Molecular Diagnostics: Where have we been and where are we going?

William T. Bellamy, Ph.D., HCLD(ABB), CC
Professor of Pathology and Genetics, Director, Molecular Diagnostics Division
University of Arkansas for Medical Sciences
Little Rock, AR
Disclosures

No conflicts of interest.
Questions Facing Labs

• What tests should we offer?
• How do we validate new tests?
• How do we keep up with new evolving technologies?
• What will we be reimbursed?
Molecular Diagnostics

Detection and/or quantification of specific DNA or RNA molecules.

Qualitative Assays
High sensitivity
Is it present?

Quantitative Assays
Detection cutoff > qualitative broad dynamic range
How much is present?

Information Assays
Is a genetic variant present?
Evolution of Molecular Testing

• One gene at a time
  – Southern blots
  – RFLP analysis
  – Single-plex PCR

“One Test, One Gene”

• Multiple genes at a time
  – Multiplex PCR
• >20,000 genes at a time
  – DNA/RNA Microarrays
  – Next Gen Sequencing

“One Test, Multiple Genes”
Molecular Diagnostics (Early Applications)

- Genetics: FRAX, CF
- HemePath: B and T cell gene rearrangements, FV
- ID: CT/NG, HIV viral load
- Pharmacogenomics: None
- Solid Tumors: None
Molecular Diagnostics (Current Applications)

- Genetics
  - Mitochondrial,
  - Autism
  - Seizure,
  - Developmental delay
  - Failure to thrive
    - Panels >250 genes, whole exome, whole genome
- Hemopath
  - Panels and whole exome by NGS
    - AML,
    - MDS,
    - B & T gene rearrangements
- ID
  - Gram positive
  - Gram negative
  - Bacterial ID
  - Fungus
  - Antibiotic resistance
  - Deep sequencing by NGS
- Pharmacogenetics
  - CYP450 2D6, 2C9, 2C19, IL28b
- Solid Tumors
  - NGS whole exome
  - Clinical exome
  - Panels for 20 to >400 genes
Molecular Testing

• Diagnostic
  – What does the patient have?

• Prognostic
  – Will the disease progress or recur?

• Predictive
  – Is the patient likely to develop the disease or will the therapy likely work?

• Monitoring
  – Is the therapy working?
What Tests Should We Offer?
DNA alterations

- Deletion/Insertion
- Amplification
- Translocation

**Example:**
- 22q11.2 region – DiGeorge syndrome
- 17q21.1 (ERBB2) – Breast cancer
- t(11;22)(q24;q12) – Ewing’s sarcoma
DNA alterations

**Point Mutation** (Single base pair change)

CCTGAGGAG → CCTGTGGAG

Example: hemoglobin, beta – sickle cell disease

**Deletion/Insertion** (Frame Shifts)

GAATTAAGAGAAGCA → GAAGCA

Example: epidermal growth factor receptor – lung cancer

**Sequence Repeats**

TTCCAG…(CAG)_60…CAGCAA

Examples: huntingtin – Huntington disease
          MSI – Colon Cancer
PCR-based Methods

Fluorescence in situ hybridization (FISH)

Next-Generation Sequencing (NGS)

Point Mutations
Small insertions/deletions
Large duplications/deletions
Trisomies/monosomies
Altered ploidy
Level of Service

• Basic
  – Labs with little experience in molecular diagnostics
  – Lack resources to conduct extensive test validation
  – Perform only FDA-cleared/approved in vitro diagnostic tests (IVDs)
  – Verification of test performance in the lab
Level of Service

• Intermediate
  – Labs with more experience in molecular diagnostics
  – Greater resources available for test validation
  – Perform FDA-cleared/approved and use LDTs with ASRs
  – Validation required if IVD is modified (e.g. different sample type)
Level of Service

• Advanced
  – Labs with the highest level of experience in molecular diagnostics
  – Sufficient resources to design and validate LDTs
  – Serve as resources for basic and intermediate service level labs
Planning the Molecular Test Menu

- Clinical utility/validity (medical need)
- Testing Platform
- Personnel and training (Expertise)
- Test complexity and validation
- Regulatory concerns (LDT vs. IVD)
- Reimbursement
Clinical Indication for Testing

• What is the diagnostic question?

• Do the assay results correlate to disease or condition?

• Does the assay support or surpass other diagnostic methods?

• Clinical utility – Intended use
  — What is the significance of the assay results for medical decision making?
  — How will the care of the patient or public be affected by this test result?
Clinical Utility/Validity

• How will the findings change patient care or physician treatment decisions?

• What is the current testing for this condition?
  – Methodology
  – Gold Standard
  – Accuracy

• Role for Laboratory Test Utilization Committees
In-house or send-out?

• Sending out is not necessarily more expensive

• What is goal for TAT? Consider clinicians’ needs.
  — Sending out may mean shorter or longer TAT, depending on the test, test volumes, batch sizes
  — Send out TAT may be unacceptable (warfarin, MRSA)
  — Sending out may have a better TAT (low volume viral tests, etc.)

• How does the test fit into the workflow?

• Will the test stand alone on a new platform, or easily adaptable on an existing platform?

• Is the send-out lab licensed, reputable, reliable?
Know what volume of testing to expect

• Gather information…
• Check your monthly send-out reports and trends
• Get to know what professional guidelines say: “Learn”
• Know your clinical departments, their needs, strengths, ordering habits
• Consult with physicians in various disciplines: “Listen”
Choosing a Test Platform
What is the Best Method?

- Diagnostic Sensitivity
- Diagnostic Specificity
- Type of Mutation
- Distribution of Mutation
- Analytical Sensitivity
- Analytical Specificity

Decision
Choosing test platforms
“Point-of-Care” Instruments

BioFire (Idaho Technologies) Film array

Roche (IQuum) Liat Analyzer

Cepheid GeneXpert™
High Through-put Platforms – Viral load

Abbott M2000

Roche Ampliprep & Taqman 24
Next Generation Sequencing Platforms

**Illumina HiSeq 2000**
- 300 – 600 Gb
- 6 – 11 days

**Illumina HiSeq 2500**
- 100 Gb
- 27 hours (Rapid Run)

**Illumina MiSeq**
- 1.5 Gb
- 1 day

**Ion Torrent PGM**
- 10 Mb – 1 Gb
- 6 hours

**Platform-Specific Differences:**
- Differences in read length, error rate, sequence bias
- Potential of sequencer choice to influence findings/results
- Important to recognize and understand the limitations and caveats of the platform used
NGS Strategies

**Whole-genome sequencing**
- Most comprehensive genomic analysis
- Hypothesis-independent
- Extensive data analysis

**Whole-Exome Sequencing**
- Targets coding regions
- Enables increased sequencing depth

**Targeted Sequencing**
- Hypothesis-driven
- Most sensitive approach
- Easiest data interpretation
New Era in Health Care: Issues for Molecular Diagnostics

• Increase FDA-cleared/approved/waived tests
• FDA regulatory oversight over LDTs
• Increased costs for validation
• Decreased or no reimbursement
• Aging technical workforce
• Decreased technical staff skills
• Space needs
• Increased calls for “near patient” or “Point of Impact” diagnostic testing
Molecular CPT Codes

• Tier 1 (more commonly performed tests)
  — >100 new codes (81161-81355)
  — Human Leukocyte Antigen (HLA) typing (81370-81383)

• Tier 2 (less commonly performed tests)
  — 9 new codes/levels (81400-81408)
  — Levels based on complexity of work; >300 named analytes

• Molecular NOS code (81479) for unlisted analytes, emerging tests without established utility, tests that “don’t fit”

• Genomic sequencing codes (81410-81471) added January 1, 2015 (7 additional NGS tests added for 2016)
Laboratory Staff Skill Sets

• Molecular testing is considered as high complexity testing under CLIA

• Basic principles
  — Molecular biology
  — Genetics

• Understanding of clinical context

• Technical competence & good laboratory techniques
  — Many are interested but not able to pipette consistently very small volumes (e.g. 1-2 uL)
Same Reagents, Different Hands

Good Technique

Poor Technique
Personnel Challenges

• Aging workforce (average age in lab >50 years)
• Decreasing number of graduates in training programs
• Low number of technologists trained in molecular diagnostics
• Competition from biotech programs
Personnel Needs

- Individual Skills
  - For low volume laboratories, mostly manual procedures
  - Good organizational skills
  - Detail oriented
  - Manual dexterity
  - Good eyesight
The future of Molecular Diagnostics?!