Molecular Diagnostics

510(k) Workshop 2011

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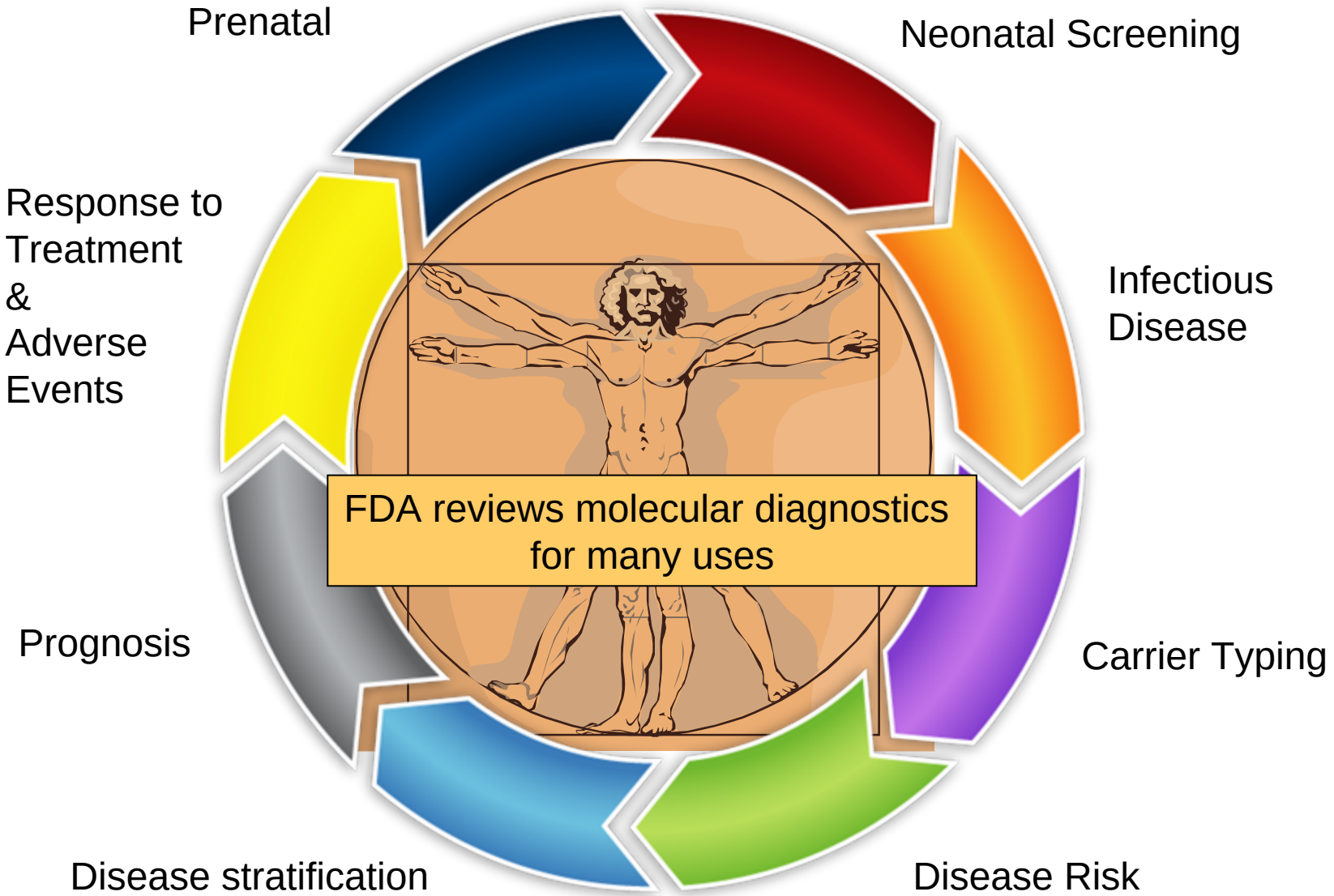

Prenatal
Neonatal Screening
Response to Treatment & Adverse Events
Infectious Disease
FDA reviews molecular diagnostics for many uses
Prognosis
Carrier Typing
Disease stratification
Disease Risk

FDA reviews tests for safety and effectiveness:

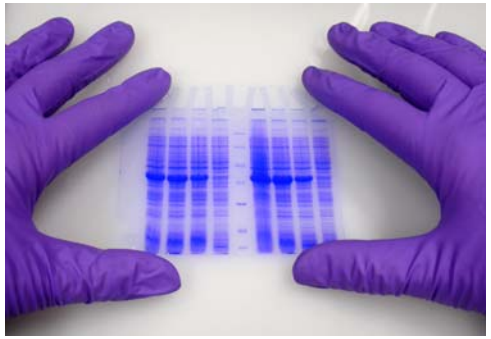
Genetic tests should demonstrate:

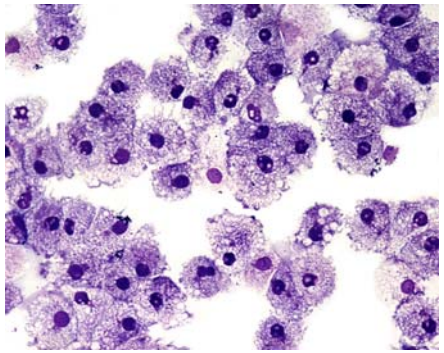
- Analytical Reliability
- Clinical significance
- Benefits outweigh the risks

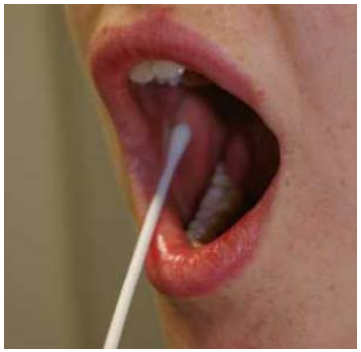
Patients and Physicians should know:

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

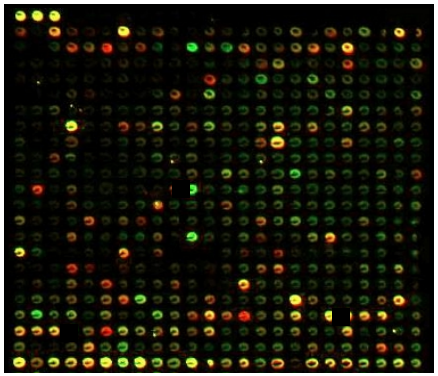
- Genetic Tests can be moderate risk (class II) or high risk (class III)
- Variety of Analytes
  - DNA
  - RNA
  - Gene Signatures ("score")
- Variety of Specimens
  - Whole blood
  - Buccal swab
  - Tumor Tissue
- Variety of Methodologies
  - PCR platforms
  - Microarrays
  - FISH







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
- Nucleic acid sample collection kits are regulated by type are also regulated.

Analytical Validation

Accuracy:

- Real clinical samples for everly claimed allele
- Span range of results (e.g., % tumor, % mutation), as applicable
- Compare to reference method/bi-directional sequencing
- Different approaches when bi-directional sequencing not sensitive enough
- Can conduct testing in-house
- 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
- Can use pooled extracted analyte in some cases
- May need separate extraction study
- One site can be in-house
- Additional stuides such as Instrument to instrument and Lot-to-Lot

Analytical Validation

Analytical Sensitivity:

Dependent on intended use and specimen type

For qualitative hereditary genotyping-

- **Lowest and highest concentration of input sample**

For qualitative tests with underlying quantitative component-

- **Limit of Blank –**
  - **No template**
  - **DNA without allele of interest**
- **Limit of Detection –**
  - **Minimum and Maximum Input DNA**
  - **Minimum % mutation detected in a background of Wild type**
  - **Minimum % tumor proportion**

Analytical Validation

Other analytical performance studies as necessary:

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

Analytical Validation

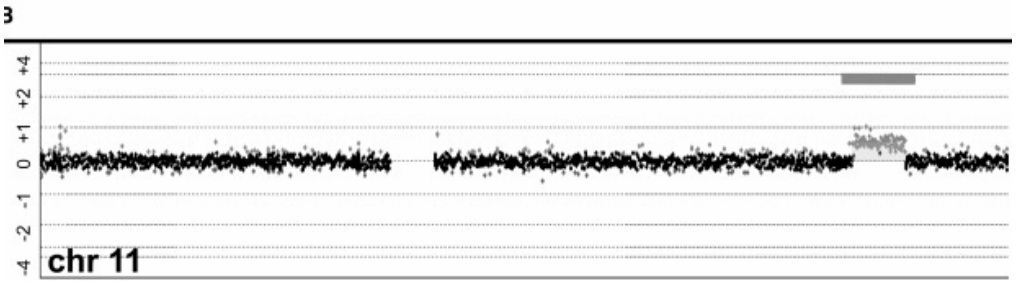
Genotyping assays with tumor tissue presents unique challenges. For example:

- **May need controls to identify "functional" template**
- **Exclusivity for multiple alleles**
- **% tumor proportion studies: may want to make macrodissection recommendations to improve performance**
- **Accuracy – comparator bidirectional sequencing likely not as sensitive**

Other Challenges with Analytical Validation

- Difficulty obtaining clinical samples for rare alleles
- Multiplex assays often require complex validation
- Bi-directional sequencing is the comparator – can be difficult for somatic mutations, deletions, translocations
- Lack of reproducibility/ High analytical variability
- Analytes are not stable
- Lack of 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 3 clinical sites
- Clinical specificity studies 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 and ensure study design is appropriate**
- **Demonstrate benefit beyond current practice**
- **Study design should support the Indications for Use**
- **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 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
  - reflective of current target population and treatments
  - 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

Required when therapeutic decisions are made/optimized on the basis of a test result:

Drug usage depends on the biomarker/test results (if the test doesn't work, the drug could be improperly administered)

- **Specific drug target required (e.g., EGFR testing for EGFR mAbs)**
- **Unique findings related to response (e.g., kras mutations)**
- **Dosing (Warfarin and CYP450)**
- **Distinct populations having adverse events (Abacavir and HLA-B*5701)**

Companion Diagnostics

- CoDx are combination products involving two Centers
- Regulatory requirements for devices and drugs are different
  - -Different review obligations
  - -Different timing requirements
- Requires concurrent FDA approval of both the device and the drug
- Co-labeling of the drug and device
- CoDx require FDA review approval – even if the CoDx is an LDT
- CoDx can be required e.g., EGFR testing for treatment with Erbitux (Cetuximab)
- CoDx can be recommended e.g., UGT1A testing for adverse events associated with Irinotecan

Companion Diagnostics

- Sponsors have options for regulatory oversight during test validation:
  - Submit an IDE for the device
  - Submit the device information in the IND
- Very important for sponsor to consider timing: don't want IND to be held up by outstanding device 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

Predictive Companion Diagnostics are generally Class III Devices

- **Carry the same risk profile as the drug**
- **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**

Companion Diagnostics

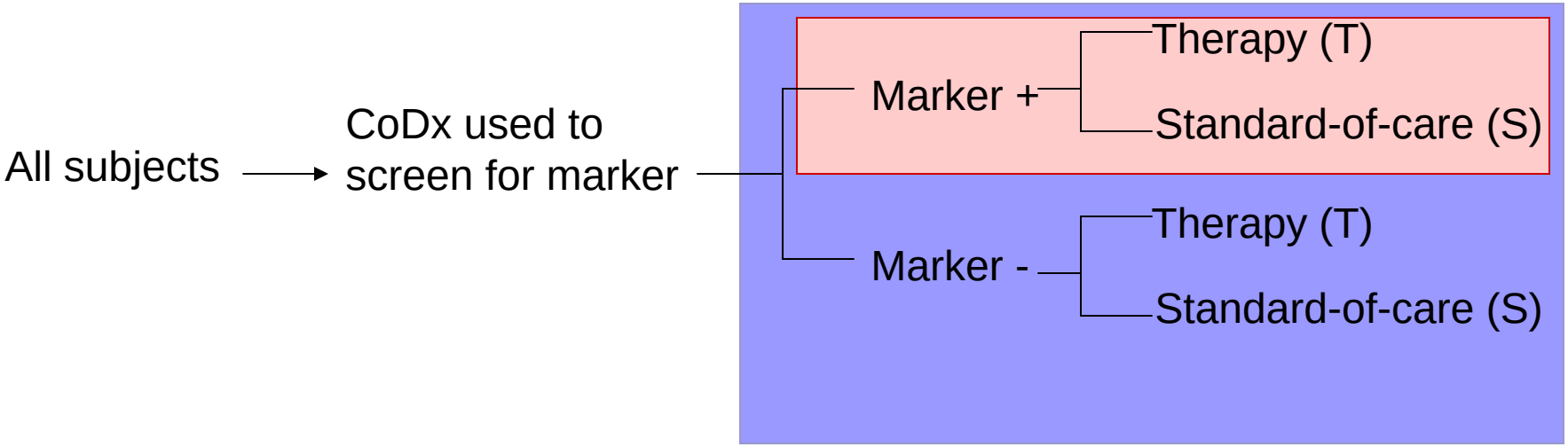
- For CoDx, the clinical validity is supported by the drug trial.
- Training Set should be distinct from validation sample set
- Analytical validation precedes clinical validation
- (Sponsors are strongly advised to have a fully designed device prior to phase III)
- Studies are prospective but some might be supported by retrospective studies (need to include CDER/CBER in discussion)
- The test is typically validated in the phase III study

Clinical Trial Designs

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.

Randomization is stratified by marker

"Predictive" claims for companion diagnostics rely on understanding the effect of the drug in both biomarker positive and biomarker negative patients.



Targeted Design

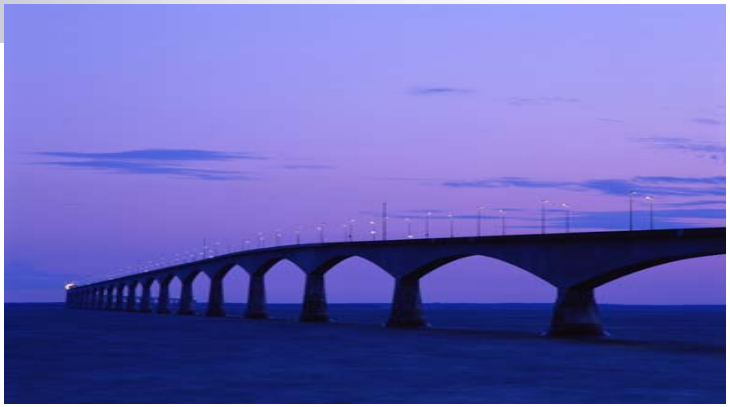
– Enroll a subgroup defined by marker; Test is not studied for effectiveness. Test claim limited to 'selection'.

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**
- **Will 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)

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

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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