FDA Update on NGS Review

Jennifer S. Dickey Ph.D. RAC

October 17, 2014

Disclaimer

The views expressed during this presentation are those of the presenter and do not necessarily reflect the policy or position of the US FDA or the US government.

Outline

- FDA clearance of NGS platform and reagents
- Using the clearance of the panel variant and clinical sequencing assays as a case study
- 2 Challenges in NGS Validation
  - Clarifying database quality needs to establish clinical validity
  - Developing reference materials to assess analytical performance

FDA NEWS RELEASE

For Immediate Release: Nov. 19, 2013
Media Inquiries: Susan Laine, 301-796-5349, susan.laine@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

FDA allows marketing of four "next generation" gene sequencing devices
Two devices aid in screening and diagnosis of cystic fibrosis

Today the U.S. Food and Drug Administration allowed marketing of four diagnostic devices that can be used for high throughput gene sequencing, often referred to as "next generation sequencing" (NGS). These instruments, reagents, and test systems allow labs to sequence a patient's DNA (deoxyribonucleic acid).

The new technology also gives physicians the ability to take a broader look at their patients' genetic makeup and can help in diagnosing disease or identifying the cause of symptoms.

"NGS is changing the way we look at genomics," said Alberto Gutierrez, Ph.D., director of the Office of In Vitro Diagnostics and Radiological Health in FDA's Center for Devices and Radiological Health. "Before NGS, sequencing genes associated with a particular disease was a long and costly process. Today, we have the capability to read and interpret large segments of DNA very quickly in a single test and this information-rich technology is becoming more accessible for use by physicians in the care of their patients."

Two of the newly cleared devices are used to detect DNA changes in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which can result in cystic fibrosis (CF), an inherited chronic disease that affects the lungs, pancreas, liver, intestines, and other organs of those who inherit a faulty CFTR gene from both parents.

Recent Clearances of NGS Devices

- K123989, NGS platform → De Novo 510(k)
- K133136, NGS kit reagents → De Novo 510(K)

- K124006, Variant panel assay → 510(k)
- K132750, Clinical sequencing assay → 510(k)

Illumina MiSeqDx Platform

21 CFR 862.2265 High throughput genomic sequence analyzer for clinical use

- Class II exempt from the premarket notification requirement subject to the limitations in 21 CFR 862.9

- The MiSeqDx Platform is a sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of instrument specific reagents and flow cells (MiSeqDx Universal Kit 1.0), imaging hardware, and data analysis software.
- The MiSeqDx Platform is intended for targeted sequencing of human genomic DNA from peripheral whole blood samples.
- The MiSeqDx Platform is not intended for whole genome or de novo sequencing.

21 CFR 862.2265 – Special Controls

- The labeling for the instrument system must reference pre-analytical and analytical reagents to be used with the instrument system and include or reference legally marketed analytical software that includes sequence alignment and variant calling functions.

- The labeling for the instrument system must include a description of the following information:
  i. The specimen type(s) validated as an appropriate source of nucleic acid for this instrument.
  ii. The type(s) of nucleic acids (e.g., germline DNA, tumor DNA) validated with this instrument.
  iii. The type(s) of sequence variations (e.g. single nucleotide variants, insertions, deletions) validated with this instrument.
  iv. The type(s) of sequencing (e.g., targeted sequencing) validated with this instrument.
  v. The appropriate read depth for the sensitivity claimed and validation information supporting those claims.
  vi. The nucleic acid extraction method(s) validated for use with the instrument system.
  vii. Limitations must specify the types of sequence variations that the instrument cannot detect with the claimed accuracy and precision (e.g., insertions or deletions larger than a certain size, translocations).

21 CFR 862.2265 – Special Controls

Performance characteristics of the instrument system must include:

A. Reproducibility data using multiple instruments, operators, sites. Samples tested must include all claimed specimen types, nucleic acid types, sequence variation types, and types of sequencing. Variants queried shall be located in varying sequence context (e.g., different chromosomes, GC-rich regions). Device results shall be compared to high confidence reference sequence data.

B. Accuracy data for all claimed specimen types and nucleic acid types generated by testing a panel of well-characterized samples to query all claimed sequence variation types, types of sequencing, and sequences located in varying sequence context (e.g., different chromosomes, GC-rich regions). The well-characterized sample panel shall include samples from at least two sources that have highly confident sequence based on well-validated sequencing methods. At least one reference source shall have sequence generated independently of the manufacturer with respect to technology and analysis. Percent agreement and percent disagreement with the reference sequences must be described for all regions queried by the instrument.

21 CFR 862.2265 – Special Controls

Performance characteristics of the instrument system must include (cont.):

C. If applicable, data describing endogenous or exogenous substances that may interfere with the instrument system.

D. If applicable, data demonstrating the ability of the system to consistently generate an accurate result for a given sample across different indexing primer combinations.

– The upper and lower limit of input nucleic acid that will achieve the claimed accuracy and reproducibility.

MiSeqDx Universal Kit

21 CFR 862.3800 Reagents for molecular diagnostic instrument test systems

- Class I exempt: general controls, including current good manufacturing practices

- The MiSeqDx Universal Kit 1.0 is a set of reagents and consumables used in the processing of human genomic DNA samples derived from peripheral whole blood, and in the subsequent targeted re-sequencing of the resulting sample libraries. User-supplied analyte specific reagents are required for the preparation of libraries targeting specific genomic regions of interest.
- The MiSeqDx Universal Kit 1.0 is intended for use with the MiSeqDx instrument.

Recent Clearances of NGS Devices

- K123989, NGS platform → De Novo 510(k)
- K133136, NGS kit reagents → De Novo 510(K)

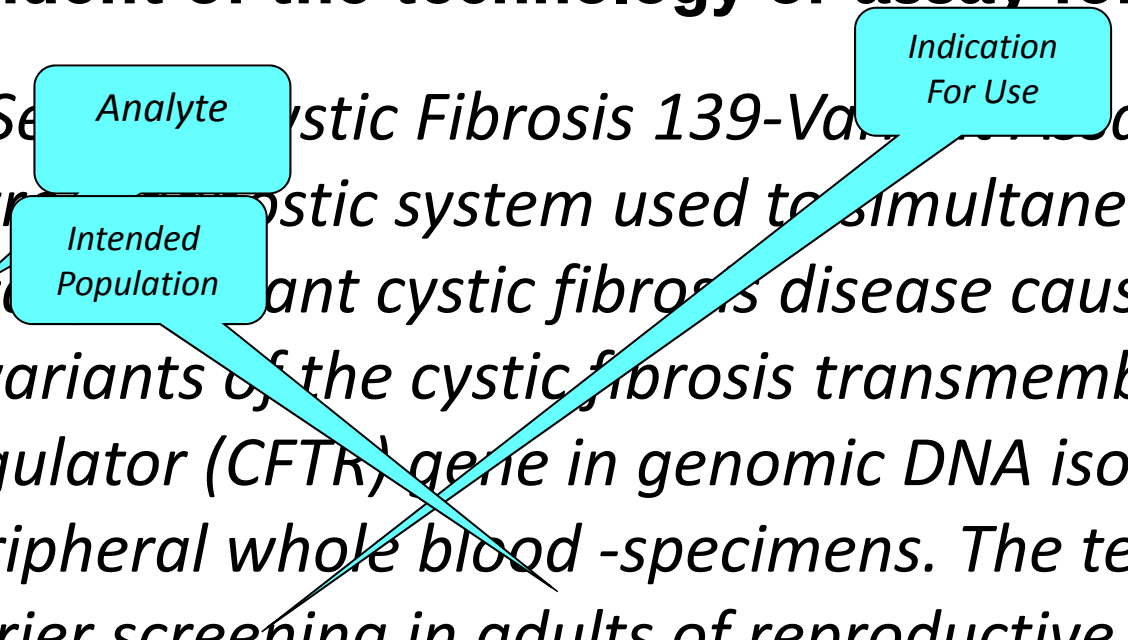
- K124006, Variant panel assay → 510(k)
- K132750, Clinical sequencing assay → 510(k)

Major Elements of IVD Submission

a) Intended use/indications for use
b) Analytical (and Pre-analytical) Validation
c) Clinical Validation
d) Device description
e) Instrument and software validation, if applicable
f) Labeling (package insert)

Intended Use

Review path, types of studies depend on IU claims; Independent of the technology or assay format.



The Illumina MiSe[q] [Cys]tic Fibrosis 139-Va[ri]ant Assay is a qualitative in vitr[o] [diag]nostic system used to simultaneously detect 139 clinic[ally] relevant cystic fibrosis disease causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood -specimens. The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis.

Analyte

Variant Panel Assay

- Discrete number of variants
- Variant types well understood (SNV, indel)
- Known clinical significance
- Assay reporting limited to the variants claimed

Clinical Sequencing Assay

- Large number of possible variants
- Different types of variants possible
- Unknown clinical significance
- Assay reporting requires trained interpretation

Strength of NGS

Public health need to also know about rare variants with known clinical significance but few samples

Major Elements of IVD Submission

a) Intended use/indications for use
b) Analytical (and Pre-analytical) Validation
c) Clinical Validation
d) Device description
e) Instrument and software validation, if applicable
f) Labeling (package insert)

Analytical validity—does my test measure the analyte I think it does? Correctly? Reliably?

Analytical Validation

Variant Panel Assay

- Accuracy of common variants established with clinical samples
- Accuracy of extremely rare variants established with plasmid blends
- Assay reproducibility established with only a subset of variants

Clinical Sequencing Assay

- Accuracy in claimed genomic region (clinical samples)
- Accuracy considered both variant calls and WT calls
- Validation of possible **types** of variants claimed
- Validation of assay performance in “tricky” regions

Major Elements of IVD Submission

a) Intended use/indications for use
b) Analytical (and Pre-analytical) Validation
c) Clinical Validation
d) Device description
e) Instrument and software validation, if applicable
f) Labeling (package insert)

Clinical validity—does my test result correlate with the expected clinical presentation? How reliably?

Clinical Validity

Variant Panel Assay

- Clinical Validity established for already cleared variants
- Clinical Validity for new variants established with a well documented/curated database

Clinical Sequencing Assay

- No clear-cut clinical validity
- Assay reports variants from reference with no annotations on significance
- Large burden on assay interpretation

Strength of NGS

Public health need to gather data to understand genotype/phenotype link in complex disease

Major Elements of IVD Submission

a) Intended use/indications for use
b) Analytical (and Pre-analytical) Validation
c) Clinical Validation
d) Device description
e) Instrument and software validation, if applicable
f) Labeling (package insert)

Labeling

Variant Panel Assay

- Labeling to validate with a second method variants analytically validated only in plasmid (no clinical samples)

Clinical Sequencing Assay

- "The test is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations."

Challenge of NGS

"The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent"

Challenge 1: Clarifying database quality needs to establish clinical validity

- Existing databases – fragmented, different levels of data quality, curation, evidence, data formats, accessibility, etc.

- Support database building efforts / unified resource of clinically relevant variants
  - "Regulatory quality" information of disease-associated genetic variation to be used in lieu / as a part of clinical evaluation
  - Address knowledge gaps

- ClinVar / ClinGen
    - NCBI, NHGRI, ACMG, ICCG

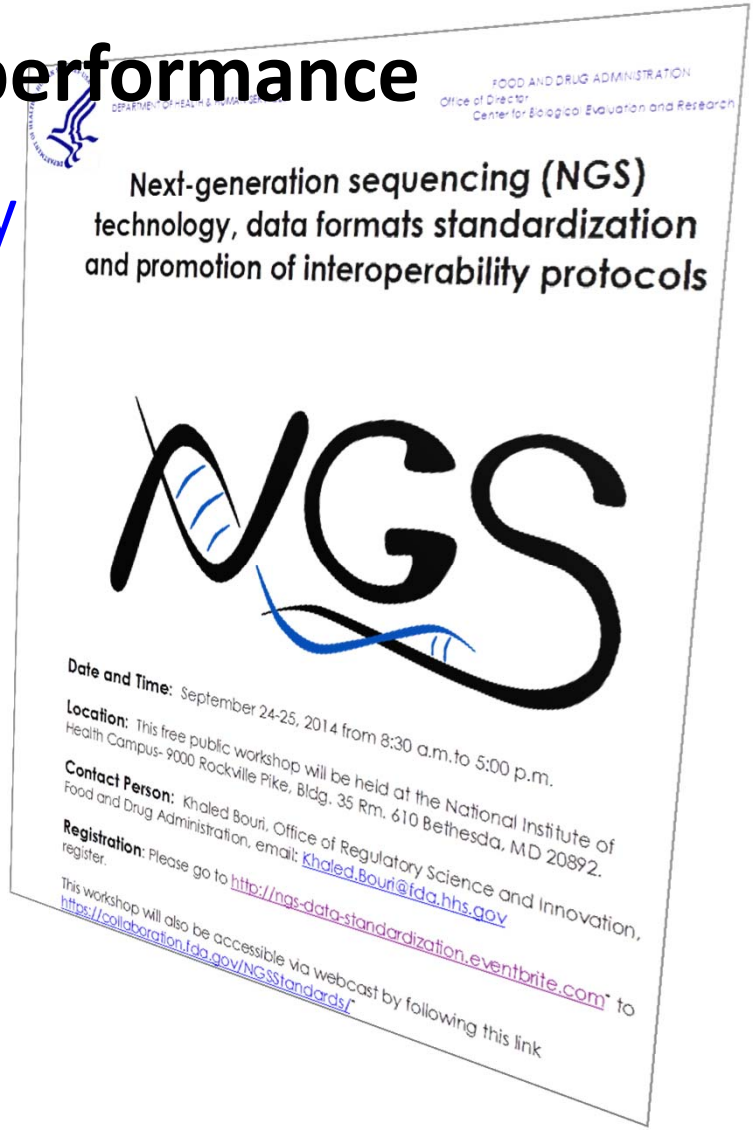
"Clinical grade" databases - possible best practice features

- **Standard nomenclature** for gene name, genomic coordinates, nucleotide change, amino acid change, etc (variant name, associated nomenclature, characteristics)
- **Annotation history** - complete provenance tracking of a start-to-finish analytical pipeline, a record of versions of databases and tools that are used in the analytical pipeline, pipeline parameters, cutoffs used for the particular assay quality filter, any changes in versioning of annotation
- **Clinical consequences of a variant** –
  - **Evaluation of research literature** - SOP in place for evaluation of research literature; a pre-curated literature knowledge base
  - **Pathogenicity** - SOP in place for evaluation of evidence used in the pathogenicity determination; inclusion of clinical/phenotypic characteristics as part of the variant assessment process
  - **Curation process –** hierarchy of qualified curators with appropriate background; SOP in place for curation process and curator training

Adapted from Deloitte report, 2014

Challenge 2: Developing reference materials to assess analytical performance

- Support development of clinically relevant variant resource
- Development of reference materials – GIAB (human, microbial RMs)
- CDC-facilitated effort to address non-standardized file formats in the clinical arena
- Upcoming FDA-hosted public conference



FOOD AND DRUG ADMINISTRATION
Office of Director
Center for Biologics Evaluation and Research

Next-generation sequencing (NGS) technology, data formats standardization and promotion of interoperability protocols

NGS

Date and Time: September 24-25, 2014 from 8:30 a.m. to 5:00 p.m.

Location: This free public workshop will be held at the National Institute of Health Campus- 9000 Rockville Pike, Bldg. 35 Rm. 610 Bethesda, MD 20892.

Contact Person: Khaled Bouri, Office of Regulatory Science and Innovation, Food and Drug Administration, email: Khaled.Bouri@fda.hhs.gov

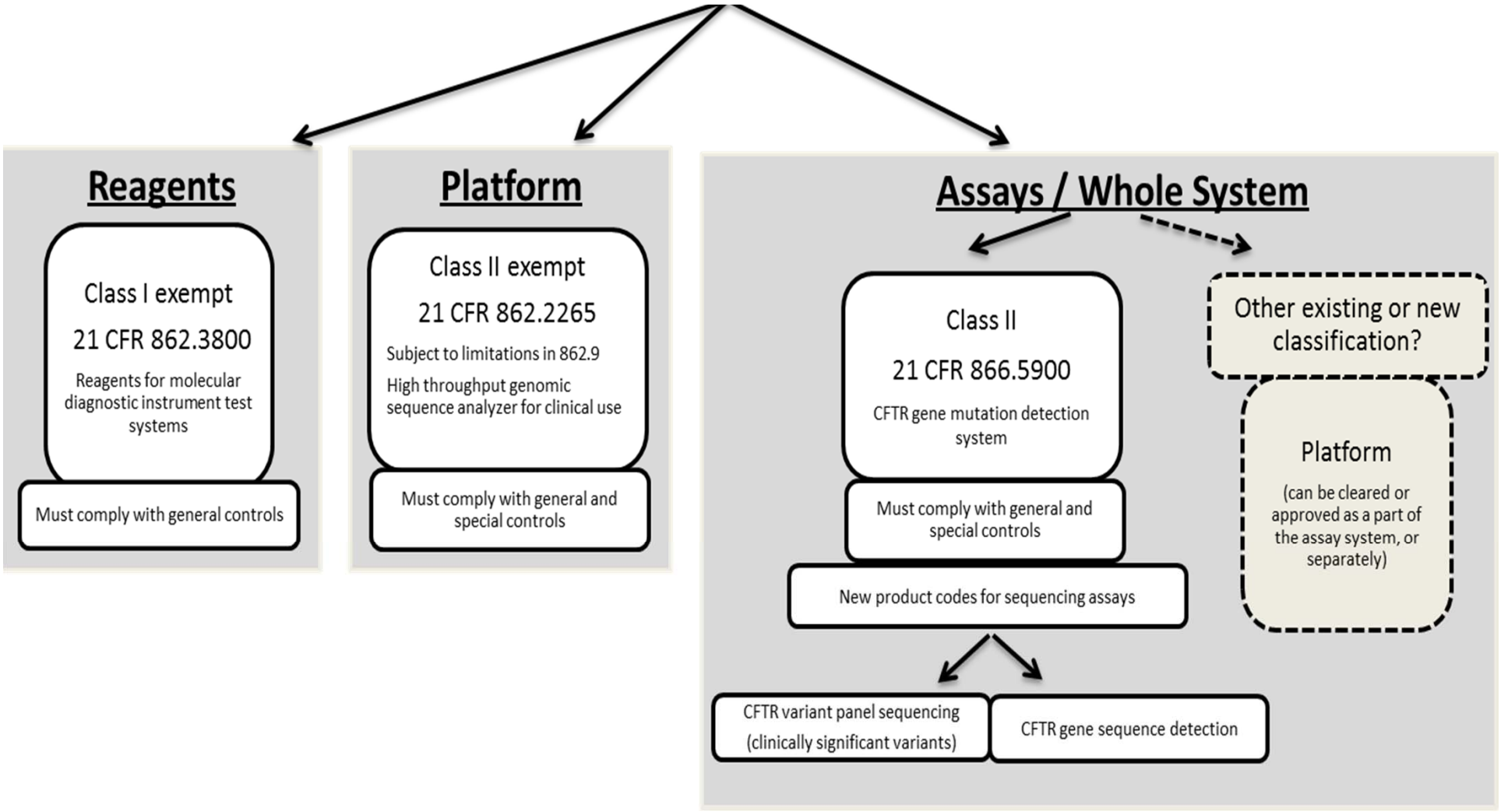
Registration: Please go to http://ngs-data-standardization.eventbrite.com* to register.

This workshop will also be accessible via webcast by following this link https://collaboration.fda.gov/NGSStandards/

The Genome in a Bottle Consortium

- FDA collaborating with the National Institute of Standards and Technology (NIST) through GIAB – developing reference materials, reference data, and reference methods to assess NGS performance
- Building pieces of framework needed to ensure the validation and quality of NGS-based tests:
  - Validation of sequencing platforms, associated algorithms
  - Performance assessment and quality control of NGS-based clinical diagnostic assays
- Methods developed to characterize the initial materials can be used to characterize other human or microbial materials

Current NGS Picture



From Bijwaard et al, submitted

More Information on NGS Regulation

FDA Website (Nucleic Acid Based Tests):
http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm

Decision Summaries:

Reagents:

http://www.accessdata.fda.gov/cdrh_docs/reviews/K133136.pdf

Instrument:

http://www.accessdata.fda.gov/cdrh_docs/reviews/K133136.pdf

Assays:

http://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf
http://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf

Thank You!

Jennifer Dickey

Jennifer.dickey@fda.hhs.gov