Demystifying the Development and Implementation of Molecular Tests in a Clinical Laboratory

The Simple, Sensible, Salient & Still Spell Binding Seven Questions About Laboratory Developed Tests

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Learning Objectives

• Describe different types of in-vitro diagnostic tests
• Identify differences between laboratory developed tests (LDT) and In-Vitro Diagnostic (IVD) tests
• List the benefits of running IVDs and LDTS
• Explain how LDTs and IVD tests are designed and regulated in the US
Agenda

1. Where do we start: Basics of Diagnostic Test Terminology
2. What is a Laboratory Developed Test (LDT) and an In-Vitro Diagnostic (IVD)?
3. Why use LDTs? Why use IVDs?
4. Who uses LDTs? Who uses IVDs?
5. When can I develop these tests?
6. How do LDTs compare to IVDs?
7. Can a lab protect their Intellectual Property?
<table>
<thead>
<tr>
<th>In Vivo</th>
<th>In Vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IVDs</strong></td>
<td><strong>LDTs</strong></td>
</tr>
<tr>
<td>Tests performed on body fluids (blood, urine) or cells / tissues (pap smear, biopsy)</td>
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<tr>
<td>Detect and/or quantify levels of desired bio markers (e.g., enzymes, protein) to diagnose cancer, body function disorders, cellular malfunctions</td>
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</tr>
<tr>
<td>Technology <strong>DRIVEN BY PRODUCTS</strong> from simple (clinical chemistry / ELISA) to complex (flow cytometry)</td>
<td>Technology <strong>DRIVEN BY PROCESS</strong></td>
</tr>
</tbody>
</table>

**In Vivo**
- Physiology
  - EKG / BP
- Imaging
  - X-Ray / CT
  - MRI / PET
- Technology **DRIVEN BY PHYSICIANS**
## Laboratory Developed Test v. In Vitro Diagnostic Test

<table>
<thead>
<tr>
<th><strong>IVD test registered with FDA</strong></th>
<th><strong>LDT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed for sale to diagnostic laboratories, health clinics, or consumers</td>
<td>Developed by individual laboratories; not transferred, licensed, or sold</td>
</tr>
<tr>
<td>Standardized instrument qualification procedures and training required</td>
<td>Instrument qualification and training requirements established by individual laboratories</td>
</tr>
<tr>
<td>Must be pre-validated with a data analysis and bioinformatics report</td>
<td>Often developed in-house by necessity—no standard assay available</td>
</tr>
<tr>
<td>Must be clinically validated</td>
<td>Must be clinically verified and can be implemented quickly for emergency use (must be CLIA compliant)</td>
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</tbody>
</table>
Molecular Tests Can Include A Wide Variety Of Assays For Different Applications

Examples of what molecular tests can detect or measure:
- Respiratory pathogens
- Genetic variants
- Genes and proteins linked to rare diseases
- Metabolites
- Pathogens
- Oncology markers
- Gene expression levels
- Pharmacogenomic markers (companion diagnostics)
Reasons for Developing an IVD

- Broader distribution and increased revenue
- Design controls
- Easier to establish as the gold standard
- FDA guidance for use as a companion diagnostic
- Complete Clinical Validation prior to marketing (focus on clinical validity)
- Adverse event reporting
- Manufacturing controls
Reasons for Developing an LDT

Why develop an LDT?

- No IVD test available
- Cannot scale up IVD test for desired throughput
- LDT would be an improvement over an existing assay
- No IVD test aligns with desired procedure or utility
- LDT can help a laboratory remain competitive
- No IVD test meets performance requirements
- LDT can be developed more rapidly than IVD test
## Advantages of IVDs

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Example</th>
</tr>
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<tr>
<td>Quality System</td>
<td>Test is subject to a number of requirements including design controls, manufacturing controls and handling complaints.</td>
</tr>
<tr>
<td>Simplified inventory control</td>
<td>Only need to order the manufactured tests for anticipated use rather than LDTs which require inventory of the actual test, but also all of the components necessary to produce the test – reduced documentation.</td>
</tr>
<tr>
<td>Technical support</td>
<td>Customer can go to supplier’s technical support to troubleshoot and also replace faulty products.</td>
</tr>
<tr>
<td>Clinical validity</td>
<td>Clinical validation of test to ensure that it identifies, measures or predicts if a clinical condition or predisposition is present or absent prior to marketing.</td>
</tr>
<tr>
<td>Broad distribution</td>
<td>Many laboratories can utilize the test providing greater amounts of use data which can increase (or possibly decrease) confidence in the test.</td>
</tr>
</tbody>
</table>
## Advantages of LDTs

<table>
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<tr>
<th>Advantage</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control over content</td>
<td>Laboratories can select specific and relevant target(s).</td>
</tr>
<tr>
<td>Rapid adaptation</td>
<td>LDTs can be developed and modified relatively quickly to respond to market needs, such as outbreaks and rare diseases.</td>
</tr>
<tr>
<td>Lower cost per test</td>
<td>Technological advances have made complex analyses faster and more affordable.</td>
</tr>
<tr>
<td>Consolidation into a single test</td>
<td>Testing for multiple analytes provides more data per sample and may enable faster diagnosis.</td>
</tr>
<tr>
<td>Laboratory qualification</td>
<td>Laboratories and their quality systems are qualified rather than individual tests.</td>
</tr>
</tbody>
</table>
## Typical LDT Process From Planning To Launch

### Key questions:
- Why run an LDT?
- What is the test / panel?
- Is test set-up properly and performing as expected?
- Are test results clinically accurate?
- What are best practices for LDT introduction?

### Considerations:
- CLIA certification
- CAP (2-year cycle)
- JCAHO
- State-specific regulations

### Potential drivers:
- Lack of alternative test
- Technological requirements (automation vs. manual)
- Clinical, economic, or operational improvements
- Reimbursement

### Planning
- Examine available tests, technology options, and resources

### Test configuration
- Assess available test menu
- Select targets
- Customization
- Interpretation
- Reporting

### Analytical validation
- Validate analytical performance based on published clinical literature or CLSI* criteria
  - Sensitivity
  - Specificity
  - Reproducibility
  - Accuracy
  - Interference tests
  - Split samples for clinical validation

### Clinical verification
- Run clinical samples to assess accuracy
  - Samples previously characterized by another laboratory (blind)
- Compare results to check concordance

### Test launch*
- Announce test to providers
- Describe test utility in educational content/forums
- Publish peer reviewed papers (if consistent with IP strategy)

*Note: CLSI* stands for Clinical and Laboratory Standards Institute.
## Regulation

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<th><strong>LDT</strong></th>
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<td>Diagnostics include &quot;reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease.&quot;</td>
<td>FDA claims that it <em>could</em> regulate LDTs, but is exercising <em>enforcement discretion</em></td>
</tr>
<tr>
<td>Regulated by FDA as a Device when marketed to any third party – may require PMA or sponsor can use 510(k) pathway if suitable predicate device exists</td>
<td>Under the 1988 CLIA Amendments, all laboratories that test patient specimens must obtain a certificate of compliance or accreditation <em>in order to bill CMS for their services</em></td>
</tr>
<tr>
<td>The sponsor can use non-approved/non-cleared FDA IVD for RUO or IUO, but MUST: (i) use proper labeling and (ii) refrain from use in clinical diagnostic procedures</td>
<td><em>CLIA focuses on the laboratory and its personnel – not the test</em></td>
</tr>
<tr>
<td>FDA has authority to use Emergency Use Authorization (EUA) to provisionally approve IVDs during an emergency (e.g., pandemic)</td>
<td>LDTs are categorized into different levels of complexity (next slide)</td>
</tr>
<tr>
<td></td>
<td>For a limited time, FDA was requiring EUAs for SARS-CoV-2 LDTs – power revoked by HHS in August 2020</td>
</tr>
</tbody>
</table>

* [https://www.fda.gov/medical-devices/in-vitro-diagnostics/laboratory-developed-tests](https://www.fda.gov/medical-devices/in-vitro-diagnostics/laboratory-developed-tests)
### Test Complexity and Examples

<table>
<thead>
<tr>
<th>CLIA categorization</th>
<th>Waived tests</th>
<th>Moderately complex tests</th>
<th>Highly complex tests</th>
</tr>
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</table>
| Description         | • Simple to perform  
                      • Low risk of interpretation error  
                      • Little clinical significance  
                      • Many sold over the counter (OTC) for consumer use |
|                      | • Usually performed with automated clinical laboratory equipment |
|                      | • Electrolyte profiles  
                      • Chemistry profiles  
                      • Complete blood count  
                      • Urinalysis  
                      • Urine drug screen  
                      • Automated immunoassays |
|                      | • Cytology  
                      • Immunohistochemistry  
                      • Peripheral smears  
                      • Flow cytometry  
                      • Gel electrophoresis  
                      • Most molecular diagnostic tests like RT-PCR, gene chip arrays, multiplexed analyses, dot blots, viral loads, expression arrays and CGH arrays |

**Examples**

- Pregnancy tests
- Strep tests
- Dipsticks – Urine tests
- Tests to detect drugs of abuse
- Glucometers and other simple devices
- Electrolyte profiles
- Chemistry profiles
- Complete blood count
- Urinalysis
- Urine drug screen
- Automated immunoassays
- Cytology
- Immunohistochemistry
- Peripheral smears
- Flow cytometry
- Gel electrophoresis
- Most molecular diagnostic tests like RT-PCR, gene chip arrays, multiplexed analyses, dot blots, viral loads, expression arrays and CGH arrays

[https://hub.ucsf.edu/clinical-laboratory-testing](https://hub.ucsf.edu/clinical-laboratory-testing)
Intellectual Property Issues

Obtaining IP Protection

• Challenging if not impossible to protect:
  • Use of conventional platform technologies
  • Use of known/previously published biomarkers

• May be able to obtain patent protection for:
  • Novel platform technologies
  • Improvements to conventional platform technologies
  • Novel biomarkers
    • Mayo v. Prometheus Supreme Court case makes protection of novel biomarkers quite challenging
    • Without detail more “well-understood, routine, conventional” elements, the claims are not protectable because they recite only a high-level relationship

• One option – consider trade secret protection
Intellectual Property Issues

Pre-Commercialization Concerns

• Regardless of issues from Mayo case, patent infringement is still a concern
• As early as possible in the development process, engage counsel to conduct a freedom-to-operate (FTO) search and opinion
• Results of FTO search may necessitate preparation of invalidity/non-infringement opinions
  • FTO search may also guide patent-prosecution strategy
  • FTO search may also reveal the need to in-license one or more patents
LDTs in the COVID-19 Pandemic

- U.S. Centers for Disease Control and Prevention (CDC) created an LDT for SARS-CoV-2 within 10 days of genome sequencing
- University of Washington and Broad Institute each developed their own LDTs by end of January 2020
- U.S. labs also developed antibody-based assays
  - Limited deployment before FDA required EUA
What can we look for?

**Viral Elements**

- mRNA genome (29,811 base pairs)
  - 80% identical to SARS 1, 50% identical to common cold
  - Unique primers designed to detect specific regions (PCR, Crispr etc.)
  - Requires two >10bp primers (ideally 18-22bp) to detect and amplify effectively
- Viral proteins (29 different types)
  - 4 external structural proteins
    - Most abundant is Nucleocapsid
    - Largest, most active & most unique is Spike (S1 ACE2 receptor binding; S2 TMPRSS cleavage site)
  - 25 other proteins

**Immune Reaction**

- Circulating Antibodies (B Cell)
  - Simple to analyze (e.g. present antigen; measure bound antibody from serum)
  - IgG: late but long lasting presence
  - IgM & IgA: early but brief presence
- Circulating Activated T Cells
  - Complex to analyze (e.g. Elispot: stimulate sample with antigen; quantify cytokine response (IFNγ, TNFα, or IL2 +)
- Systemic biometric dynamics
  - Oxygenation / Circulation (Pulse & Blood pressure)
  - Coagulation
  - Fever
  - Sense of Smell (Anosmia)
  - (VOC) Volatile Organic Compounds
The History and Progression of COVID-19 Diagnostics

**Symptomatic Dx**
- Lung CT Broncheolar Lavage

**Active Disease Diagnosis**
- PCR Viral Antigens
- Individual or Pooled PCR Rapid Antigens

**Screening**
- Rapid Antigens Screening

**Surveillance**
- Air monitoring
- Breath tests
- COVID sniffing dogs
- Scratch & Sniff tests
- Wastewater PCR & NGS

**Hospitals**
- Central Lab – Public Health, National, Regional, Research

**Invasive - Lungs**
- Semi-invasive: Nasopharyngeal Swabs
- Minimally invasive – Anterior Nares / Mid Turbinate Swabs

**Home / Self Tests**
- Passive & Non-invasive: Saliva / Breath

**Costs**
- $1,000+
- $75-200
- $10-20
- <$5

Mara.Aspinall@healthcatalysts.com
TestingCommons.com Review of COVID Tests

Molecular, Antigen, Serology, & T-Cell Tests, Patient Management, Combination Respiratory Panels & Collection Kits
Schedule IV notifications & Umbrella Molecular EUAs

1. n/a after 10/7/20 when HHS/FDA announced policy to not require authorization for any LDT
2. 19% of tests with approval internationally have been granted EUA by the US FDA

Questions: mara.aspinall@asu.edu
US FDA Emergency Use Authorizations

- Molecular
  - 88% RTqPCR
  - 9% Isothermal
  - 3% Sequencing
  - 1% CRISPR
- Antigen
  - 88% Lateral Flow
  - 8% Chemiluminescence
  - 4% other
- Antibody
  - 60% ELISA
  - 31% Lateral Flow
  - 6% Chemiluminescence
- Other
  - Collection Kits
  - Flu/RSV Panels
  - Patient Management

Questions:
mara.aspinall@asu.edu

Pandemic Total through 6/30/21
Tests in Development Worldwide

Molecular
- 40% RTqPCR
- 23% Isothermal
- 16% CRISPR
- 14% Sequencing

Antigen
- 56% Lateral Flow
- 18% ELISA
- 10% Breath
- 16% Other

Antibody
- 63% Lateral Flow
- 25% ELISA
- 12% Other

Other
- Breath / VOC
- Mass Spec
- Raman Spec

Questions: mara.aspinall@asu.edu

Pandemic Total through 6/30/21
...all because Delta (δ) is now 99.7% US cases

Source: https://covid.cdc.gov/covid-data-tracker/#variant-proportions accessed 9/20/21
SARS-CoV-2 Variants: Five Questions

1. Make tests less accurate? No
2. Increase cases and deaths? Yes
3. Make treatments ineffective? Some
4. Vaccine effectiveness? Reduced
5. Raise hurdle for herd immunity? Yes

Source: Janet Iwasa, University of Utah: Nature Vol 595 7/2/21
SARS-COV-2 is not the worst epidemic threat possible

Transmissibility ($R_0$)

Case Fatality Rate

Source: David McCandless, Informationisbeautiful.net
Conclusion

• LDTs and IVDs are central to clinical patient care as well as medical research and development
  • Both types of tests will help make personalized medicine a reality for patients

• LDTs are an important locus of diagnostic innovation

• LDTs leverage a regulatory system that provides labs the ability to quickly adapt to changing needs

• LDTs are tied to laboratory processes while IVDs are tied to laboratory tests
  • Labs need to be cognizant of the processes and procedures that must be in place to correctly prepare, develop, perform and document the assay
Appendix

Specific Validation Comparisons
**Validation requirements for LDTs and IVD tests**

<table>
<thead>
<tr>
<th><strong>Utility</strong></th>
<th>IVD FDA test validation*</th>
<th>LDT validation*</th>
</tr>
</thead>
</table>
| Reproducibility (CV) | High and low controls:  
- Intra-run precision (10 or more samples)  
- Inter-run precision (10 days) | High and low controls:  
- Intra-run precision (10 or more samples)  
- Inter-run precision (10 days) |
| Analytical sensitivity | Determine LOD with serial low-end dilutions | Determine LOD with serial low-end dilutions |
| Analytical specificity | Identify interferents (mucus, normal flora, etc.) | Varies with sample type |
| Analytical range | Validate established package insert cutoff with 10 or more samples | Establish normal range using samples from a mixed male and female cohort |
| Clinical sensitivity | Verify performance per package insert with samples from patients with and without disease | Verify performance with samples from patients with and without disease |
| Clinical specificity | Verify performance per package insert with samples from patients with and without disease | Verify performance with samples from patients with and without disease |
| Method correlation study (R², slope) | Not usually applicable (refer to IVD label) | Comparison with a different platform |
| Interpretation | IVD label | Criteria established by laboratory |
| Documentation for inspector | • QC  
• Calibration  
• PT  
• Reviewed, updated, and approved procedures  
• training records  
• personnel qualifications | • Procedures  
• Utility (defined by laboratory) |

* IVDs are regulated by FDA and need to be registered with FDA. Validation procedures and lab requirements are determined by the laboratory based on accrediting body, state and/or local policies and regulations.
### Accreditation and validation parameters for LDT and IVD tests

<table>
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<tr>
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<th>LDT validation*</th>
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<tr>
<td><strong>Accreditation</strong></td>
<td>CLIA + CAP* or JCAHO*</td>
<td>CLIA + CAP or JCAHO</td>
</tr>
<tr>
<td><strong>Assay reagents</strong></td>
<td>IVD Kit</td>
<td>LDT Kit</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>Provided</td>
<td>Provided</td>
</tr>
<tr>
<td><strong>Calibrators</strong></td>
<td>Provided</td>
<td>Provided</td>
</tr>
<tr>
<td><strong>Calibration verification (linearity)</strong></td>
<td>Third party† every 6 months</td>
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</tr>
<tr>
<td><strong>Proficiency Testing</strong></td>
<td>Third party, 2-3 tests per year</td>
<td>Third party, 2-3 tests per year</td>
</tr>
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* CAP: College of American Pathologists  
** JCAHO: Joint Commission on Accreditation of Healthcare Organizations  
† Third party calibration (CAP, American Petroleum Institute, Maine Standards, American Association of Bioanalysts)