



# Linking the Scientific and Regulatory Environments for PHEMCE

Robert W. Fisher  
07 January 2016



U.S. Food and Drug Administration  
Medical Countermeasures Initiative



# FDA and PHEMCE<sup>1</sup>

- 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)

<sup>1</sup>Public Health Emergency Medical Countermeasures Enterprise

# Medical Countermeasures Initiative (MCMi)

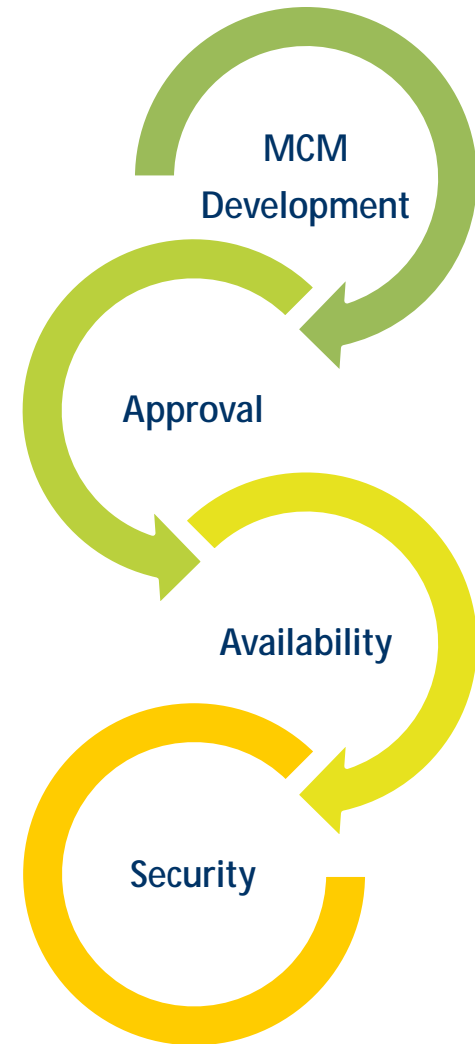
Promote development and  
availability of safe, effective  
medical countermeasures

# FDA MCMi

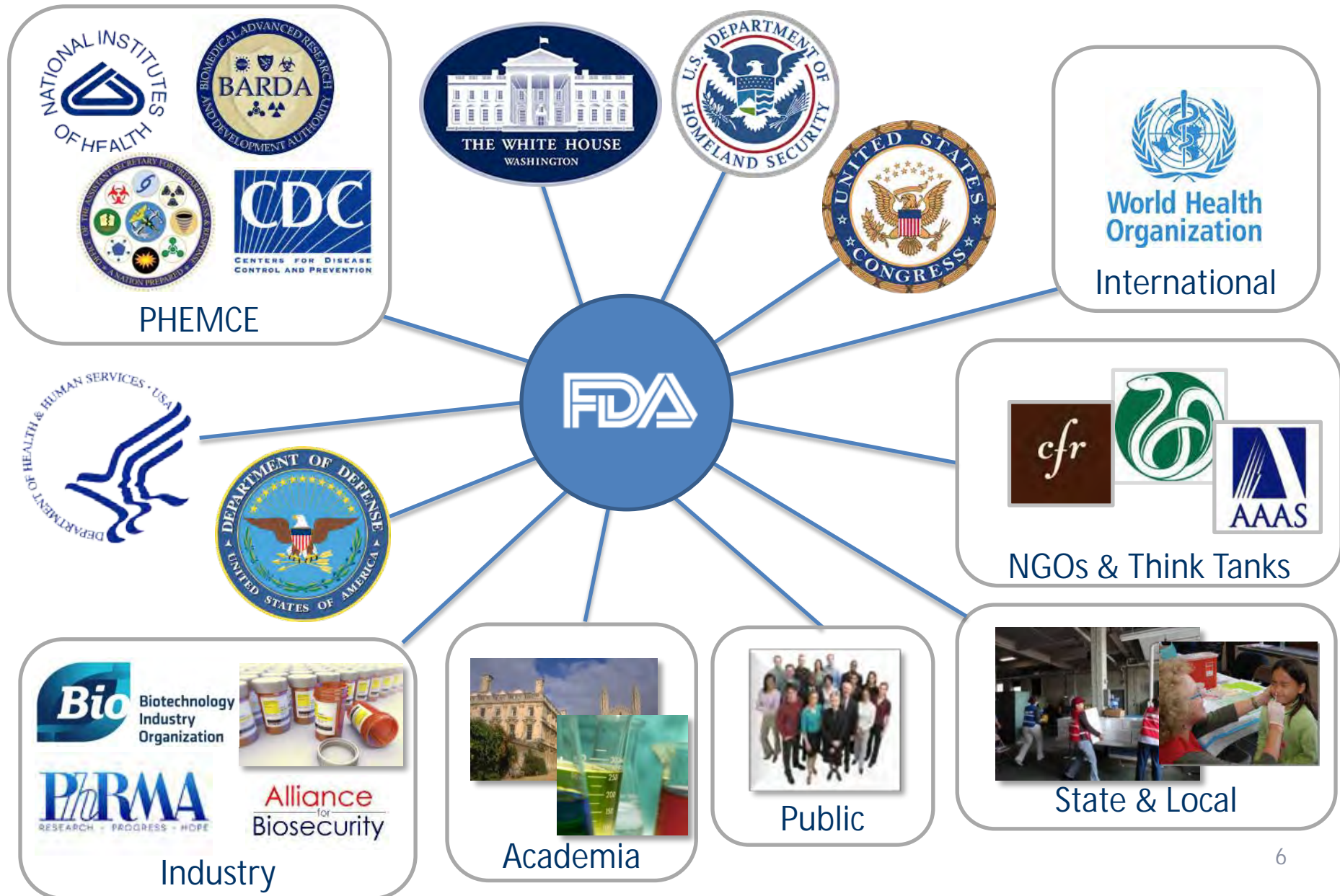
- Launched August 2010 in response to PHEMCE review of the U.S.'s readiness for public health emergencies
- FDA-wide initiative to coordinate medical countermeasure development, preparedness, and response
- FDA's MCMi:
  - Establishes clear regulatory pathways for MCMs
  - Supports regulatory decision-making 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



# 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 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!

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<http://www.fda.gov/medicalcountermeasures>

[AskMCMi@fda.hhs.gov](mailto:AskMCMi@fda.hhs.gov)

 @FDA\_MCMi



**U.S. Food and Drug Administration**  
Medical Countermeasures Initiative



# Resources

- MCMi Regulatory Science program
  - <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMRegulatoryScience/default.htm>
- Extramural research funding and current projects
  - <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMRegulatoryScience/ucm391617.htm>
- Animal Rule information and guidance
  - <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMRegulatoryScience/ucm391604.htm>
- MCMi news and events (workshops, etc.)
  - <http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/AboutMCMi/ucm262925.htm>

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## **CBER MCM Research and a Case Study: Prolonging Anthrax Vaccine Shelf Life**

# Scope of CBER's MCM-Related Regulatory Science Program

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## **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

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## Issues addressed

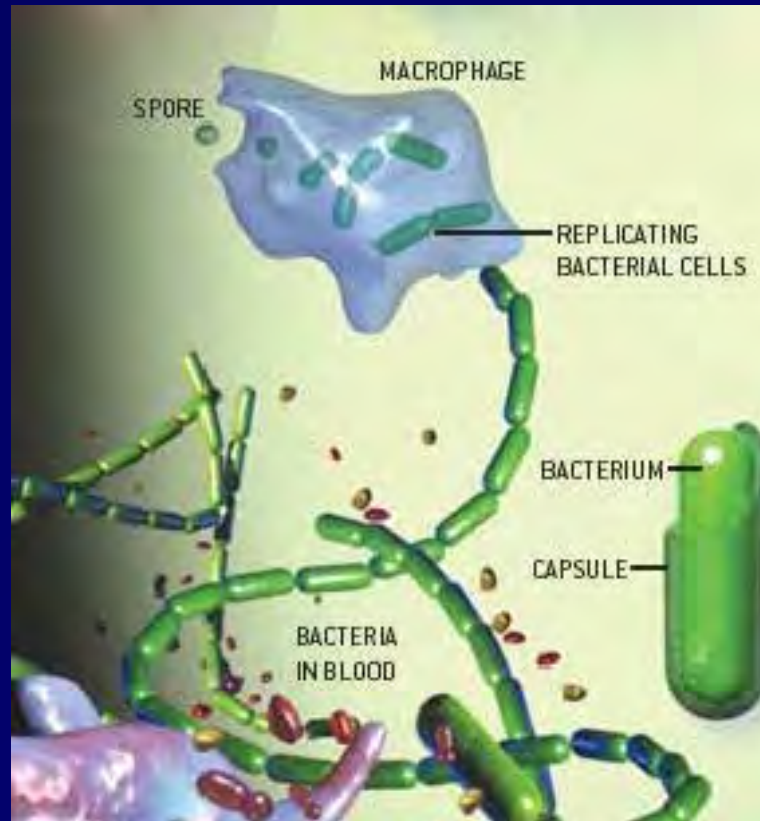
- Manufacturing
- Product quality
- Assay development, especially potency and other lot release assays
- Animal models
- Biomarkers/correlates of protection
- Clinical trial design
- Post-marketing safety

## Case study: Prolonging anthrax vaccine shelf life

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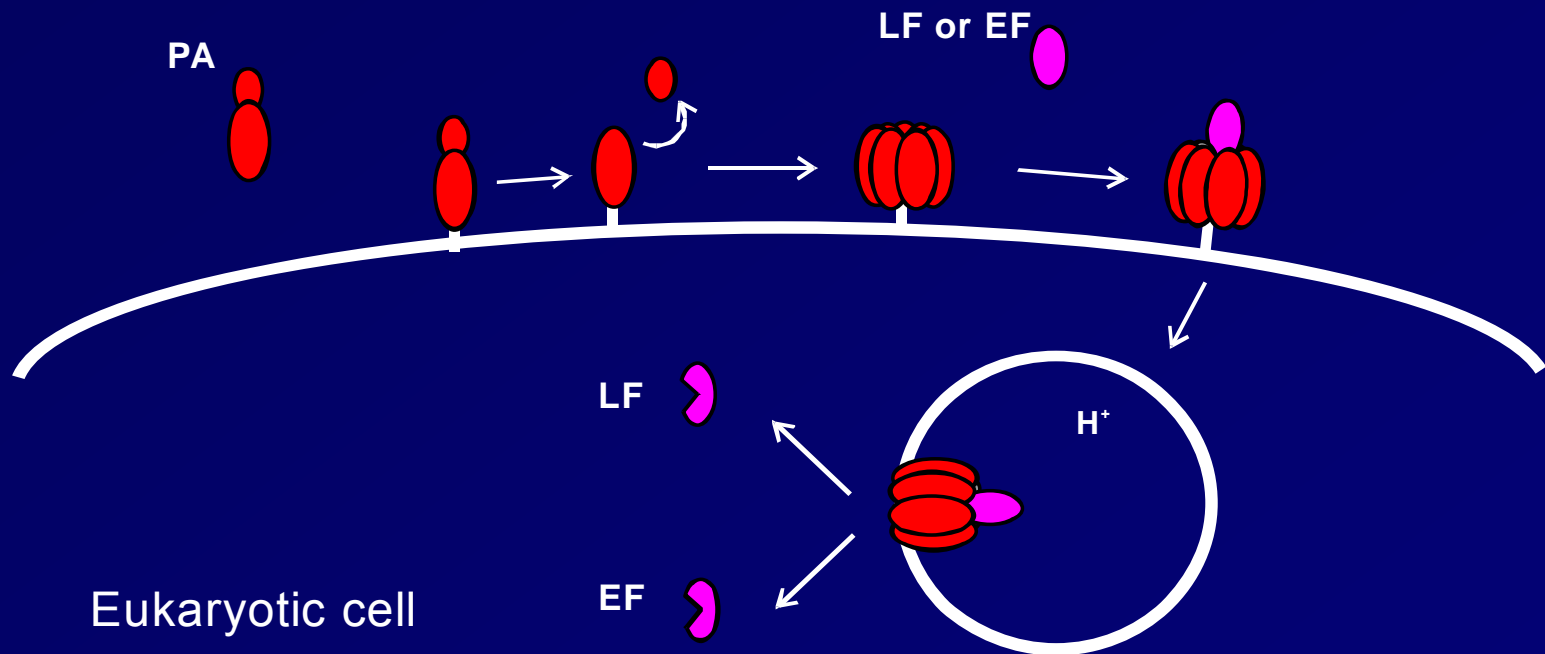
- Anthrax is one of the most feared bioweapons
- Efforts are underway to develop new generation anthrax vaccines
- Not expected to be used for routine immunization of the general population
- Stockpiled for use in an emergency, so stability is key

# Anthrax



From Collier and Young, *Sci. Am.* March 2002

# Mechanism of action of anthrax toxin



# New generation anthrax vaccine design

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- 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

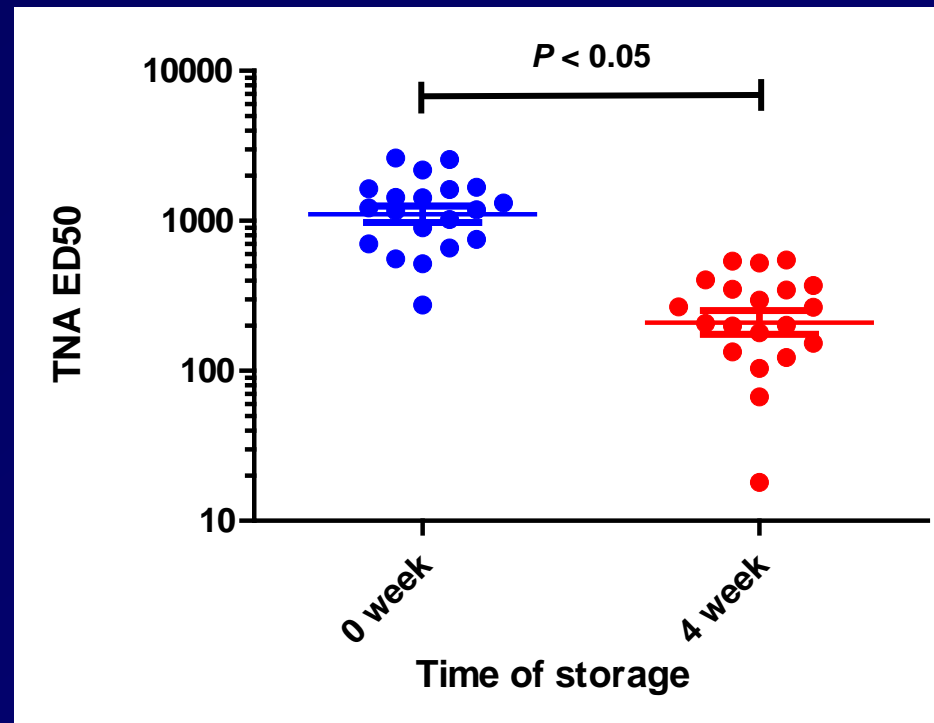
# rPA vaccines

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- 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

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- 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

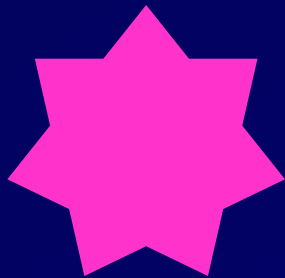
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- What changes in rPA occur upon storage?
  - Structural changes
  - Compositional changes

Do changes occur long-term that affect immunogenicity?

# Formulation of rPA vaccine

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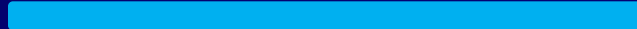
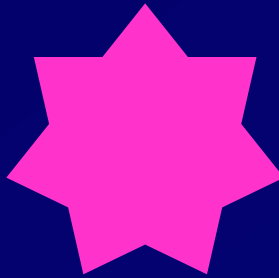


+



rPA

aluminum adjuvant



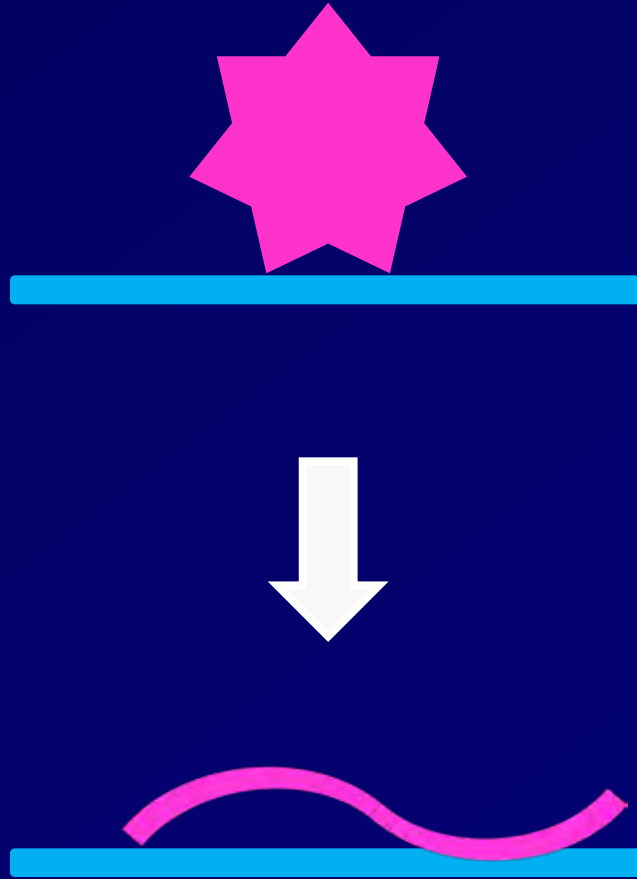
## Structural changes during storage detected by:

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- Melting point analysis
- Intrinsic protein fluorescence
- Immunogenicity of specific regions of the protein

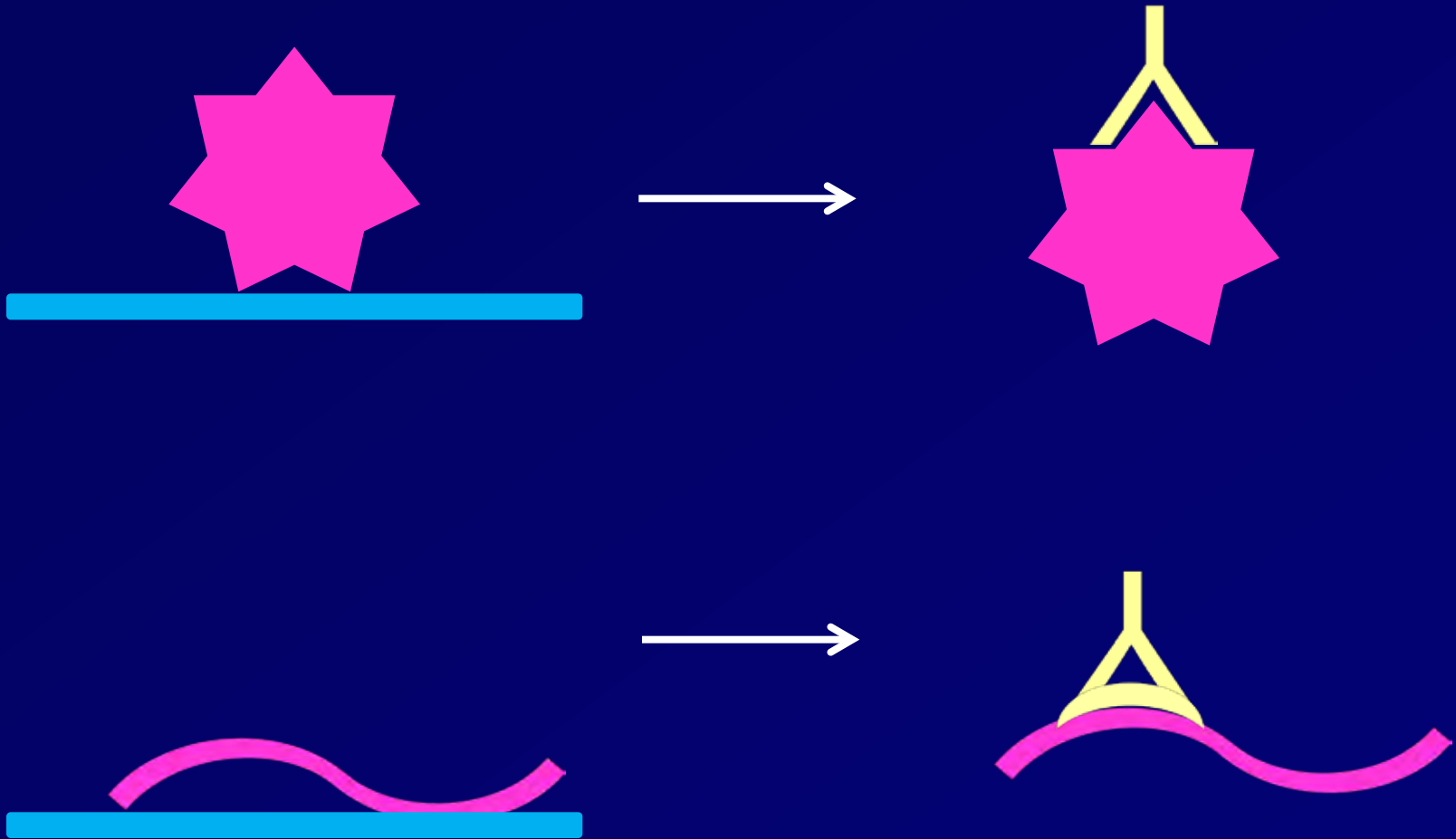
Over time rPA denatures on aluminum hydroxide adjuvant

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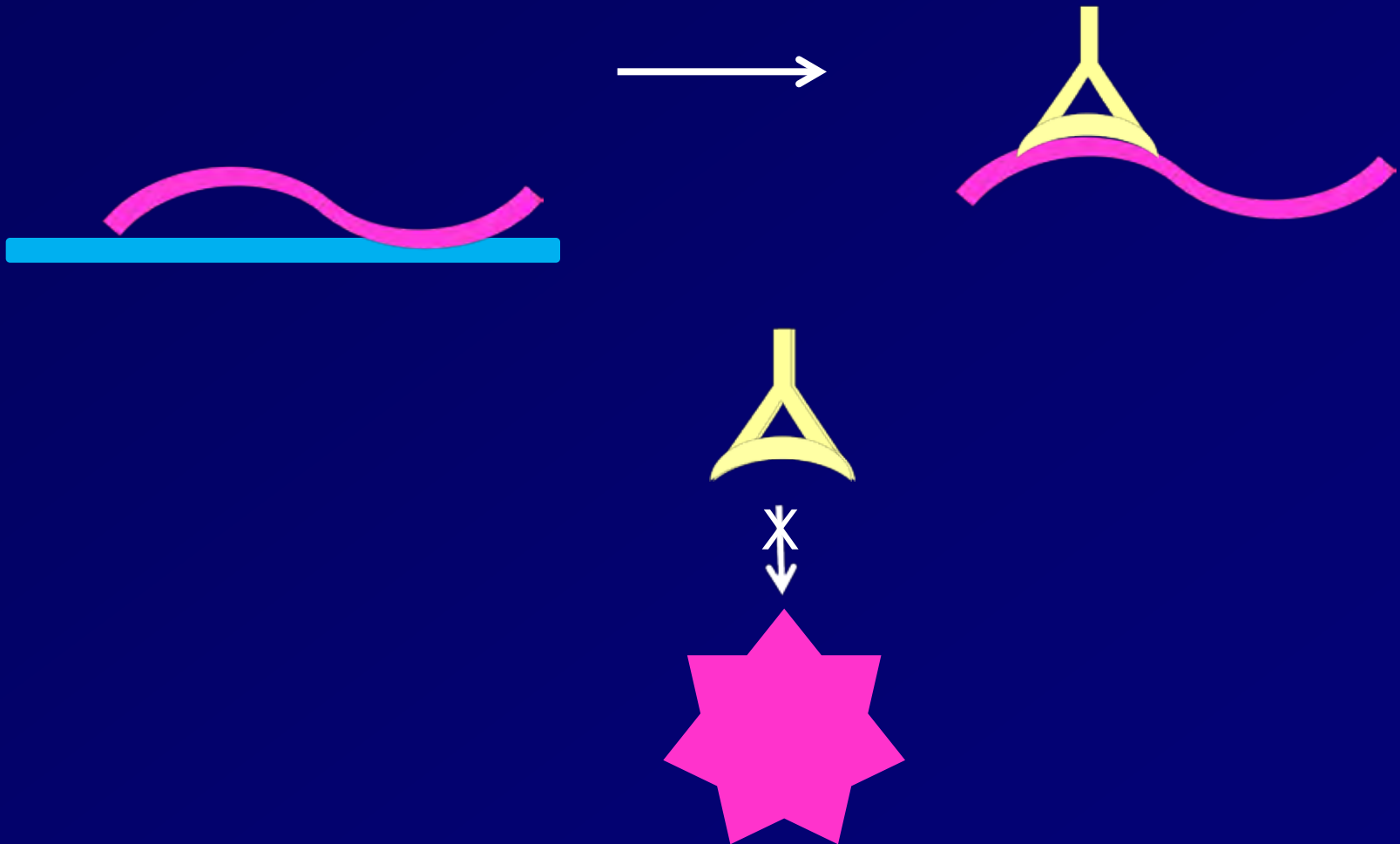
# Antibody population induced depends on antigen conformation

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Antibody to buried epitopes may not recognize native antigen

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## Effect of adsorption onto aluminum adjuvant

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- 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

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

Do changes occur long-term that affect immunogenicity?

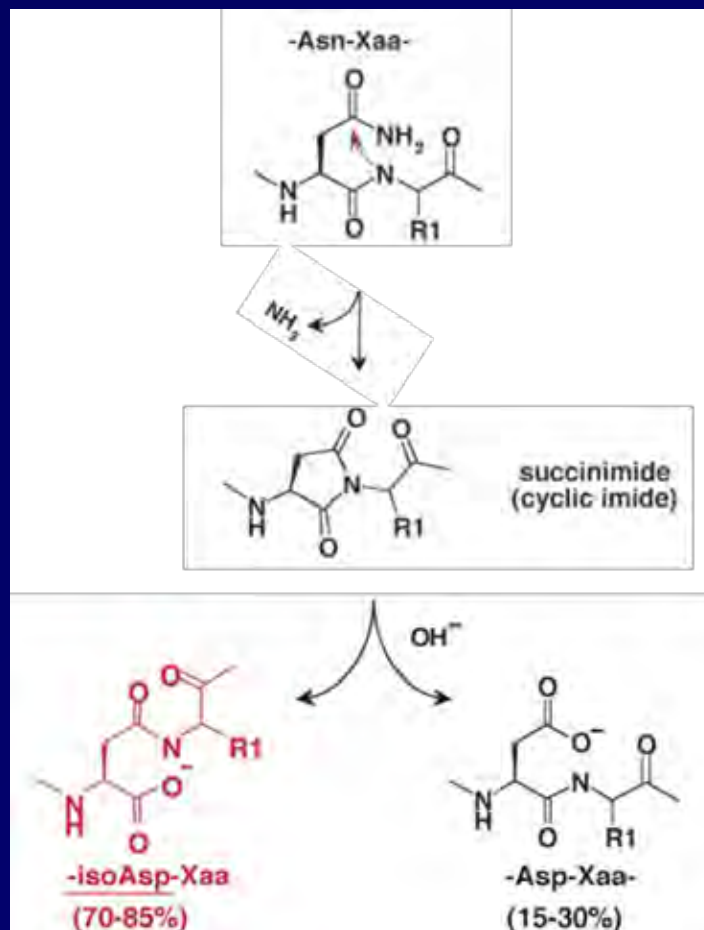
# Understanding the molecular basis for rPA vaccine instability

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

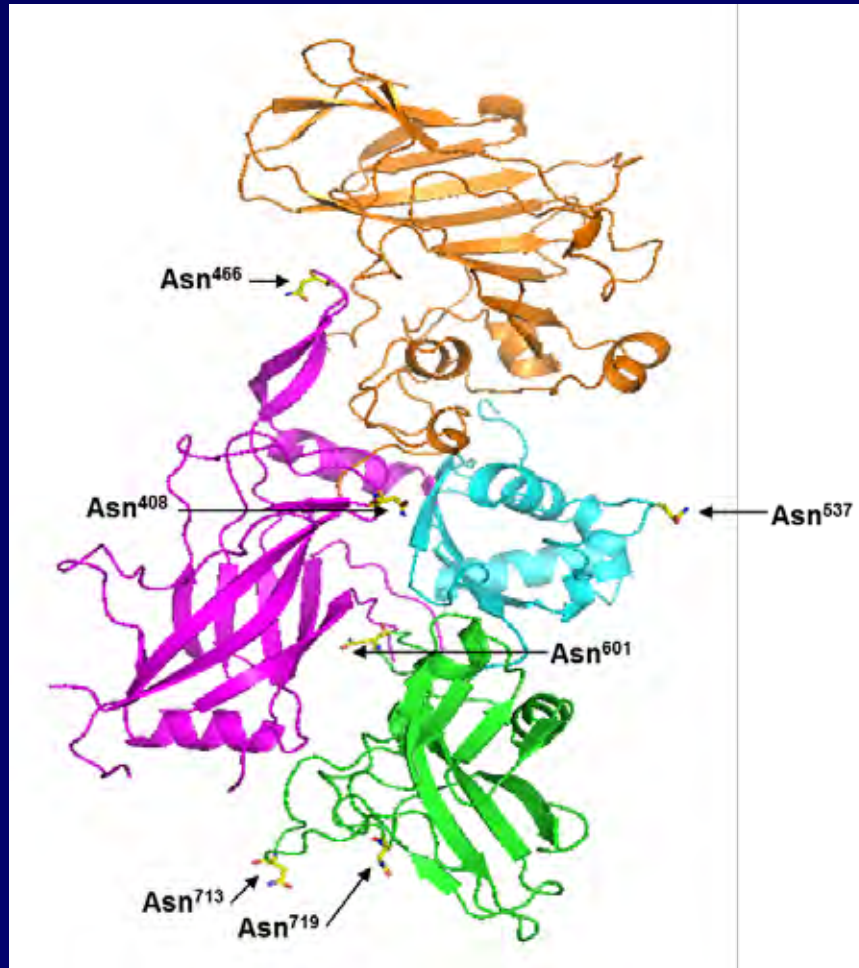
Do changes occur long-term that affect immunogenicity?

# Deamidation of Asn residues in proteins



Adapted from Reissner, K.J. and Aswad, D.W., *Cell. Mol. Life Sci.* 60:1281-1295 (2003)

# Deamidation-prone Asn residues of PA

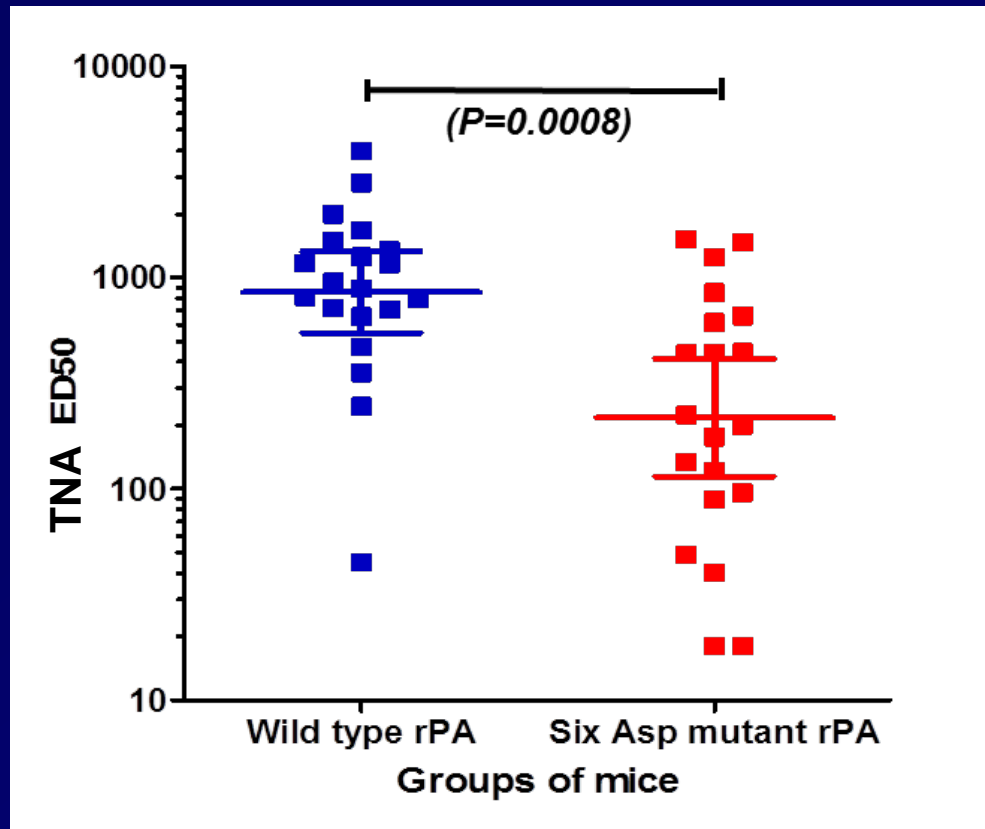


# Does spontaneous deamidation of Asn residues play a role in the instability of rPA vaccines?

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- 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



## Possible causes of low immunogenicity

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

# Conclusions

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- 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

# Acknowledgements

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Rocio Dominguez-Castillo, CBER

Juan Amador-Molina, CBER

Bruce Meade, Meade Biologics

# Determining the Dose of MCM Products in Special Populations

Kevin M. Krudys

Team Leader, Division of Pharmacometrics

Office of Clinical Pharmacology

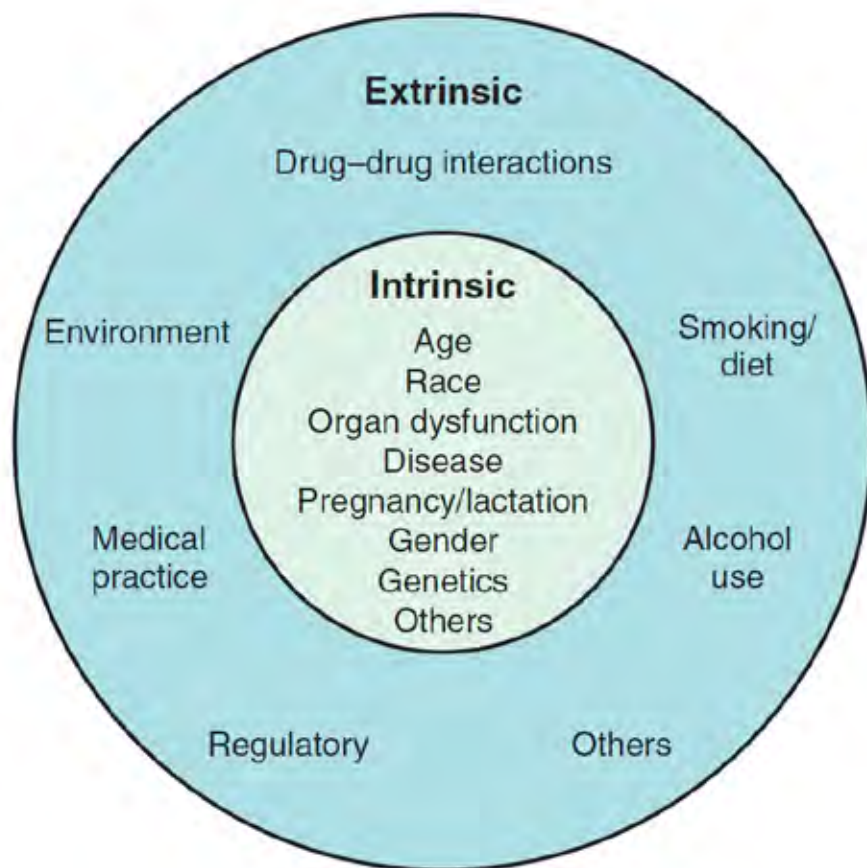
Center for Drug Evaluation and Research

January 7, 2016

## Selection of an Effective Dose in Humans

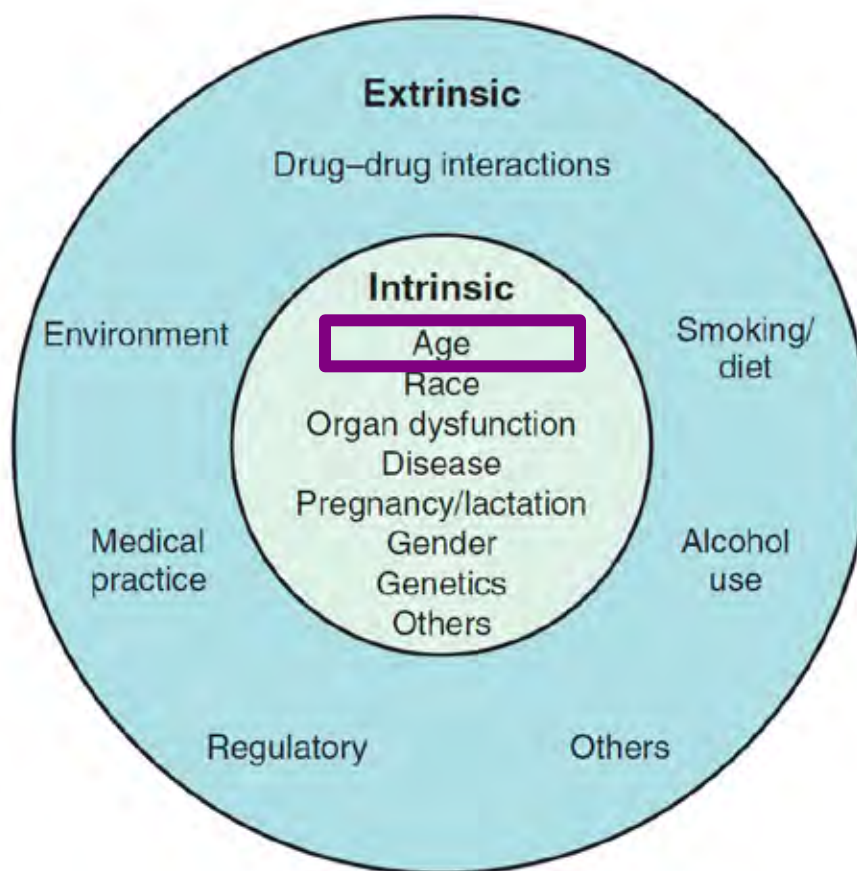
- Under the Animal Rule, a thorough understanding of the PK and PD data for the investigational drug or biologic is essential in selection of a dose regimen expected to be effective in humans.
- Clinical trials in healthy humans should evaluate safety and PK data over a range of doses
- Multiple approaches 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

# 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



Ø **Learn** from adult population PK analysis

- § The relationship between PK parameters vs body weight
- § Inter-subject variability

Ø **Simulate** pediatric PK profiles using different dosing regimens

- § Various combinations of dose and body weight band

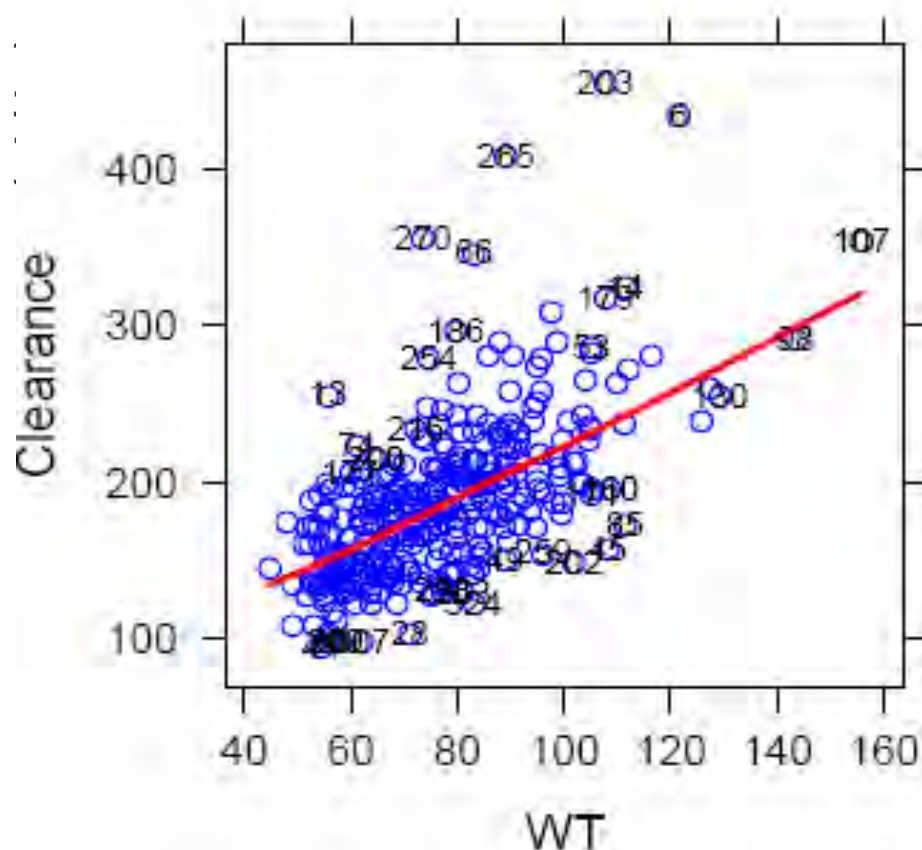
Ø **Select** a pediatric dosing regimen

- § 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

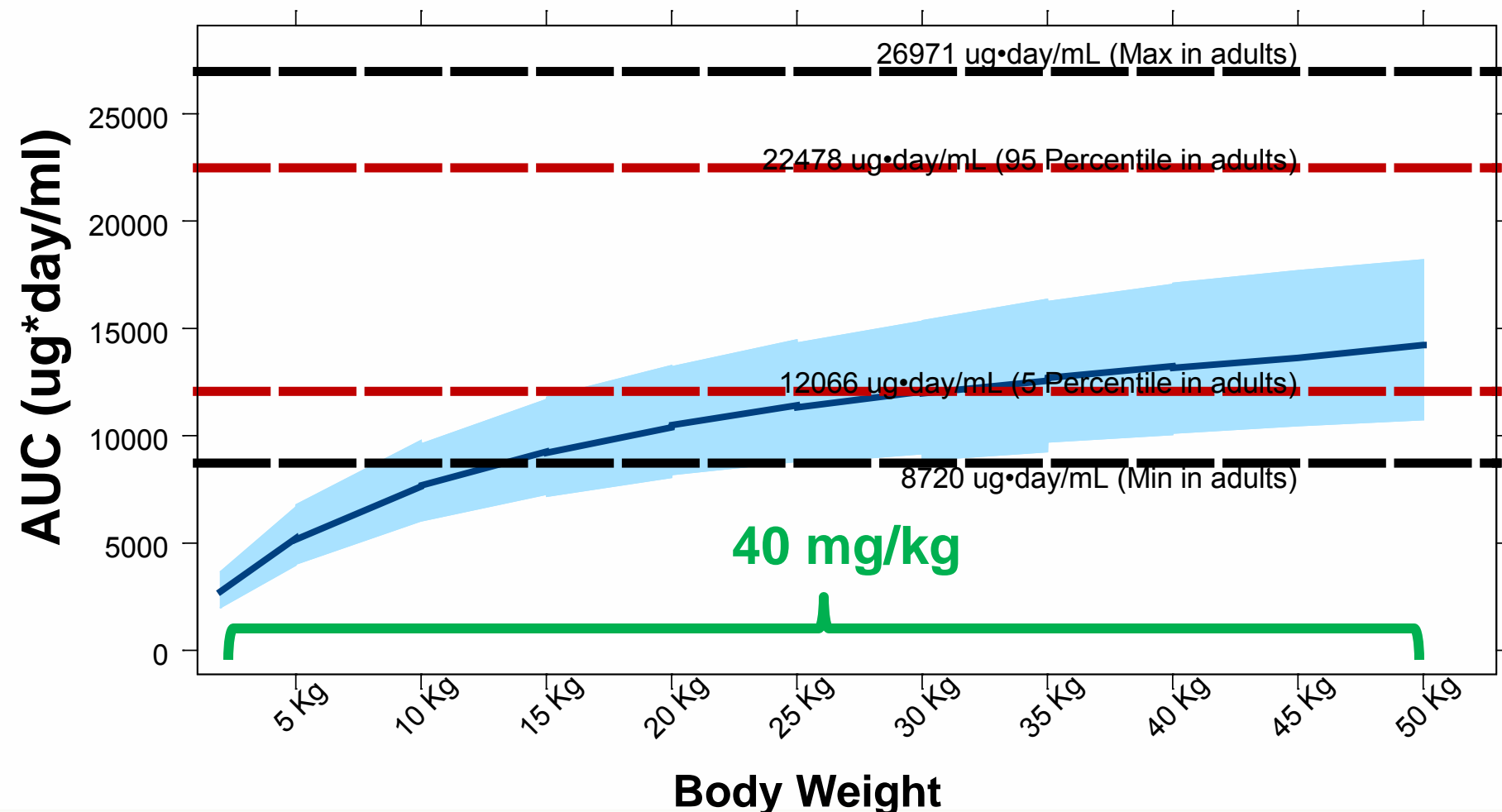
### Healthy adult PK @ 40 mg/Kg



**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

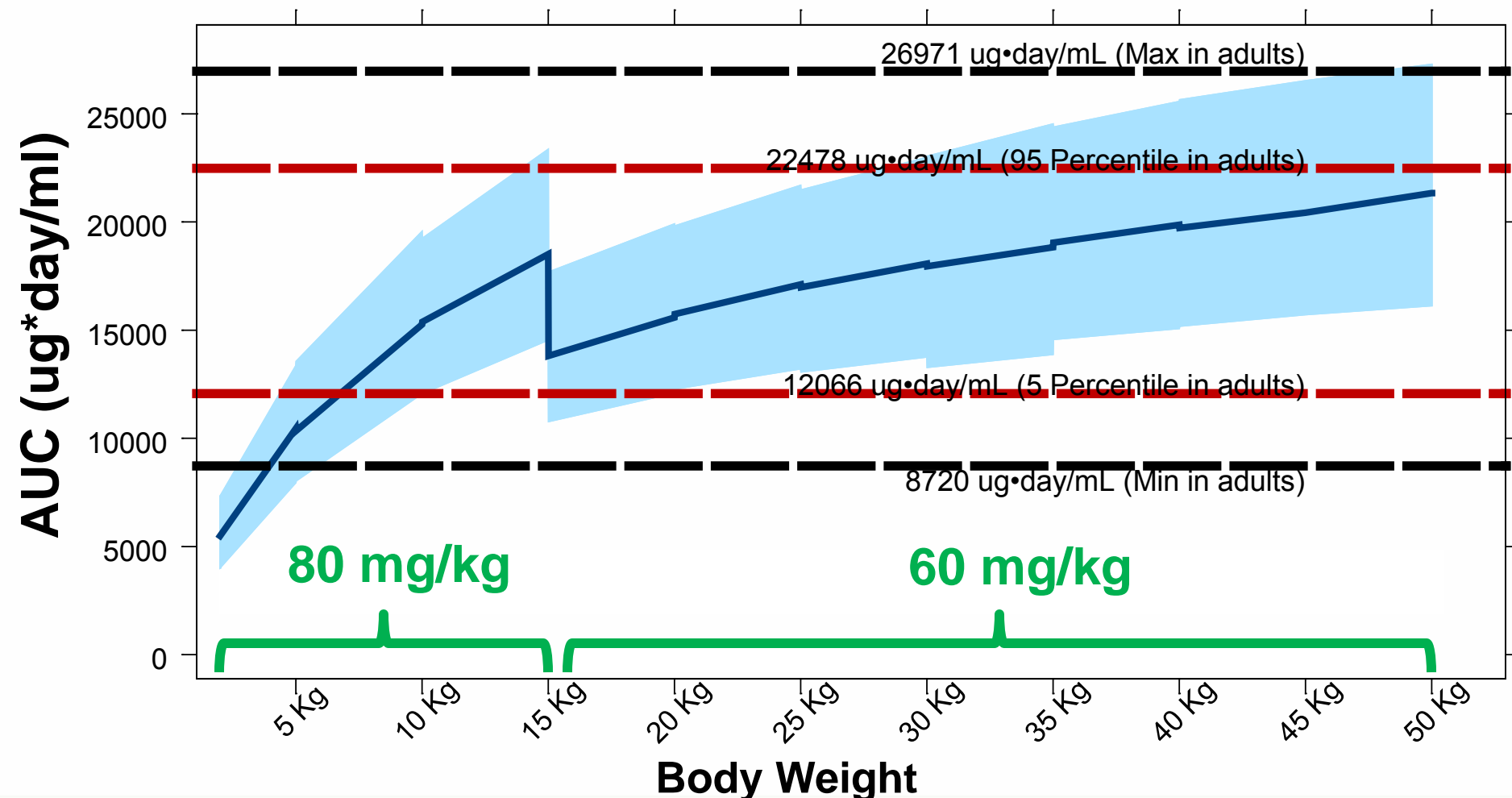
# Simulated $AUC_{inf}$ in Pediatric Population following Adult Dosing Regimen of 40 mg/kg



## Proposed Pediatric Dosing

Body Weight	Pediatric Dose
>50 kg	40 mg/kg
> 15 kg to $\leq$ 50 kg	60 mg/kg
$\leq$ 15 kg	80 mg/kg

# Simulated $AUC_{inf}$ in Pediatrics following the Proposed Dosing Regimen

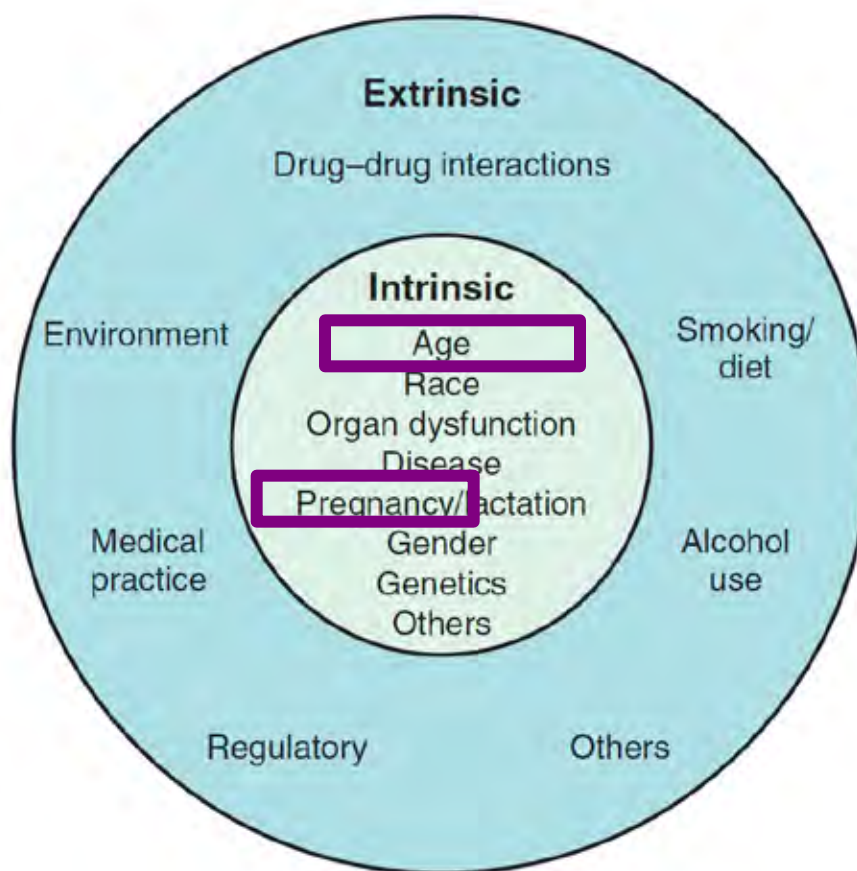


# Summary

- 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

Body Weight	Pediatric Dose
>50 kg	40 mg/kg
> 15 kg to $\leq$ 50 kg	60 mg/kg
$\leq$ 15 kg	80 mg/kg

## 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**

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

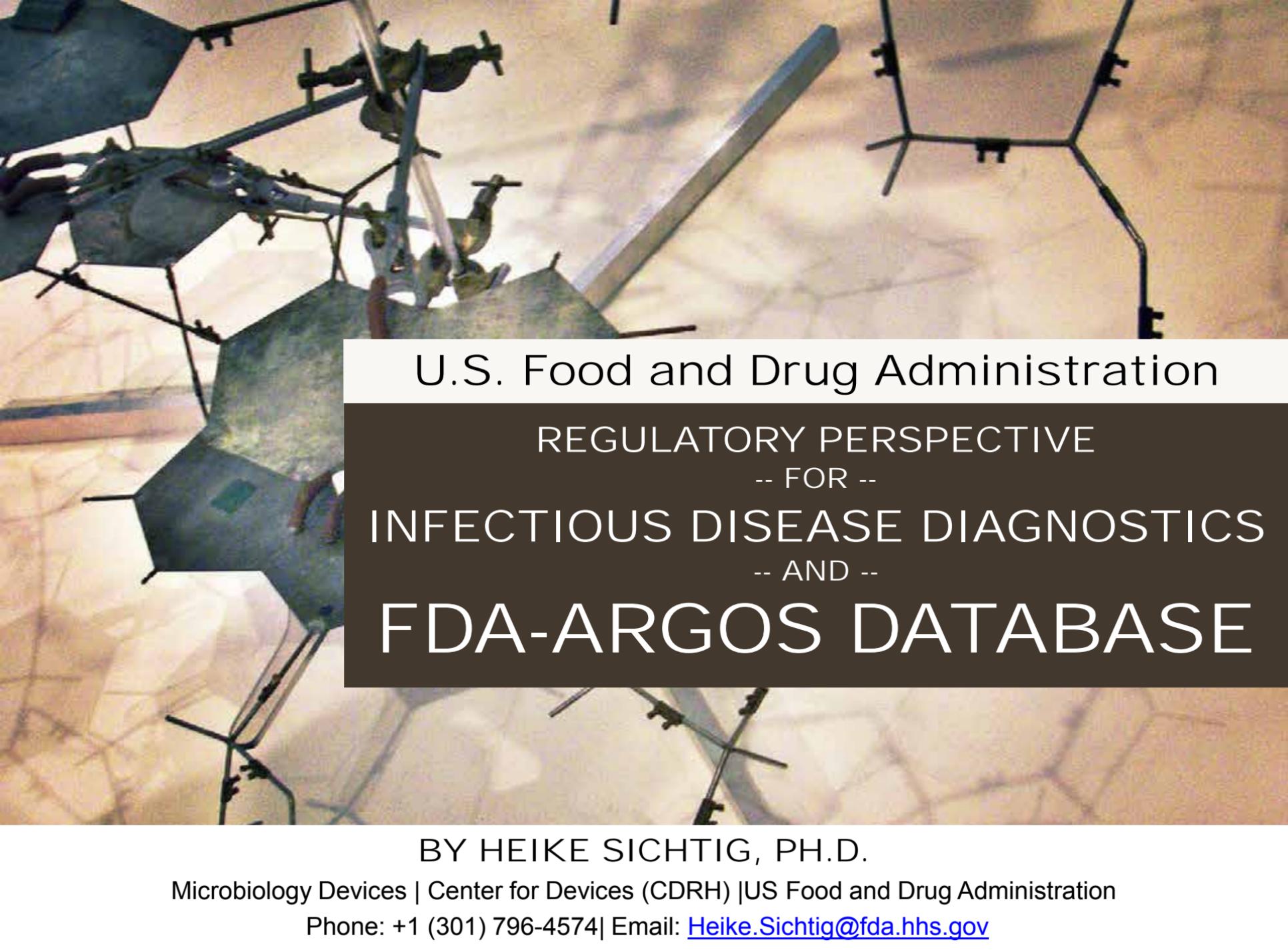
# Amoxicillin Dosing Recommendations: Pediatrics

**Pediatric Recommended Dose: 25 mg/kg every 8 hours**

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

## 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 in special populations should be considered
- Quantitative methods, such as PK modeling can be used to derive dosing of MCM products in special populations



U.S. Food and Drug Administration  
REGULATORY PERSPECTIVE  
-- FOR --  
INFECTIOUS DISEASE DIAGNOSTICS  
-- AND --  
FDA-ARGOS DATABASE

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# CDRH MCM Mission Space

## 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**.



# 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

**Opinions are my own**



# Risk Based Regulation of IVDs

Class I most 510(k) exempt



Risk

Knowledge

Class III - PMA

## Knowledge Mitigates Risk

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

**General Controls**

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

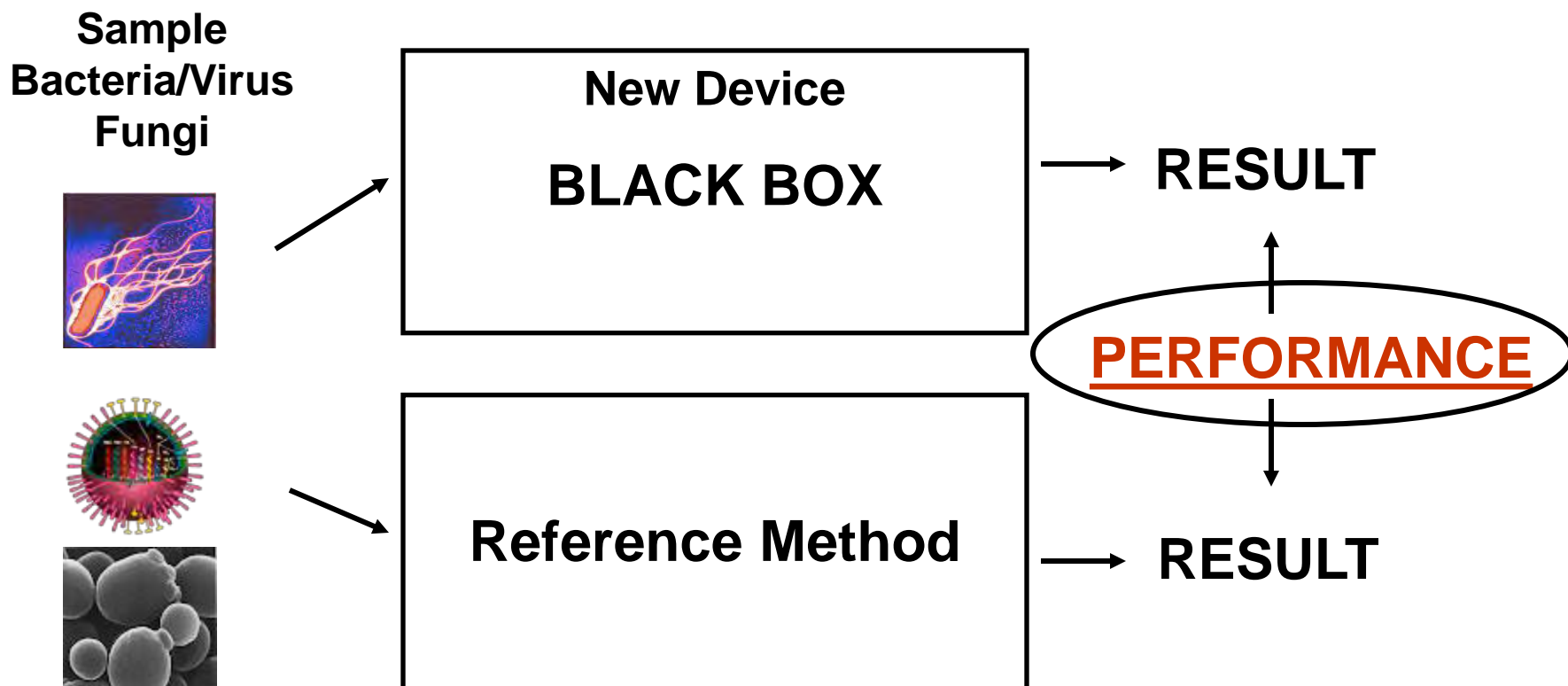
**Special Controls**

Class III - High or unknown  
likelihood of harm  
Significant Risk

**Pre-market Approval**

# 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**

**FDA-ARGOS**



FDA dAtabase for  
Regulatory Grade  
micrObial Sequences

(NCBI BioProject 231221)



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.

Microscopic detection and morphological identification of parasites from clinical specimens are the gold standards for the labors.

## Proposed Information Needed For FDA Marketing Authorization of Infectious Disease NGS-Based Test/Assay



Targeted NGS

Agnostic  
(Metagenomics)  
NGS

Intended Use  
Test  
Methodology  
Ancillary  
Reagents  
Controls  
Interpreting Test  
Results

Test/Assay Description

Pre-  
analytical  
Factors  
Performance  
Metrics

Test/Assay Validation

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

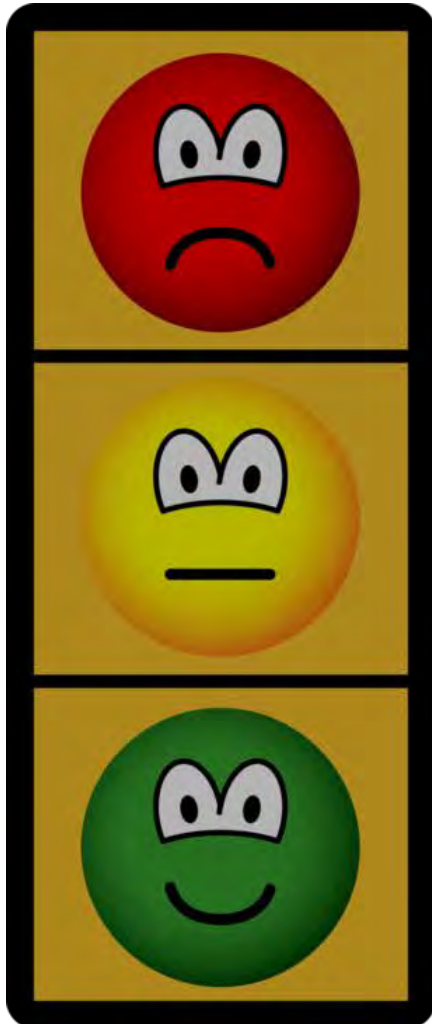
Analytical Performance

Description of  
Instrumentation  
Description of  
Computational  
Pipeline  
Description of  
Database

Instrumentation/Software

IRB Review  
and Approval  
Study Design  
Elements

Clinical Evaluation



## Current Challenge:

**U.S. Marketing Authorization of NGS-Based Diagnostics in the Microbiology Laboratory**

Next-Generation Sequencing (NGS) technologies including:  
 - Single-Molecule Sequencing (SMS)  
 - Nanopore Sequencing  
 - Capillary Electrophoresis (CE)  
 - Pyrosequencing  
 - Semiconductor Sequencing  
 - Whole-Genome Sequencing (WGS)  
 - High-Throughput Sequencing  
 - Massively-Parallel Sequencing  
 - Short-Read Sequencing  
 - Nanochannel Sequencing

## Current Need

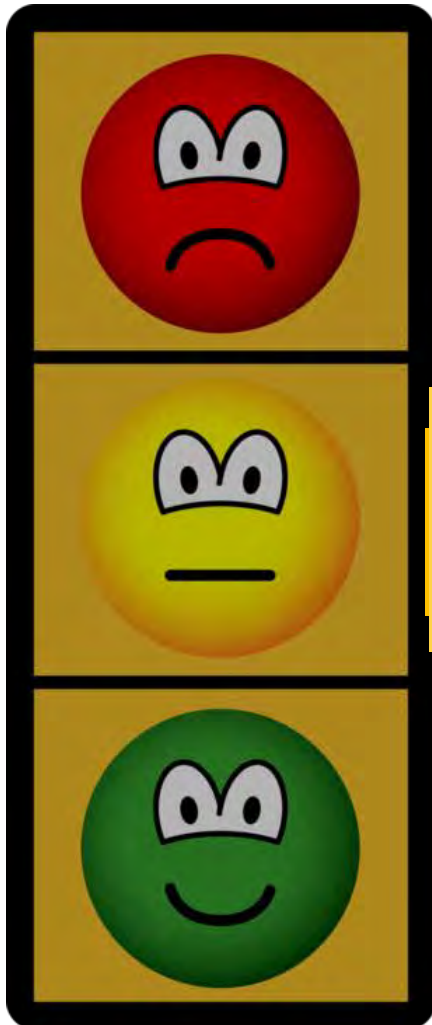
### Robust, Standardized, and High Quality Microbial Sequence Database in the Public Sector



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

*Cover illustration*

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## **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.

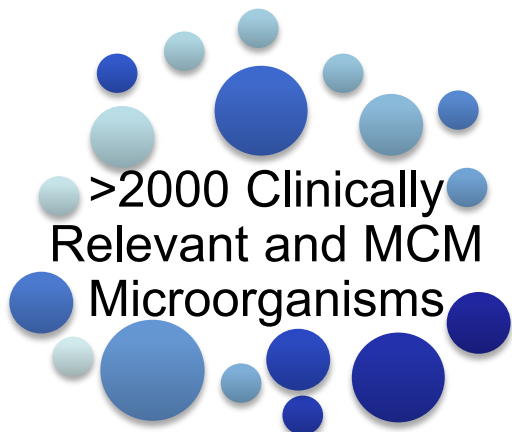
Geographically diverse isolate collection from agencies, public health labs, clinical labs and repositories.

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



## 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](https://www.ncbi.nlm.nih.gov/bioproject/231221)
- FDA Web interface to aggregate data
- Base modification detection

**FDA-ARGOS**



FDA dAtabase for  
Regulatory Grade  
micrObial Sequences

(NCBI BioProject 231221)



## **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 MUST GO

*Stopping Ebola is Everybody's Business*



Brought to you by  
Monrovia City Corporation



Take the pledge to protect yourself,  
your family, and your community.

## EBOLA PILOT SEQUENCING

**FDA**  
d**A**tabase for  
R**e**gulatory G**r**ade  
micr**O**bial S**e**quences

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# Ebola Virus, Makona

Patient No.	Age	Hospital	Date of Sampling	Outcome	Complete Genome
C5	16 F	Gueckedou	March 19	Survived	KJ660348
C7	47 F	Gueckedou	March 20	Died	KJ660347
C15	28 F	Kissidougou	March 17	Survived	KJ660346

Virus info obtained from N. Engl. J Med  
paper (Baize, et al 2014)



**Figure 1.** Map of Guinea Showing Initial Locations of the Outbreak of Ebola Virus Disease.

The area of the outbreak is highlighted in red. The main road between the outbreak area and Conakry, the capital of Guinea, is also shown. The map was modified from a United Nations map.



# 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
  - Clontech 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

FDA ARGOS ID	Isolate Description	NCBI BioSample	NCBI SRA	GenBank
FDA_ARGOS_EBOV1	EBOV/Makona-C05 2014, P1A, 9dpi, RNA, 90 ul, PHAC harvest	SAMN03611814	SRX1023888, SRX1023889, SRX1023890	KT013254
FDA_ARGOS_EBOV2	EBOV/Makona-C05 2014, P1B, 13dpi, RNA, 90 ul, PHAC harvest	SAMN03611815	SRX1024946, SRX1024947, SRX1024948	KT013255
FDA_ARGOS_EBOV3	EBOV/Makona-C07 2014, P1A, 9dpi, RNA, 90 ul, PHAC harvest	SAMN03611816	SRX1025888, SRX1025889	KT013256
FDA_ARGOS_EBOV4	EBOV/Makona-C07 2014, P1B, 13dpi, RNA, 90 ul, PHAC harvest	SAMN03611817	SRX1025960, SRX1025961, SRX1025962	KT013257
FDA_ARGOS_EBOV5	EBOV/Makona-C15 2014, P1A, 14dpi, RNA, 90 ul, PHAC harvest	SAMN03611818	SRX1025963, SRX1025964, SRX1025965	KT013258
FDA_ARGOS_EBOV6	EBOV/Makona-C15 2014, P1B, 15dpi, RNA, 90 ul, PHAC harvest	SAMN03611819	SRX1025966, SRX1025967, SRX1025968	KT013259

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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Additional slides

# **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](mailto: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 RMs (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