Molecular Diagnostics: 
Local vs. Central Lab

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- Scientific and Clinical Validity
- Innovative Research
- Technical Optimization
- FDA Approval - or - Lab Developed Test
- Proof of Clinical Utility
- Decision on Local vs Central Lab
- Approval for payment by payors
- Clinical Use

Life cycle of diagnostic tests
How does MGH decide where to do lab tests?

1. Is the test turnaround time dependant? Regardless of volume or unit cost some tests must be available. For example carboxyhemoglobin for acute CO poisoning and other STAT tests that need to be done rapidly to permit clinical operations to function.

2. Is the specimen unstable and must be performed promptly regardless of clinical urgency? Example: Ionized calcium.

3. Is it a proprietary test or does it require patented/copyrighted interpretive software? We can’t perform these tests: Example: Fibrosure for liver fibrosis.

4. Do we have the medical and technical expertise to perform and support the test: For example, nobody on our faculty knows enough about kidney stone analysis to interpret the test results so we send them out.

5. Then it is a make or buy decision. Moot if labs do 3rd party billing. Currently we send out 2,187 specific assays (n=161,000/yr; $7,000,000/yr)

From Kent Lewandrowski, MD, MGH Associate Chief, Laboratory & Molecular Medicine
Potential Advantages of Central Lab

Standardized methodology

?Peer reviewed methods

High volume

Build large multicenter comparison database for classifier development

?Cheaper (volume/fixed cost of equip)

?Turnaround time (runs every day)

High level of expertise
Examples of central better than local

Exotic lab send outs for low volume tests,
  Rare genetic diseases
  Rare infectious agents

Analysis of clinical trial samples
Example of a Central Lab

**BRCA1,2 Breast Cancer risk (Myriad)**

Myriad 25

**BRACAnalysis®**

The BRACAnalysis® test assesses a person’s risk of developing hereditary breast or ovarian cancer based on the detection of mutations in the BRCA1 and BRCA2 genes.

**BRACAnalysis CDx**

BRACAnalysis CDx is an FDA-approved companion diagnostic test for germline BRCA1 and BRCA2 mutations intended to be used as an aid in treatment decision making for LynparzaTM (olaparib), a PARP inhibitor.

Successful, developed database correlating outcome with individual mutations

Patented **BRCA1** and **BRCA2**— others couldn’t perform the test
He noted
• There are no method claims
• Does not involve patents on new applications of knowledge about BRCA1 and BRCA2
• Does not consider the patentability of DNA in which the order of the naturally occurring nucleotides has been altered
Potential Advantages of Local Lab

Custom methodology (LDT)
FDA Approval not necessary (may change!)
Build comparison database for classifier development from local population samples
Cheaper (Non-profit, no logistics)
Turnaround time (no transportation)
Data automatically entered into LIS
Pathologist integrates results with pathology and clinical data
Training of residents/faculty
Familiarity breeds innovation
Examples of local molecular tests better than central

Common genetic diseases (Factor V Leiden)

Mutational analysis of tumors (high volume hosp)

Common infections
Pathology = Molecular Diagnostics

Research
Training
Practice

MGH Fellowships:
  Molecular Pathology
  Informatics
MGH **Local** Molecular Tests (Micro)

7 platforms
15 tests

**Roche TaqMan**
- HIV
- HCV
- HBV
- CMV

**Seimens**
- HCV genotype

**Cepheid GeneXpert**
- Influenza A/B and RSV
- Cdiff toxin (stool)
- Enterovirus (on CSF)
- MRSA/ MSSA (nasal swabs to detect colonization)
- MTb (and Rifampin resistance) from sputum/BAL

**Focus 3M**
- HSV 1 and HSV 2 (CSF)

**Hologic/ Panther system**
- Chlamydia/Gonorrhea (Urine and cervical swabs)

**Biofire**
- Ebola (emergency use) nucleic acid test

**BD Max**
- Multiplex stool parasite panel
- Multiplex Stool bacterial pathogen panel

*Courtesy of Eric Rosenberg, MD PhD*
MGH Local Testing for Drugable Mutations in Cancer

• SnapShot
• Next Generation Sequencing
  ArcherDX
  Illumina

John Iafrate, MD, PhD
SNAPSHOT Overview

- Multiplex PCR
- Single Base Extension Reaction
- Capillary Electrophoresis

Loci of interest

- ddNTP
- ddNTP
- ddNTP

Electrophoretic Output

Lung cancer

EGFR mutation Glu746_Ala750del (c.2235_2249del)

Dias-Santagata et al EMBO Mol 2010
### Proportion of Mutations By Gene Across Disease Groups

| # | PIK3CA | PTEN | AKT1 | KRAS | NRAS | BRAF | EGFR | ERBB2 | APC | CTNNB1 | TP53 | IDH1 |
|---|--------|------|------|------|------|------|------|-------|-----|--------|------|------|
|   |        |      |      |      |      |      |      |       |     |        |      |      |
| Breast    | 285    | 32   | 19   | 910  | 107  | 196  | 311  | 23    | 42  | 73     | 448  | 155  |
| Bladder   |        |      |      |      |      |      |      |       |     |        |      |      |
| Colorectal |        |      |      |      |      |      |      |       |     |        |      |      |
| Endometrial |      |      |      |      |      |      |      |       |     |        |      |      |
| Esophageal |        |      |      |      |      |      |      |       |     |        |      |      |
| Head & Neck |      |      |      |      |      |      |      |       |     |        |      |      |
| Gastric   |        |      |      |      |      |      |      |       |     |        |      |      |
| GI Other  |        |      |      |      |      |      |      |       |     |        |      |      |
| Glioma    |        |      |      |      |      |      |      |       |     |        |      |      |
| Heme      |        |      |      |      |      |      |      |       |     |        |      |      |
| Lung      |        |      |      |      |      |      |      |       |     |        |      |      |
| Melanoma  |        |      |      |      |      |      |      |       |     |        |      |      |
| Other     |        |      |      |      |      |      |      |       |     |        |      |      |
| Ovary     |        |      |      |      |      |      |      |       |     |        |      |      |
| Pancreatic |        |      |      |      |      |      |      |       |     |        |      |      |
| Sarcoma   |        |      |      |      |      |      |      |       |     |        |      |      |
| Thyroid   |        |      |      |      |      |      |      |       |     |        |      |      |

*Courtesy Darrell Borger*
Next Generation Sequencing (NGS) Clinical Cancer Genotyping

**Clinical** targeted sequencing of FFPE DNA

- 1000+ genes (~2.6 Mb)
- >100X coverage 10 bp into intron
- 5-10 Gb data per tumor-normal pair
- 5% analytical sensitivity
- 3-4 week turnaround time
- $700 raw reagent cost
- SNV, indel, copy number
**Example of a Local Lab**

**Prosigna (Nanostring)**

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**Every Prosigna Score is generated by a proprietary algorithm**

- The Prosigna Score is a numerical value on a 0-to-100 scale that correlates with the probability of distant recurrence within 10 years.
- The gene expression profile of a patient's tumor is compared with each of the 4 PAM50 prototypical molecular profiles to determine the degree of similarity. The results in combination with a proliferation score and tumor size produce an individualized Prosigna Score.

**Intended use/indications for use**: The Prosigna Breast Cancer Prognostic Gene Signature Assay is an in vitro diagnostic assay which is performed on the NanoString nCounter® Dx Analysis System using FFPE breast tumor tissue previously diagnosed as invasive breast carcinoma. This qualitative assay utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease. The Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:
Nanostring Platform

FDA Approved Test for
Breast Cancer Prognosis (Prosigna)
NanoString® Technique

• High sensitivity
  > microarrays
  = RT-PCR, **without** amplification
• Quantitative
  Counts individual mRNA molecules

Fluorescent “Bar tags” detected and counted

Geiss et al
Nature Biotech 2008
Chronic Antibody-Mediated Rejection in Nonhuman Primate Renal Allografts: Validation of Human Histological and Molecular Phenotypes

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Presented at the ATC 2016/Banff 2017
Under review AJT
Endothelial genes correlate with C4d, DSA, cg, g, ptc

Inflammation-related genes correlate with t, i, ti

Ben Adam et al submitted
Best performers in repeated ROC analysis: VWF, DARC, CAV1

Ben Adam et al submitted
3 gene set distinguishes AMR

- ABMR (n=38)
- Borderline (n=21)
- Mixed (n=27)
- Native (n=15)
- No rejection (n=32)
- Other (n=8)
- Suspicious (n=15)
- TCMR (n=41)

| Group 1       | Native | No rejection | Borderline | Other | TCMR | Suspicious | Mixed | ABMR |
|---------------|--------|--------------|------------|-------|------|------------|-------|------|
| Native        | p=0.001 | p<0.001     | p<0.001    | p<0.001 | p<0.001 | p<0.001   | p<0.001 | p<0.001 |
| No rejection  | p=0.001 | ns           | ns         | ns     | ns   | ns         | ns    | ns   |
| Borderline    | p<0.001 | ns           | ns         | ns     | p=0.016 | p<0.001   | p<0.001 | p<0.001 |
| Other         | p<0.001 | ns           | ns         | ns     | ns   | ns         | ns    | p<0.001 |
| TCMR          | p<0.001 | ns           | p=0.016    | ns     | ns   | ns         | ns    | p=0.004 |
| Suspicious    | p<0.001 | p<0.001      | p<0.001    | ns     | p=0.004 | p<0.001   | p<0.001 | p<0.001 |
| Mixed         | p<0.001 | p<0.001      | p<0.001    | p<0.001 | p<0.001 | p<0.001   | ns    | ns   |
| ABMR          | p<0.001 | p<0.001      | p<0.001    | p<0.001 | p<0.001 | p<0.001   | p=0.011 | ns   |
Innovative Research → New Test → Analytic and Clinical Validation

Manufactured Test Approval by Regulators (e.g., FDA)

Laboratory Developed Test (LDT)* Single laboratory

Clinical Utility Proved

Approval by Payers (e.g., CMS)

Clinical Use

*Federal regulations pending in US
| mRNA Test Complexity |
|-----------------------|
| **Examples**          |
| Single Gene           | Gene Set                                      | Classifier                  |
| Granzyme B EBER       | ENDAT (Halloran) ABMR/TCMR score (Halloran)   | Prosigna (Nanostring)       |
|                       | Eculizumab Response (Lefaucheur)              | Molecular Microscope        |
|                       |                                                | Transcriptome Sciences      |
| **Technique**         |
| PCR                   | PCR                                            | Affymetrix Nanostring       |
| In situ hybridization | Affymetrix                                    |                            |
|                       | Nanostring                                    |                            |
| **Interpretation**    |
| Value vs. disease control (mean ± std dev) | Value vs. disease control (mean ± std dev)   | Pattern vs large data set of classified samples |
| Presence/Absence      | Geometric mean vs comparison group            | Archetypes, PCA, random forest... |
Steps for Molecular Dx in Transplantation

• Prove clinical utility
• Link results to specific therapy

• Optimize and simplify techniques
  
  Platform (Affymetrix, Nanostring, PCR...)

• Develop LDT or FDA approved tests

• Show cost effectiveness
• Get Payors to pay

• Then decide the optimal way to provide the test
  • local vs central