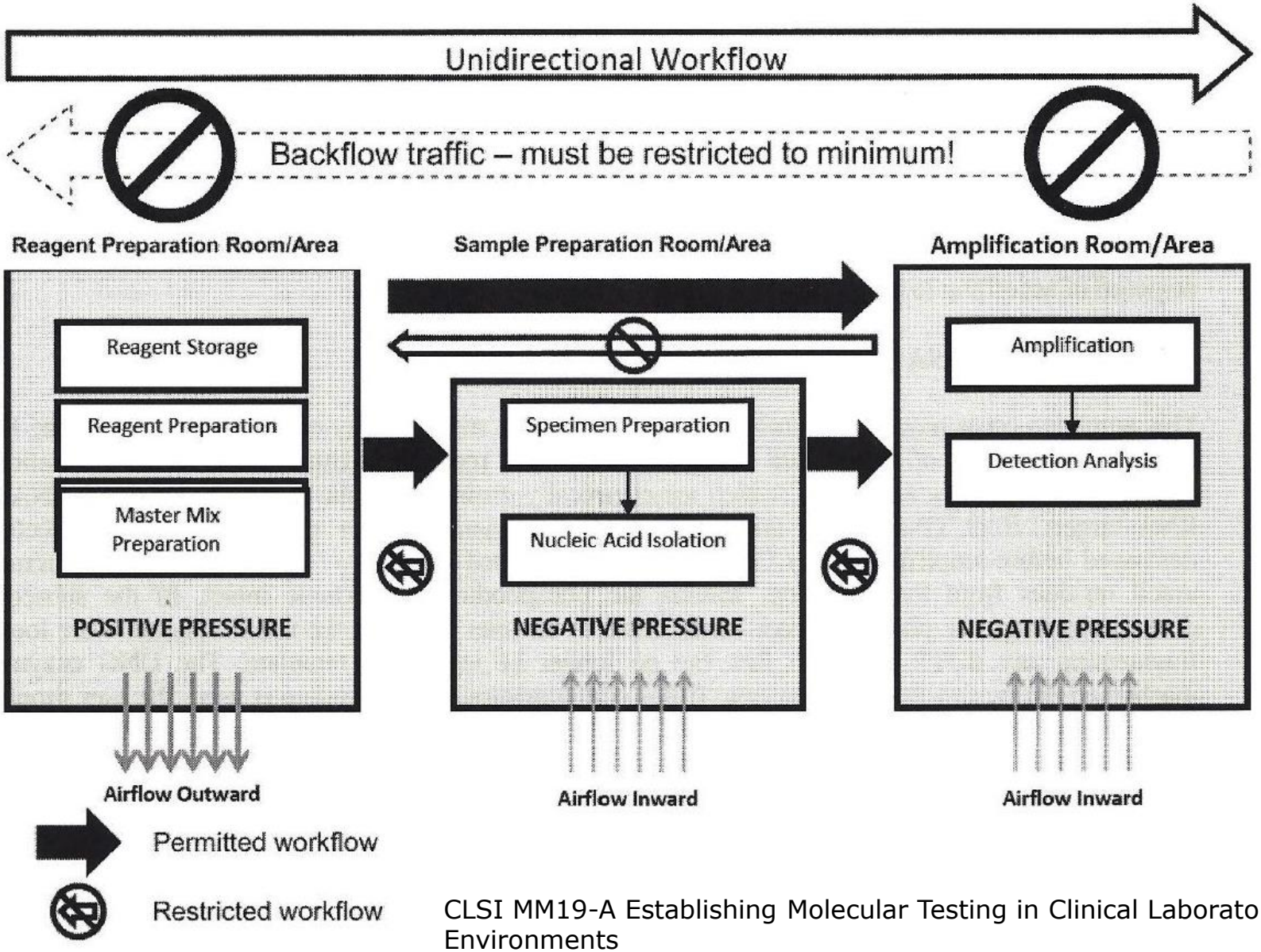
Setting Up a Molecular Laboratory

- Mechanical barriers to prevent contamination
- Spatial separation of pre- and post-amplification work areas
  - Area 1 – Reagent preparation
  - Area 2 – Specimen/control preparation, PCR set-up
  - Area 3 – Amplification/product detection, plasmid preparation
- Physically separated and, preferably, at a substantial distance from each other

Unidirectional Flow

- Both personnel, including cleaning personnel, and specimens
- Amplification product-free to product-rich
- Remove PPE before leaving one area
- Avoid or limit reverse direction
- Reusable supplies in the reverse direction need to be bleached.





CLSI MM19-A Establishing Molecular Testing in Clinical Laboratory Environments

Features of the 3 Areas

- Each area has separate sets of equipment and supplies
  - Refrigerator/freezer (manual defrost)
  - Pipettes, filtered tips, tubes, and racks
  - Centrifuge, timers, vortex
  - Lab coat (color-coded), disposable gloves, safety glasses, and other PPE
  - Cleaning supplies
  - Office supplies
  - Ventilation system
- Dead air box with UV light – serves as a clean bench area

Features of the 3 Areas

- Air pressure
  - Reagent Prep – Positive
  - Sample Prep - Negative
  - Postamplification - Negative
- Reagent Prep – Single entrance, reagents used for amplification should not be exposed to other areas
- Specimen Prep – Specimens should not be exposed to post-amplification work areas
- Size of each area should consider space for equipment and bench space needed for preparation

Laboratory Design Example 1

Mitchell P. S. et al. Nucleic Acid Amplification Methods: Laboratory Design and Operations, 2004, In "Molecular Microbiology: Diagnostic Principles and Practice, edited by D. H. Persing et al" 99. 85-93.

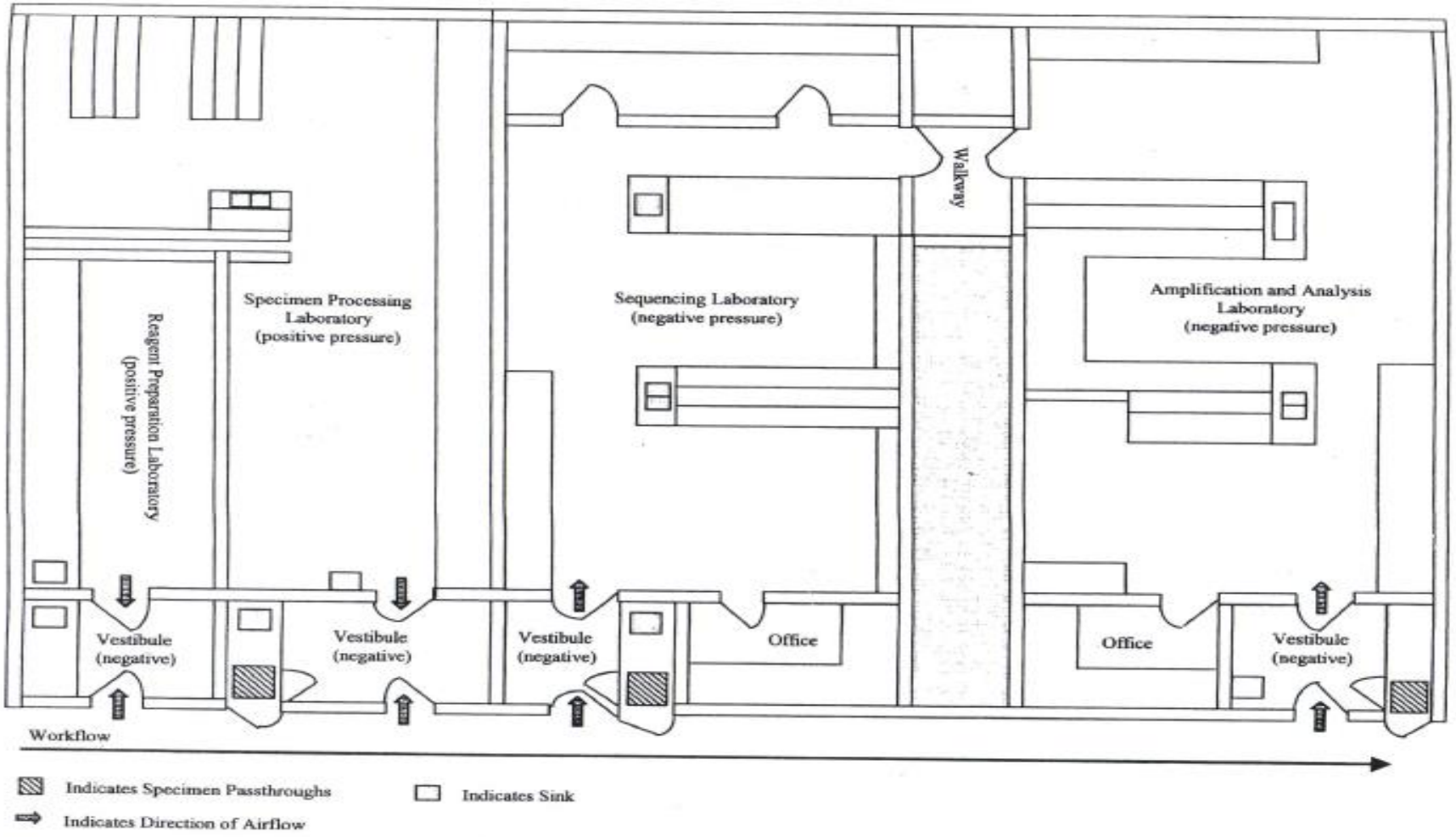
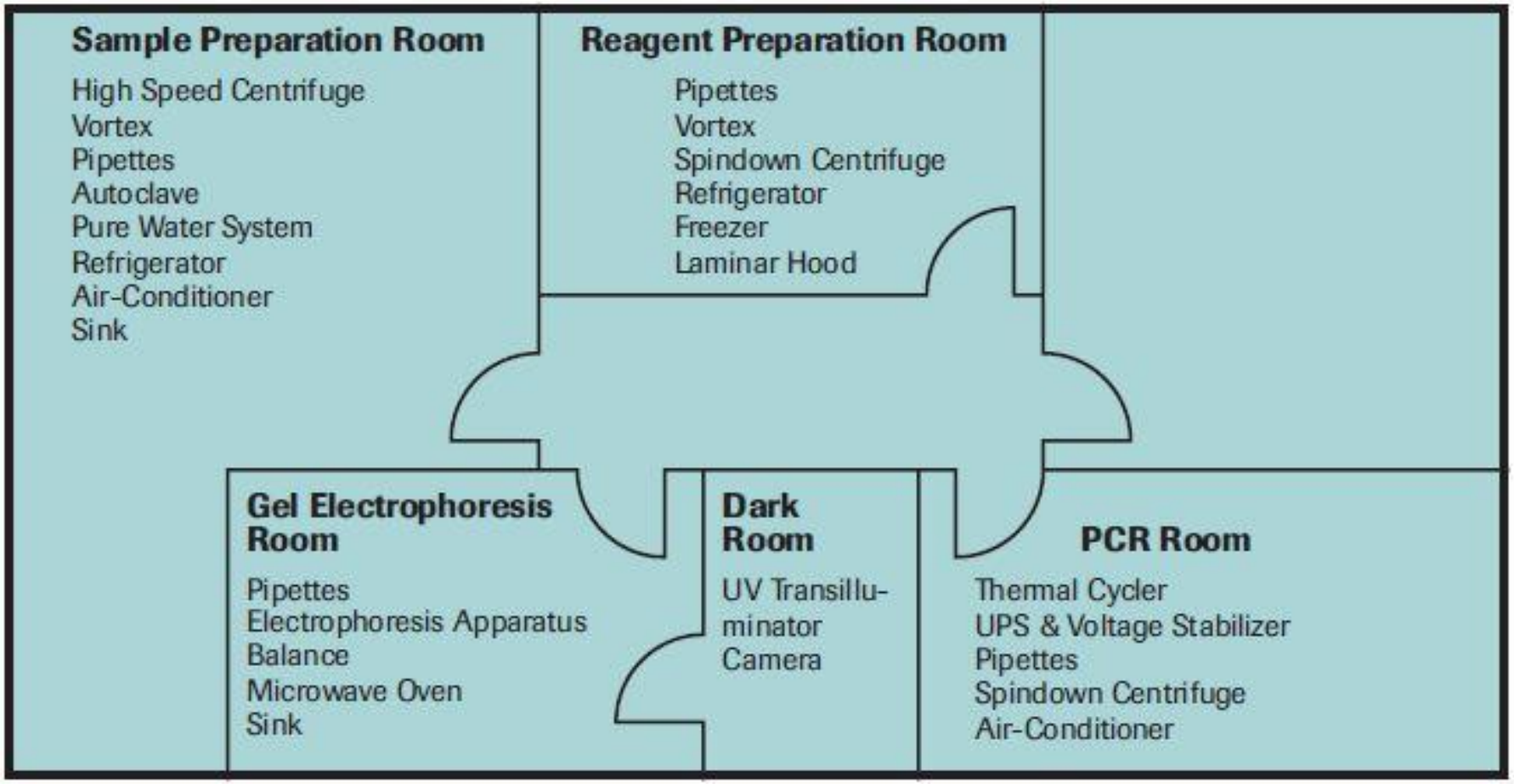


Figure 1. Recommended design for a molecular microbiology facility.

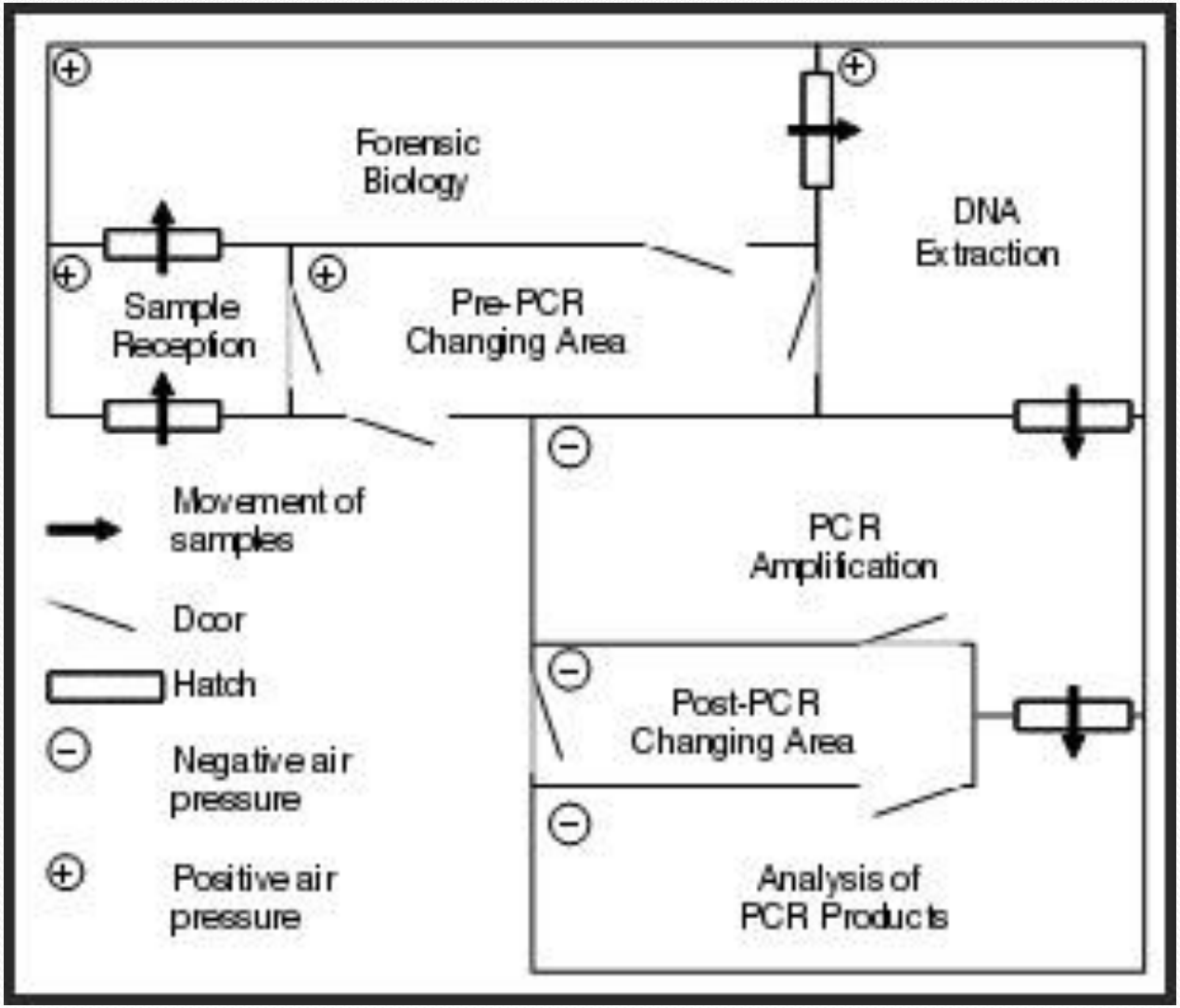
Laboratory Design Example 2

http://www.roche-applied-science.com/campaigns/DeveloperTips/pcr/Physical-separation.html



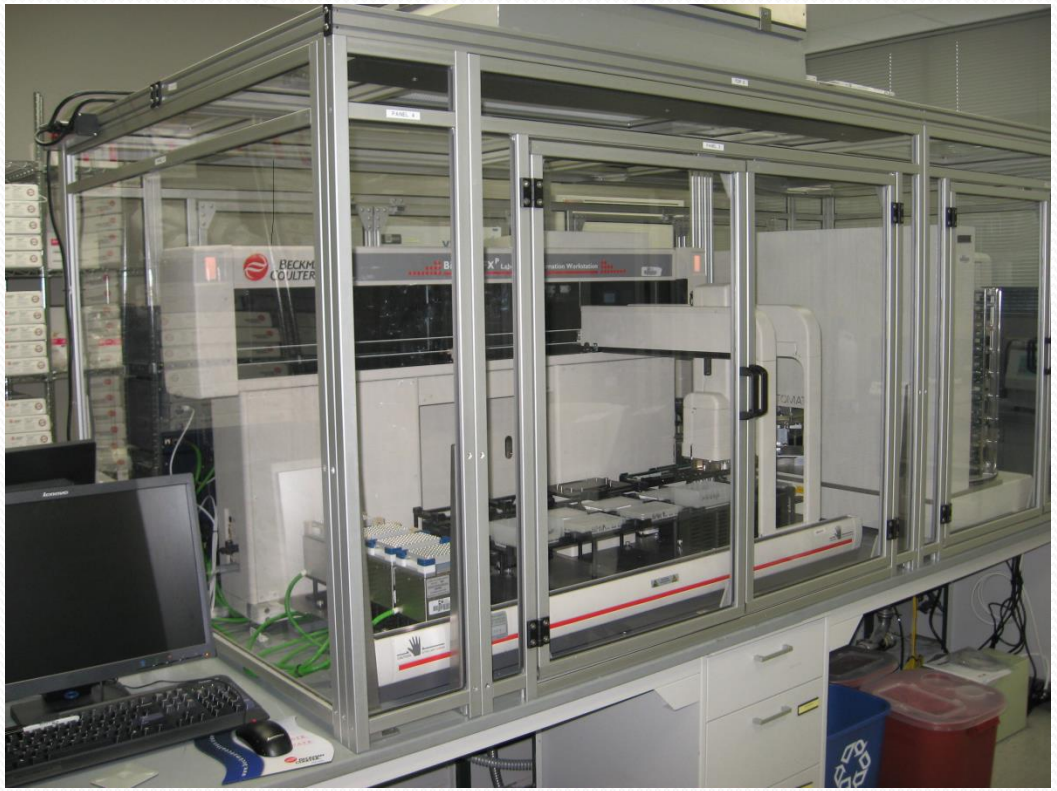
Laboratory Design Example 3

http://fx.damasgate.com/the-pcr-laboratory/



Two Areas Only

- Area 1 – Reagent prep, specimen prep, and target loading – use of laminar-flow hoods
- Area 2 – Amplification/product detection



Alternative to Spatial Separation

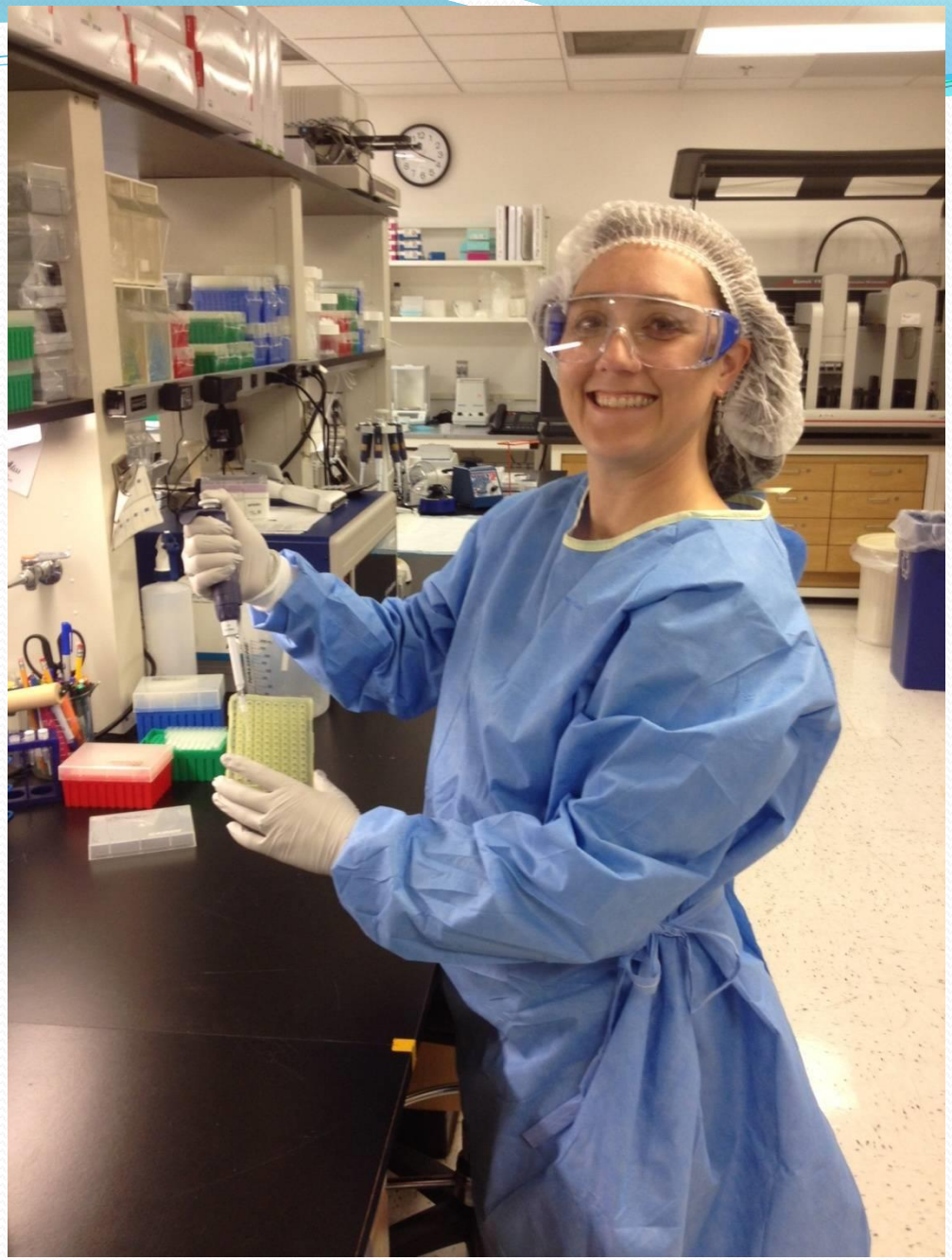
- Class II biological safety cabinet
- Dedicated areas for each work phase
- Unidirectional
- Automated specimen processing station/closed-tube amplification and detection system

Other Laboratory Design Considerations

- Temperature and humidity requirements
- Exhaust ventilation
- Water quality
- Electric outlet
- Back-up power system
- Eye wash
- Ergonomic assessment

Laboratory Practices

- Use of positive displacement pipettes and disposable filtered pipette tips
- Avoid production of aerosols when pipetting
- Use of sterilized single-use plasticware
- Use of cleanroom sticky floor mats
- Minimizes the risk of amplicon carry-over on clothing, hair and skin
  - Hairnet
  - Dedicated safety glasses
  - Disposable labcoat/gown, color-coded preferred
  - Gloves, need to change periodically
  - Shoe covers



More Laboratory Practices

- Clean punches between samples
- Use of nuclease free or autoclaved water
- Aliquot oligonucleotides – multiple freeze thaws will cause degradation
- Always include a blank (no template) control to check for contamination
- Use of electronic data system (flow of paper)
- Wipe test (swab test)
  - Monthly
  - Detect, localize, and remove contamination
  - Identify the source of the contamination

Decontamination Approaches

- Clean the work area & equipment routinely
  - Clean the PCR workstation at the start and end of each work day/run (UV light, 70% ethanol, fresh 10% sodium hypochlorite, DNA Away)
  - Clean the exterior and interior parts of the pipette
  - Clean the equipment
  - Clean the doorknobs, handle of freezers

Chemical and Enzymatic Controls

- Work stations should all be cleaned with 10% sodium hypochlorite solution (bleach), followed by removal of the bleach with ethanol and water.
- Ultra-violet light irradiation
  - UV light induces thymidine dimers and other modifications that render nucleic acid inactive as a template for amplification
- Enzymatic inactivation with uracil-N-glycosylase
  - Substitution of uracil (dUTP) for thymine (dTTP) during PCR amplification
  - New PCR sample reactions pre-treated with Uracil-N-glycosylase (UNG) – contaminating PCR amplicons are degraded leaving only genomic DNA available for PCR

When is a Validation/Verification Study Required?

- Introduce a new testing system
  - New analyte
  - Analyte previously measured/detected on an alternate system
- An analyte added to a test system
- A modification to a test system
- Applies to
  - Unmodified, FDA-cleared or approved method
  - Modified, FDA-cleared or approved method
  - In-house method
  - Standardize method such as textbook procedure
- Determine analytic performance of an assay

Quality Control Plan

Monitor all steps of analytical procedure

- Types of Control
- Frequency and Number of Controls
- Evaluation of Controls and Calibrators

Types of Controls

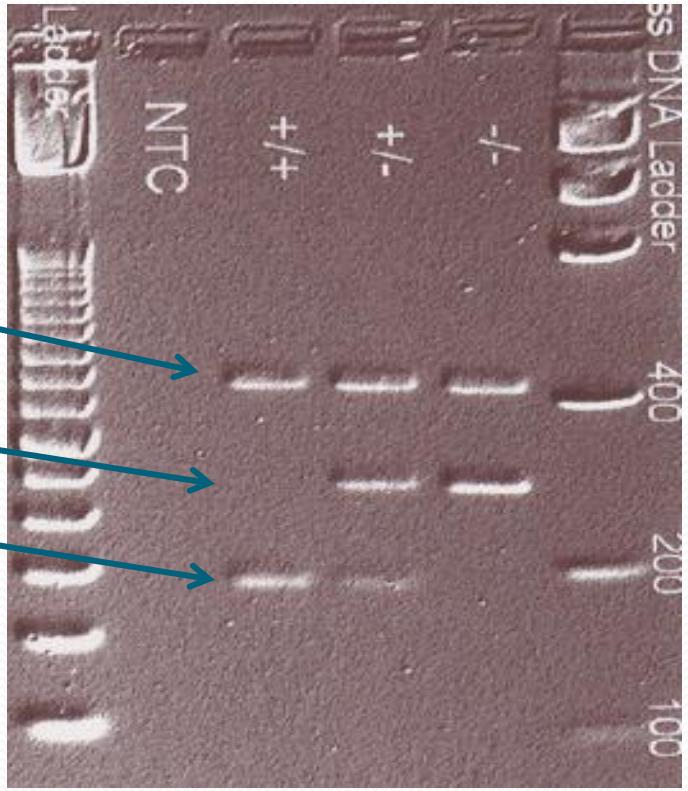
- Internal Control
  - Internal positive amplification controls to detect failure of DNA extraction or PCR amplification
    - Reagent or equipment issues
    - Integrity of DNA sample
    - Presence of inhibitory substance
- External Control
  - Positive control
  - Negative control (normal, wild type)
  - No template control (extraction blank)
  - Blank

Internal Controls

Tetra-primer ARMS-PCR

Simultaneous amplification of:

- Positive amplification control
- Mutation allele
- Reference allele



Alternative to tetra-primer ARMS is to include an additional primer set to amplify a different control sequence

Reference gene (e.g. RNaseP)

External Controls

- Positive and negative controls:
  - Inhibitors
  - Component failure
  - Interpretation of results
  - Sources:
    - Residual DBS
    - PT samples
    - QC materials
- No template controls and Blanks:
  - Nucleic acid contamination during extraction
  - Nucleic acid contamination during PCR

Frequency and Number of External Controls

- Based on risk
- Ideally should represent each target allele and include in each run, but may not be feasible when:
  - Highly multiplex genotypes
    - Systematic rotation of different alleles as positives
    - Specimens representing short and long amplification products to control for differential amplification
  - Rare alleles
- Quantitative PCR
  - External controls should represent more than one concentration, covering the analytical measurement range
  - Daily run or with each runs
- After equipment maintenance, new operator, new reagent lot/shipment

Calibrators

- Calibrator copy levels should cover analytic cut-offs

Evaluation of Controls and Calibrators

- Pass/Fail Criteria – established during validation study
  - Parameters
    - Specific PCR product bands
    - Specific DNA fragments
    - Quantity or Ct of reference gene
    - Quantity or Ct of targeted marker
    - Slope, R2, and Y-intercept of Calibrator curve
  - Threshold
    - Presence or absence of DNA bands
    - Above or below LoB
    - Above or below cut-offs
    - Within Mean± 2SD, Mean ± 3SD, or Mean± 10%
  - % of controls acceptable
  - Impact the entire run or only affected samples

Allele drop-out (ADO)

- The failure of a molecular test to amplify or detect one or more alleles
- Potential causes:
  - DNA template concentration
    - Incomplete cell lysis
    - DNA degradation
  - Non-optimized assay conditions
  - Unknown polymorphisms in target sites
  - Reagent component failure
  - Interfering substance, http://www.aphl.org/aphlprograms/newborn-screening-and-genetics/Pages/Assuring-Laboratory-Quality.aspx
- Major concern for screening laboratories
  - Confirmation of mutation inheritance in families may not an option

False Amplification

Potential causes:

- Non-optimized assay conditions
- Unknown polymorphisms in target sites
  - Gene duplications
  - Oligonucleotide mis-priming at related sequences
    - Psuedogenes or gene families
- Oligonucleotide concentrations too high
- Nucleic acid cross-contamination

What to do if control fails?

Quality Indicator

Measurement to monitor and record specific activities as part of the quality management system

- Turnaround Time
- % of failed runs
- Population medium
- Calibrator parameters
- Graph to identify trend or shift
- Monitor frequency and acceptable range

Proficiency Testing

- Assessment of the Competence in Testing
- Required for all CLIA/CAP certified laboratories
- Performed twice a year
- If specimens are not commercially available alternative proficiency testing program has to be established (specimen exchange etc.)

Molecular Assay Proficiency Testing Material Sources

- CDC NSQAP
- UKNEQS
- EuroGentest
- CAP
- Maine Molecular
- SeraCare
- Corielle
- ECACC
- In-house samples
- Round-robin with other NBS laboratories

Sample Acceptance and Tracking

- Special specimen acceptance criteria?
- Assign a unique code to each patient
- Use two patient-identifiers at every step of the procedure
- Develop worksheets and document every step
- LIMS interface and Positive ID

Reagents

- Labeling Reagents:
  - Content, quantity, concentration
  - Lot #
  - Storage requirements (temperature etc.)
  - Expiration date
  - Date of use/disposal
- Know your critical reagents (enzymes, probes, digestion and electrophoresis buffers) and perform QC checks as appropriate

Critical Molecular Assay Components

- Nucleic Acids: Prepare aliquots appropriate to workflow to limit freeze-thaw cycles
  - Primers and probes
  - dNTPs
  - Genomic DNA
    - 4-8°C
    - -15 to -25°C
- Enzymes
  - Benchtop coolers recommended
- Fluorescent reporters
  - Limit exposure to light
  - Amber storage tubes or wrap in shielding (foil)

Other QA/QC Considerations

- Specimen storage
- Laboratory Cleanliness, and Waste Disposal
- Instrument Maintenance and Calibration
- Instrument/Method Comparison
- Document Management
- Personnel Training and Competency
- Periodic Review of QA/QC
- COOP Plan

New CAP Requirement on TAT

- **CBG.20140 Out-of-Range/Invalid Results Phase II**

There is a policy for reporting positive (out of range) or invalid results to the submitting location and other appropriate entities to allow for patient follow-up within a timeframe appropriate to ensure maximum health benefit.

NOTE: Positive results include those results that are outside of the expected range of testing results established for a particular condition. Invalid results include situations where the laboratory is unable to complete the screening process due to an unsuitable specimen, test, or incomplete information. The findings must be communicated in a manner consistent with the urgency of the intervention needed. For situations requiring repeat screening or confirmatory testing, the laboratory must clearly communicate the timing of the actions to be taken.

Results must be reported to the submitting location (at minimum) within 7 days of specimen receipt and within 3 days for specimens received for tests requiring additional action (e.g. invalid or positive).The records should indicate when results were reported and who received the results. In cases where the testing laboratory is responsible for documenting that a return specimen has been received and analyzed, appropriate records should attest to specimen receipt, testing and result reporting.

Take Home Messages

- Separate laboratory spaces for Reagent Prep, Sample Prep, and Amplification and Detection
- Precautions and special laboratory practices must be made to minimize the risk of contamination
- A Quality Control Plan to monitor the quality of testing process and detect errors should be in placed for each new test before it's implemented.
- Continuous quality improvement is essential