

Hunting Microbes in International Laboratories in Plain Sight

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Diplomate American College of Veterinary Microbiologists
(1984)

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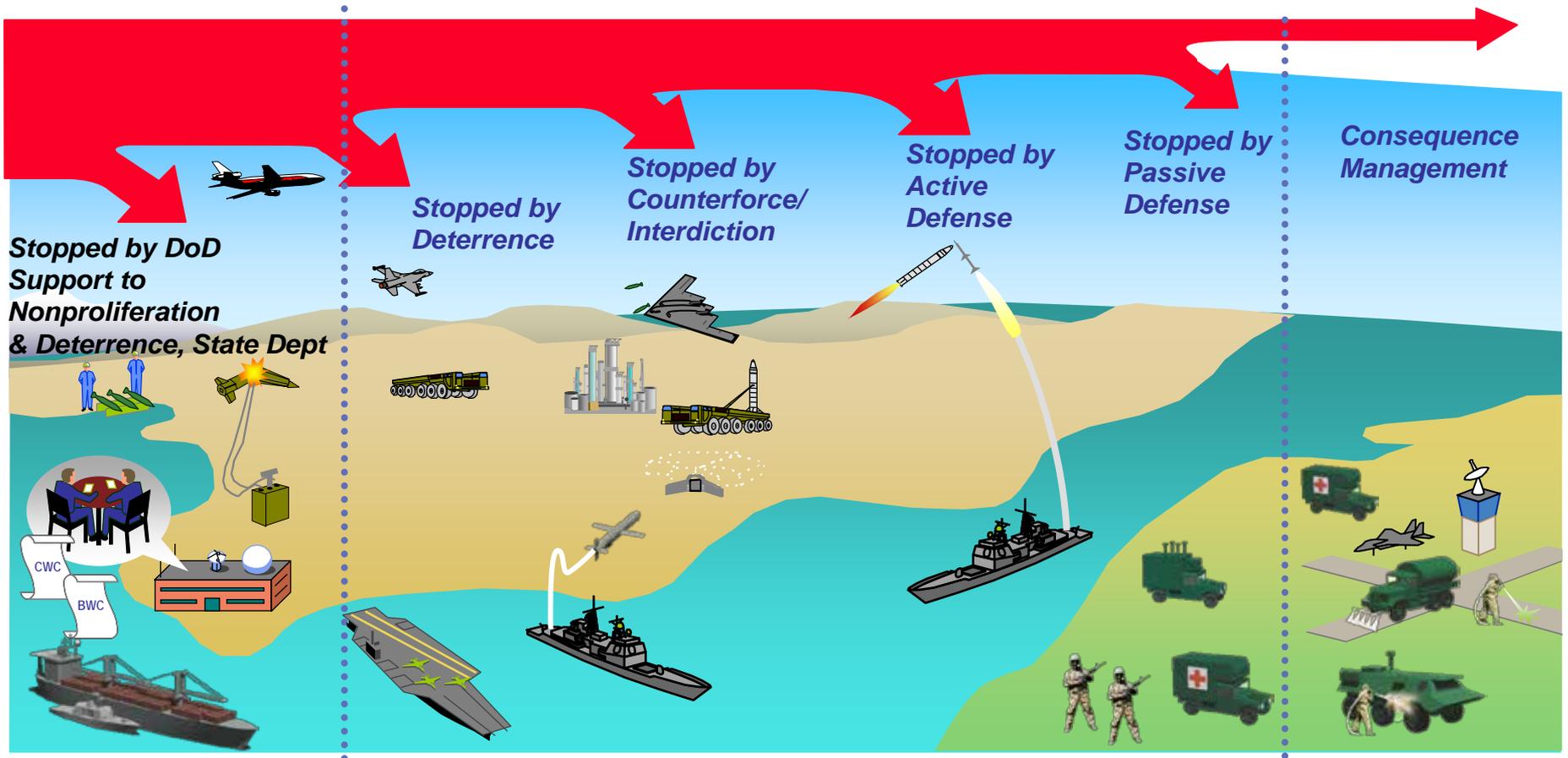
2015 Texas Laboratory Response Network Meeting
February 24 – February 26, 2015
Mayan Ranch Conference Center
PO Box 577, Bandera, TX 78003

National Strategy for Combating WMD

Nonproliferation

Counterproliferation

Consequence Management



Outline

- **The Law and Regulations**
- **Koch's Postulates**
- **Kiel's Postulates**
- **Where do you get a Select and Other Agent Collection?**
- **What Chem/Bio Facilities look like: Lab or Production?**
- **What the "Field" Looks Like: Iraq Prewar Preparations**
- **What happens if you blow up a production facility?**
- **Post War Iraq**
- **The Cooperative Biological Engagement Program (CBEP)**
- **Hunting Anthrax and Other Microbes in Azerbaijan**
- **The Right and Wrong of models: Garbage in, Garbage out**
- **The FINAL EXAM: Looking at what looks like anthrax: Is it?**
- **The FINAL ANSWERS: What you should have learned?**
- **The End: TGIO**

The White House
Office of the Press Secretary
For Immediate Release
July 02, 2010

Executive Order 13546-- Optimizing the Security of Biological Select Agents and Toxins in the United States

By the authority vested in me as President by the Constitution and the laws of the United States of America, it is hereby ordered as follows:

Section 1. Policy. It is the policy of the United States that:

- (a) A robust and productive scientific enterprise that utilizes biological select agents and toxins (BSAT) is essential to national security;
- (b) BSAT shall be secured in a manner appropriate to their risk of misuse, theft, loss, and accidental release; and
- (c) Security measures shall be taken in a coordinated manner that balances their efficacy with the need to minimize the adverse impact on the legitimate use of BSAT.

BARACK OBAMA

THE WHITE HOUSE,
July 2, 2010.

The Law and Regulations

Export Control Regulations (ITAR: International Traffic in Arms Regulations)

- 15 CFR 730-774; controlled technologies are at 15 CFR 774, supplement I
- 22 CFR 120-130; controlled technologies are at 22 CFR 121.1

Exports to countries which the Secretary of State has determined to have repeatedly provided support for acts of international terrorism (such as Cuba, Iran, Iraq, Libya, etc)*

*These are not complete lists of countries and they change on a daily basis!

Controls export of “defense articles and defense services”

- Includes data and Intellectual Property as well as physical devices & software
- Provides and maintains the U.S. Munitions List (in conjunction with DOD)



Department of Defense
INSTRUCTION

NUMBER 5210.89
April 18, 2006

(USDR)

SUBJECT: Minimum Security Standards for Safeguarding Biological Select Agents and Toxins

- References: (i) DoD Directive 5210.88, "Safeguarding Biological Select Agents and Toxins," February 11, 2004
- (ii) Title 42, Code of Federal Regulations, Part 73, "Department of Health and Human Services, Possession, Use, and Transfer of Select Agents and Toxins, Interim Final Rule," current edition
- (iii) Public Law 107-188, "Public Health Security and Bioterrorism Response and Preparedness Act of 2002," June 12, 2002
- (iv) Title 7, Code of Federal Regulations, Part 331, "Animals and Plant Health Inspection Service," and Title 9, Code of Federal Regulations, Part 121, "Department of Agriculture, Agricultural Bioterrorism Protection Act of 2002; Possession, Use, and Transfer of Biological Agents and Toxins, Interim Rule," current edition
- (v) through (v), see Enclosure 1

1. PURPOSE

This Instruction:

- 1.1. Implements security policy and assigns responsibilities under Reference (i).
- 1.2. Establishes minimum standards for securing and safeguarding biological select agents and toxins (BSAT) in the custody or possession of the Department of Defense.
- 1.3. Establishes the criteria for personnel regarding BSAT, including requirements for the Biological Personnel Reliability Program (BPRP).
- 1.4. Permits BSAT to be used for bona fide research and other peaceful purposes. Ensures the security of BSAT from attack, theft, wrongful use, and inappropriate transfer to unauthorized personnel, organizations, and/or laboratories.

United States Government Policy for
Institutional Oversight of Life Sciences Dual Use Research of Concern

Key Dates

Release date: September 24, 2011
Effective date: September 24, 2011

Relevant Notices

See the U.S. Government Science, Safety, Security (S3) website at: <http://www.s3e.gov/s3/issai/issai.cfm>

Issued By

The United States Government

Overview

Despite its value and benefits, certain types of research conducted for legitimate purposes can be utilized for both benevolent and harmful purposes. Such research is called "dual use research." Dual use research of concern is a subset of dual use research defined as: "life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, material, or national security." The United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern articulates the practices and procedures required to ensure that dual use research of concern is identified at the institutional level and risk mitigation measures are implemented as necessary.

For more information about this Policy and other policies regarding dual use research of concern, visit the U.S. Government Science, Safety, Security (S3) website at: <http://www.s3e.gov/s3/issai/issai.cfm>

All provisions in this Policy supersede those contained in the previous draft policy published on February 22, 2011 (Federal Register 76 (86): 42369-42372). This Policy and the United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern, which was released on March 29, 2012 (<http://www.s3e.gov/s3/issai/issai/issai.cfm?document=issai/issai.cfm-032812.pdf>) are complementary and emphasize a culture of responsibility by reminding all involved parties of the shared duty to uphold the integrity of science and prevent its misuse.

“War demands secrecy; science thrives on openness. How can a free society balance those competing demands?”

Sherwood Boehlert (R-NY)
House Science Committee
Chairman
October 10, 2002

Koch's Postulates (abbreviated):

The Microbe Must...

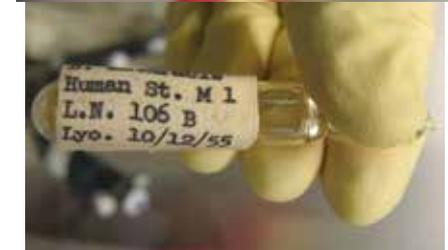
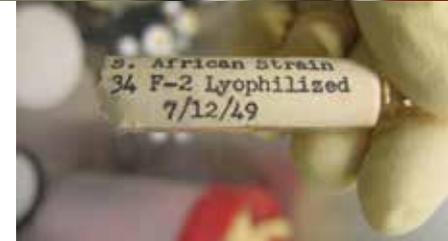
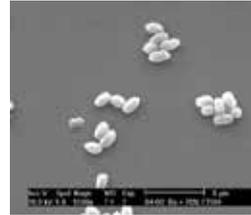
(formulated in 1884 and jointly with Friedrich Loeffler, based on earlier concepts of Jakob Henle)

- Be found in animals or humans with disease, not in the healthy
- Be isolatable from diseased hosts (in pure culture: desirable)
- After isolation cause disease in a healthy host
- Be re-isolatable from the inoculated, diseased host (identical?)

Kiel's Postulates of Biosurveillance and Consequence Management

- Biosurveillance is more than waiting for an outbreak or cleaning up after one
- No infectious disease outbreak does not mean no infectious agent
- You find what you look for, especially with PCR
 - Always, always use 3 orthogonal techniques to identify an agent
 - If you use only two, have a coin ready to flip to decide
- Never forget there are always things you don't know you don't know
 - **“There are more things in heaven and earth,
Horatio, than are dreamt of in your philosophy,”
Hamlet, Shakespeare**

Where the USAF/AFRL found their Select Agents and Related Microbes and Toxins: Especially Dangerous Pathogens (EDPs)



- **Anthrax**

- **172 isolates**

- 2 USDA collections (going back to 1945)
 - Remainder of Iowa State University collection
 - University of Scranton collection
 - Limited number from LSU collection
 - from Lawrence Livermore National (DOE) Lab
 - Texas field isolates from 2005 and 2007
 - K strain from Baghdad, Iraq
 - Vaccine strains and non-Select forms

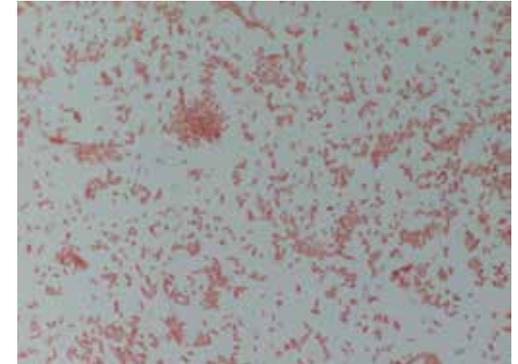
- **Brucellosis**

- Strain 19, exempt but **can cause Brucellosis in humans**
 - RB51, vaccine strain safe for humans: maybe not (See CDC) (Special USDA license)

USAF AFRL Bio Agents: Especially Dangerous Pathogens (EDPs)

• Plague

- Original strain for killed vaccine production, but pathogenic (first thought by CDC to be attenuated vaccine strain: has **pgm**, pigmentation, chromosomal gene, of a high pathogenicity island that encodes in part a siderophore, an iron chelator, and transporter, and **lcr (lcrG)**, low calcium response gene in virulence plasmid pYV: 70-kb, and virulence plasmid (pCD1) that encodes Yersinia outer membrane proteins (Yop) which inhibit phagocytosis and inflammation)
- Master seed stock from Greer Laboratories killed vaccine last manufactured in 1997



• Q Fever

- Killed vaccine from Australia (imported Q-Vax, Special USDA License)
- Isolated *Coxiella burnetii* DNA from ticks from South Texas



• Tularemia

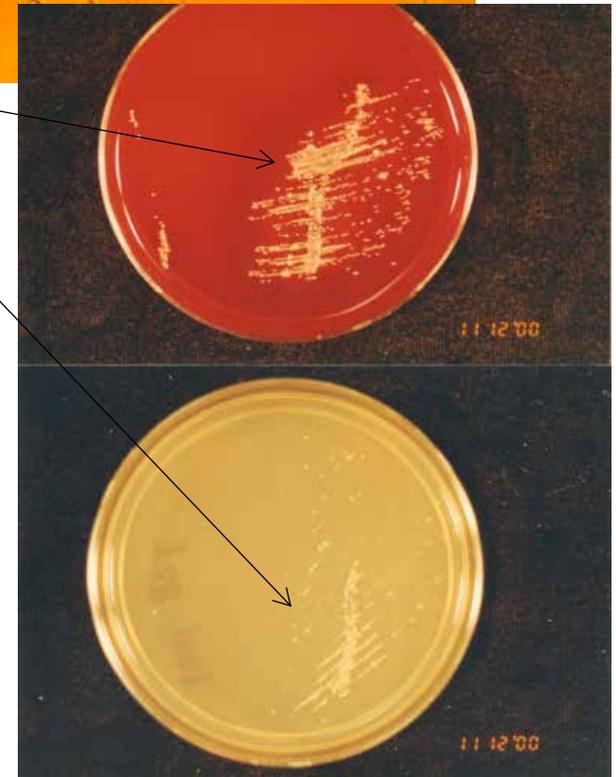
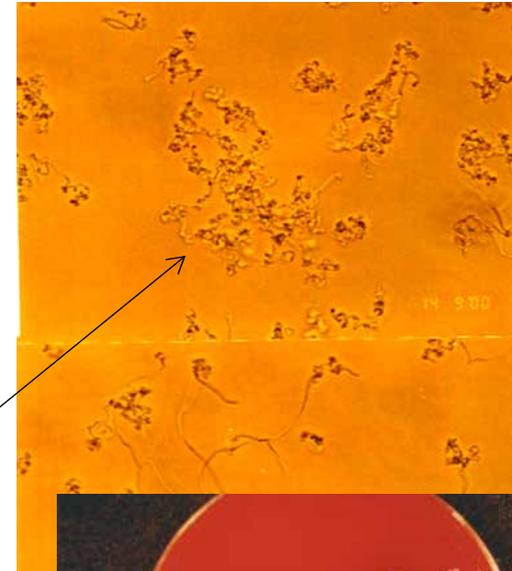
- Live Vaccine Strain
- DNA from Type B field isolate from Houston, TX
- Schu 4 Type A killed antigen from Critical Reagents Repository at Aberdeen Proving Ground, also in BEI Resources (ATCC) collection



Other Agents of Interest in USAF AFRL Collection

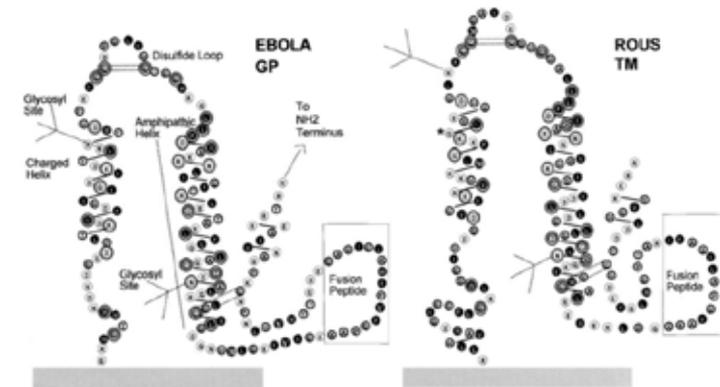
- **Cat Scratch Fever Bacteria** (*Bartonella henselae*)
- **Trench Fever Bacteria** (*Bartonella quintana*)
- **Shiga toxin and Shiga-Like Toxins 1 and 2**
- **Aflatoxin**
- Various hemorrhagic and neurotoxic **snake venoms**

- Genetically modified organisms: **GMO**
 - anthrax:** Parker, J.E., Kiel, J.L., Gifford, H., Alls, J.L., and Morales, P.J. U.S. Patent 7,279,320 B1. Curlicue Vaccine Strain of *Bacillus Anthracis*, 9 Oct 2007; **before this patented strain there was only one other patent** by Mock, M., Cataldi, A. (Buenos Aires, Argentina), and Pezard, C. Institut Pasteur, Paris, France, US Patent 5,840,312. RECOMBINANT BACILLUS ANTHRACIS STRAINS UNABLE TO PRODUCE THE LETHAL FACTOR PROTEIN OR EDEMA FACTOR PROTEIN, Nov. 24, 1998; **latest one:** Patent application: Protease-deficient bacillus anthracis, Andrei P. POMERANTSEV, Stephen H. Leppla; WO 2013019946 A3, CN103975072A, EP2739746A2, US20140314716, Publication date: Feb 7, 2013; Filing date: Aug 2, 2012; Priority date: Aug 2, 2011



USAF AFRL Bio Agents

- **Vaccinia** (Smallpox simulant)
 - Wyest vaccinia: smallpox vaccine strain
 - Merial Vaccinia/Rabies recombinant (Special USDA License)
- **VEE**
 - Killed Viral Equine Encephalitis Virus vaccine
 - Cloned functional NSP-4 from VEE
 - TC-83 (vaccine strain, but can cause human encephalitis)
- **Rous Sarcoma** simulant for Ebola (has similar surface glycoprotein)
 - South American (Arenavirus) Viral Hemorrhagic Fever killed virus
 - Cloned envelope protein gene of Ebola in expression vector (to work with an Ebola target without having to have a BSL-4 facility) (Chinese researchers took this approach in 2005)
- **Botulinum toxin**
 - Heavy and light chains separately
 - Holotoxin below Select Agent action level
 - Estimated human median lethal dose (LD-50) of 1.3–2.1 ng/kg intravenously or intramuscularly and 10–13 ng/kg when inhaled
 - The exempt amount is about 714 human lethal doses by inhalation
- **Staphylococcal Enterotoxin B**
 - Holotoxin below Select Agent action level



William R. Gallaher, Cell, Vol. 85, 477–478, May 17, 1996

not regulated if the aggregate amount under the control of a principal investigator does not, at any time, exceed 0.5 mg.

Brooks City-Base Air Handling: If You Saw this Would You Be Suspicious?



BCB: Glove Box for “Temporary” BSL-4: How Sophisticated Does One Have to Be to Work with Hemorrhagic Viruses Anyway?



ESTRUCTURA DEL LABORATORIO



PLAN FEDERAL DE SALUD



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SALUD
de la NACIÓN

LABORATORIOS DE BIOSEGURIDAD
NIVEL II, III Y IIIA

2/22/2015



ANLIS: Outside



"Virology Lab" now with windows installed and fence removed

**Post WWII Era
to Today**

ANLIS: Inside



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LABORATORIOS DE BIOSEGURIDAD
NIVEL II, III Y IIIA

2/22/2015

Argentina
un país en serio

Laboratorio de Animales
ABSL3.



ANLIS: Small Animal Facilities: Mice to Rabbits

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NIVEL II, III Y IIIA
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Argentina
un país en serio

The ANLIS: Nuts and Bolts



2/22/2015

Spiez Laboratory, Switzerland (European Point of View)

- The term biosafety covers two aspects: **Biosafety and Biosecurity**
- The WHO defines **Biosafety** as the “**containment principles, technologies and procedures** that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release”.
- **Biosecurity**, on the other hand, refers to “institutional and personal security measures aimed at preventing the loss, theft, misuse, diversion or intentional release of valuable biological material”.



The Spiez Laboratory Interim Answer until it Built its BSL3/4: The Cabinet



At the end of 2003, the Swiss Federal Office for Public Health (SFOPH) authorized the SPIEZ LABORATORY to undertake activities associated with Risk Group 3 bacteria, enabling it to carry out detection procedures for anthrax, plague and tularemia. The facility was installed in the restricted-access High-Tox building of the SPIEZ LABORATORY, which facilitated the implementation of the biosafety concept. All procedures are strictly governed by detailed regulations and revalidation work is regularly carried out

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Spiez Laboratory: BSL3/4

National Reference Centre for Anthrax (NANT): The Reference Centre was founded in 2001 and directed by the Institute for Veterinary Bacteriology of the University of Bern until the end of 2013. At the beginning of 2014, its mandate was transferred to the SPIEZ LABORATORY: Biology section of the SPIEZ LABORATORY is the specialist agency for the protection against biological threats and dangers and is specialised in analysis and diagnosis of highly pathogenic germs and toxins. Within the context of its referral function as NANT, referral diagnostics is provided for the following bacteria that are relevant to bioterrorism: <http://www.labor-spiez.ch/en/index.htm>.

Bacillus anthracis (anthrax); *Francisella tularensis* (tularemia); *Yersinia pestis* (plague); *Brucella* spp. (brucellosis); *Clostridium botulinum* (botulism)



**April 2008
Spiez BSL3/4 under construction**



**BSL3/4
Now Open
(2010
CBMTS VIII
Tour:
complete)**

WPAFB BSL-3: Being Installed and Inside



2/22/2015

What was Expected: Sprayer Release of Anthrax Spores (Simulant: *Bacillus thuringiensis*)



History: Iraqi Wars (Pre and Post) and Other Preparations

- Fielded the first live *Bacillus anthracis* isolation and identification kit in the Iraqi Theater just in time for the First Iraqi War (1991):



- Prepared for destruction of production facilities, Second Iraqi War (2003)

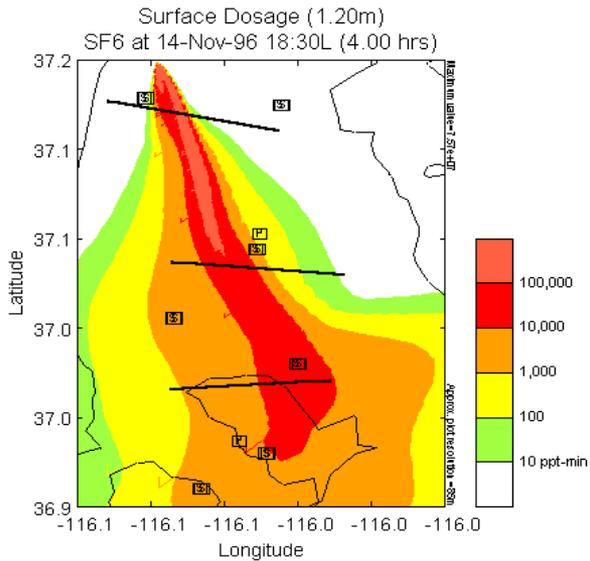


Full Scale Test of An Agent Defeat Weapon: *CrashPAD*



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Incinerating a Deep Chem/Bio Facility (Test)



2/22/2015

Postwar: Problems with Iraq: Looting and Chaos



Entering Baghdad



What they found: destroyed weapons and old biological equipment (safety hoods)

For more details read “Notes from the Shadows” (2015) by J L Kiel

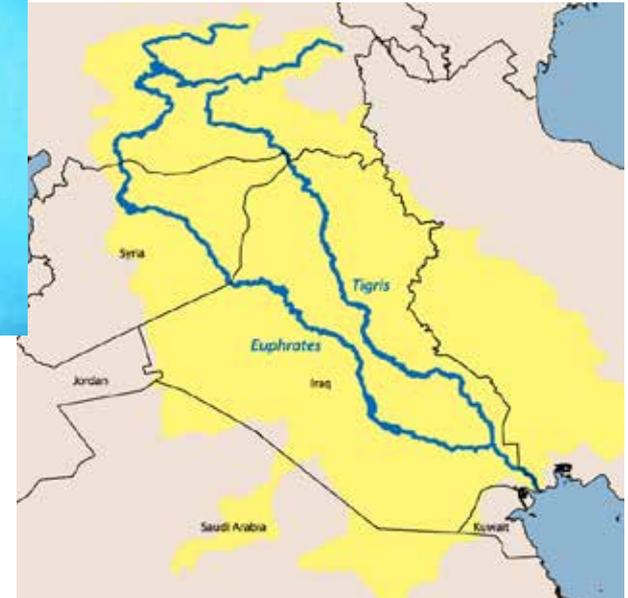
http://www.amazon.com/Notes-Shadows-Master-Black-Dragon-ebook/dp/B00RF1YWTW/ref=sr_1_1?s=books&ie=UTF8&qid=1421069996&sr=1-1&keywords=Notes+from+the+Shadows.

Problems with Iraq: It's not so easy to know you have been exposed to an EDP!

- PCR for botulinum toxin, using *Clostridium botulinum* DNA contamination as a marker
 - Always positive, no negative controls worked in Baghdad
 - Cause: dust from bottom mud of drained marshes of Tigris and Euphrates River blowing in the wind contaminated with *Cl. botulinum* (natural background)
- Equivocal PCR results from *Bacillus anthracis* from Al Kindi (Vet School)
- What was Iraqi Intelligence Service (Secret Police) doing with goats across from Vet School (and burning and burying them)? 0037 and 0038 Bacillus ?



2/22/2015



Suspicious Bacillus spp.: The Story of K Strain

- “K strain”: from the Bacterial Research Laboratory, Zoonoses Unit, of the Baghdad Veterinary College, originally a gift from Al Kindi Co. for Production of Veterinary Vaccines & Drugs, and collected by United Nations Monitoring, Verification and Inspection Commission (UNMOVIC) in March 2003
- **Last anthrax bacillus out of Iraq before the 2nd war**
- **Reported to be 34F2 Sterne spore vaccine strain (NMRC)**
 - **Mixture of various strains and closely related species** (the looking for different colony morphotypes on a single isolation plate and separating them for culture and genetic analysis was used here and later to “solve” the Amerithrax case in 2008 (genotypes/morphotypes A1, A3, D, and E): On 6 August 2008, the US Attorney declared Bruce Ivins to be the sole perpetrator in the Amerithrax case; on 19 February 2010, the FBI formally closed the case)
- **Iraqis also declared they had Pasteur Vaccine 2 (17JB) from Central Veterinary Laboratory, Weybridge, UK, even though it was never found**



2/22/2015

Looking at *Bacillus anthracis* from Iraq: Genotype/Phenotype

CDC Collaboration: Do we really know what we have been exposed to?

	KM-10B (<i>B. anthracis</i>)	K5A (<i>B. anthracis</i>)
		
Airforce PCR (chromo, pX01,pX02)	+, +, -	+, +, -
LRN PCR (chromo, pX01, pX02)	+, +, +	+, +, -
Old School pX01 PCR (<i>cya</i> , <i>lef</i> , <i>pagA</i>)	ND	+, +, +
Old School pX02 PCR (<i>capA</i> , <i>capB</i> , <i>capC</i>)	+fb, +, +fb	- , - , -
MLVA8	<i>vrrA</i> = 313, <i>vrrB1</i> = 229, <i>vrrB2</i> = 162, <i>vrrC1</i> = 583, <i>vrrC2</i> = 532, <i>CG3</i> = 158, pX01 = 129; pX02 = unknown allele call Closest MLVA8 GT matches are GT59 & GT61. (Sterne GT's; Group A3.b)	<i>vrrA</i> = 313, <i>vrrB1</i> = 229, <i>vrrB2</i> = 162, <i>vrrC1</i> = 583, <i>vrrC2</i> = 532, <i>CG3</i> = 158, pX01 = 129; pX02 - Closest MLVA8 GT matches are GT59 & GT61. (Sterne GT's; Group A3.b)
Gamma Phage	+	-
CP51 Phage	+	-
Penicillin	S	S
Source	Rodent liver	Not Available
Other	Greenish, β-hemolytic colonies	

In collaboration with
Alex R. Hoffmaster, Ph. D.
 Chief, Zoonoses and Select Agent
 Laboratory Bacterial Zoonoses
 Branch, CDC

MLVA =multi-locus variable-number of tandem [consecutive] repeat analysis)

+fb = faint band, matches B.a. amplicon size

2/22/2015

Confusion over Field Diagnosis and Identification of EDPs: Iraq and Anthrax Situational Awareness

1. Temperate bacteriophages lay silent within *Bacillus anthracis* and near-relative *Bacillus cereus* ATCC#4342, without killing the microbes but capable of transferring genes between strains and species
2. “Sophisticated” genetic engineering was simply a result of sloppy microbiology yielding cross contamination and genetic promiscuity mediated by the viruses: People in other countries will work in labs and production facilities that are “unacceptable in the US”
3. In 2011, mystery of why Iraq was using 17JB anthrax in its vaccine production facility was solved and was not very nefarious.
 - a. Iranian investigators, “The phenotypic and genotypic characterization of *Bacillus anthracis* isolates from Iran,” used Pasteur 17JB and Sterne 34F2 for comparison to the other strains
 - a. A standard strain in Iran (and other Middle Eastern and Developing Countries):
 - b. UN publication: “Manual for the production of anthrax and blackleg vaccines” (ISN 0254-6019), published in 1991.
 - a. Recommends using the 17JB which is pathogenic for guinea pigs (the standard animal to test anthrax vaccines for animals, since Max Sterne) as the challenge strain to test if the vaccine is protective of guinea pigs. This strain is less pathogenic for humans and large animals (safer for less sophisticated labs and production facilities)

Where we are Today: Are we too “Molecular”?



**The “Smart Ticket”:
Immunochromatograph**

A “smart ticket” type test for anthrax (positive for a strain from China)

LT Reagents



R.A.P.I.D. LT



RAZOR



2/22/2015

3/12/2015

What Have We Learned About Attribution: Forensic Genomics? Not Much



United States Government Accountability Office
Report to Congressional Requesters

December 2014

ANTHRAX

Agency Approaches to Validation and Statistical Analyses Could Be Improved

“The lack of an understanding of how bacteria change (mutate) in their natural environment and in a laboratory is a key scientific gap that remains and could affect testing conducted in future incidents. Specifically, the significance of using such mutations as genetic markers for analyzing evidentiary samples to determine their origins is not clear. This gap affects both the development of genetic tests targeting such mutations and statistical analyses of the results of their use on evidentiary samples. “

“.....**validation** as a process by which a test procedure is evaluated **to determine its efficacy and reliability for analysis.... Validation confirms by examination, from laboratory experiments, and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled. Successful validation offers some assurance that a given genetic test is sufficiently robust to provide reproducible results, regardless of the practitioner, agency, contractor, or laboratory applying it to a sample. Validation is frequently used to connote confidence, but it may also be thought of as defining the limitations of a method.”**

“**Verification**, confirms by objective evidence, from laboratory experiments, **that the given test meets the user’s specific requirements, such as criteria for accuracy.... If the verification testing were not to produce consistent results, then the scientist or the laboratory would have to return to the optimization phase to further refine the method and materials and then revise the test’s standard operating procedure (SOP) accordingly. Verification of the acceptance criteria must include repeated testing to account for measurement uncertainty, and confidence in performance....”**

GAO-15-80

<http://www.gao.gov/products/GAO-15-80>

2/22/2015

What Have We Learned About Attribution: Forensic Genomics? Not Much



United States Government Accountability Office
Report to Congressional Requesters

December 2014

ANTHRAX

Agency Approaches to Validation and Statistical Analyses Could Be Improved

GAO-15-80

“We found that **conditions causing the rise of the genetic mutations in the evidence were not known** before or after validation or during the subsequent statistical analysis of the results of the repository screening (Ames Ba from USAMRIID RMR-1029 derived and non-derived sources). During the investigation, it was not known what conditions would have promoted or inhibited the presence of the genetic mutations at detectable levels. Such knowledge would have indicated whether they were associated with the evidence itself or with the culture practices normally used in a laboratory.”

“.....including their mutation rates and genome “hotspots” for mutation, so that their “relatedness” can be measured. In this context, an expert who reviewed this report stated that computational methods are also needed to reconstruct (or assemble) genome sequencing data so that the relationship between markers that are not independent, as is common in asexually reproducing bacterial genomes, can be inferred.....DHS has funded research that is intended to provide a better understanding of how morphological variants, or mutations, could emerge and evolve in bacterial genomes”

“The laboratory mutation rate of B. anthracis is $\approx 2 \times 10^{-5}$ (Keim et al. 2001) and the natural mutation rate is $\approx 2 \times 10^{-10}$ (Van Ert et al. 2007), assuming little or no recombination, which may not be the case (Ko et al. 2004); however, we have previously shown that nitration conditions in vitro easily generate profound mutations (Kiel et al. 2002).” Kiel et al, Folia Microbiol. **53** (6), 472–478 (2008)



Measuring Cooperative Biological Engagement Program (CBEP) Performance

Capacities, Capabilities, and Sustainability Enablers for Biorisk Management and Biosurveillance

Stephanie Young, Henry H. Willis, Melinda Moore, Jeffrey Engstrom



Prepared for Cooperative Biological Engagement Program

Approved for public release; distribution unlimited

2/22/2015

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“.....remnants of the Soviet Union’s biological weapon complex presented a significant threat to U.S. and international security.broader Cooperative Threat Reduction (CTR) program, the Biological Threat Reduction Program (BTRP) addressed proliferation risks associated with biological agents, related materials, and technical expertise associated with the defunct biological weapon program. Under its authorities to operate in the former Soviet Union, BTRP worked to destroy bioweapon production facilities, consolidate collections of dangerous pathogens, and support peaceful research activities to employ personnel with knowledge.global health security challenges arise from developments far broader than state-sponsored biological weapon programs..... the Cooperative Biological Engagement Program (CBEP).....partners with about 20 countries in different regions around the world and **works with them to address diverse threats to international security, including terrorist organizations seeking to acquire pathogens of security concern; human, animal, and agricultural facilities operating with inadequate safety and security safeguards; and the spread of diseases with potential security or economic consequences.”**

- “
- *Strengthen enduring partner capabilities for biorisk management.* This objective refers to both biosafety and laboratory biosecurity control measures, as well as associated risk assessment and oversight functions. We developed separate logic models for biosafety and biosecurity, which reflect both common elements and the components that make each unique.
 - *Strengthen enduring partner capabilities for biosurveillance.* “



What We Started With in Azerbaijan

Department of Defense Cooperative Biological Engagement Program (CBEP: DTRA)



Where we are in Azerbaijan Today



2/22/2015

Anthrax: Follow up on Spatial Analysis Based on Old FSU Disease Surveillance Records in Azerbaijan: Models are only as good as the data: Garbage in, Garbage out

How good was the ... analysis at predicting the next outbreak of anthrax? Why new data is better than old.

Complete miss

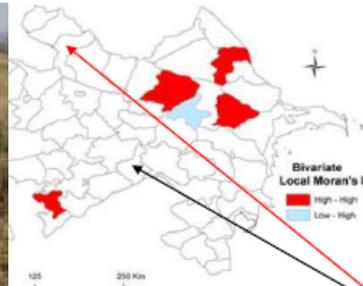
50/50 hit

Anthrax

.....the admitted accuracy of mathematical processes is allowed to throw a wholly inadmissible appearance of authority over the results obtained by them. Mathematics may be compared to a mill of exquisite workmanship, which grinds your stuff to any degree of fineness; but, nevertheless, what you get out depends on what you put in; and as the grandest mill in the world will not extract wheat flour from peascods, so pages of formulae will not get a definite result out of loose data.

Thomas Huxley

British Biologist: Born: 4 May 1825, Ealing, Middlesex; Died: 29 June 1895 (age 70), Eastbourne, Sussex



1: The top map displays a 22.5% percent annual increase in anthrax inside the buffer compared to decrease outside. The bottom map displays an 18.4% percent annual increase in brucellosis inside the buffer compared to an 8.9% increase outside.



The Test: Is it Anthrax or Not?

A



B



C



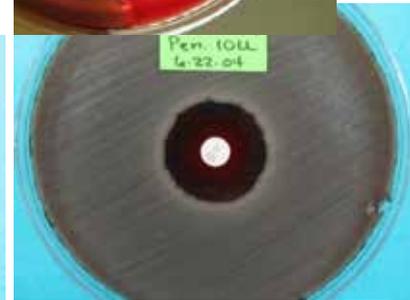
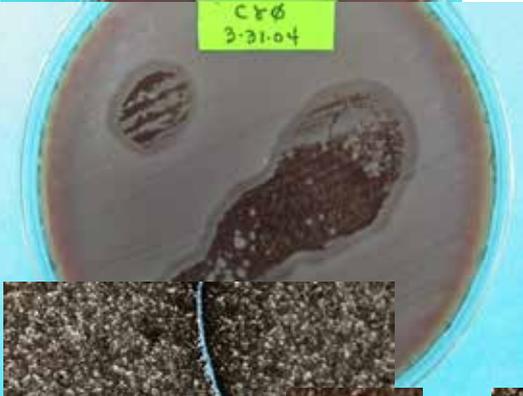
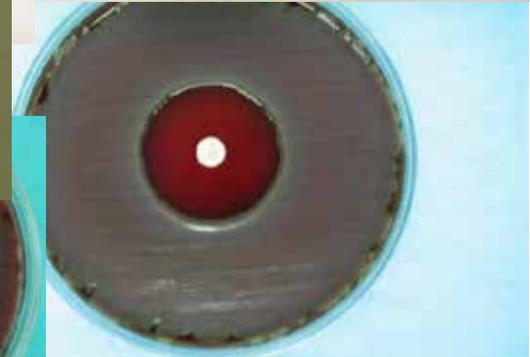
D



Cro
3-31-04



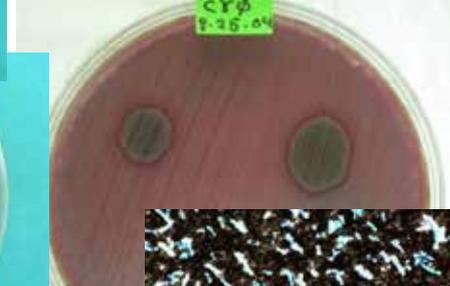
Pen. 10LL
4-22-04



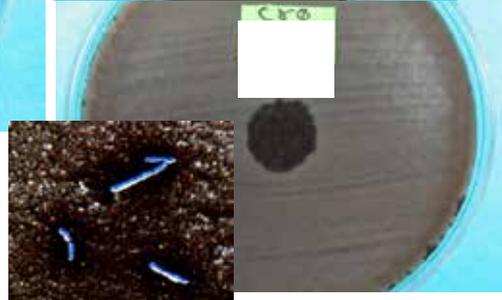
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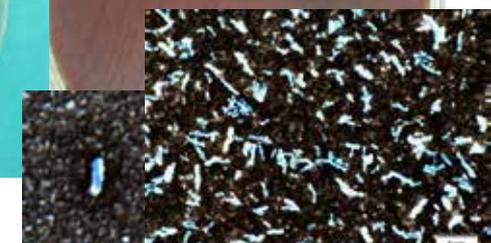
Cro
11-20-02



Cro
1-25-04



2/22/2015

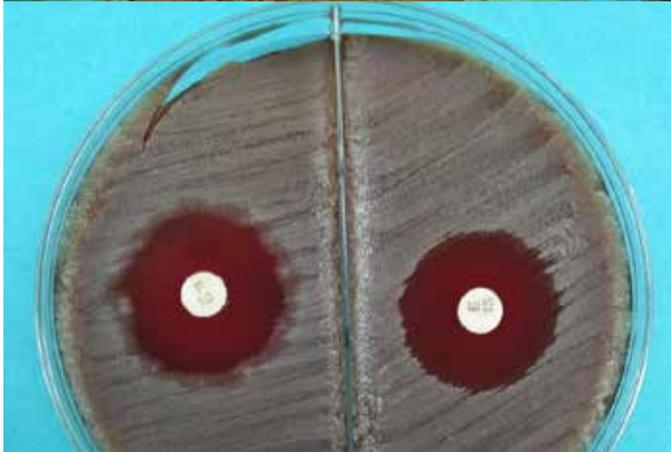


The Test: Last Chance: Is This *Bacillus anthracis*?

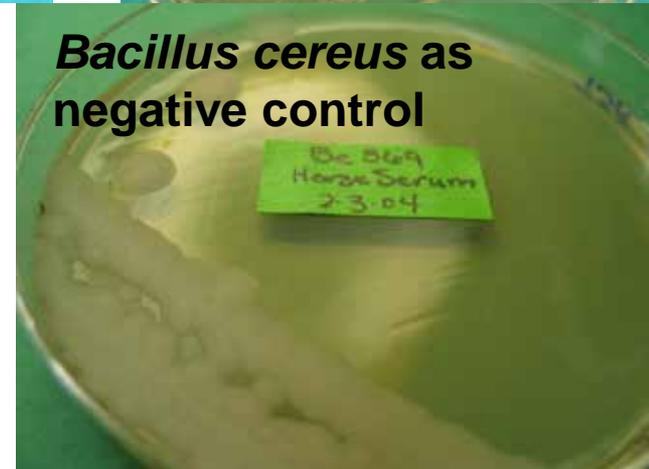
E



Hot Anthrax for Comparison
(Horse serum and carbon dioxide=capsule)



Bacillus cereus as
negative control



FINAL ANSWERS: What you should have learned?

- "Once you eliminate the impossible, whatever remains, no matter how improbable, must be the truth." Sir Arthur Conan Doyle in "A Scandal in Bohemia", spoken by the character Sherlock Holmes (and by Mr. Spock, "Undiscovered Country", Star Trek)
- Highly infectious agents can circumnavigate the globe and be present everywhere without us knowing it and "Suddenly Emerge" as highly pathogenic
- An invasive species may precede appearance as an emerging pathogen by years, if not decades
- The difference between endemic and epidemic (enzootic and epizootic) is environment
- Trust your intuition, if it appears to be "like" a known infectious disease then don't disregard the pathogen just because you can't find it or identify it with your known techniques for that pathogen, look for a different but related one (your ultimate lesson here is **discernment**)

Sunrise in Baku, Azerbaijan On the Hunt for Microbes Questions (TGIO)?

“Quantity has a quality all its own”—Old Russian Proverb



Working with 10 million human lethal doses of anthrax spores at Brooks Air Force Research Laboratory



Highly recommended reading:

"Spillover: Animal Infections and the Next Human Pandemic" (2012) by David Quammen

"Pathogenic Ecology" (2015) by J L Kiel

http://www.amazon.com/Pathogenic-Ecology-Black-Dragon-Trilogy-ebook/dp/B00S47D2BU/ref=sr_1_1?s=books&ie=UTF8&qid=1421069933&sr=1-1&keywords=Pathogenic+Ecology

John L. Kiel

**"Military Microbe
Scout and Hunter"**