NCI-FDA-NIST Workshop on Standards in Molecular Diagnostics

Whole Genome, High Density Platforms, Standards, and New Approaches to Evaluation of Molecular Assays

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December 7, 2012
Rockville, MD

Disclaimer

- I will touch briefly on various data, performance, and interpretation issues
- Thoughts presented here are preliminary and do not represent finalized FDA policy

- Pre-submission for outstanding questions

NGS challenges

- Rapid development of NGS technologies –
  - Paradigm-shifting, disruptive technology
  - Moving towards wider clinical use
  - Predicted change in how clinical practice / dx will be performed
  - Looking at the whole genome instead of just a few mutations in one gene
  - Raising new policy / regulatory issues

Overview

- Regulatory requirements and FDA evaluation of genomic tests (IVDs)
  - Performance information
    - Pre-analytical, analytical, and clinical performance
- Current developments
  - High density platforms / NGS / WGS
  - Challenges

Genomic Tests

- DNA, RNA-based; single marker, multiple marker tests
- Genotyping for
  - ✓ Carrier screening (e.g., CFTR)
  - ✓ Aid in diagnosis (e.g., FVL/FII/MTHFR, CFTR)
- Drug metabolism
  (e.g., CYP2C9, VKORC1)
- Markers for
  - ✓ Disease risk, prognosis
  - ✓ Therapeutic decisions – prediction, adverse events, dosing
- Infectious diseases
  - ✓ Detection, genotyping, viral load

Performance of FDA Approved/Cleared Genomic Tests Publicly Available

- Decision summaries of 510(k)s
  http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm
- SS&ED of PMAs
  http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMA/pma.cfm

What does FDA review for genomic tests?

- Intended use/indications for use
- Device description (platform, software)
- Pre-analytical
- Analytical Performance
- Clinical Performance
- Instrumentation, software validation (if applicable)
- Labeling (package insert)

Analytical Performance

- Does my test measure the analyte I think it does?
- Correctly?
- Reliably?

Non-NGS Genomic Tests…
Analytical Performance: Accuracy

- Evaluating by -
  - Comparison to a reference method
    e.g., bi-directional DNA sequencing
  - Comparison to a clinical truth
- Real clinical samples
  - Multiple clinical samples per allele
- Cover every claimed allele/result, genotype, subtype/class

* Accuracy - closeness of the agreement between the result of a test and result of reference method.

Non-NGS Genomic Tests…

Analytical Performance: Accuracy

-- example (accuracy data per allele vs. per test?)

Alleles	**% Agreement**
Total	98.40
Alleles 1 - 20	100.00
Allele 21	87.50
Allele 22	66.67
Allele 23	96.55

Non-NGS Genomic Tests…
Analytical Performance: Precision

- Studies to demonstrate intended users can get reliable results
- All sources of variability should be identified and assessed
- Use clinical samples where possible
  - Adequate coverage of all genotypes/tumor types
  - In limited cases (i.e., very rare alleles) may use contrived samples
  - Samples should mimic the molecular composition and concentration of real clinical samples
- All analytical steps of the assay should be included

Test Performance Evaluation

- **Analytical performance** - does my test measure the analyte I think it does? Correctly? How reliably?

- **Clinical performance** - does my test result correlate with target condition of interest in a clinically significant way?

Clinical Performance

- When sufficient information, well-known association between genetic variants and medical condition –
  - For each claimed allele:
    - Peer-reviewed articles
    - Genotype - phenotype
  - Example - CFTR mutation panel – ACMG/ACOG, literature
- When not enough information, well-known association between genetic variants and medical condition –
  - Likely require clinical studies
  - Example - mutations in a novel gene to predict risk of developing cancer

Summary: What Does FDA Review for Genomic Tests?

Safety and effectiveness based on:

- Satisfactory analytical performance
- Clinical performance in the context of use
- Labeling compliant with labeling regulations for IVDs (21 CFR 809.10)
- Other factors such as ability to repeatedly manufacture the device to specifications

Challenges – High Density Platforms

- Unlimited results, open to interpretation --> clinical significance?
- Adequate demonstration of analytical reliability of all possible outputs?
  - Cannot expect clinical samples that span all possible variation in the genome for studies
  - Accuracy – reference method? What is “truth” analytically?
  - Difficult to capture all sources of analytical variation
  - Expected accuracy / precision?
- Controls? Standards?

Public Meeting to Discuss WGS / NGS

News & Events (Medical Devices)
Workshops & Conferences (Medical Devices)
Past Workshops & Conferences

Ultra High Throughput Sequencing for Clinical Diagnostic Applications - Approaches to Assess Analytical Validity, June 23, 2011

The Food and Drug Administration (FDA) is announcing a public meeting Ultra High Throughput Sequencing for Clinical Diagnostic Applications - Approaches to Assess Analytical Validity.

The purpose of the meeting is to discuss challenges in assessing analytical performance for ultra high throughput genomic sequencing-based clinical applications.

- Topics:
  - Technical performance evaluation of sequencing platforms
    - Accuracy evaluation, validation samples, analytical standards
  - Bioinformatics
    - Data format, storage, data analysis

http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm255327.htm

Applications / Scenarios of use (ACMG)

- Targeted; WES; WGS

- Considered in the clinical diagnostic assessment of a phenotypically affected individual when:
  - Strongly implicated genetic etiology, phenotype does not correspond with a specific disorder.
  - A patient presents with a genetic disorder with high degree of genetic heterogeneity / multiple gene possibilities for the phenotype.
  - A likely genetic disorder but specific genetic tests available for that phenotype have failed to arrive at a diagnosis.

  Plus:
  - Actionable targets in cancer (tumor/normal pairs)

Workflow / Modules

- Pre-analytical (sample collection, extraction / quantification, amplification)
- Library construction
- Amplification
- Detection - sequence generation
- Analysis –
  - Base calling (quality score)
  - Alignment
  - Variation identification

⇨ Confirmation

- Reagents
  - assay-specific
  - general
- Library preparation
- Detection (sequencing)
  - Instrument
  - Software
- Analysis
  - Formats?

⇨ Possible exchange between modules?

⇨ General vs specific clinical application modules

Analytical validation strategies?

- Need to develop efficient approaches for analytical validation of NGS / highly multiplexed genetic tests.
  - Cannot expect clinical samples covering every variation (traditional approach: explicitly validate each marker used in generating test result)
- Possible approach - select and validate adequate subset of genetic markers → inference that platform as a whole analytically valid -
  - Enrich with analytically challenging markers?
  - Include markers from relevant disorders?
  - Homopolymeric regions, indels, repeats, CNVs, redundant sequences, samples across the genome / chromosomes, etc?
- Perform confirmatory testing of the results?

Analytical validation strategies? (cont.)

→ Minimum percentage of genome that needs to be covered to understand the platform performance as a whole?

→ Perform confirmatory testing of the results?

- Comparator / reference methods; orthogonal methods?
- Use well characterized samples (eg, NA12878)?

Needs / possible reference materials

- SNVs
- CNVs, structural variants

- Characterized human reference materials? Cell lines?
  - Family trio? (1000 genomes samples, etc)
- Synthetic materials, spiked in controls? (-> cancer, ratios)

✓ Existing data?

✓ Data formats??

Analytical evaluation – criteria?

- Completeness, quality scores, sequencing depth / coverage, % correct base calls, haplotype error, quality likelihood, confidence levels?

- Variant calling – number of expected calls, base substitutions, insertions, deletions, inversions?
- Unbiased allele sampling; distribution of calls along chromosomes?
- Base quality scores?
- Easy-to-call vs difficult regions – different performance expectations?

Clinical evaluation / interpretation

- Platform
- Specific intended uses

- Confirmation of results?
- Data bases?
- Clinical interpretation by certified professionals?

Where do we go from here?

- Move towards FDA-regulated systems
  - Novel / flexible approach to validation
  - System needs to be analytically validated
  - Interpretation – expert, database development
- Work across government, academia, professional societies, industry, etc, towards validated technologies and best practices
- Build databases to increase generalizable knowledge

Collaborations / leveraging resources

- Collaborations across agencies - within FDA, with other federal partners, academia, MDIC
- Standardization efforts –
  - NIST – “genomes in a bottle”
  - CDC-led initiatives (GeT-RM, Nex-StoCT2)
  - NCBI
  - collaborating w/ CFSAN, NCTR
- Other initiatives –
  - CAP
  - ACMG (policy statement, April 2012)
  - CLSI, others

Looking Forward

- FDA's process is evolving
- Each new submission may raise different regulatory and scientific issues
- Contact the Agency early and often!

Summary

- Established way of regulating
  - Molecular / genetic tests
- Future?
  - WGS…