Molecular Diagnostics

510(k) Workshop 2012

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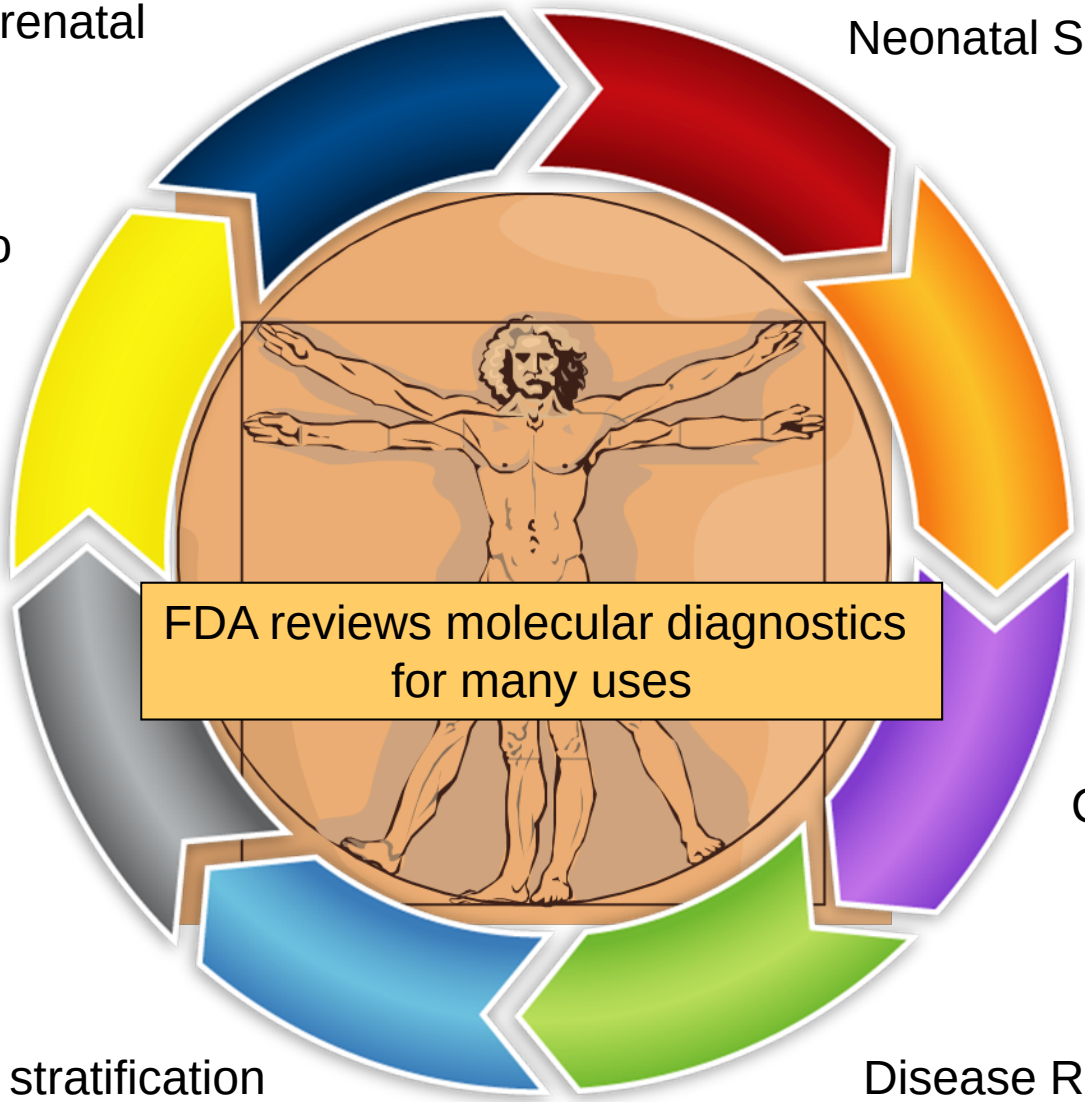

Prenatal
Neonatal Screening
Response to Treatment & Adverse Events
Infectious Disease
FDA reviews molecular diagnostics for many uses
Prognosis
Carrier Typing
Disease stratification
Disease Risk

FDA reviews tests for safety and effectiveness:

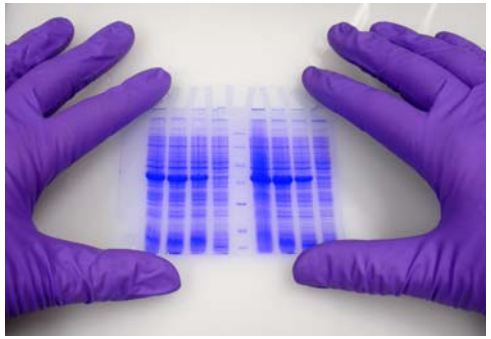
Genetic tests should demonstrate:

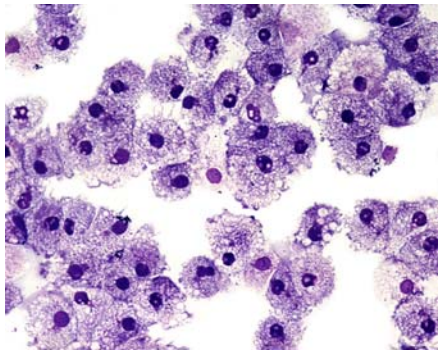
- Analytical Reliability
- Clinical significance
- Benefits outweigh the risks

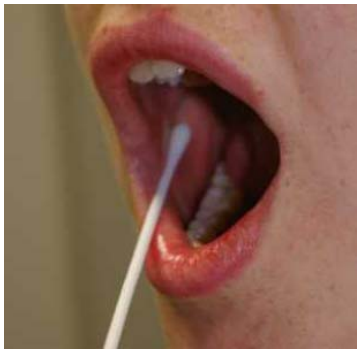
Patients and Physicians should know:

- How to interpret the information
- Clinical value of the information
- Limitations of the information

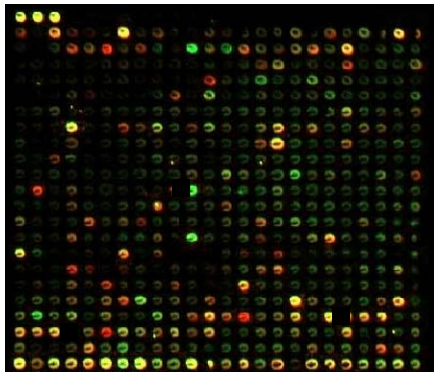
- Genetic Tests can be moderate risk (class II) or high risk (class III)
- Variety of Analytes
  - DNA
  - RNA
  - Gene Signatures ("score")
- Variety of Specimens
  - Whole blood
  - Buccal swab
  - Tumor Tissue
- Variety of Methodologies
  - PCR platforms
  - Microarrays
  - FISH







Courtesy: National Human Genome Research Institute & The University of North Carolina DNA Day program



Analytical Validation Studies

- Test = specimen ⟹ result (validate all steps)
- Pre-analytic steps are part of assay
  - e.g., bisulfite modification, melanin extraction, WGA
- Validation with each specimen type
- Have pre-specified acceptance criteria
- All studies should follow protocol in labeling
- Studies should demonstrate robustness at clinical cut-off, as needed
- Several options for pre-extraction reagents:
  - Provide reagents as part of assay
  - Recommend specific extraction kit
    (must be labeled appropriately, reg 21 CFR 862.2310 Clinical Sample concentrator)
  - Evaluate 3 methods, provide quality/quantity specs in labeling

Reagents, Instruments and Software

- Avoid Research Use Only (RUO) labeled instruments & components
- Require FDA review and clearance to market for clinical use
  - cGMP/QSR manufacturing required
  - 21 CFR 862.2570 Instrumentation For Clinical Multiplex Test Systems
- Follow the FDA guidance for premarket submission requirements for devices containing software and off-the-shelf software
- Note: collection kits and extraction kits are not General Purpose Reagents
- Microdissection instruments labeled RUO

Analytical Validation

Accuracy:

- Real clinical samples for everly claimed allele
- Span range of results (e.g., % tumor, % mutation), as applicable
- Compare to bi-directional sequencing
- With somatic genotyping in tumor tissue – pyrosequencing may be acceptable comparator
- Repeat testing once unless stated otherwise in label
- Report results before and after repeat testing for invalid and no calls, but miscalls not repeated

Analytical Validation

Precision/Reproducibility:

- 3 sites, 2 operators at each site, multiple days, multiple runs, duplicate
- Use clinical samples
- Use pre-extraction methods based on labeling
- May need separate extraction study
- Additional studies such as instrument to instrument and Lot-to-Lot

Analytical Validation

Analytical Sensitivity:

- **Dependent on intended use and specimen type**
- **Several factors with molecular assays**
  - **Minimum and Maximum Input DNA**
  - **Minimum % mutation detected in a background of Wild type**
  - **Minimum % tumor proportion**
  - **No template**
  - **DNA without allele of interest**

Analytical Validation

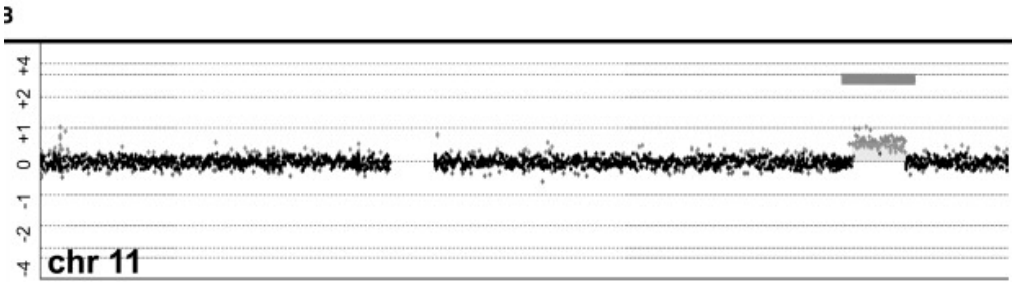
Other analytical performance studies as necessary:

- Specimen handling
- Primer-Probe specificity
- Cross-reactivity/exclusivity
- Interfering Substances
  - Co-adminstered drugs
  - Common endogenous and exogenous substances
  - Challenges associated with sample type
    - Hemolysis, lipemia
    - Necrotic tissue, fatty tissue
- Stability studies (reagent and specimen)
- Guard band studies

Challenges with Analytical Validation

- Specimen handling variability
- Difficulty obtaining clinical samples for rare alleles
- Multiplex assays often require complex validation
- Lack of reproducibility/ High analytical variability
- Analytes are not stable
- Lack of comparators, calibrators and standards
- Whole genome technologies present unique challenges to validation strategies

Cytogenetic Arrays



- Used to detect chromosomal abnormalities (copy number changes (CNV) (gains/losses)) in the DNA of a patient
- Survey the entire genome, unlimited results, open to interpretation
- Analyte is the whole genome, measuring range equivalent to detection claims for gains and deletions across the whole genome
- Analytical validation with large pool of banked samples (cell lines and clinical)
- Samples represent gains and deletions across entire genome
- Samples should include syndromes, challenging features, and specific claims such as mosaicism and uniparental disomy
- Samples also must support resolution claims across the genome
- Compare all results to a medically established validation method (FISH, Karyotyping, MLPA, PCR)

Cytogenetic Arrays

- Reproducibility studies: ~100 samples covering gains and losses across the genome; 3 sites, 2 operators at each site, 3 non-consecutive days.
- Clinical studies with prospectively collected samples from 2 or more clinical sites
- 'Expected values' with apparently healthy individuals
- Results limited to the level of validation
- Results limited to the indications for use
- Restrict use to certain professionals

Clinical Validation Molecular Dx

Key Points:

- Determine how it will be used in clinical setting ensure study design is appropriate
- Study design should support the Indications for
- Consider possible confounding covariables
- Risk Analysis: wrong results / effects of discordance
- Pre-specified clinical and statistical analysis plan
- Establish clinical performance of device compared to an endpoint or appropriate surrogate

Clinical Validation Molecular Dx

- Training Set(s), Validate IVD on independent dataset
- Analytical validation precedes clinical validation.

  (Fully designed device prior to phase III)
- Clearly state how performance will be calculated
- Sufficiently large sample size
- Retrospective samples selected using inclusion and exclusion criteria

Clinical Validation

- Retrospective samples can be used for some indications
  - be able to avoid bias due to missing samples, excluded cases, etc.
  - use a sample collection protocol
  - avoid convenience sampling
  - be able to ensure age and storage don't impact results
  - <u>reflective of current target population and treatments</u>
  - adequately annotated with necessary information
  - have appropriate outcome data on population, timepoints etc.

Clinical Validation

When peer-reviewed literature is used to support each claimed allele

- Should be summarized and organized
- Describe genotypes and associated phenotypes
- Information about prevalence in diseased and carrier population summarized by ethnicity
- Biological in vitro data about effect may be useful
- Statistical analysis plan

Statistical Plan

- Study results:
  - How results are reported to sponsor
  - How results are analyzed
  - Describe statistical tests
  - Describe how discrepant results are handled
- Definition of true positive, true negative, equivocal, and inconclusive results
- Primary endpoints
- "Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests" http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.htm

Companion Diagnostics

When safety or efficacy of a therapeutic relies on the result of a test:

(if the test doesn't work, the drug could be improperly administered)

- **CoDx generally considered significant risk devices (Class III/PMA)**
  - **Used to make treatment decisions**
  - **Carry the same risk profile as the drug**
- **Co-development is a device and drug/biologic collaboration**
  - **Cross-labeling**
  - **Concurrent FDA approval**
  - **CoDx require FDA review approval – even if the CoDx is an LDT**

Guidance document "In Vitro Companion Diagnostic Devices"
http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf

Co-Approval Successes

Date	Therapeutic	CoDx	Indication
2010-Oct 20	Herceptin* (trastuzumab)	Dako HercepTest and HER2 FISH pharmDx Kits*	-**HER2 overexpressing** breast cancer and metastatic gastric or gastroesophageal junction adenocarcinoma (*Co-approval for new indication)
2011-Aug 17	Zelboraf (vemurafenib)	Cobas 4800 BRAF V600 Mutation Test	-unresectable or metastatic melanoma with **BRAF V600E mutation** as detected by an FDA-approved test
2011-Aug 26	Xalkori (crizotinib)	Vysis ALK Break Apart FISH Probe Kit	-locally advanced or metastatic non-small cell lung cancer (NSCLC) that is anaplastic lymphoma kinase **(ALK)-positive** as detected by an FDA-approved test

Companion Diagnostics

- For CoDx, the clinical validity is supported by the drug trial.
- Training Set should be distinct from validation sample set
- Analytical validation precedes clinical validation
- (Sponsors are strongly advised to have a fully designed device prior to phase III)
- Mid-trial test changes problematic
- KEY ISSUE: When CoDx used to identify a distinct group of patients, the pharma sponsor needs to ensure that the same patient population can be identified after drug approval.

Companion Diagnostics

- Sponsors have options for regulatory oversight during test validation:
  - Submit an IDE for the device
  - Submit the device information in the IND
- Consider timing issues
- IDEs have been useful when the device manufacturer wants to remain separate from the Pharma company.
- When submitting to the IND include IDE relevant elements
  - SOPs for testing labs and reagent control
  - Number intended to screen
  - Number of testing sites
  - Whether there will be a charge implemented for the test
  - See IDE websites for additional info

Companion Diagnostics

- **Modular PMA review process: analytical, manufacturing, and clinical performance submitted in modules - useful for companion diagnostics.**

  Guidance document "Premarket Approval Application Modular Review"http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089767.pdf

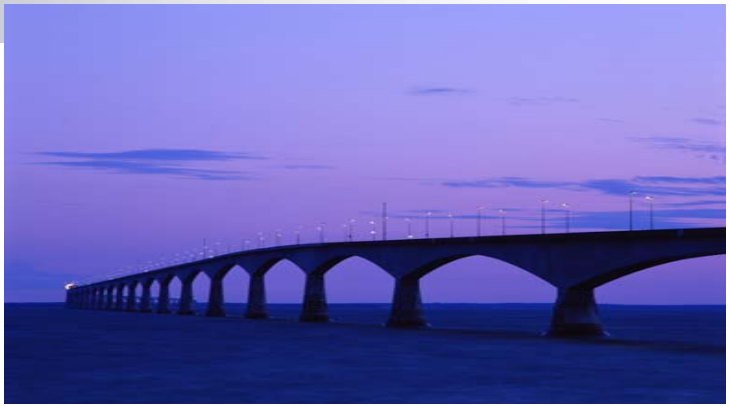
- **Master device files an option for proprietary drug data**

Bridging Studies:

Bridging studies may be necessary in certain situations:

- **Test used in drug trials not the marketed version**
- **Changes to test can change enrollment**
- **Need plan for sample acquisition, storage, and access for re-test analyses (SAVE both screen negative and screen positive)**
- **For studies that lack both marker – and +, analytical performance at cut-off is critical**
- **What if re-analysis using market test results provides different conclusions? Degree of discordance will be a review issue**
- **May need to provide evidence of analytical performance between old and new test.**

Bridging Studies:



- Need well annotated records for bridging studies (e.g., demographics, previous treatments and factors that affect the test such as %tumor content)
  - Factors that affect efficacy
  - Factors that affect test performance
- Need to control for bias due to lost samples
- Need both screen negative and screen positive
- Ensure storage conditions don't impact assay

Most Common Pitfalls

- Lack of samples available for re-test
- Inadequate annotation
- Storage factors (sample degradation)
- Lack of single validated assay/Assay design changes
- Design changes
- May involve more than one test
- Cannot account for post-trial discordance
- Lack of reproducibility
- Use of RUO instruments
- Pre-screening by enrollment sites (check prevalence at testing sites)

Tackling additional issues…

- Adding new analytes to multiplex test
  - Cannot treat the addition of the analyte as if it is a stand alone assay
  - Must demonstrate how well the assay now functions in toto
  - Re-establish the clinical and analytical performance of the previously cleared analytes in the new assay configuration

    October 13, 2011 FDA public meeting: Advancing Regulatory Science, Highly Multiplexed Microbiology / MCM Devices, (link: http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm267410.htm

- Expanding claimed mutations to test
  - Poorly understood/rare/pan-ethnic data not well known
  - not enrolled in clinical trial

Tackling additional issues…

- **Technological advances**

  June 23, 2011 FDA public meeting: Ultra High Throughput Sequencing for Clinical Diagnostic Applications - Approaches to Assess Analytical Validity
  http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm255327.htm

- **Practice of medicine ahead of clinical validated tests**

Examples of Genotyping Molecular Diagnostics

- **Drug Metabolizing Enzyme Genotyping Systems (Product codes NTI, ODW, ODV)**
- **CFTR Gene Mutation Detection Tests (Product code NUA)**
- **Factor V & Factor II Leiden Mutations, Genomic DNA PCR Test (Product Codes NPQ, NPR)**
- **Third Wave Technologies UGT1A1 Assay**
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K051824.pdf
- **cobas 4800 BRAF V600 Mutation Test**
  http://www.accessdata.fda.gov/cdrh_docs/pdf11/P110020b.pdf
- **Vysis ALK Break Apart FISH Probe Kit**
  http://www.accessdata.fda.gov/cdrh_docs/pdf11/P110012b.pdf
- **Vysis CLL FISH Probe Kit**
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K100015.pdf

Examples of "Omics" Molecular Diagnostics

Cleared 4 IVDMIA (one of which is a proteomics assay) in the de novo process

- Affymetrix GeneChip Microarray Instrumentation System
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K042279.pdf
- Agendia MammaPrint Assay
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K062694.pdf
- XDx AlloMap
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K073482.pdf
- Vermillion OVA1 (Protein-based IVDMIA)
  http://www.accessdata.fda.gov/cdrh_docs/reviews/K081754.pdf

Guidance and References for Molecular Diagnostics

http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm070274.htm

- Identification of IVDMIA (In Vitro Diagnostic Multivariate Index Assays)
- Special Controls Guidance document: Instrumentation for clinical multiplex test systems.
- Gene Expression Profiling Test System for Breast Cancer Prognosis
- Class II Special Controls Guidance Document: CFTR Gene Mutation Detection Systems
- Factor V Leiden DNA Mutation Detection Systems - Guidance for Industry and FDA Staff
- Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System
- Pharmacogenetic Tests and Genetic Tests for Heritable Markers
- Protein-Based Multiplex Assays: Mock Presubmissions to the US Food and Drug Administration" Regnier et al., Clin Chem 2009

Thank you

Questions?

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