U.S. Food and Drug Administration

# Enabling Sequence-based Technologies for Micro Diagnostics:

## FDA Division of Microbiology Devices Perspective

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# Disclaimer

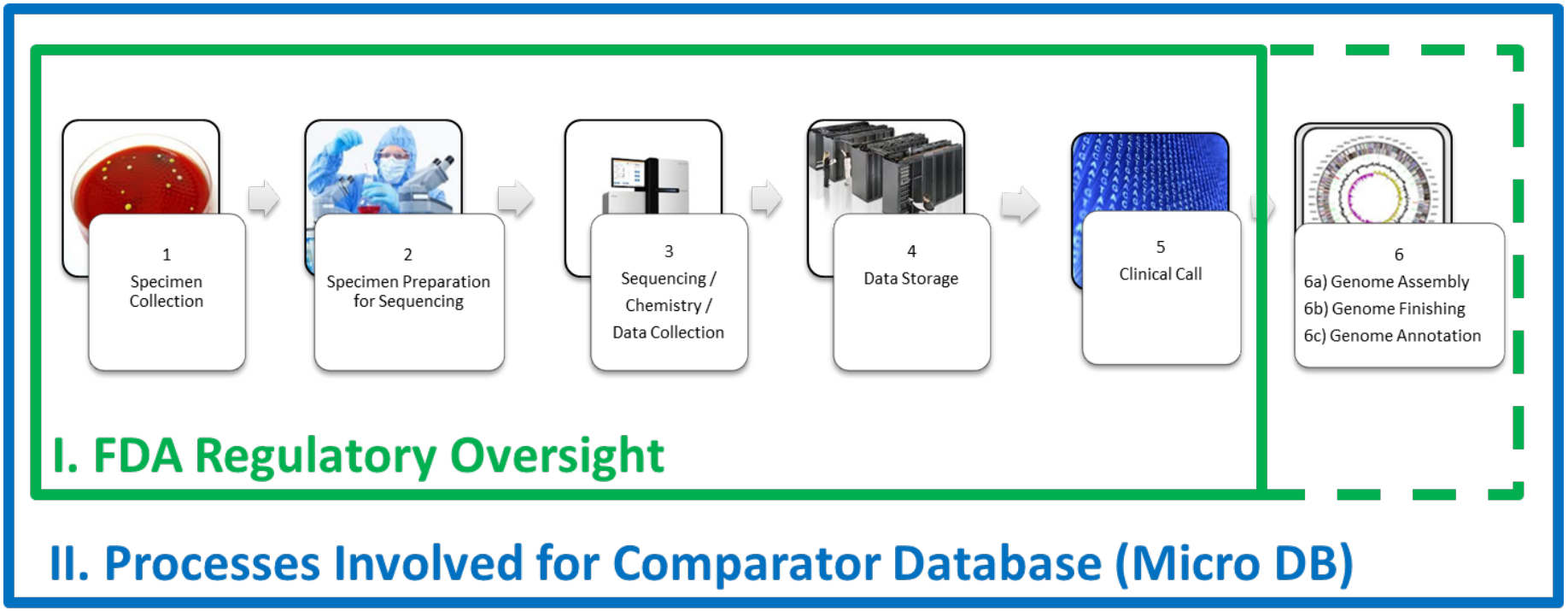
- Sequence-based diagnostic devices for the Microbiology Laboratory are raising new policy / regulatory issues; thoughts presented here are preliminary and do not represent finalized FDA policy

- Pre-submission for outstanding questions

# Microbial Dx Sequence Based Technologies

1. Possible approaches to validation studies for High Throughput Sequencing (HTS) systems
   - Metagenomic vs. Targeted (custom amplicon)
   - Use of sequence outputs in combination with database to evaluate performance
   - HTS as a comparator for regulatory submissions
2. Inter-Agency Working Group on Feasibility
3. NIST Microbial Reference Materials

# 1. Potential Validation Strategies



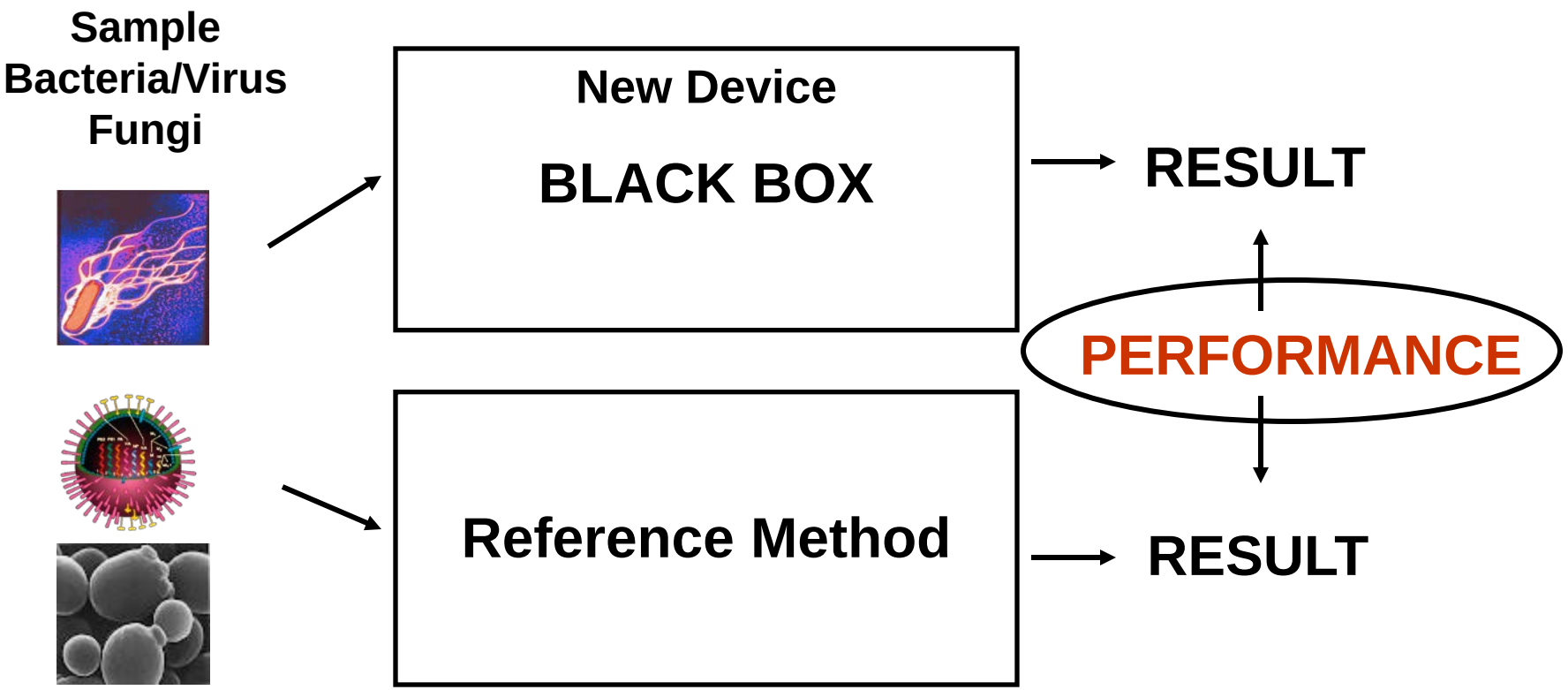

- System approach – collection to result
- Impact of format on validation strategies
  - Metagenomic vs. Targeted (custom amplicon)

# System Approach ??

- Sampling
- “pre-analytical” Steps
  - Lysis
  - Nucleic Acid Isolation
  - Library construction
- Amplification
- Sequence Detection/Read
- Assembly
- Database Query/Algorithm
- Database

# Evaluation of Diagnostic Devices

## FDA's general concept of diagnostic device evaluation




**Problem: each possible organism needs confirmation by reference method (ref. positive or negative)**

# Metagenomic Sequencing Validation Studies

## Clinical Evaluation (multisite)

Clinical Performance

- Multisite (end-user)
- Designed to test the Data Analysis Pipeline – Final call determination
- Performance could be established through comparison to database

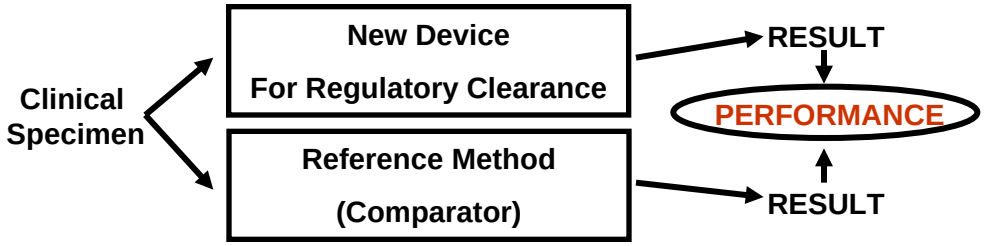
Precision/Reproducibility

- Panel of representative microorganisms
- Microbial Reference Standards (NIST)

## Analytical Studies (in-house)

- Specimen Type and Handling
- Library Prep
- LOD *
- Inclusivity *
- Interference
  - Specimen
  - Chemistry
- Contamination/Carry-over

# HTS as a Comparator




- Cornerstone of all microbiology regulatory submissions
- Accomplished using a comparator device
  - Can be a composite of multiple technologies
  - Burden increases with increase in analytes (especially relevant for multiplexed technologies)
  - May not be uniform

A standardized method that can accurately establish the presence/absence of a microbe in a given sample would be ideal

# 2. Inter-Agency Working Group on Feasibility

**Approach:**

- Formed a diverse working group **FDA, NIH-NCBI, NIAID, DTRA, LLNL, and CDC**
- Conducted small pilot study to generate information to evaluate quality of existing sequences in the public domain (In Progress)
  - Identify the pre-existing high-quality deposits, and build from there
  - Will use information to set quality bar for sequence outputs for our ongoing sequencing efforts
  - Utilized existing standards (if available) for technical and isolate metadata –no need to re-invent
    - Attention given to connecting antimicrobial resistance phenotype to genomic deposits – clinical collection site

# High-level Results Summary

1. **Unbiased benchtop NGS** is currently unable to make confident species calls from mock human samples at clinically-relevant titers
   - Clutter mitigation and/or targeted amplification will be needed

   **Highly-informative regions were seen at 10 GE/ml using AmpliSeq (LLNL/NMRC)**

2. **Sample carry-over** is a real concern, despite barcoding and aggressive cleaning protocols

   **Likely to be one of the biggest hurdles for NGS as a clinical diagnostic.**

3. **Human sequence** present in some microbial genomes can cause exciting results

   **LLNL analyzed all human data in NCBI to extract many kmers that are not in the reference human genome -> scalable way of filtering microbial genomes**

4. **Screening** against one reference human genome may be insufficient

   Some sequencing reagents cause false positive microbial hits if not vigorously masked (phiX174 control, Illumina adapter that has high homology to *E. coli,* NIST controls, etc.)

   **Aggressive screening of artificial constructs for all reference genome databases.**

5. **Restricted reference databases** provide a false sense of confidence about results

   Opportunities for false positives, false negatives, and missed detection due to limited taxonomic and/or strain coverage

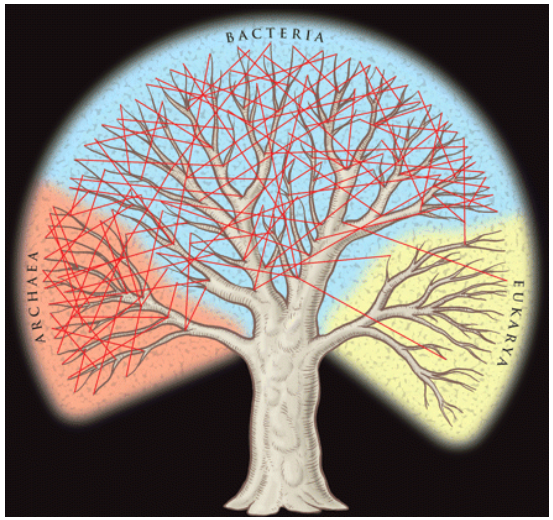
   **“Drive-by” NGS analyses are not a long-term solution to the very real problems of genome reference DB curation**

# Looking ahead: Predictions for Reference Databases

- **Multiple levels of Reference DBs likely**
  - **“High quality” genomes only**
    - **For validation and clinical use**
  - **“High quality” + other available genomes**
    - **For testing and development**
  - **Requires definition of “high quality” that *must* include some draft genomes**
- **Extensive screening required**
  - **Human and other hosts; chimeras**
  - **Artificial constructs**
- **Separate bacterial, viral, fungal reference DBs**
- **Publicly available (NCBI/EMBL/DDBJ)**

# Current Need

## Robust, Standardized, and High Quality Microbial Sequence Database in the Public Sector

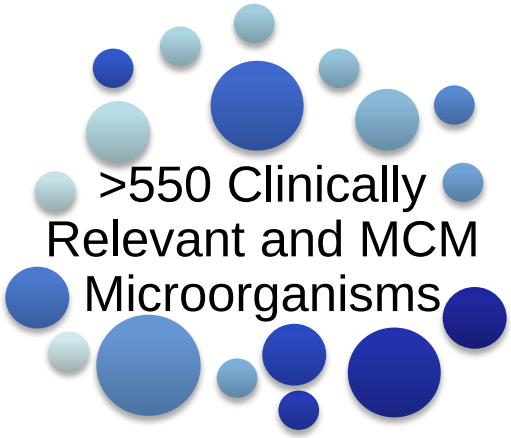



*Cover illustration*


- Representative Samples
- Metadata
- High quality raw sequences
- Assemblies
- Annotation
- Public Domain

# 3. Microbial Reference Database (MicroDB)

- Identify “gaps” and target sequencing efforts (Funding awarded by FDA/OCET)
  - Raw reads, assemblies, annotations, metadata sent to NCBI and accessible by the PUBLIC
  - Traceable results that could be reevaluated as necessary





Collaborations with Clinical Labs and Repositories

- Children’s National Hospital
- DoD Critical Reagents Program (CRP, USAMRIID)
- FDA-CFSAN, FDA-CBER, FDA-CDER
- DHS National Biodefense Analysis and Countermeasures Center (NBACC)
- The Rockefeller University
- Culture Collections: ATCC, DSMZ

Sequencing Center (UMD IGS)

- Hybrid Approach (PacBio and Illumina)
- Deposit of Raw Reads at NCBI (SRA)
- Deposit of Assemblies at NCBI
- Deposit of Annotations at NCBI
- FDA Interface to Access Data

# 4. NIST Microbial Reference Materials

- Characterizing Reference Materials for Clinical Applications of Genome Sequencing
- Reference Material (RM) “Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process”
- Genomes for NIST RMs
  - **Staphylococcus aureus – FDA interest**
  - **Salmonella typhimurium – FDA interest**
  - **Pseudomonas aeruginosa – high GC**
  - **Clostridium sporogenes - low GC**
- Contact: Nathaniel Olson, Ph.D. and Justin Zook, Ph.D. from NIST

# Conclusions

- To realize HTS as a regulated device for microbial diagnostics it is necessary to ensure that comparator information used for interpretation of results is of suitable quality and include appropriate metadata. Efforts are underway…
- There will be difference in the validation strategies for targeted vs. metagenomic sequencing applications
- Will enable a streamlined approach for regulatory evaluation
  - HTS may be another tool for commercial developers to utilize in the evaluation of other types of devices, especially for multiplexed devices
- Highlighted as a need by commercial developers and clinical end-users
  - For clinical adoption really need a simple solution (minimize bioinformatic staffing, etc.)
- FDA Division of Microbiology Devices will continue with current efforts to augment existing sequence information in the public domain

# Thank You

**FDA Micro Team**

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# Enable regulatory and clinical pathway for sequencing based devices in the Microbiology Laboratory

FDA OIR Micro Team