Linking the Scientific and Regulatory Environments for PHEMCE

Robert W. Fisher
07 January 2016
FDA and PHEMCE\(^1\)

- **PHEMCE**: protecting the U.S. from threats
  - Chemical, biological, radiological, nuclear (CBRN)
  - Emerging infectious diseases
- **FDA**: ensuring that medical countermeasures (MCMs) to counter these threats are safe, effective, and secure
  - Drugs, vaccines, diagnostic tests, personal protective equipment (PPE)

\(^1\)Public Health Emergency Medical Countermeasures Enterprise
Medical Countermeasures Initiative (MCMi)

Promote development and availability of safe, effective medical countermeasures
FDA MCMi

- Launched August 2010 in response to PHEMCE review of the U.S.’s readiness for public health emergencies
- FDA-wide initiative to coordinate medical countermeasure development, preparedness, and response
- FDA’s MCMi:
  - Establishes clear regulatory pathways for MCMs
  - Supports regulatory decision-making through the development of tools, standards, and approaches to assess MCM safety, efficacy, and quality
  - Establishes effective policies and mechanisms to safeguard and facilitate rapid access to MCMs
  - Is managed by FDA’s Office of Counterterrorism and Emerging Threats
OCET Responsibilities

• Coordinates MCMi
• FDA point of entry on policy, planning for:
  – Global health security
  – Counterterrorism
  – Emerging threats
• Identify and resolve complex scientific and regulatory challenges for MCMs
• Lead emergency use activities
• Develop and implement preparedness plans & programs
External Stakeholders

- NGOs & Think Tanks
- International
- Industry
- Academia
- Public
- State & Local
Building on Success

• Established agreements between FDA and its international counterparts that enabled information-sharing and effective collaboration
• Extended the expiry dating of certain lots of oral doxycycline for the prevention of anthrax disease held by state and local public health preparedness stakeholders
• Funded the establishment of a centralized repository of bacterial pathogens with well-characterized antimicrobial resistance profiles (in collaboration with CDC) representing more than 160 pathogens
Regulatory science case studies

- Anthrax vaccine stability: Drusilla Burns
- MCM dosing in special populations: Kevin Krudys
- Infectious disease diagnostics & FDA: Heike Sichtig
Thank you!

Robert W. Fisher

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202-329-3957

http://www.fda.gov/medicalcountermeasures

AskMCMi@fda.hhs.gov

@FDA_MCMi
Resources

• MCMi Regulatory Science program

• Extramural research funding and current projects

• Animal Rule information and guidance

• MCMi news and events (workshops, etc.)
  – http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/AboutMCMi/ucm262925.htm
CBER MCM Research and a Case Study: Prolonging Anthrax Vaccine Shelf Life
Scope of CBER’s MCM-Related Regulatory Science Program

Agents/Diseases
- Anthrax
- Botulism
- Tularemia
- Smallpox
- Viral hemorrhagic fevers
- Pandemic Influenza
- Emerging Infectious Disease

Chemical/Radiological/Nuclear Threats
- Cell therapies
Scope of CBER’s MCM-Related Regulatory Science Program

Issues addressed

• Manufacturing
• Product quality
• Assay development, especially potency and other lot release assays
• Animal models
• Biomarkers/correlates of protection
• Clinical trial design
• Post-marketing safety
Case study: Prolonging anthrax vaccine shelf life

• Anthrax is one of the most feared bioweapons

• Efforts are underway to develop new generation anthrax vaccines

• Not expected to be used for routine immunization of the general population

• Stockpiled for use in an emergency, so stability is key
Anthrax

From Collier and Young, *Sci. Am.* March 2002
Mechanism of action of anthrax toxin

Eukaryotic cell

PA \rightarrow LF or EF \rightarrow H^+
New generation anthrax vaccine design

- Based on PA (usually a recombinant form, rPA)
- Elicits toxin neutralizing antibodies
- Toxin neutralizing antibodies correlate with protection
- Neutralizing antibodies will be used as a measure of protection to assess the efficacy of new rPA vaccines
rPA vaccines

- Development is simple in concept but difficult in execution
- Development has stalled because of lack of stability
Toxin neutralizing titers of mice immunized with adjuvanted rPA vaccine
Understanding the molecular basis for rPA vaccine instability

- What changes in rPA occur upon storage?
  - Structural changes
  - Compositional changes

Do changes occur long-term that affect immunogenicity?
Understanding the molecular basis for rPA vaccine instability

• What changes in rPA occur upon storage?
  
  - Structural changes
  
  - Compositional changes

Do changes occur long-term that affect immunogenicity?
Formulation of rPA vaccine

rPA + aluminum adjuvant
Structural changes during storage detected by:

- Melting point analysis
- Intrinsic protein fluorescence
- Immunogenicity of specific regions of the protein
Over time rPA denatures on aluminum hydroxide adjuvant
Antibody population induced depends on antigen conformation
Antibody to buried epitopes may not recognize native antigen
Effect of adsorption onto aluminum adjuvant

- Dynamic structural changes in the protein occur upon storage on aluminum hydroxide adjuvant leading to loss of folded structure

- Conformational epitopes that may represent important neutralizing epitopes are lost
Understanding the molecular basis for rPA vaccine instability

• What changes in rPA occur upon storage?
  - Structural changes
  - Compositional changes

Do changes occur long-term that affect immunogenicity?
Understanding the molecular basis for rPA vaccine instability

- What changes in rPA occur upon storage?
  - Structural changes
  - Compositional changes

Do changes occur long-term that affect immunogenicity?
Deamidation of Asn residues in proteins

Deamidation-prone Asn residues of PA
Does spontaneous demidation of Asn residues play a role in the instability of rPA vaccines?

- Use site-specific mutagenesis to change six deamidation-prone Asn residues of rPA to Asp
- Purify the “genetically deamidated” mutant protein
- Examine its immunogenicity
Immunogenicity of rPA and six-Asp rPA

(P=0.0008)
Possible causes of low immunogenicity

- Conformational differences between WT and six-Asp rPA mutant
- Loss of immunodominant B-cell epitopes
- Differences in eliciting T-cell help
Conclusions

- Multiple factors may play a role in rPA vaccine instability
  - Significant structural changes that affect immunogenicity can occur when proteins are bound to aluminum adjuvant
  - Non-enzymatic protein modifications occur slowly over time that affect immunogenicity

- Use of adjuvants that allow retention of structure and use of conditions that slow deamidation might prolong vaccine lifetime
<table>
<thead>
<tr>
<th>Acknowledgements</th>
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<tbody>
<tr>
<td>Anita Verma</td>
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<tr>
<td>Leslie Wagner</td>
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<tr>
<td>Miriam Ngundi</td>
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<tr>
<td>Scott Stibitz, CBER</td>
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<tr>
<td>Beth McNichol, CBER</td>
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<tr>
<td>Juan Arciniega, CBER</td>
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<td>Rocio Dominguez-Castillo, CBER</td>
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<td>Juan Amador-Molina, CBER</td>
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<td>Bruce Meade, Meade Biologics</td>
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Determining the Dose of MCM Products in Special Populations

Kevin M. Krudys
Team Leader, Division of Pharmacometrics
Office of Clinical Pharmacology
Center for Drug Evaluation and Research
January 7, 2016
Selection of an Effective Dose in Humans

• Under the Animal Rule, a thorough understanding of the PK and PD data for the investigational drug or biologic is essential in selection of a dose regimen expected to be effective in humans.

• Clinical trials in healthy humans should evaluate safety and PK data over a range of doses.

• Multiple approaches to human dose selection are possible, with varying levels of uncertainty.
But Is This The Right Dose for **YOU**?

- Differences in response to medical products can be attributed to intrinsic and extrinsic factors.
- For example, PK interactions with medical products concomitantly used in the clinical scenario.
- Quantitative methods, such as PK modeling can be used to derive dosing of MCM products in special populations.

Huang et. al., Clinical Pharmacology and Therapeutics 2008
Case Study #1: Pediatric Dosing of Raxibacumab for Inhalation Anthrax
Starting Assumptions and Question

- 40 mg/kg dosing regimen may provide an acceptable benefit/risk profile for adult patients.
- Adult and pediatric patients are similar in terms of:
  - Disease progression
  - Response to the treatment
  - Exposure-response (E-R) relationship

*What pediatric dose of Raxibacumab is predicted to match adult exposure at 40 mg/kg?*
Workflow to Determine the Pediatric Dose

- **Learn** from adult population PK analysis
  - The relationship between PK parameters vs body weight
  - Inter-subject variability

- **Simulate** pediatric PK profiles using different dosing regimens
  - Various combinations of dose and body weight band

- **Select** a pediatric dosing regimen
  - Match the exposure (e.g., AUC*) observed in adults at 40 mg/Kg
  - Simple to implement

* AUC: Area under the concentration curve
Raxibacumab Clearance vs. Body Weight in Adults

Healthy adult PK @ 40 mg/Kg

Assuming the observed relationship between PK and body weight in adults is applicable to pediatric population

- Mainly eliminated by non-specific proteolysis
- Very unlikely to be eliminated by kidney due to its large size
Simulated AUC\textsubscript{inf} in Pediatric Population following Adult Dosing Regimen of 40 mg/kg

- Min in adults: 8720 ug\textsuperscript{•}day/mL
- 5 Percentile in adults: 12066 ug\textsuperscript{•}day/mL
- 95 Percentile in adults: 22478 ug\textsuperscript{•}day/mL
- Max in adults: 26971 ug\textsuperscript{•}day/mL

Body Weight

- 5 Kg
- 10 Kg
- 15 Kg
- 20 Kg
- 25 Kg
- 30 Kg
- 35 Kg
- 40 Kg
- 45 Kg
- 50 Kg
### Proposed Pediatric Dosing

<table>
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<th>Body Weight</th>
<th>Pediatric Dose</th>
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<td>&gt; 50 kg</td>
<td>40 mg/kg</td>
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<tr>
<td>&gt; 15 kg to ≤ 50 kg</td>
<td>60 mg/kg</td>
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<tr>
<td>≤ 15 kg</td>
<td>80 mg/kg</td>
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Simulated $\text{AUC}_{\text{inf}}$ in Pediatrics following the Proposed Dosing Regimen

- **80 mg/kg**
- **60 mg/kg**

**AUC (ug•day/ml)**

- 26971 ug•day/mL (Max in adults)
- 22478 ug•day/mL (95 Percentile in adults)
- 12066 ug•day/mL (5 Percentile in adults)
- 8720 ug•day/mL (Min in adults)
Summary

• Assumptions
  – 40 mg/kg may be safe and efficacious in adult patients
  – Extrapolation from adults to children
    • Disease, exposure-response, PK variability
    • Relationship between PK parameters and body weight

• Criteria
  – Match the observed exposure in adults at 40 mg/kg
  – Simple to implement

• Pediatric dosing

<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Pediatric Dose</th>
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<tbody>
<tr>
<td>&gt;50 kg</td>
<td>40 mg/kg</td>
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<tr>
<td>&gt; 15 kg to ≤ 50 kg</td>
<td>60 mg/kg</td>
</tr>
<tr>
<td>≤ 15 kg</td>
<td>80 mg/kg</td>
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Case Study #2: Dosing of Amoxicillin for Post-Exposure Inhalation Anthrax

Non-Labeled Dosing of Amoxicillin for Post-Exposure Inhalation Anthrax

- **Decision:** Dosing in the event of an intentional release of or accidental exposure to penicillin-susceptible strains of *B. anthracis*
- Amoxicillin may be considered when other antibacterial drugs are not as safe to use
- Dosing recommendations are based on the following:
  1. Maintain plasma concentrations above an MIC of 0.125 mcg/mL
  2. Dosing intervals of less than 8 hours are not practical
  3. Consistent dosing recommendations regardless of pregnancy status
  4. Same dosing frequency in adult and pediatric patients

Approach to Amoxicillin Dosing Recommendations

- Pharmacokinetic data in adults, children and pregnant women were obtained from various drug applications and literature*

- A population pharmacokinetic approach was used to characterize the concentration time-course of amoxicillin
  - Such an approach can be used to simulate dosing regimens that may not have been studied previously

- Simulations were performed at different dose levels (e.g., 500 mg and 1000 mg) and frequencies (e.g., 8, 6 and 4 hours)

Amoxicillin Dosing Recommendations: Pregnancy

**Adult Recommended Dose: 1000 mg every 8 hours**

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<th>Pregnancy Status</th>
<th>Trough (mcg/mL) Median [5th to 9th]</th>
<th>Time Above MIC (.0125 mcg/mL)</th>
<th>100% of dosing interval</th>
<th>75% to 100% of dosing interval</th>
<th>&lt; 75% of dosing interval</th>
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<td>2nd Trimester</td>
<td>0.20 [0.06 – 0.53]</td>
<td>77%</td>
<td>23%</td>
<td>0%</td>
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<tr>
<td>3rd Trimester</td>
<td>0.29 [0.10 – 0.71]</td>
<td>90%</td>
<td>10%</td>
<td>0%</td>
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<td>Postpartum</td>
<td>0.29 [0.12 – 0.75]</td>
<td>93%</td>
<td>7%</td>
<td>0%</td>
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<tr>
<td>Non-Pregnant Adults</td>
<td>0.50 [0.16 – 1.36]</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
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</table>
Amoxicillin Dosing Recommendations: Pediatrics

Pediatric Recommended Dose: 25 mg/kg every 8 hours

<table>
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<tr>
<th>Age Group (years)</th>
<th>Trough (mcg/mL) Median [5th to 9th]</th>
<th>Time Above MIC (.0125 mcg/mL)</th>
<th>100% of dosing interval</th>
<th>75% to 100% of dosing interval</th>
<th>&lt; 75% of dosing interval</th>
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</thead>
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<td>12 to ≤ 16</td>
<td>0.52 [0.15 – 1.68]</td>
<td>98%</td>
<td>2%</td>
<td>0%</td>
<td></td>
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<tr>
<td>6 to ≤ 12</td>
<td>0.53 [0.15 – 1.60]</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td></td>
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<tr>
<td>2 to ≤ 6</td>
<td>0.44 [0.12 – 1.24]</td>
<td>95%</td>
<td>5%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>1 month to ≤ 2 years</td>
<td>0.57 [0.16 – 1.88]</td>
<td>97%</td>
<td>3%</td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions

• A thorough understanding of the PK and PD data for the investigational drug or biologic is essential in selection of a dose regimen expected to be effective in humans.

• The impact of intrinsic and extrinsic factors on dosing is special populations should be considered

• Quantitative methods, such as PK modeling can be used to derive dosing of MCM products in special populations
Medical Countermeasure Approvals

- Diagnostics for CBRN threats, Pandemic Influenza and Antimicrobial Resistance

Enabling Access to Available Medical Countermeasures

- Emergency Use Authorizations (EUAs) for diagnostic tests for Ebola virus, Enterovirus D68 (EV-D68) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV)
- Pre-EUA submission process for prepositioning (DoD, BARDA, CDC and industry)

Responding to Emerging Public Health Threats

- Issuing EUAs for diagnostic tests for MERS-CoV, EV-D68 and EVD

Facilitating Medical Countermeasure Development

- Multiplex and Microbial Sequencing In Vitro Diagnostics Action Team

Regulatory Advice and Guidance

MCMi Regulatory Science Program
Multiplex and Microbial Sequencing

In Vitro Diagnostics Action Team

This Action Team facilitates the development of multiplex and microbial DNA sequence-based *in vitro* diagnostic tests. Such diagnostics could be used to test for **multiple pathogens simultaneously** from a single clinical specimen, providing valuable information when responding to a **public health emergency**.
• 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

Opinions are my own
Risk Based Regulation of IVDs

Class I - Low likelihood of harm
- General Controls
  - register & list (21CFR §807)

Class II - Moderate likelihood of harm or risk can be mitigated
- Special Controls

Class III - High or unknown likelihood of harm
- Significant Risk
  - Pre-market Approval

Knowledge Mitigates Risk

Class I most 510(k) exempt
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)
FDA Current Thinking
Infectious Disease NGS Dx

Emphasis from scientific and clinical community leaders for guidance on infectious disease

- **Infectious Disease NGS Dx**
  - Very different from human NGS:
    - Absolute need for immediate and actionable result
    - Broad range of specimen types (e.g., urine, blood, CSF, stool, sputum, and others)
    - Large diversity of the infectious disease agents possible present within one specimen
    - Dynamic nature of infectious disease agents

- **Public Workshop held on April 1, 2014** with FDA discussion paper

- **FDA Regulatory-Grade Microbial Database**

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1. **Clinical applications and public health needs:**
   Identified specific applications where high throughput sequencing could be used for diagnosis of infectious diseases and markers of antimicrobial resistance from isolates.

2. **Device validation:**
   Developed and specified standards for the microbial genome sequencing process (from sample collection to result reporting), introduced best practices for sample/library preparation, variant identification, genome annotation, output de-convolution/results interpretation, and reporting.

3. **Reference databases:**
   Developed quality criteria for reference databases and database itself (FDA-ARGOS).

4. **Streamlined clinical evaluations/trials for microbial identification:**
   Established a new comparator paradigm for NGS as the reference method to augment or replace existing reference testing methods.
Development and Evaluation of a Panel of Filovirus Sequence Capture Probes for Pathogen Detection by Next-Generation Sequencing


Rapid Whole-Genome Sequencing for Detection and Characterization of Microorganisms Directly from Clinical Samples

Henrik Haman, Dinithi Sapura, Thomas Schiratori-Ponton, Ola Lund, Christina Aaby Svendsen, Nils Fredriksen-Mellot, Frank M. Aarestrup

Whole genome sequencing (WGS) is becoming a routine tool for clinical microbiology. If applied directly on samples, this could further reduce diagnosis times and thereby improve control and treatment. A major bottleneck is the time required for sample preparation and DNA extraction. This study was conducted to evaluate the suitability of WGS as a routine tool for clinical microbiology.

NOSOCOMIAL INFECTION

Tracking a Hospital Outbreak of Carbapenem-Resistant Klebsiella pneumoniae with Whole-Genome Sequencing

Evan S. Smirnov, Adrian M. Zelazny, Pamela J. Thomas, Freda Stock, NISC Comparative Sequencing Program, David K. Henderson

The Gram-negative bacteria Klebsiella pneumoniae is a major cause of nosocomial infections, primarily among immunocompromised patients. The emergence of strains resistant to carbapenems has led to increased treatment options, making infection control critical. In 2011, the U.S. National Institute of Health Central Clinical experienced an outbreak of carbapenem-resistant 6 pneumonia that affected 16 patients. Of those who died, whole-genome sequencing was performed on respiratory isolates to gain insight into why the outbreak progressed despite efforts to implement infection control procedures. Integrated genomic and epidemiologic analysis traced the outbreak to three independent transmissions from a single patient who was discharged 3 weeks before the next admission of a second affected patient.

NIH Public Access

Author Manuscript

Published in final edited form as: Genet Med. 2013 September; 15(9): 733–747. doi: 10.1038/gim.2013.92

ACMG clinical laboratory standards for next-generation sequencing

Heidi L. Rahm, PhD, Sherri J Bate, PhD, Pinar Bayrak-Toydemir, PhD, Jonathan S. Berg, MD, Kerry K. Brown, PhD, Joshua L. Deignan, MD, Michael J. Freifeld, PhD, Birgit H. Funke, PhD, Madhun R. Hegde, PhD, Elaine Lyon, PhD, and the Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee

Validation of high throughput sequencing microbial forensics applications


Molecular diagnosis in clinical parasitology: When and why?

Samson SY Wong, Kitty SC Fung, Sandy Chau, Rosana WS Pun, Sally C Y Wong, and Kwok-Yung Yuen

Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, Pok Fu Lam, Hong Kong, Department of Pathology, United Christian Hospital, Kowloon Tong, Hong Kong, Corresponding author: Kwok-Yung Yuen, Email: keylong@hku.hk
Proposed Information Needed For FDA Marketing Authorization of Infectious Disease NGS-Based Test/Assay

- Intended Use
- Test Methodology
- Ancillary Reagents
- Controls
- Interpreting Test Results

- Test/Assay Description
- Pre-analytical Factors
- Performance Metrics

- Limit of Detection
- Inclusivity
- Interfering Substances
- Precision (Reproducibility and Repeatability)
- Carry-over and Cross-contamination
- Stability

- Analytical Performance
- Description of Instrumentation
- Description of Computational Pipeline
- Description of Database

- Instrumentation/Software
- IRB Review and Approval
- Study Design Elements
- Clinical Evaluation

Targeted NGS
Agnostic (Metagenomics) NGS
Current Challenge: U.S. Marketing Authorization of NGS-Based Diagnostics in the Microbiology Laboratory
Current Need

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

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

Cover illustration
(Copyright © 2009, American Society for Microbiology. All Rights Reserved.)
Current Challenge: U.S. Approval/Clearance of NGS-Based Diagnostics in the Microbiology Laboratory

FDA Regulatory Science Efforts: Add 2000 high-quality MCM and Clinically-Relevant Pathogen Sequences
FDA ESTABLISHED AND IS EXPANDING A PUBLICALLY AVAILABLE, MICROBIAL GENOMIC REFERENCE SEQUENCE DATABASE (FDA-ARGOS) THAT MEETS REGULATORY GRADE QUALITY CRITERIA

Critical to developers seeking to validate their candidate high-throughput sequencing-based in vitro diagnostic assays.

Collaborating with DoD, NCBI and U-MD Institute for Genome Sciences.

Geographically diverse isolate collection from agencies, public health labs, clinical labs and repositories.

Around 2,000 isolates will be sequenced with the FDA-ARGOS project.

Antimicrobial resistance (AMR) isolates to include metadata, sequencing and registration of isolates.
FDA ARGOS DATABASE (@NCBI PRJNA231221)

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

>2000 Clinically Relevant and MCM Microorganisms

Collaborations with Agencies, Clinical Labs and Repositories
- DoD (CRP, USAMRIID, MCS/JVAP)
- Public Health Agency Canada, Public Health Agency England
- Bernard Nocht Institute for Tropical Medicine, Germany
- National Center for Biotechnology Information (NCBI)
- National Institute of Allergy and Infectious Diseases (NIAID)
- FDA-CFSAN, FDA-CBER, FDA-CVM
- Lawrence Livermore National Lab, Los Alamos National Lab
- DHS National Biodefense Center (NBACC)
- Children’s National Hospital, GWU, others
- Rockefeller University, ATCC Culture Collection

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
Project Approach

• Hybrid Sequencing Approach
  – Illumina PE HiSeq4000 (~300x cov of 5Mbp genome)/MiSeq
  – PacBio RS II (P6-C4, ~100x cov of 5Mbp genome)
  – 3-tiered viral approach (shotgun, amplicon, RACE)
  – Raw reads -> NCBI SRA

• Assembly/ Annotation
  – PacBio-only, Illumina-only, Hybrid
  – Assembly QA/QC --> “best” assembly selection
  – Automated genome annotation
  – Assembled & annotated genomes -> Genbank
    • NCBI BIOPROJECT ID: PRJNA231221

• FDA Web interface to aggregate data
• Base modification detection
Current Challenge:
U.S. Approval/ Clearance of NGS-Based Diagnostics in the Microbiology Laboratory

FDA Regulatory Efforts:
Add 2000 high-quality MCM and Clinically-Relevant Pathogen Sequences

Public Health Need:
Robust, Standardized, and High Quality Microbial Comparator Sequence Database
EBOLA PILOT SEQUENCING

Heike Sichtig, Ph.D. | Microbiology Devices | Center for Devices (CDRH) | US Food and Drug Administration | Phone: +1 (301) 796-4574 | Email: Heike.Sichtig@fda.hhs.gov
## Ebola Virus, Makona

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Hospital</th>
<th>Date of Sampling</th>
<th>Outcome</th>
<th>Complete Genome</th>
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<tr>
<td>C5</td>
<td>16</td>
<td>F Gueckedou</td>
<td>March 19</td>
<td>Survived</td>
<td>KJ660348</td>
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<tr>
<td>C7</td>
<td>47</td>
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<td>KJ660347</td>
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<tr>
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<td>F Kissidougou</td>
<td>March 17</td>
<td>Survived</td>
<td>KJ660346</td>
</tr>
</tbody>
</table>

IGS Ebola Sequencing Approach

- **Amplicon**
  - 96 amplicons, ~450bp each with 60-100bp overlaps
  - 2x amplicon coverage of the genome
  - Secondary PCR adds adaptors and barcodes

- **Shotgun**
  - cDNA: Nugen Ovation V2 from 5ng total RNA
  - Library: Nugen Ovation Ultralow Library V2

- **5’ RACE**
  - Clonetech SMARTer RACE 5’/3’ kit

- All three sequenced on Illumina MiSeq
Assembly & Analysis

• Amplicon primers trimmed
• Assembled with SPAdes
• Consensus polished with shotgun data
• 5’ RACE stitched on by Minimus
• Variants called by GATK
# PHAC Ebola P1 Isolates

<table>
<thead>
<tr>
<th>FDA ARGOS ID</th>
<th>Isolate Description</th>
<th>NCBI BioSample</th>
<th>NCBI SRA</th>
<th>GenBank</th>
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<td>SRX1023888,</td>
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P1A = 350 ul of original clinical isolate added to 5 mL DMEM medium then added to Vero E6 cells; P1B = 500 ul of P1A solution added to 4.5 mL DMEM medium
Regulatory Grade Sequencing

**Ebola Makona (PHAC)**
- Sequenced and Submitted to NCBI DB
  - C05
    - P1A: Amplicon, Shotgun, RACE
    - P1B: Amplicon, Shotgun, RACE
  - C07
    - P1A: Amplicon, RACE
    - P1B: Amplicon, Shotgun, RACE
  - C15
    - P1A: Amplicon, Shotgun, RACE
    - P1B: Amplicon, Shotgun, RACE
- Received for Sequencing
  - Clinical C05, C07, C15
  - P2 of C05, C07 and C015
  - P1A of C07 for shotgun sequencing

**Challenge Stocks (PHE)**
- In the Pipeline for Sequencing
  - P2s
    - 2 Ebola stocks
    - 2 Sudan stocks
    - 1 Bundibugyo stock
    - 1 Tai Forrest stock
    - 2 Marburg stocks
  - P1s
    - 2 Ebola stocks
    - 2 Sudan stocks
    - 1 Bundibugyo
    - 1 Marburg Angola

**Contact PI for Sequencing Requests**
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DOD-USAMRIID
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DTRA
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Tom Slezak, Patrick Chain

NBACC
Nick Bergman

ATCC
Liz Kerrigan

NIST
Scott Jackson, Jason Kralj

DoD-MCS/JVAP
Lucy Ward

NIH-NIAID
William Dowling

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Bernard Nocht Institute for Tropical Medicine, Germany
Stephan Günther

Public Health England
Kevin Richards, Christine Bruce, Kim Couch

IRF-Frederick
Jens Kuhn

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MORE BACKGROUND
Summary on FDA Regulatory Efforts

NGS for Infectious Disease Diagnostics

• FDA ARGOS Database (@NCBI PRJNA231221)
  – Public Regulatory-Grade Microbial Genomic Reference Database
  – Expansion as a community effort
  – Manuscript in preparation

• Microbial NGS Leapfrog Guidance (DRAFT)
  – FDA Microbial NGS Workshop (APRIL 1, 2014)
  – Targeted sequencing and Agnostic (metagenomic) sequencing

• Interagency Work Group on NGS Feasibility
  – Clinical Metagenomics Study, Results to be published

• NIST Collaboration on Microbial Reference Materials

FDA Pre-submission Process for Feedback
  – Pre-submission template for infectious disease NGS-based diagnostics available (Contact Heike.Sichtig@fda.hhs.gov)
NIST Collaboration on Sequence-based Microbial Reference Material for NGS Validation

• Reference Material for Challenging Microbes Generated
  – List of candidate organisms (~1500 vials of gDNA):
    • Salmonella typhimurium LT2 (environmental isolate, CFSAN lab strain)
    • Staphylococcus aureus (clinical isolate from FDA ARGOS, Children’s National)
    • Escherichia coli (clinical isolate from FDA ARGOS, Children’s National)
    • Clostridium sporogenes (environmental isolate, CFSAN lab strain)
  – Sequencing and characterization ongoing

• FDA-NIST Workshop on Mixed Sample RM (Oct 27/28)
  – Input on defining reference materials for generation of reference data and methods
  – Material will be critical to address the challenges associated with mixed pathogen detection in complex (clinical) samples using agnostic (metagenomic) and targeted sequencing