

# Validation and Verification Of Molecular Diagnostic Tests

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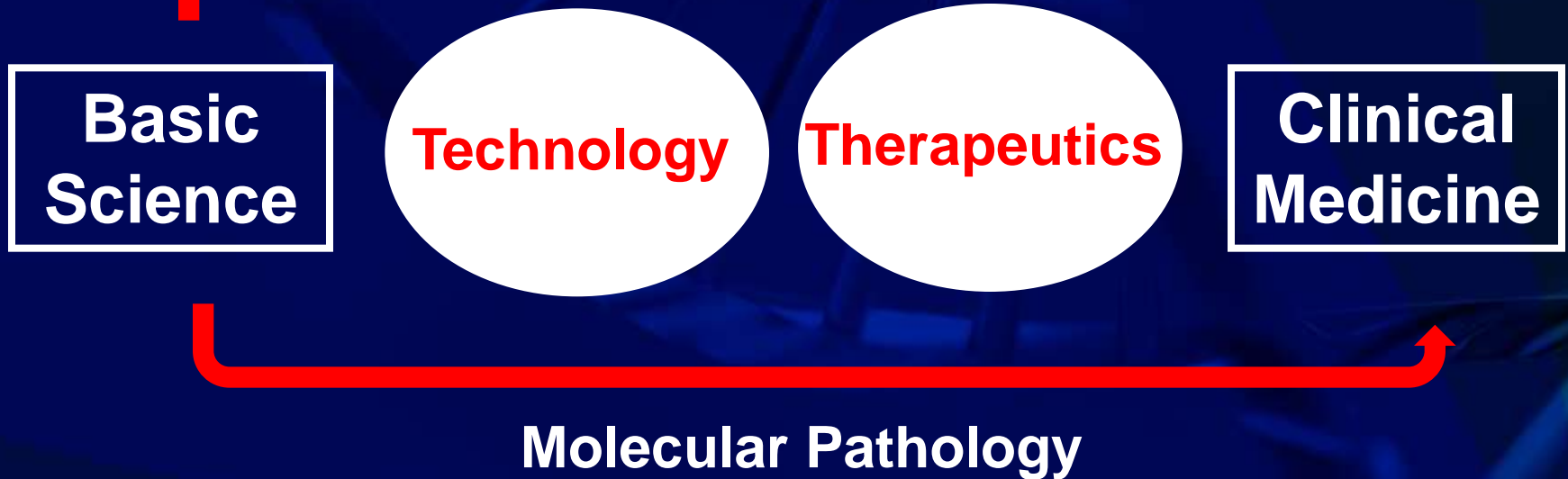
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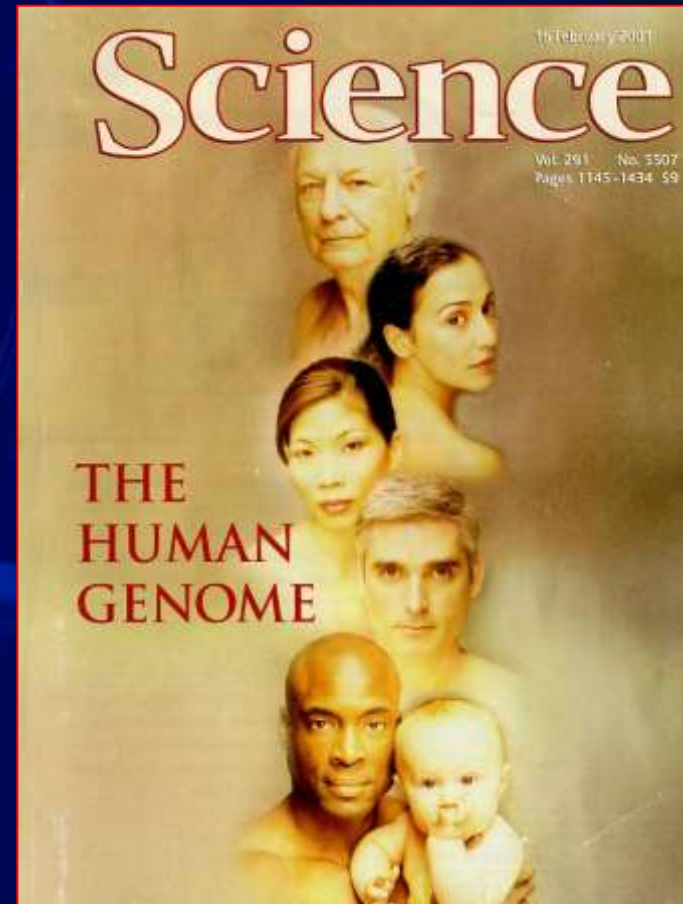
The following information does not reflect the opinions of the College of American Pathologists or the U.S. Food and Drug Administration, they are only my interpretation of the regulations.

# Why do we need new tests?

Translational Research



# The Human Genome Project



Introduced genetics to the public and increased the need for more molecular testing capabilities.

# Promises of the Human Genome

**Diagnostic - better**

**Prognostic - more powerful**

**Predictive - preventative**

**Therapeutic – more  
personalized**



# Clinical Laboratory Improvement Amendments

- Regulates all clinical testing in the U.S.
- Passed by Congress in 1988
- Published in 1992 and revised in 2003
- To ensure accuracy, reliability, and appropriateness of results

## **CLIA Recognizes Three Main Types of Tests**

- FDA approved or cleared w/o modification
- FDA approved or cleared w/ modification
- Tests that are not FDA approved or cleared

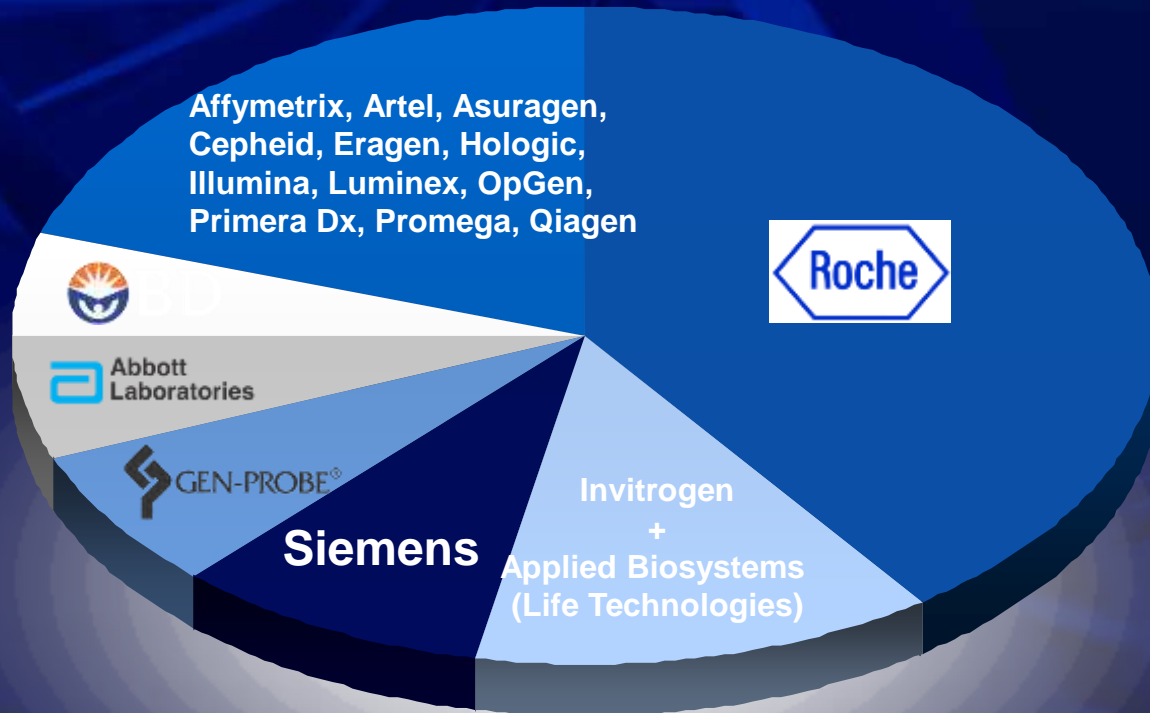
**Many molecular tests are not commercially available or FDA approved/cleared because the target is rare and the market is not profitable.**

# What is the molecular diagnostics playing field like?

- Many players
- Technical issues
- Clinical issues
- Operational issues
- Reimbursement issues
- Ethical issues



# Some of the Players in Nucleic Acid Testing



# Where Does The New Test Request Come From?

- Recent article
- Recent meeting
- New program initiative at the institution
- Electronic media of one sort or another

# Why Do These Requests Come?

## Clinical necessity

- **Genetics**
  - identification of new diseases
  - previously unavailable tests
  - carrier detection/risk assessment
- **Infectious diseases**
  - difficult to culture organisms
  - quantitative analysis/genotyping
- **Heme/Oncology**
  - confirmation
  - minimal residual disease
  - therapeutics
- **Identity testing**
  - chimerism
  - parentage
  - specimen identification
  - twinship
- **Pharmacogenomics**
  - Metabolism
  - Targeted therapy

# Does This New Test Have Value?

**What is the mission of the institution?**

- **Hospital vs reference lab**
- **Academic vs private**
- **Small vs large**

# Does This New Test Have Value?

**What type of patient population do you serve?**

- **Ethnicity/race**
- **Pediatric vs geriatric**
- **Outpatient vs inpatient**
- **Women's health**



# Does This New Test Have Value?

Who is the client?



**Patient/Family**



**Provider**



**Pathologist**



**Payor**

# Does This New Test Have Value?

- Do we currently send this out?
- Do we have the equipment/staff?
- Can we meet the expected TAT?
- What is reimbursement like?

# Staffing

**Skill Level**



**Qualifications**



**CME**

**Satisfaction**



# Cost/Reimbursement

Payor mix???

Reimbursement?

CPT coding

Knowing when to negotiate pricing

Test cost to the laboratory vs institution



# The Pathway To Funding

Test coding

87491  
Chlamydia  
trachomatis

Procedural or stacked  
coding

83890 Molecular  
isolation

83898  
Amplification

83892 Enzymatic  
digestion

83894 Separation



# What It Takes To Bring A New Test In House

Commitment



Space



Equipment



Staff



Start-up \$\$



**You need a plan!**

# Why A New Test?

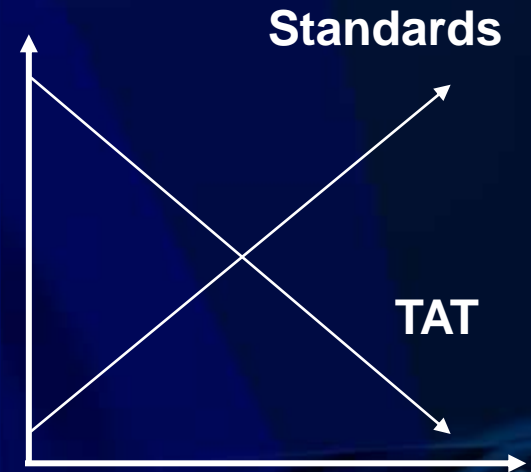
## Laboratory Performance



Support

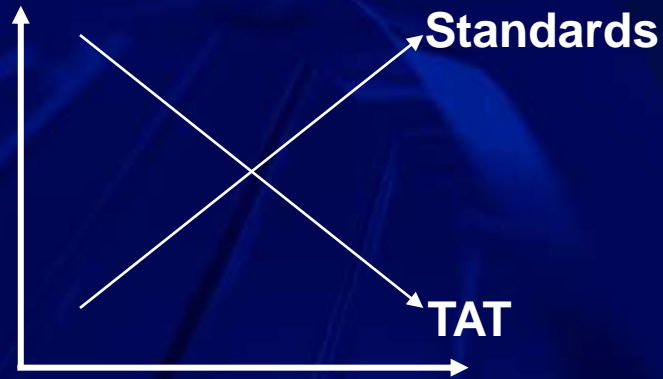
Client?

Analytical



# Why A New Test?

## Analytical Performance



Clinical Interaction



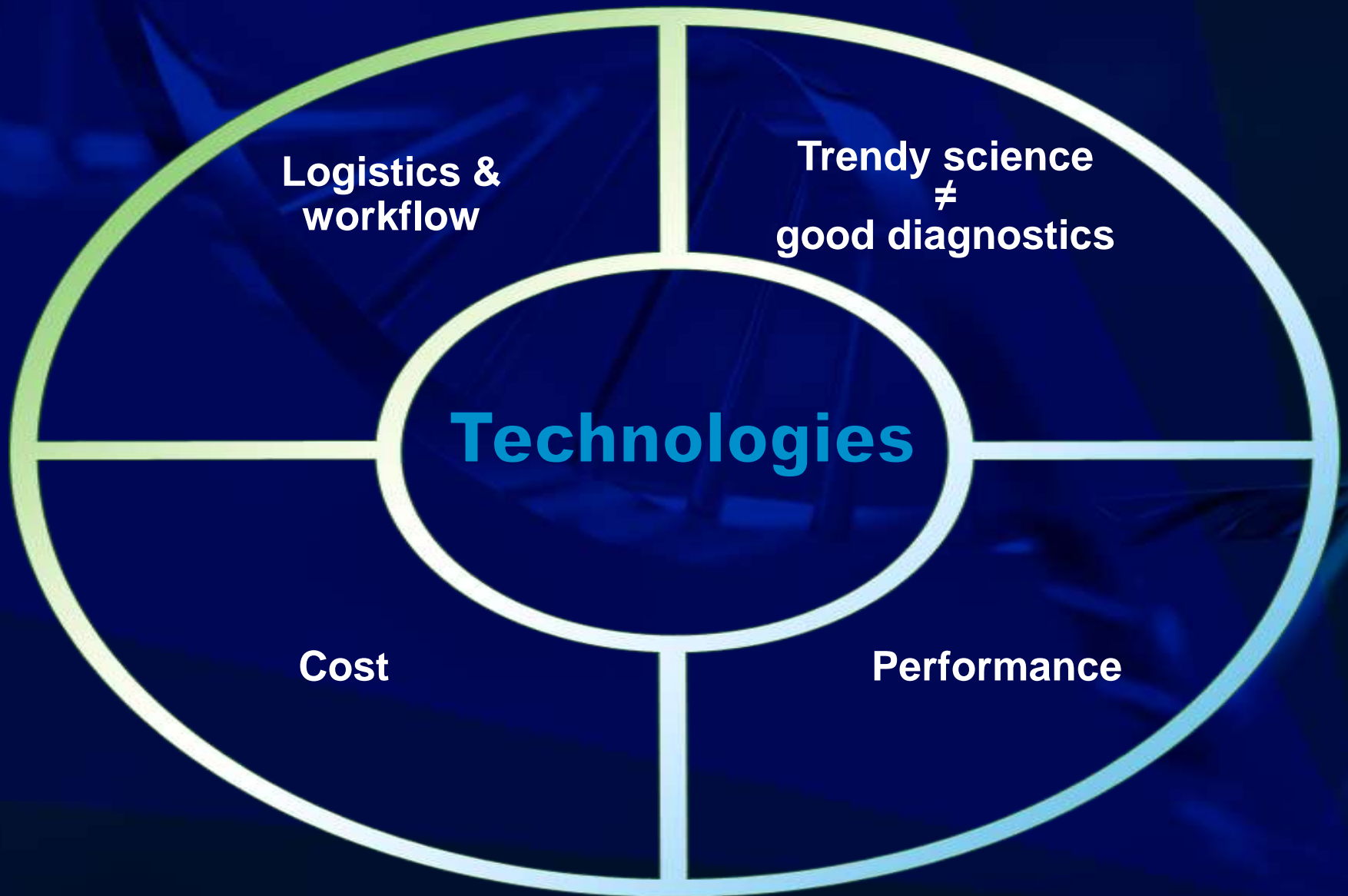
Technologies



Specimen types

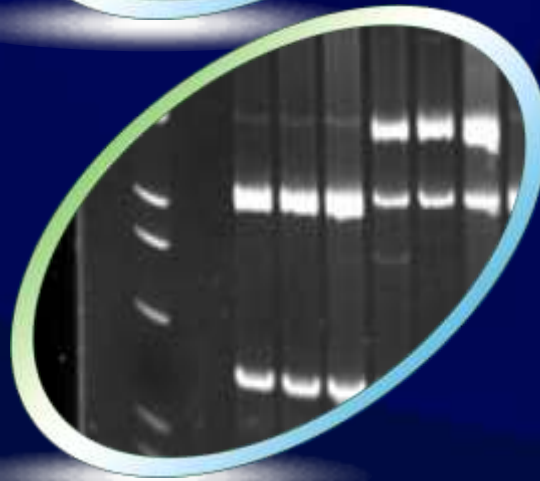


# Why A New Test?





# Trends In Molecular Diagnostic Technologies



**Cost**

**Throughput**

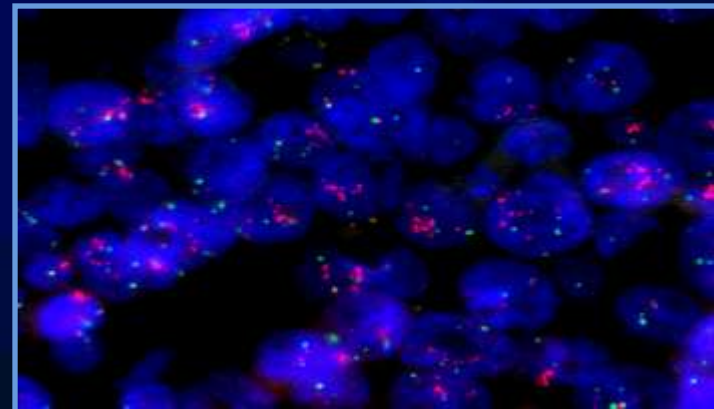
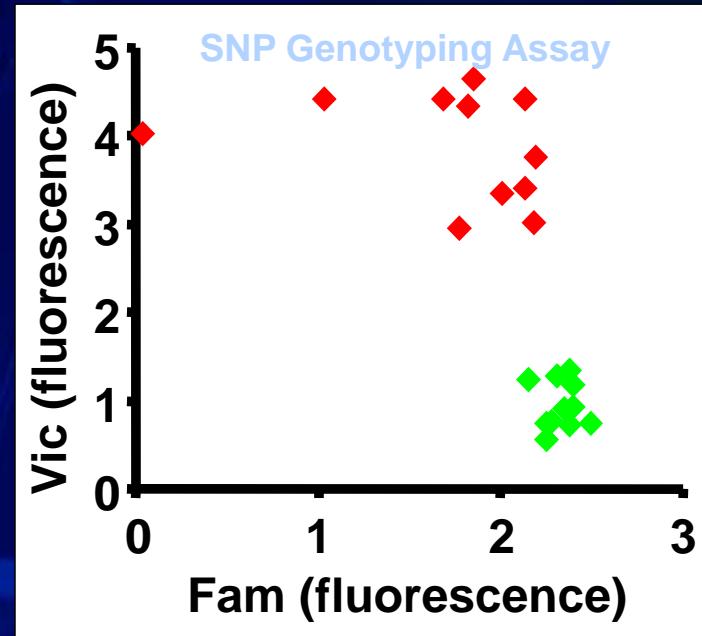
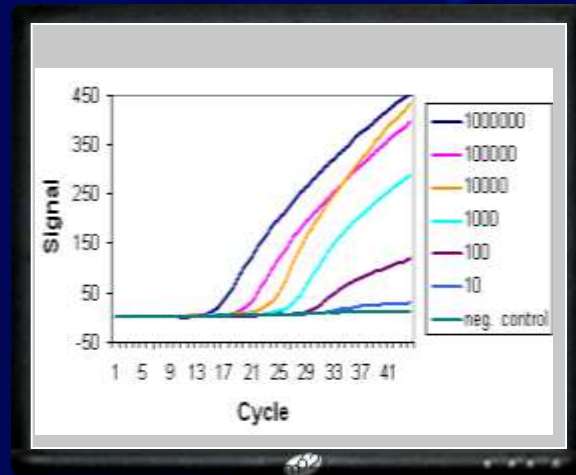
**TAT**



# Trends In Molecular Diagnostic Technologies

- Labor intense – **more automated**
- Low Throughput – **mid to high throughput**
- No Standardization - **CLSI Guidelines**
- Costly – **Less expensive**
- High complexity – **More turn key**
- Staffing – **Less expertise required**

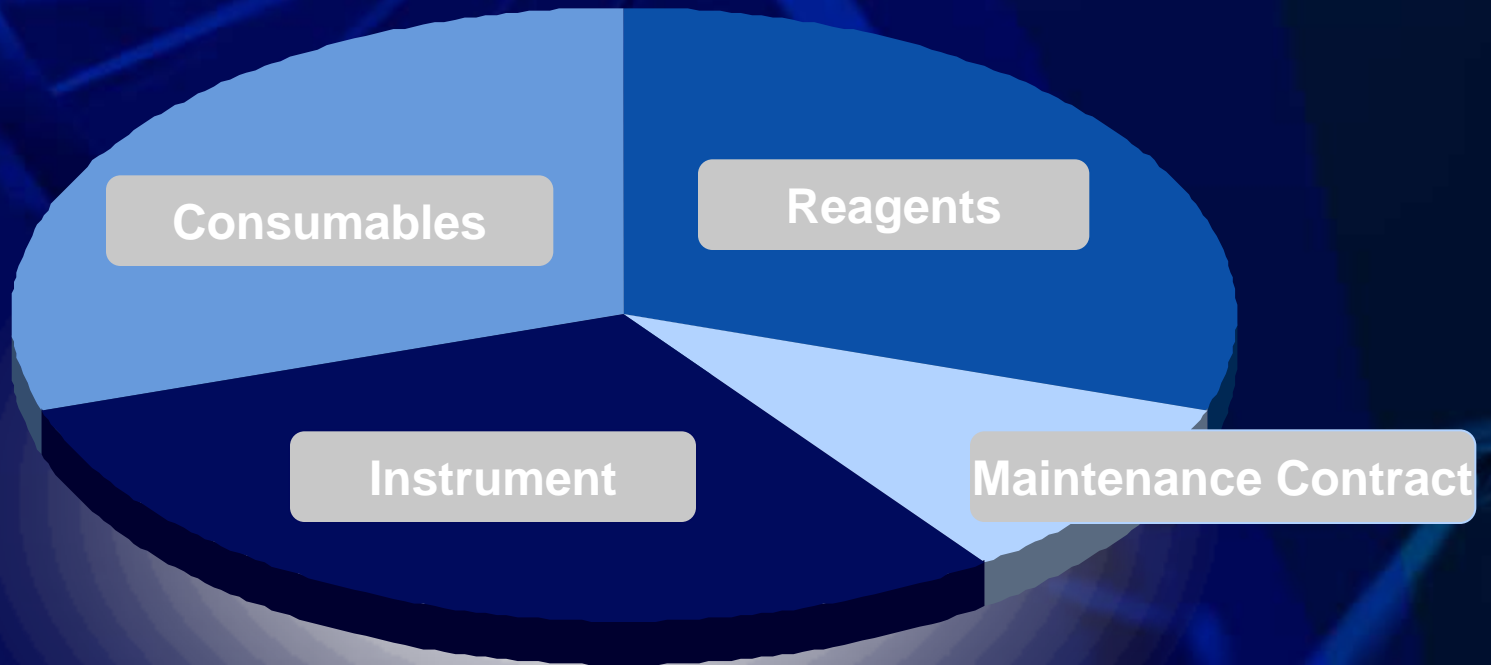
# Trends In Molecular Diagnostic Technologies



# DHMC Molecular Diagnostic Technologies

- **Gel Electrophoresis Methods**
  - Southern blot
  - PCR-based Restriction Fragment Length Polymorphism (RFLP) Analysis
- **Real Time PCR**
  - Allele specific PCR (dyes)
  - Allelic discrimination probes (Taqman)
- **DNA Sequencing**
  - Applied Biosystems
  - Beckman Coulter
  - Primera Dx
  - Pyrosequencing
- **Array**
  - Illumina
  - Luminex Bead Arrays
  - Nanosphere Verigene System
  - Roche AmpliChip
  - Hologic-TWT Invader Assays

# Cost



# Let's Offer The Test

- Identified need and market
- Identified platform with acceptable performance and TAT
- Adequate staffing and space
- What to do next: (Lab and IT)
  - Validation or verification
  - Update lab handbook/requisitions
  - Billing
  - Reporting
  - Educational materials



## FDA Approved Assay

- “In vitro diagnostic devices (IVDs) that include instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article intended for use in the diagnosis of disease or other conditions, or in the cure, treatment, or prevention of disease in man.”
- In the U.S., manufacturers are regulated by the FDA’s Center for Devices and Radiological Health (CDRH)

## Path to Market for IVD's

- 510(k) – premarket notification showing equivalency to existing device for safety and effectiveness for intended use – FDA Cleared
- Premarket approval (PMA) – no other existing device (novel agent, new method, analyte poses a major health threat), must establish clinical, relevance – FDA Approved
- Labeled “for in vitro diagnostic use”

# Laboratory Developed Tests (LDTs)

- Used for patient management
- Developed in a CLIA certified laboratory
- Consist of:
  - Modified FDA approved or cleared assay
  - Tests not subject to FDA clearance or approval
  - Test systems where performance is not provided by the manufacturer
- Modifications:
  - Test components (extraction, amplification, detection)
  - Procedure
  - Cutoff values
  - Specimen type or collection device (CAP allows for disclaimer if specimen type is rare)

# Analyte Specific Reagent (ASR)

- Published in the Code of Federal Regulations in 1997
- To ensure components were made consistently over time (GMP)
- ASRs are **NOT** diagnostic tests
- ASRs are key components: “antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which through specific binding or chemical reaction with substances in a sample, are intended for use in a diagnostic application for identification and quantification of an individual substance or ligand in biological specimens.”

## ASR Disclaimer

- Federal Regs require this be added to report: “This test was developed and its performance characteristics determined by [laboratory name]. It has not been cleared or approved by the U.S. Food and Drug Administration.”
- CAP recommends adding: “The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 as qualified to perform high complexity clinical laboratory testing.”

## Research-Use-Only (RUO)

- Not intended for diagnostic purposes
- Not regulated
- No performance claims or reference values
- Can not bill CMS for tests that use RUOs
- CAP allows RUO reagents to be used as components of tests when FDA approved or cleared or ASR products are not available
- Discretion of lab director



# Investigational-Use-Only (IUO)

- Products in phase of development that requires clinical investigational use for submission to FDA
- Only for use in clinical trials

# Validation/Verification

- Validation: proving that a procedure or instrument used works as expected and achieves the intended results or performance characteristics.
- Verification: For FDA cleared or approved processes, the lab must demonstrate that it can obtain performance characteristics similar to those claimed by the manufacturer.
- Clinical and Laboratory Standards Institute (CLSI)

# DHMC Molecular Test Menu 2011

## GENETICS

AAT  
aCGH  
**CF**  
FII  
FRAX  
FV  
HFE  
MTHFR  
SRY

## HEMEPATH

Bcl-2  
Bcr/Abl  
Chimerism  
FLT3  
IgH  
JAK2  
NPM1  
PML-RARA  
TCR

## IDENTITY

M/F Contam.  
Spec. ID  
Twinship

## INFECTIOUS DISEASES

BKV  
B. holmesii  
B. parapertussis  
B. pertussis  
**GBS**  
**HCV Quant**  
HCV Geno  
**HIV Quant**  
**HPV**  
**MRSA**  
Parvo B19

## ONCOLOGY

BRAF  
C-kit  
Colon MSI  
EGFR  
ELM4-ALK1  
**HER2**  
KRAS  
MGMT  
N-Myc  
Pancreas miRNA  
TTF-1

## Personalized Medicine

**UGT1A1**  
CYP2C9  
CYP2C19  
**CYP2D6**  
GSTP1  
IL28B  
VKORC1

9 of 50 (18%) tests are FDA approved/cleared

# Verification For FDA Cleared Assays (Example)

- Accuracy: 20 pos, 50 neg, 90% agreement with reference method
- Precision (Qual): 1 pos for 20 days or duplicates for 10 days (mean  $\pm$  sd)
- Precision (Quant): 20 data points, 2-3 conc, inter-run, intra-run, between day ( $< \pm 3$  sd)
- Analytical measurement range (reportable range): 5 conc in triplicate, determine upper and lower LOQ
- Normal range (reference interval): 100 values

# For Modified FDA, LDT or ASR (Example)

- Accuracy: 50 pos, 100 neg, 90% agreement with reference method
- Precision (Qual): 1 pos for 20 days or duplicates for 10 days (mean  $\pm$  sd)
- Precision (Quant): 20 data points, 2-3 conc, inter-run, intra-run, between day ( $< \pm 3$  sd)
- **Analytical sensitivity**: 15-20 samples at low conc (90% agreement)
- **Analytical specificity**: same sample matrix but no target
- Analytical measurement range: 5 conc in triplicate, determine upper and lower LOQ
- Normal range: 100 values

# Establishing Performance Specifications for LDTs

- Comparison of methods experiment (accuracy)
- Replication experiment (precision)
- Linearity experiment for quant assays (reportable range, LLOQ)
- LOD experiment (analytical sensitivity)
- Interference/cross reactivity experiment (analytical specificity)
- Reference value study (reference range)



# Quality Control

- Assay type: Qual vs Quant
- Amplification controls (NTC, neg, pos)
- Internal control for inhibition
- Extraction control

Pre-analytic



Analytic



Post-analytic

# Hurdles To Implementation

Validation/Verification

Training

Ordering

Reporting

Billing

Central vs decentralized

Turf claims

Hours of operation

Expertise

CLIA vs Non-CLIA lab



# Clinical Service

Impact

Clinical  
presence

**Clinical  
Service**

Client  
satisfaction





**Deliver the value!**

What if.....



# DHMC Molecular Pathology Laboratory and Translational Research Program

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