Roadmap to Strategic Molecular Testing Integration

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Clinical Lab Consulting, LLC.

- Founded by members, physicians, pathologists, Ph.D.s, managers and technologists, who are committed to assisting laboratories in providing the highest quality diagnostic laboratory services.
- Our members are experts in the field of Clinical Laboratory Science and have a record of accomplishment for developing some of the most successful clinical laboratories in the country.
- CLC provides consulting in all laboratory services, well known and specialized in molecular diagnostics.
- For more go to Clinicallabconsulting.com
MDx Represents the Fastest Growing and Most Profitable Segment of Lab Services

2004
50,867 tests

2005
58,485 tests

2006
66,764 tests

~30 %
growth over 2 years

Averaged annual billable molecular tests / laboratory

Source: Molecular Diagnostics State of the market 2007, Washington G-2 Reports
US Molecular Clinical Diagnostic Market

Molecular Diagnostics Revenue Forecasts 2002–2012

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
Infectious Disease is currently a high proportion of MDx Testing today

- Infectious Disease: 71%
- HIV: 32%
- STDs (CT/NG): 15%
- HPV: 11%
- HCV: 24%
- Others: 18%
- Cancer: 11%
- Pharmacogenomics: 11%
- Prenatal and Newborn: 7%

Source: US Molecular Diagnostics Market, Frost and Sullivan 2006
US Molecular Clinical Diagnostic Market

Revenue Forecasts for Infectious Disease Segment

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
Oncology and Pharmacogenomic tests will increase in contribution to both test and revenue numbers in the future.

Source: US Molecular Diagnostics Market, Frost and Sullivan 2006
US Molecular Clinical Diagnostic Market

Approximate Average Reimbursement Rate 2005

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
Molecular Diagnostics Today

- Molecular Genetic
- Infectious Disease
- Molecular pathology (solid tumors and hematopathology)
- Cytogenetics
- Flow cytometry
- Forensic molecular medicine
- HLA and typing
- Personal Medicine and theranostics
Advantages of Molecular Testing

- Rapid and Robust
- Sensitive
- Specific
- Can be performed from all specimen types
- Independent of specimen viability
- Well controlled
- Open to news and updates
- Diversity
Disadvantages of Molecular Testing

- Highly complex technology
# Myths About the Costs of Molecular Diagnostics

<table>
<thead>
<tr>
<th>Barriers to Molecular</th>
<th>Opportunities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myth 1:</strong> Molecular requires 3 separate rooms</td>
<td><strong>Contamination control and automation has eliminated 3 room concept</strong></td>
</tr>
<tr>
<td><strong>Myth 2:</strong> Don’t have the expertise</td>
<td><strong>Coast lower as competition increase</strong></td>
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<tr>
<td><strong>Myth 3:</strong> Molecular costs too much</td>
<td><strong>Training or retraining personnel for molecular testing available</strong></td>
</tr>
<tr>
<td><strong>Myth 4:</strong> I don’t have the volumes to bring MDx in-house.</td>
<td><strong>Start with IVDs</strong></td>
</tr>
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</table>

**How can I afford Molecular?**

**Can you afford not be in Molecular?**

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Personnel Shortage

Source: Washington C-2 Reports
Current Molecular Applications

- Blood Banking
- Infectious Disease
- Sexually Transmitted Disease (STD)
- Genetic Testing
- Molecular Oncology
- Pharmacogenomics
Blood Bank and Tissue Screening

- In addition to 7 immuno-chemistry assays and 1 serology assay, 3 MDx tests are used
- HIV, HCV and WNV (qualitative)
- HBV is also used in some countries
- HIV and HCV used for tissue screening
- In transplants, CMV and EBV viral load
Importance of MDx in Blood Bank

**WINDOW TIMES FOR CATCHING PATHOGENS**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Method</th>
<th>Window Detection Period (days post infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B surface antigen (HBsAg)</td>
<td>Immunoassay (JNJ)</td>
<td>10-40</td>
</tr>
<tr>
<td>Hepatitis B surface antigen (HBsAg)</td>
<td>Immunoassay (Abbott)</td>
<td>10-40</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>NAT (single donor testing)</td>
<td>10-40</td>
</tr>
<tr>
<td>Hepatitis C antigen</td>
<td>Immunoassay</td>
<td>10-40</td>
</tr>
<tr>
<td>Hepatitis C NAT</td>
<td>NAT</td>
<td>10-40</td>
</tr>
<tr>
<td>HIV-1</td>
<td>Immunoassay</td>
<td>10-40</td>
</tr>
<tr>
<td>HIV-1</td>
<td>NAT</td>
<td>10-40</td>
</tr>
</tbody>
</table>

Source: AABB and Gen-Probe
Molecular Infectious Disease

- HIV viral load and genotyping (Currently available as IVD)
- HCV viral load and genotyping (Currently available as IVD and ASR)
- HBV viral load and genotyping (Currently available as ASR)
- Mycobacterium (ASR, some probes IVD)
- Other infectious pathogens (IVD and ASR)
Sexually Transmitted Disease

- **C. trachomatis** (TMA, PCR, HC2, BD)
- **N. gonorrhoeae** (TMA, PCR, HC2, BD)
- **HPV** (HC2 only IVD available, ASR)
- **HPV genotyping** (ASR)
- **HSV 1&2 by PCR** (ASR)
- **GBS by PCR** (IVD and ASR)
- **Other STD infectious agents**
Genetic Testing/Screening

- **Diagnostic (mutation detection)**
  - Factor V Leiden, F2, MTHFR,
  - FRAXA, HD, DMD,
  - Hemochromatosis
  - Cystic Fibrosis (diagnostic)

- **Prognostic/genetic risk screening**
  - Cystic Fibrosis carrier screening
  - Thrombotic risk screening (FV, F2)
  - Familial breast and ovarian cancer risk screening (BRCA1, BRCA2)
  - Screening for DNA MMR gene in Colorectal Cancer
Molecular Oncology

- Oncology or solid tumor markers
- Hematopathology or leukemia and lymphoma

1. Diagnostic
2. Prognostic and therapeutic monitoring
Molecular Oncology

1. Diagnostic
   - K-ras, and p53 in pancreatic and lung cancer
   - Leukemia and Lymphoma (BCR/ABL, JAK2, flt3)
   - Other chromosomal abnormalities

2. Prognostic and therapeutic monitoring
   - Her2neu in breast cancer
   - BRCA1 and BRCA2 in breast and ovarian cancer
   - Microsatellite instability in HNPCC
Pharmacogenomics

- Screening for genetic polymorphism of the enzyme responsible for drug metabolism
- Drug response and adverse drug reactions
- CYP2D6, poor, intermediate, extensive and ultrametabolizer
- TPMT polymorphism in leukemia and autoimmune disease dosage treatment
- Other drugs metabolized by CYP2C9*2 & CYP2C9*3
- VKORC1 for coumadin dosage
Bringing Molecular Dx in house

Barriers to overcome
- Test volumes too low
- Space
- Expertise
- Costs

Reasons to bring MDx in-house
- Increased in send outs
- ↑Demand for new MDx tests
- ↓Decreased MDx cost
- Technical and clinical support
- Improve profitability
- Foundation for growth
- Capital/Financing Available

Source: US Molecular Diagnostics Market, Frost and Sullivan 2006
Financial Goals for New to Molecular Labs

- All institutions are paying for some molecular tests today
- Bringing in Molecular testing will contribute to improving the profitability of the institution
- Molecular Dx will continue to grow in percentage of total laboratory tests
- Infectious disease (CT/GC, HPV) will remain a foundation for many molecular laboratories.
Start with Basic Molecular Dx

- Start with FDA approved tests
- Offer high volume (CT/GC, HPV) tests
- Chosen an initial MDx vendor (go with reagent rental)
- Negotiate the price (consult experienced experts)
- Go with one platform with a brought menu (closed system)
- Higher consultant to training staff in MDx techniques (cheaper then to enroll then in the MDx course)
- Collaborate with other labs (consulting until you establish your team)
- Use hired vendor to train your sale reps on upsaling MDx
Increasing Your Molecular Dx Menu

- Continue expanding the menu
  - Plasma: Viral Load (HIV, HCV, CMV)
  - Other OBGYN tests: HSV, GBS
  - MRSA
  - Genetics: Factor II Prothrombin, Factor V Leiden, MTHFR, CF
- Again go with IVDs first, if not use ASR (no RUO or LDT)
What Tests to Start With

Demand and market will dictate what test to bring or expand

Source: 2006 / 2007 Survey of 600 US Molecular Laboratories
The Evolution of a Molecular Diagnostic Laboratory

- More Automation with brought menu (closed systems less space)
- Less labor and errors (interfacing with LIS)
- Lower volume, high impact tests
  - Molecular oncology (BCR/ABL, JAK2)
  - CYP450 2C9 and VKOR
- Reimbursement based on methods
- Grouped CTP codes
- ASR and home brew methods are added
- Report formatting
Increasing marketing and sales efforts to attract new clinicians (use vendors to help)

- Use available brochures and literature
- Use guidelines and recommendations (ACOG, AMCG, AAP, AMP)
- Provide clinicians with the specialized training for the interpretation (education using brochures and pamphlets)
- Set up presentations for clients
How to Compete

Patient Requirements

• Early Detection
• Diagnosis
• Stratification
• Treatment
• Monitoring

Physician Requirements

• Rapid Identification
• Screening
• Esoteric Testing
• Disease Monitoring

Laboratory Requirements

• Rapid TAT
• Small volume
• Maximum flexibility
• Medium TAT
• Large volume
• Low prevalence
• Cost effective
• Medium TAT
• Cost effective
• Various levels of complexity
• Medium TAT
• Medium volume
• High positivity
• Consistency
US Molecular Clinical Diagnostic Market

Revenue Forecasts 2002–2012

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
Revenue Forecasts for Infectious Disease Segment

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
# US Revenues for the Infectious Disease Testing Segment 2005

<table>
<thead>
<tr>
<th>Segment</th>
<th>2005 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>32.1</td>
</tr>
<tr>
<td>HCV</td>
<td>23.6</td>
</tr>
<tr>
<td>STDs</td>
<td>14.9</td>
</tr>
<tr>
<td>HPV</td>
<td>11.0</td>
</tr>
<tr>
<td>Others*</td>
<td>18.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

* Includes CMV, HBV, WNV, GAS, GBS etc.

Source: US Molecular Diagnostics Market, F743-55, Frost and Sullivan
Summary

The costs of molecular are real, but so are the Opportunities

1. Molecular Diagnostics is improving patients lives
2. Molecular Diagnostic testing will continue to grow:
   • MDx delivers actionable healthcare information to answer unique clinical questions
   • More FDA approvals and more options
3. Get in the Game!
   • Infectious disease testing will continue to be the foundation for most molecular labs because of established reimbursement and clinical utility
   • Oncology and pharmacogenomic tests requests will grow however physicians will require more local support for these complex methods.
   • Molecular labs today can and will provide clinical, operational, and financial benefits to their institutions.

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Molecular Diagnostic Testing

- Molecular biology is the study of nucleic acids
  - DNA or RNA

- Molecular diagnostics is the study of nucleic acids for diagnostic purposes
  - Identification of whole genes
  - Identification of a specific sequence
  - Identification of a single mutation
Molecular Diagnostic Testing

- Nucleic acid sequences are unique
  - A single specific sequence can be detected from the massive amounts present in a whole genome
  - Humans have ~ 6 billion bp
    - 4-base \(4^4 = 256\) bp
    - 8-base \(4^8 = 65,536\) bp
    - 20-base \(4^{20} = 4,398,046,511,104\) bp
Molecular Diagnostic Technologies

Molecular testing typically consists of three parts:

1. Extraction and Purification
2. Amplification
3. Detection
Extraction: Releasing Nucleic Acid from the Cell

DNA the Molecule of Life

http://www.paternityexperts.com/img/DNA-of-life.jpg
NA Extraction

All protocols use 4 basic steps:

Step 1: Lyse
- Boiling
- Homogenization
- Sonication
- Pressure
- Detergents
- Enzymes

Step 2: Bind NA
- Silica-based particles
- Magnetic beads

Step 3: Remove contaminants
- Ethanol-based buffer

Step 4: Release purified NA
- Water
- Buffer

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http://www.chemicell.com/products/purification/dna/_img/dna.png
Amplification

1. Target amplification methods

2. Signal amplification methods
Molecular Diagnostics Target Amplification Technologies

• Molecular biologists manipulate normal DNA replication and other normal cellular processes in the laboratory to make more of the nucleic acid for subsequent analysis.
DNA Replication: The Basics

- Double stranded DNA
- Strand denaturation
- Primer annealing
- Polymerase extension

Replication is bi-directional, resulting in two new strands of DNA.
Target Amplification Methods

- Polymerase Chain Reaction (PCR)
- Real-time PCR
- Transcription mediated amplification
- Nucleic acid sequence based amplification
DNA Replication

- Double stranded DNA
- Strand denaturation
- Primer annealing
- Polymerase extension

- Template
- Primer(s)
- Nucleotides (A,C,T,G)
- Polymerase
- Buffer
The primers determine the boarders of the piece of DNA to be amplified. 

**Polymerase Chain Reaction (PCR)**

1. **Denature; 94°C**
2. **Anneal primers; 25 to 65°C**
3. **Extend; 72°C**

**35 cycles**
One cycle of ‘traditional’ PCR

- **Denature, 94°C, 10 sec**
- **Ramp Time, 20 sec**
- **Anneal, 40-60°C, 20 sec**
- **Ramp Time, 10 sec**
- **Extend, 72°C, 50 sec**
- **Ramp Time, 10 sec**

*One cycle = 2 minutes*
The Power of “2”

1st Round: 2 copies
2nd Round: 4 copies
3rd Round: 8 copies
4th Round: 16 copies
35 Rounds: Over a million copies

Template

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Detection Methods

- Detection occurs after the reaction is performed
  - End-point detection
- Methods include:
  - Agarose gel electrophoresis
  - Colorimetric reactions
Real-Time PCR

- Real-time PCR detects the amplification product as it occurs, or in “real-time”

- Three technologies were needed to accomplished this:
  - instrumentation, detection chemistries, analysis

- What are some of the differences between traditional PCR and real-time PCR?
Traditional and Real-Time PCR

- Heating blocks for heat transfer.
- Thin-walled PCR reaction tubes.
- Tubes are placed in heating blocks.
- Detection is separate.

- High velocity air for heat transfer.
- Glass capillary reaction chambers.
- Capillaries are placed in holder and act as cuvettes.
- Detection occurs simultaneously.


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Real-Time PCR Chemistries

- SYBR Green I
- TaqMan
- FRET Probes
- Molecular Beacons
- + others

http://pathmicro.med.sc.edu/p cr/SYBRGreen_small.jpg

http://www.clinical-virology.org/graphic/pcr_mb.gif

www.iba-go.com/naps/ naps_p_rg_fret.html

http://www.clinical-virology.org/graphic/pcr_mb.gif
One cycle of real-time PCR

- **Denature**, 94°C, 0 sec
- **Anneal**, 40-60°C, 5 sec
- **Extend**, 72°C, 15 sec

One cycle = 20 seconds

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PCR and Real-Time PCR

Traditional PCR = ~ 2 minutes/ 1 complete cycle
Real-time PCR = ~ 2 minutes/ 6 complete cycles

Temperature (°C)

Time (sec)
Transcription Mediated Amplification (TMA) and Nucleic Acid Sequence Based Amplification (NASBA)

Promoter-primer binds RNA

Reverse transcription of cDNA

RNAase H activity of RT degrades cRNA

Primer 2 binds cDNA

2nd strand synthesis by RT

RNA Polymerase initiates transcription

100-1,000 RNA (+) copies produced

RNA Polymerase initiates transcription

100-1,000 RNA (-) copies produced

AMV RT

RNase

Primer 2 binds RNA

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Real-Time NASBA
Signal Amplification Methods

- Branched DNA (bDNA)
- Hybrid Capture
- Invader
Branched DNA (bDNA)

1. Degrade RNases and release RNA
2. Hybridize target probes
3. Hybridize preamplifiers
4. Add substrate and measure light
Hybrid Capture

**Release Nucleic Acids**
Clinical specimens are combined with a base solution which disrupts the virus or bacteria and releases target DNA.

**Hybridize RNA**
Target DNA combines with specific RNA probes creating RNA:DNA hybrids.

**Capture Hybrids**
RNA:DNA hybrids are captured onto a microtiter well coated with capture antibodies specific for RNA:DNA hybrids.

**Label for Detection**
Captured RNA:DNA hybrids are detected with multiple antibodies conjugated to alkaline phosphatase.

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Invader Chemistry

1. Probe oligo and invader oligo bind to specific target sequence
2. Clevase recognizes this structure and cleaves the probe oligo, releasing 5' sequence
3. 5' flap sequence binds second FRET oligo
4. Cleavage releases fluorophore resulting in signal
The End