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Military Biological Weapons Programs

Geoffrey Forden
Security Studies Program, MIT
April 3, 2002

We now know what terrorists want from Biological Weapons, but what does a military want?

I will examine two programs we know a lot about:

- Voluntarily eliminated BW program in 1969 and declassified much information
- Forced to “eliminate” program and much information gained from inspectors.
Estimates of Historical Weapons Effectiveness

- Combatants
  - Precision Guided Munitions: 0.75 Casualties/ton
  - World War I Chemical Weapons: 10 Casualties/ton (0.2 deaths/ton)
  - Iran-Iraq War Chemical Weapons: ~35 Casualties/ton

- Civilians
  - 1995 Tokyo Sarin Attack (CW): ~2200 Deaths/ton
  - Atomic Bomb (Hiroshima): ~100,000 Deaths/ton
  - Anthrax Attacks (October Incident): ~1,000,000 Deaths/ton
  - Thermonuclear: ?

Thermonuclear: ?
The U.S. Biological Weapons Program
1. Artillery shells

2. Stationary generator left behind by special forces.

3. Boat mounted line spray source

4. Naval point source

5. Self-dispersing spheres

6. Flettner rotors

7. Drone mounted line spray source.
1. Artillery shells  
   Abandoned

2. Stationary generator left behind by special forces.

3. Boat mounted line spray source

4. Naval point source  
   Abandoned

5. Self-dispersing spheres  
   Obsolete

6. Flettner rotors  
   Obsolete

7. Drone mounted line spray source.
Overview of Various BW weapon Systems.
Military Uses of Biological Weapons: Serious Suggestions, Far from being actual war plans!
Using Tactical BW in General War: blunting a Soviet Attack (ca. 1958)
Using Tactical BW in General War: Blunting a Soviet Attack (ca. 1958)

Resources required for BW mission:

+ thermonuclear weapons in the north

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**NOTE:** Total ground troops in southern army front, 429,250.
Using “Strategic” BW in a Peripheral War: blunting a Soviet Attack (ca. 1958)

Assumption:
US fights a static defense until all its forces are in place. It can then launch an offensive at a time of its choosing, i.e. 7 days after BW attack.

Goals:

1) Block troop transfer from the North to the South.

2) Incapacitate enemy troops in the South to reduce their effectiveness at defense.
Resources required for BW mission:

**Brucellosis**
12-15 day delay
< 2% fatalities

**VEE**
2-5 day delay
< 2% fatalities
Iraq’s Biological Weapons Program
Timeline of Iraq’s WMD Programs

- Biological Weapons Program
- Chemical Weapons Program
- Nuclear Weapons Program
- Missile Development Program
The Different Periods of Iraq’s BW Program

Concepts of operations were formed but UNSCOM does not know much about these since they are prohibited from inquiring about military matters.

A procurement network was established (for importing needed items from outside the country) and planning took place, but not much else is known about Iraq’s activities.

This period was dedicated to weapons development, with some research.

This was primarily a training period with students sent abroad for PhDs (mostly to the UK). Existing facility was closed. During this period, Iraq isolated bacteria and viruses, investigated drying processes, taught students, and built up an institute.

During this period, the goals of the program were “dirty tricks” associated with assassination of political opponents (staining shirts with ricin) and protection of the President (food poison testing). But the program failed with people being sent to jail because of fraud, for instance. However, the people who ended up leading the BW program were associated with it at this period.
Weaponization of Iraq’s BW Agents

Iraq tried to set up a factory for these tactical warheads that could be filled with either CW or BW. They are made to look just like normal HE 155 mm rounds, presumably to prevent Iranians from taking CBW defense measures.

Iraq tried to develop remotely controlled Mirage fighters with large drop tanks filled with BW, even into the Gulf War.

25 SCUD warheads were filled with anthrax. These were filled in the period before the 1991 Gulf War.
Weaponization of Iraq’s BW Agents (Cont’)

These gravity bombs were filled with CW, as indicated by the empty circle. BW bombs had an Arabic A inside the circle.

CW markings, BW bombs had an Arabic “A”
Supergun—A new way of getting Intercontinental Distances

A supergun (termed little Babylon), with 350 mm diameter, as actually built but apparently not tested. This version was built as a fixed direction/launch angle. But it could have been weaponized to a more mobile version.
Clandestine BW Dispersal
Anthrax and Smallpox: Comparison of Two Outbreaks

Jeanne Guillemin
Senior Fellow
MIT Security Studies Program
Anthrax and Smallpox: Comparison of Two Outbreaks

- 1979 Sverdlovsk anthrax epidemic, officially explained by consumption of infected meat; military aerosol suspected
- 1972 Yugoslavia smallpox epidemic, started by a pilgrim returning from Mecca via Baghdad, site of unreported outbreak
Key Problem = Late Diagnosis

1. What are the political causes?
2. What are the medical/professional causes?
3. What are the public communication causes?
1979 Sverdlovsk Epidemic

1992-1994 investigation of an “unnatural” outbreak of inhalational anthrax
Sources of Evidence

- KGB list of 64 victims’ names and addresses
- Interviews with families/neighbors of 56 victims
- Cemetery data
- Autopsy tissue data
- Hospital records (5 survivors)
- Local hospital and factory clinic lists
- Veterinary documents/animal deaths
April 4-May 16, 1979 cases reported as due to eating infected meat over weeks.
Fatalities 64, survivors 15.
Anna Komina
Ceramics factory worker, age 54; resident of affected district

Date of onset of symptoms: April 4
Date of death: April 10
Valentin Petrovich Borisov  
Age 27, Soldier, Compound 32

Pyotr Pilyasov, Age 39  
Construction worker
June, 1992, Hospital 20, in Ekaterinburg’s southern Chkalovsky district. Team members Martin Hugh Jones, veterinarian, Alexis Shelokov, virologist, and Matthew Meselson, biochemist and team organizer, with a university host V. A. Shpetkin, and the hospital director, Margarita Ilyenko.
Street leading towards ceramics factory (smokestack in Center) where 18 workers died of anthrax, April-May 1979
1993. Interior of pipe shop of abandoned ceramics factory. Large, third-story windows on left face northwest.
Gate of Compound 19 military base, southwest Ekaterinburg. Soldier is allowing truck to enter.
Cottage in village southeast of Ekaterinburg where animals died of anthrax in 1979, starting April 5-6, and where villagers were vaccinated and quarantined.
Sverdlovsk, c.1985
Red dots=Nighttime
Locations of victims.
Addresses obtained from
KGB and other lists.
Southern cluster is in
Chkalovsky rayon.
Arrows=homes off map.
Chkalovsky District Only
(note inset of entire city)

Irregular white lines show Compounds 19 and 32.

White rectangle indicates Ceramics factory.

Red dots=daytime locations of 66 victims and 11 survivors.
Six villages southeast of Sverdlovsk where 1979 epizootic occurred. Public health measures April through May. Interviews conducted at F, Abramovo, confirmed Veterinary documents.
Research Findings

• A lethal emission of anthrax spores from Compound 19 occurred during the afternoon of April 2, 1979.
• No young people under 24 or children were affected.
• Approximately 80 people (of some 5000 exposed) became infected; 11 survived with treatment.
• An estimated gram (a trillion spores) caused the fatalities; attack rate of 1-2%; fatality rate around 80% (note late diagnosis).
• Inhalation anthrax in humans can occur as long as 43 days after exposure. (First evidence in human cases)
Soviet Public Health Response

- Urban: lab diagnosis, screening for central hospital intensive care and pediatric cases, ambulance transport, autopsy team; 4000 volunteers mobilized for disinfection and distribution of antibiotics; Moscow clinical team, vaccine campaign for 50,000; building exteriors washed.

- Rural: roadblocks, carcasses burnt, enforced human vaccination, animal sheds destroyed, 3-week village quarantine.
Diagnosis 9 days post April 2 exposure
Total 21 deaths

Moscow doctors April 12 arrival.
Total 25 deaths

17 victims die with no hospital care

City clean-up begun. 30,000 vaccinated.
April 16,
Total 42 deaths

Last recorded death May 16.
Total 66 valid cases
11 survivors
Smallpox Epidemic
Yugoslavia, 1972

Imported Virus Contagion
“Natural Outbreak”
Fig. 1.3. The clinical course of moderately severe ordinary-type variola major in an unvaccinated subject (A); inoculation smallpox (variolation) in an unvaccinated subject (B); and primary vaccination (C). (Temperature records from an illustration in Hime (1896) with modified wording.)
Fig. 23.5. Spread of smallpox in Iran, Iraq and the Syrian Arab Republic, 1970–1972. The disease was introduced from Afghanistan into Mashhad, Iran, in October 1970. There were three waves of dispersion through Iran, which lasted over a period of 22 months. By the end of 1971 smallpox had crossed into Iraq, where it spread north to Arbil and south to As Samawah. Transmission in Iraq was interrupted by June 1972. In February 1972, smallpox spread from Baghdad in Iraq to Meyadin in the Syrian Arab Republic, where a smaller outbreak occurred that was contained by June 1972.
Feb. 3-7 index case infected in Baghdad.
Feb.15-16 falls ill at home Danjani (Kosovo)

Mar.5 one of 11 infected by index case falls ill in Serbia
Mar.10 Serbian dies after infecting 42 in hospital
Mar.11, Serbia case total 10, Kosovo 12
Mar.13 physician in Kosovo sounds alert

Mar.17 diagnosis and state containment initiative
Mar.25 case total is 137
April 15 case total is 173 (123 Kosovo, 48 Serbia, 1 Vojvodina, 1 Montenegro)
Fig. 4.8. The interval between the first possible exposure to a case of smallpox imported into Europe by air and the onset of symptoms in first generation indigenous cases, in family and hospital environments. (Based on Mack, 1972.)
Fig. 23.7 Yugoslavia: number of cases of smallpox, by date of onset and locality, 1972. The first generation of cases occurred in Kosovo province and adjacent areas; the large second generation in Kosovo, Belgrade and some other places.
Public Health Response
Mar. 15 to May 9
Vaccine campaign, Quarantine, roadblocks. Belgrade team joins Kosavar local health staff (rural, many migrant workers) to begin concentric circles of Vaccinations in 25 foci, with family and village quarantine, prohibition of public meetings. 18 million (of 20.8 million citizens) were vaccinated in 3 weeks. 175 cases, 35 dead (20%) case fatality rate. 37% of cases among previously vaccinated.
Structural Sources of Late Diagnosis

- Political: military secrecy/religious repression
- Medical/Professional: lack of familiarity with disease (misdiagnosis)
- Communication: public uneducated about risk
Solutions to Late Diagnosis

1. Political-public health cooperation
2. Medical technology and education
3. Accurate public communication
US Preparedness for Biological Terrorism

Gregory Koblentz
Security Studies Program
MIT
April 3, 2002
Overview

• Background and History

• September 11 and Anthrax Letters

• Preparedness Post-September 11
Background and History

• 1996 Nunn-Lugar-Domenici Domestic Preparedness Program

• 1998 White House Initiative
  – Pharmaceutical Stockpile
  – Grants to State Public Health Agencies
  – Metropolitan Medical Response System
  – Research & Development
Figure 1. HHS Spending on Bioterrorism Preparedness and Research, 1998-2002

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Figure 2. HHS Spending on Bioterrorism Preparedness, 1998-2002

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Bioterrorism Preparedness Prior to September 11

• State and Local Laboratory, Surveillance and Epidemiological Capabilities Improving Slowly

• Pharmaceutical Stockpile in Place

• Hospitals and Healthcare Providers Neglected
The Anthrax Letters

- 5 letters each with ~2 grams of anthrax
- 23 confirmed cases: 5 fatalities
- > 10,000 on antibiotic prophylaxis
- Cost of response: $250 million
Lessons Learned

1. Expect the Unexpected
2. Doctors are the First Line of Detection
3. Early Treatment is Key
4. Lab Capacity Needs to Be More Robust
5. Coordination and Communication Problems
6. Flawed Knowledge Assessment
7. Importance of Forensics
Figure 3. HHS Spending on Bioterrorism Preparedness and Research, 1998-2003

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Figure 4. HHS Spending on Bioterrorism Preparedness, 1998-2003

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Post-September 11 Preparedness

• Existing Programs Expanded and/or Accelerated
  – Public Health Infrastructure
  – Pharmaceutical Stockpile
  – Metropolitan Medical Response System
  – Research and Development

• New Programs Established
  – Hospital Preparedness
  – Medical Responder Training
Assessment of Post-September 11 Preparedness

• National Strategy is Required
  – Interagency
  – Intergovernmental
  – Interdisciplinary
  – Public-Private

• Bioterrorism is NOT Another Emerging Infectious Disease
  – Mother Nature vs. Bin Laden
  – Criminal and National Security Implications
Public Health Surveillance: 
A local health department perspective

M. Anita Barry, MD, MPH
Director, Communicable Disease Control
Boston Public Health Commission
Objectives

- Current public health surveillance
- Characteristics of the ideal surveillance system
- Boston’s enhanced surveillance system for bioterrorism and mass casualty events
- Future plans
Types of Surveillance

- Notifiable disease reporting
- Active surveillance
- Laboratory based surveillance
- Population based surveillance
Notifiable Disease Reporting

• Health care providers are required by law or regulation to notify public health about:
  – Named pathogens
  – Specified diagnoses
  – Outbreaks or clusters of illness

• Usually a passive system, but can use enhanced passive technique

• Reporting requirements differ among states
Notifiable Disease Reporting: Why it’s incomplete

- Unaware of the requirement to report
- Confused about the mechanics of reporting
- Concern about confidentiality
- Someone else’s job
- Unconfirmed case (wrong diagnosis, no lab)
- Forgot to do it
Active surveillance

• Public health staff review records and other data on site (for example, at a hospital)
• Provides fairly complete data
• Very labor intensive and requires a sustained effort - resources become a problem
Laboratory based surveillance

- Laboratories are required to report certain positive test results to public health
- Isolated laboratory data are incomplete
  - False positives, false negatives
  - Skewed testing (publicity, specific signs and symptoms)
- Molecular microbiologic techniques enhance epidemiologic investigations
Population Based Surveillance

- Illness in closed communities (such as incarcerated populations)
- Absenteeism rates
- Insurance claims data
- Sales of specific products (such as anti-diarrheal medications)
The Ideal Surveillance System

Fast, cheap, and easy...
The Problem

• Traditional surveillance systems based on the reporting of specific diseases have limited potential for early detection of mass casualty events such as bioterrorism or pandemic influenza.
Milwaukee: Cryptosporidium Infection Related to the Public Water Supply

- Estimated 400,000 people had outbreak associated diarrhea.
- 285 laboratory confirmed cases.
- Recognition of the outbreak was delayed:
  - Non-specific nature of the symptoms
  - Limited laboratory testing
  - Infrequent use of the health care system by people with diarrhea
Identification of the Outbreak

- Shortages of over the counter anti-diarrheal medications
  - pharmaceutical sales data impacted by sales & is unlikely to detect small case numbers
- Retrospective data indicated changes in health care utilization patterns prior to identification of the outbreak
Agents of Concern: CDC Category A

- *Bacillus anthracis* (anthrax)
- *Clostridium botulinum* toxin (botulism)
- *Yersinia pestis* (plague)
- variola major (smallpox)
- *Francisella tularensis* (tularemia)
- Viral hemorrhagic fever
Agents of Concern:
CDC Category B

- *Coxiella burnetti* (Q fever)
- *Brucella* species (brucellosis)
- *Burkholderia mallei* (glanders)
- ricin toxin from *Ricinus communis* (castor beans)
- epsilon toxin of *Clostridium perfringens*
Agents of Concern: CDC Category C

- Nipah virus
- hantaviruses
- tickborne hemorrhagic fever viruses
- yellow fever
- multidrug-resistant tuberculosis
Bioterrorism Events in the United States

- 1984, The Dalles, Oregon
  - Salmonella in salad bars
  - 751 ill (45 hospitalized)

- 1996, Dallas, Texas
  - Shigella in micro-lab donuts
  - 12 ill (4 hospitalized)
Anthrax Cases, 2001

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The Ideal Surveillance System

- Sensitive (with enough specificity to make it workable)
- Timely
- Provides complete data
- Cost effective
- Linked to an effective follow-up system to interpret initial signals
Enhanced Surveillance in Boston

- Emergency department visits
- Urgent care visits
- Boston EMS calls
- Death certificates
- Poison Control Center
VOLUME SURVEILLANCE SYSTEM DESIGN: DATA SOURCES

Poison Control Center

BOSTON HOSPITALS

MDPH HEALTH ALERT NETWORK (HAN)

BPHC CDC

BPHC MORTALITY DATA (Death Certificates)

BOSTON EMS
Enhanced Surveillance in Boston: Hospitals

- Every 24 hours volume data is electronically sent by SFTP to the Boston Public Health Commission (BPHC)
- Threshold data for each site based on historical data has been calculated
- If threshold is exceeded an initial assessment is automatically sent to an onsite contact
Calculations

Binomial distribution: adjust for month and day of the week

Number of events = average daily volume by month
n = Boston population (1990 census)
p = number of events/n

Upper CI = p + ((1 - ?)(sqrt(p)(1-p)/(n))))
Upper threshold = Upper CI(n)
Enhanced Surveillance in Boston: Hospitals (Cont’d)

• If a cluster or any unusual cases of illness are identified on initial assessment, BPHC nurses/epidemiologists investigate further
• Data are typically available within 12 hours after the close of a 24 hour period
Enhanced Surveillance in Boston: Other Sites

• Poison Control Center: daily volume data being sent, thresholds being adjusted
• Boston EMS: type of calls of interest selected, automatic data transfer being developed
• Death Certificates: database developed; timeliness of data input being addressed
Enhanced Surveillance in Boston
Preliminary Findings

• System detected morbidity associated with a heat wave (retrospective)
• Volume data corresponded well with influenza activity in 1999 and 2000
• System identified changes in health seeking behavior post September 11
Volume data and influenza

• In 2000 there were 103 episodes of a site exceeding threshold.
• However, 3 or more sites simultaneously exceeded threshold on only 4 days and 2 sites on 17 days.
• Most of the time (N=54), only one site exceeded threshold on a given day.
Daily volume by site
December 1, 1999 - January 31, 2000

- Days exceeding threshold
- Peak influenza activity in the U.S. (12/26 to 1/15/00)
Volume Surveillance - 12/4 to 12/9/00

- Exceeded threshold

1st flu isolate in MA
Volume data: Findings from 9/11/02 - 11/11/02
Days exceeding threshold. No infectious disease clusters identified.
How many times did multiple sites exceed threshold on a given day?

• There were 22 episodes of a site exceeding threshold in the time period.
• For most (n=17) only a single site exceeded threshold on a given day.
• On two days, two sites simultaneously exceeded threshold.
• On one day, four sites simultaneously exceeded threshold.
Follow-Up with sites exceeding threshold and Boston Public Health Commission’s (BPHC) Response

- Persons seeking nasal swabs and antibiotics for anthrax resulted in increased activity on 10/15
- No anthrax cases or anthrax contaminated environmental specimens were identified in Massachusetts
- The BPHC posted information on anthrax including updates to BPHC’s website (www.bphc.org)
- Clinical advisories on anthrax were emailed to health care providers throughout the city
Enhanced Surveillance in Boston

**Strengths**
- Adjusts for site case mix
- Adjusts for seasonal changes
- City wide coverage
- Electronic

**Weaknesses**
- Non-specific for BT events
- Changes influenced by the business of health care
Conclusions

• Volume based surveillance is a feasible method for the early identification of a mass morbidity event
• A rapid follow-up system is a critical component to understanding initial signals
• Data from this system can be used to create educational messages for both health care providers and the public
• Additional research is needed to define the sensitivity of the individual or combined measures being used and the optimal combination to detect significant activity
Enhanced Surveillance in Boston: Lessons Learned

• Systems must be electronic
• Add on systems will not be sustainable
• Computers system go down (even for days)
  – Develop back up plans
• Don’t abandon case reporting
  – No one system is perfect
• The more complex data - the harder it will be to retrieve it manually
• Build communication networks into the surveillance system
Enhanced Surveillance in Boston: Future Plans

• Capture more granular data
  – Chief complaint data
  – Natural language programming
  – Minimize human contact

• Add additional populations and types of health care sites

• Enhance the surveillance feedback loop

• Syndromic surveillance
Syndromes That May Be Associated With Bioterrorism

- **Pulmonary**
  - Fever
  - Cough
  - Myalgias
  - Hypoxia

- **GI**
  - Fever
  - Nausea/vomiting
  - Diarrhea (+/- bloody)

- **Rash and fever**
  - Vesicular
  - Petechial

- **Neurologic**
  - cranial nerve palsies, HA, fever, confusion

- **Septic Shock**
  - DIC
  - Organ failure
Syndromic Surveillance

- ICD-9 code data or chief complaints to identify potential BT-related syndromes
  - How much is to much
  - Follow-up is critical
  - Real time data is limited
  - Sustainability
  - Validity of chief complaint data - How do different populations describe illness
Questions?
Bioterrorism Preparedness - Laboratory Analysis

Kate Ruoff, Ph.D.
Microbiology Laboratories
Massachusetts General Hospital
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Bioterrorism Preparedness - Laboratory Analysis

An account from the “real world” of the clinical microbiology laboratory
Clinical Laboratories - The Need for Preparation

- Agents likely to be used by terrorists
  - Unfamiliar, rarely encountered organisms
  - Potential for misidentification, mishandling of specimens, laboratory acquired infection
- Public health agency-sponsored training in the Northeast began in 1999
- Laboratory Response Network (LRN)
- Were we prepared in the autumn of 2001?
Autumn, 2001 - Anthrax!

- Wake-up call for clinical microbiologists
- Expect the unexpected
- Preparedness is an absolute necessity
LRN Level A Lab Preparedness

• Level A laboratory functions
  – Rule out / refer
  – Ship suspicious infectious agents to higher level labs for further study

• Level A laboratory activities
  – Formulate laboratory procedures
  – Train staff
  – Biosafety concerns

• Assistance from public health agencies
Activities of Clinical Micro Labs

• “Average” Labs
  – Microscopic examination of specimens
  – Culture of specimens and isolation of many bacterial and fungal pathogens
  – Identification and susceptibility testing

• “Advanced” Labs
  – Viruses (culture, direct detection)
  – Mycobacteria (culture, susceptibility)
  – Certain fungi (culture and identification)
  – Molecular testing
Level A Lab Example: *B. anthracis*

- Gram stain* of CSF, positive blood culture or wound culture shows large gram-positive rods

*Gram stain: Differential stain, not specific, but can be extremely helpful

Jernigan, et.al. EID 7(6); 2001
Level A Lab Example: *B. anthracis*

- Culture on blood agar*. Examine for characteristic colony morphology and lack of beta-hemolysis.

*Agents of anthrax and plague are “easy” to grow. Agents of tularemia, brucellosis are harder to recover, may require special media.
Level A Lab Example: *B. anthracis*

- Perform identification tests. For *B. anthracis*, perform motility test*

*Minimal rule out tests (minimal manipulation of potentially dangerous cultures) are recommended for Level A labs*
Level A Lab Example: *B. anthracis*

- Ruled in?
  - *Bacillus* species with characteristic colony morphology, non-hemolytic, non-motile
- REFER
  - Contact Level B lab
  - Ship suspect isolate
Level A Lab Preparedness - Where Are We Now?

• Bigger seems to be “better”
  – Wider variety of pathogens encountered; personnel experienced in working with infrequently isolated agents
  – More and/or better biosafety equipment
  – Institutional support for needed resources is more likely in larger hospitals

• Small labs can still have successful preparedness programs
Level A Lab Preparedness

- Anthrax
- Plague
- Tularemia

- Brucellosis
  - Botulism-
  - Specimen processing/shipping only

- Smallpox, VHF-
  - More guidance needed for Level A labs

- Environmental testing for *B. anthracis* spores
Clinical Lab Preparedness – Next Steps

- Extend training (category B agents)
- Enhance communication/cooperation with higher level public health labs
  - NLS
- Dissemination of some Level B procedures to select Level A labs
  - Rapid, specific tests/reagents
  - BSL3 activities in select labs
  - Surge capacity
Level A Clinical Microbiology Laboratories

- Can be instrumental in early recognition
- Must be trained, alert and vigilant
- Form partnerships with public health labs for BT preparedness assistance, BT response plans, and overall improvement of the public health system
Urban Testbed Initiative

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3 April 2001

MS-15433
This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.
Outline

- Thoughts on Urban Biodefense
- Importance of Testbeds
- MIT LL Urban Testbed Initial Approach
- MBTA subway experiments
- Algorithmic approach
- Future Work
Challenges Associated with Civilian Biodefense

- Any high-density site (city, airport, facility, building) represents a potential target

- Population to be protected is diverse (age, mobility, health)

- No environmental sensing systems will be tolerated that have high false negative or false positive rates
  - If they alarm too much or miss events, they will be ignored

- Current clinical diagnostic technologies and medical infrastructure are not suited to rapid detection of bioagent events
  - Advanced diagnostics (e.g., PCR) use is rare, even in large city hospitals
  - No medical reporting systems are in use that have real-time detection of infectious disease patterns as their objective
Biodefense development must be multi-faceted.
Needed Biodefense Investments

• Point-of-care and public health not well integrated
  – Health care system is the current detector
• System (multi-sensor) environmental monitoring development
  – Focus has been on basic technology and devices
• Characterization of environments of high-threat facilities
  – Sensor technology not universally applicable
  – Helps to set requirements
• Large-scale urban protection
  – Sparse sampling/sensing
  – Low probability event with catastrophic consequences (akin to nuclear detonation)
• Red-teaming
Environmental Monitors

- DoD environmental monitors designed for outdoor force protection
  - High sensitivity preference
  - Current cost prohibits mass-production
  - Unproven performance in urban or indoor areas where air is filled with interferents

- Urban Civil Protection has markedly different requirements from military use
  - Low false alert rate and low cost a priority
    › Lower sensitivity partial solution may be preferred
  - Wide variation in environments (e.g. stadium vs. subway)
    › Densely populated areas add to natural biological interferents
    › Airflow, HVAC are important design considerations
BAWS III Background Measurement Campaign

- **DRES, Canada**
  - 9/2000
  - (rural/arid)

- **Salt Lake City**
  - 2/2001, 2002
  - (urban/arid)

- **Dugway, UT**
  - 6/10/98 - 6/30/98
  - 10/19/98 - 10/27/98
  - (Arid)

- **Ft Leonard Wood, MO**
  - 4/8/98 - 4/18/98
  - 10/6/98 - 10/13/98
  - (Rural/Deciduous)

- **Ft McClellan, AL**
  - 3/16/98 - 3/26/98
  - (Rural/Deciduous)

- **Panama City, FL**
  - (Coastal)

- **Boston, MA**
  - 12/17/98 - 12/23/98
  - (Urban)

- **Washington DC**
  - (Urban)

- **Curtis Bay, MD**
  - 7/20/98 - 7/28/98
  - (Coastal)

- **Atlanta, GA**
  - 11/2/98 - 11/10/98
  - (Urban/Light Industry)

- **Porton Down UK**
  - 10/2000
  - (Mixed urban/coastal/rural)

- **Hawaii**
  - 6-7/2001
  - (Coastal)

- **Camp Doha, Kuwait**
  - 1999
  - (Arid desert)

- **DRES, Canada**
  - 9/2000
  - (rural/arid)

- **Salt Lake City**
  - 2/2001, 2002
  - (urban/arid)

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Testbeds as an Important Development Tool

Testbeds are needed for both public health and environmental monitoring systems
- Understand the problem and set system requirements
- Improve training
- Infuse emerging technologies in realistic settings
- Understand unique environments of various facility types
BAWS July 4, 2001 Esplanade Measurement

People sitting in tents

Increased human activity

People leaving

Boston Pops playing

Landscaping

JFT-4 trial 8 average level
MIT/LL Urban Testbed Project Goals

- Define a system architecture for facility defense using environmental monitors

- Understand the natural air composition and the response of existing instruments in those facilities

- Develop decision logic methodology that is extensible to other urban defense problems
Urban Testbed Status

- Project funding began in June, 2001
- Coordination with Boston-area authorities for the past 1-2 years
  - MA Bay Transportation Authority (MBTA), Boston Emergency Management Authority (BEMA), MA Emergency Management Authority (MEMA), MA Dept of Public Health, National Guard, Logan airport, others
- BAWS measurements at Boston Marathon, July 4th celebration
- Measurements in MBTA subway station; sensors being installed in a station.
  - Particle counters, airflow, temperature, humidity, train motion.
  - Periodic measurements in other locations or with sensors that cannot be installed for long periods.
- Develop alerting algorithm approach
- Controlled chamber releases
- Discussing measurements in other Boston locations
Subway Protection Considerations

- Threat has been established
  - Aum Shinrikyo Tokyo Sarin gas release
  - Numerous entry points and hiding places
  - Train “piston effect” moves air through the system
- System is spatially distributed
  - Many low cost sensors preferred over few high cost sensors
  - Release point cannot be anticipated apriori
- Important to find dual-use applications for system
- Principal response actions
  - Stop trains (plug tunnels?)
  - Activate vent fans?
  - Evacuate and prevent additional access
Station Particle Counts

Train Traffic significantly alters particle counts

Diurnal Cycle significantly alters particle counts
Particle counter sensors degrade quickly due to laser optics contamination. Full instrument sensitivity regained after cleaning.
Multiple sensors required to agree and sensitivity reduced to reduce risk of false alert.

Sensor Cluster

Alerting Logic

Multi-station evidence correlation

Inter-station Transport Model

Possible bioattack alert

Operations Center, Police, Fire

Possible Actions
- Additional sampling and testing
- Activate vent fans
- Stop trains
- Evacuate
- Tunnel plugs

Outside weather

Particle Counter

Anemometers

Temp, humidity

Train motion

Fusion of co-located sensors (cluster)
Health Care Provider and Public Health Integration

**Currently**

1. Patients → Physician
   - Laboratory Diagnosis
   - 1-5 days

2. Physician → Public Health
   - Sporadic reporting, multi-day delay

3. Public Health → Prevent Future Outbreaks
   - Manual Disease Pattern Detection
   - Multiple days

**Needed**

1. Patients → Physician
   - Public Health “Weather” Report
   - On-site Diagnostics
   - < 30 minutes

2. Physician → Public Health
   - Routine symptomatic data
   - Automated Disease Pattern Detection
   - < 1 day

3. Public Health → Proactive Treatment of Current Outbreak
   - Laboratory Confirmation
   - < 1 day

**Collateral benefit outside of biodefense**

New England Bioterrorism Preparedness Workshop
TJD 16
Concept:

- Implement advanced point-of-care diagnostics (including but not limited to gene-chips), into IT networked system
- Enables rapid determination of biological attack
- Benefits natural infectious disease diagnosis, effective treatment
Boston Area Agencies with Biodefense Responsibilities

Existing Structure

Suggested Added Organizations

FBI
FEMA
CDC

NIH

Medical, Bio, Public Health Facilities, e.g., Whitehead, HSPH, Partners Hospitals

FBI
FEMA
CDC

National Guard
Command Center

BEMA
Boston Emergency Management Agency
Command Center

Boston Fire Department & EMS

Federal / Research Facilities e.g., MIT/LL, MITRE

MBTA

Police Public Health

Logan Massport

DARPA
DTRA
Bio JPO

DoE

FAA

NIH

Existing Structure

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DTRA
Bio JPO

DoE

FAA
Summary

- Civilian bioterrorism defense requires that the environment of high-threat locations be well understood
  - Environment drives sensor & system design

- Initial testbed being installed at Boston subway station

- Measurements to date point out deficiencies of current sensors & software

- Modern recognition/data fusion techniques being applied to data

- Measurements at additional Boston threat locations under discussion
Facility Defense Against Aerosol Attack

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3 April 2002

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.
Outline

• Facilities and attack scenarios

• Sensing an attack

• Facility protection techniques
Types of facilities

• Simple structures
  – Residences, barracks

• Buildings with ventilation system
  – **Multiroom office building**
  – Large open space (arena, terminal, …)

• Subway

• Outdoor sites
  – Stadium
  – Public gathering
  – Military operations
Simplified Ventilating System

Exhaust

Fresh air intake

Louver

Return fan

Temperature & humidity

Zone 1: Occupied space

"6 exchanges/hr"

Zone 2

Mixing box

Standard filters:

- 30% pleated
- 80% electrostatic

10-20%

80-90%
Types of Attacks

- **External attacks**
  - Nearby cloud release
  - Burst release into air intake

- **Internal attacks**
  - Burst release into air return
  - Burst release into a large open space
  - Low level continuous release

- **Small amounts of agent are substantial threats**

  1 gram bioagent uniformly dispersed into $10^8$ liter building ($100m \times 100m \times 10m$);
  Corresponds to lethal exposure ($100 \text{ ppl} \times 10 \text{ liter/min} \times 10 \text{ min}; 10^{10} \text{ particles /gram}$)
Modeling an Attack

Burst release in an interior room

- Bioagent - 15 grams over 5 sec
- Room-Hall coupling - 10%

Lumped parameter models are well established instantaneous and uniform concentration within each room.

Initial particle dispersal and deposition are more complicated to model.
Emergency Management Measures

- **Information**
  - Observing suspicious activity
  - Knowing who to treat
    - Primarily, but not exclusively, bio agents
    - Records of access (badge swipes, tickets, ...)
    - Voluntary response to public announcement
    - Physical examination
  - Preserving forensic evidence

- **Plan of action**
  - HVAC emergency management decision tree
    - Suspicious event near air intake -> shut down intake
    - Suspicious event inside building -> full fresh air
  - Communication channels
  - Evacuation plan
    - Orderly movement to controlled safe area, avoid cross contamination
Outline

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques
Rationale for Sensing

• Issue alarm
  – initiate facility response
  – high $\text{Prob}_{\text{detection}}$; low $\text{Prob}_{\text{false alarm}}$; wide range of agents

• Identification of agent
  – initiate medical treatment

• Mapping of contamination zone

• Assessing decontamination ("all-clear")
# State-of-the-Art Bio / Chem Sensors

## Biological
- Culturing
- Immunoassay strips
- PCR / DNA analysis
- Particle number, size
- UV laser fluorescence

## Chemical
- Detection tickets
- Gas chromatography / mass spec
- Ion mobility spec
- Surface acoustic wave

<table>
<thead>
<tr>
<th>Sample based</th>
<th>Continuous monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culturing</td>
<td>Detection tickets</td>
</tr>
<tr>
<td>Immunoassay strips</td>
<td>Gas chromatography / mass spec</td>
</tr>
<tr>
<td>PCR / DNA analysis</td>
<td>Ion mobility spec</td>
</tr>
<tr>
<td>Particle number, size</td>
<td>Surface acoustic wave</td>
</tr>
<tr>
<td>UV laser fluorescence</td>
<td></td>
</tr>
</tbody>
</table>

**REC BioHAZ**

**Graseby GID-2A**

**BAWS-III**

- intake
- outflow
- Ethernet radio port
- LED indicator
Sensor Architectures for Building Defense

- **Distributed**
  - Trigger and Sampler
  - Distributed in each room
  - Sample carried to identifier in central location

- **Centralized**
  - Trigger head in each room
  - Aerosol transport in ducts
  - Centralized laser, sampler, and identifier
  - Trigger, Sampler, and Identifier located in central location

- **Aerosol transport system in ducts**

- **High Cost** ←
- **Low Cost** →

MIT Lincoln Laboratory
Atmospheric Aerosol Content


*LD50/10min
# normal blg ventilation
False Trigger Rate

• Sensor will trigger less frequently when operated at higher threshold.

e.g. BAWS-III operating within Lincoln Lab

Threshold (ppl)

1.0E-03 1.0E-02 1.0E-01 1.0E+00 1.0E+01

10 30 50 70 90 110

False triggers / hour

Simulated $B_g$ Release

Measured Background

Extrapolation to High Thresholds:

1 / 6 day 1 / 60 day 1 / 360 day
1 / 19 day 1 / 120 day 1 / 500 day
200 ppl 500 ppl 1000 ppl
Indoor Standoff Aerosol Detection

- Any point sensor is limited by aerosol transport in large open space.
- Need to detect the release promptly at a specific point

Bio sensor concept:

Minimum for detecting 1000 ppl threat

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Dwell time</th>
<th>Range cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic</td>
<td>0.1 sec</td>
<td>1 m</td>
</tr>
<tr>
<td>UV LIF</td>
<td>10 sec</td>
<td>3 m</td>
</tr>
<tr>
<td>Diff SWIR</td>
<td>10 sec</td>
<td>2 m</td>
</tr>
</tbody>
</table>

50 m range, eyesafe laser; 100 lux lighting
Outline

- Facilities and attack scenarios
- Sensing an attack
- Facility protection techniques
Facility Protection Measures

- Physical security
  - Protect fresh air intakes (location, access, surveillance)
  - Personal screening (may be difficult in civil defense)

- Ventilation system protection
  - Passive air filtration
    › Upgrade filters (best ASHRAE filters > 95%)
    › Overhauling the system (HEPA / carbon)
  - Positive pressure to overcome infiltration
  - Sensor triggered airflow control
Passive Air Filtration

- In-line passive filtration is well established
  - HEPA filters remove >99.97% suspended particles > 0.3 um.
  - Activated carbon filters adsorb most chemical vapors

- Substantial cost to overhaul existing ventilation system
  - Purchase and replacement of filters
  - Increased blower motors for higher pressure drop
  - Reinforced ductwork
  - Very little infiltration is allowable (gasket seals, overpressure)
  - Increased energy costs

- Research topics
  - Low pressure drop filter structures
  - In-line sterilization (UV, radiation, thermal,...)
Facility Defense Effectiveness

<table>
<thead>
<tr>
<th>Protection Measure</th>
<th>Estimated Exposure Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Unprotected” building</td>
<td>1</td>
</tr>
<tr>
<td>Upgraded standard filters (or in-room HEPA)</td>
<td>10-100</td>
</tr>
<tr>
<td>In-line HEPA filters</td>
<td>100-1000</td>
</tr>
<tr>
<td>In-line HEPA filters with overpressure and triggered airflow control</td>
<td>&gt; 1000</td>
</tr>
</tbody>
</table>
Summary

• Most buildings with ventilation systems are vulnerable to aerosol attack via a number of scenarios.

• Without deployed sensors, an attack may go undetected resulting in higher exposure and lack of treatment to exposed occupants.

• There are some simple measures that can be used to increase situational awareness and provide limited protection.

• A substantial degree of protection can be achieved at substantial cost with sensor triggered airflow control and HEPA/carbon filters. In this case, sensors may be operated at higher thresholds.
Aerosol Triggers

Thomas H. Jeys

New England Bioterrorism Preparedness Workshop

3-4 April 2002

This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.
Biosensor Architecture

- **Trigger (< 60 s)**
  - Continuous operation
  - Alert of potential threat aerosol
- **Collector (5 min)**
  - Activated by trigger
  - Provide sample of aerosol particles

- **Identification (15 min)**
  - Preliminary identification of agent

- **Confirmation (4 – 24 hr)**
  - Final identification of agent
  - “Gold Standard” tests
  - Performed in laboratory (TAML)
Bio-Aerosol Triggers

• **Raw Particle Counters**
  – Small, low cost
  – Nondiscriminatory - very high false trigger rates

• **Fluorescent Particle Counters**
  – **Ultra Violet Aerodynamic Particle Sizer (UVAPS)**
    Trigger for Biological Integrated Detection System (BIDS)
    Manufactured by TSI Inc. (St. Paul, MN)
    Fluorescence Aerodynamic Particle Sizer (FLAPS)
    Different trigger algorithm than UVAPS
    Trigger for Canadian Integrated Biological Agent Detection System (CIBADS)
  – **Biological Agent Detection Sensor (BAWS)**
    Trigger for Joint Biological Point Detection System
    Manufactured by Intellitec (Deland, FL)
Biological Agent Warning Sensor (BAWS)

- Army Advanced Technology Demonstration
  - Began BAWS development in 1996

- Four design generations developed

- Extensively tested
  - Performance
  - Environmental

- Integrated into the Joint Biological Point Detection System
  - Development transitioned to JBPDS in 1999.

BAWS III

<table>
<thead>
<tr>
<th>Size</th>
<th>0.8 ft³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>19 lbs</td>
</tr>
<tr>
<td>Power</td>
<td>35 W</td>
</tr>
</tbody>
</table>
BAWS Concept

Pulsed Ultraviolet Laser

Spectral Filter

Photo-detector

Fluorescence Emission and Elastic Scattering

Agent Containing Particle

Detected Signals

UV

Visible

Elastic

Time

Particle Emission Spectrum

Relative Signal

Wavelength (nm)

Fluorescence

Elastic Scattering

Tryptophan

NADH

Flavins

UV

Visible

266 330 450 560

Particle Discrimination

Dirt

Agent

UV - Elastic

UV - Visible

UV - Visible
Joint Biological Point Detection System

- Automated suite of sensors for detection and identification of biological attacks
  - Trigger – BAWS
  - Collector – Wetted Wall Cyclone
  - Identifier – Immunoassay
  - Confirmatory Samples
The Atmospheric Aerosol Composition

Aerosol Size Distribution

Composition of Coarse (>1 micron) Aerosol

<table>
<thead>
<tr>
<th>Organic Aerosols</th>
<th>Particles per Liter</th>
<th>Inorganic Aerosols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man Made</td>
<td>0 – 2000</td>
<td>100 – 10,000</td>
</tr>
<tr>
<td>Fungi</td>
<td>0 – 100</td>
<td>Clays, Sands, Composites</td>
</tr>
<tr>
<td>Bacteria (culturable)</td>
<td>0 – 1</td>
<td></td>
</tr>
<tr>
<td>Pollen</td>
<td>0 – 1</td>
<td></td>
</tr>
</tbody>
</table>
- Most sand particles do not fluoresce and are “invisible” to BAWS
Field Trials

27Oct99 - 9Nov99
BAWS/JBPDS Mini Field Trials

BAWS ROC Curve

Detection Probability
False Alarm Rate

Bioagent Cloud
Wind
Man Portables 1 2 3 4
Fixed Sites 5 6 7 8
Shelters 9 10 11 12
JBPDS
Reference Sensors
Response of BAWS Array to Agent Aerosol

East End of Grid
JFT-4

Alarm On

Center of Grid

West End of Grid

Time
Response of BAWS to Interferent and Agent Aerosol

Sensor at East End of Grid

- **Agent Signal**
- **Truth**
- **Particle Concentration**

**Sensor Alarm Period**

**Time (H:M:S):**
- 15:30:00
- 16:00:00
- 16:30:00
- 17:00:00

**Sensor Signal**

- **Agent Signal**
- **Truth**
- **Particle Concentration**

**Truth Signal and Particle Concentration**

**Sensor Alarm Period**

- 16:50:00
- 17:00:00
- 17:10:00
- 17:20:00

MIT Lincoln Laboratory
Live Agent Tests of BAWS

- Comparison of BAWS response to real agents and simulant agents
  - Simulant Agents: BG, *Erwinia herbicola*, Ovalbumin, MS2
  - Three Real agents

- Results: BAWS detects live agents as well as, or better than, simulant agents
  - Equivalent sensitivity
  - Equivalent discrimination
BAWS Performance Testing

- **Joint Field Trials**
  - JFT 3, Dugway Fall ‘96
  - JFT 4, Dugway Fall ‘97
  - JFT 4.5, Dugway Spring ‘98
  - JFT 6, DRES Canada Fall ‘00
- **Army ATD Field Trials** Spring ‘99
- **Joint Biological Point Detection System Field Trials**
  - Mini Field Trials Fall ‘99
  - Gamma-Killed Bio-Agents Spring ’99
  - PPQT Spring ‘00
  - Live Agents Summer ‘00
  - Porton Down, UK Fall ’00
  - Ambient Breeze Tunnel, Battelle Spring ’01
  - Operational Assessment 2 Fall ’01
- **Background Measurements**
  - USA tour ’98 – ‘99
  - Kuwait Spring ’99
  - Altitude study Fall ’00
  - Salt Lake City Spring ’01
  - Hawaii Summer ’01
Simulation of BAWS Response to Agent Attacks in Different Environments

- Background Measurement Data (1 week at each site)

- BAWSIII Sensor Response to BG

- Alarm Algorithm

- False Trigger Rate (per day)
  - Kuwait
  - Dugway UT
  - Atlanta GA
  - Cambridge MA
  - Fort Leonard Wood MO

- False Alarm Rate (per day)
  - Sensitivity (ACPLA)
Detector Position vs. False Trigger Rate

- **England (Sep ‘00)**
  - One week of measurements
  - 21 agent simulant challenges
  - 8 interferent challenges

- **Sensor Performance vs. sensor height**
  - BAWS at 2-m and 13-m height
  - Ten times fewer false triggers at 13-m height
Summary

- BAWS developed for early warning of a biological agent attack
  - continuously operating point detector
  - small size, low weight, low power consumption

- Generic detection (not identification) of threat aerosol
  - Individual detection of aerosol particles
  - Discrimination of threat particles from non-threat particles
  - Sensitive, low false alarm rate, fast response

- Subjected to extensive testing
  - Performance
  - environmental

- BAWS integrated into JBPDS
Emerging Technologies in Sample Analysis

4 April 2002

New England Bioterrorism Preparedness Workshop
MIT Lincoln Laboratory

Bernadette Johnson
(781)981-1902
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This work was sponsored under Air Force contract F19628-00-C-0002. The views expressed are those of the Author and do not reflect official policy or position of the United States Government.
Outline

• Current techniques in sample analysis
  – Clinical (subject of yesterday’s talk)
  – Environmental

• Challenges associated with environmental sampling

• Examples of technologies in use and in development
CDC’s Sample Analysis Guidelines
(example: B. Anthracis)

- Persons suspected of exposure/infection
  - Cultures of blood and spinal fluid
  - Cultures of tissues or fluids from affected areas
  - Microscopic examination
  - PCR
  - Nasal swab (occasionally for exposure, but not for diagnosis)
  - Antibody testing (exposure, not validated for diagnosis)

- Environmental contamination
  - Cultures of air samples, surface swabs, suspicious powders
  - Microscopic examination of suspect material
  - Evaluation of growth properties of suspect agent
  - PCR
  - DFA (direct fluorescent assay) to detect key bacterial proteins
  - Specialized tests, such as immunoassays (SMART)
How Do These Techniques Compare?

**Rapid ID**

**Immunoassays**
- Selectivity from high affinity binding of antibody to agent-specific structures
- ~15 minutes

**Orthogonal ID Confirmation Technologies**

**Polymerase Chain Reaction (PCR)**
- Selectivity from sequence-specific DNA/RNA recognition
- Enzymatic amplification provides superb sensitivity
- 1-4 hrs

**Culture-based assays**
- Traditional method since Pasteur – still “gold standard” for ID
- Viable organisms replicated in culture and identified using biochemical assays and microscopy
- 1-3 days

**Response Time**

**Sensitivity/Accuracy**
Examples of In-use and Developmental Immunoassay Devices

Ticket cartridges and reader for lateral-flow immunoassay in Joint Biological Point Detection System (JBPDS)

Dendrimer-Based Alert Ticket (ARL)

Response Equipment Co.
Bio-HAZ Biodetector

Upconverting Phosphors (SRI)
Features of Immunoassay Analysis

- Can be used on environmental samples with little or no preparation

- Readout is fast (~ 15 minutes) and simple (colorimetric or fluorimetric)

- Sensitivity modest (~10,000 - 100,000 particles)
  - Depends on antibody-antigen binding affinity and readout scheme

- Specificity reasonably good
  - Depends on antibody construct and antigen specificity

- Current IAs are not multiplexed; development of protein microarrays may lead to sensitive, multi-assay analysis tools
Examples of Existing Protein Microarrays

- Phylos (2000 element)
- Ciphergen (multiple classes of proteins)
- Zyomyx (10,000/cm²)

- Protein microarray technology development driven by drug screening and disease-marker investigations
  - Diagnostics (clinical and environmental) still developmental
Developmental Antibody-Based Sensor: CANARY

Concept

B cell emits ~200 photons within 30 seconds after bioagent binding

Prototype microcentrifuge device

Tests Against Killed Tularemia
(Collab. with NMRC)

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# Inactivated Tularemia Particles
- 600
- 60
- 0

Status of B-Cell Lines

**Complete**
- FMDV
- VEE
- Vibrio cholera
- Orthopox viruses
- Yersinia pestis
- Brucella spp
- Franciscella tularensis

**In development**
- Coxiella burnetti
- Bacillus anthracis
- E. coli O157:H7

Francisella tularensis

BioWorkshop 8
BJ 4/4/02
PCR-Based Analysis Tools

- Systems being developed (and deployed) that provide agent ID within 30 minutes of introduction of prepared sample

Semi-automated field-portable PCR devices

Example of handheld PCR device

RAPID - Idaho Technologies

SmartCycler XC System - Cepheid

HANAA - Handheld Nucleic Acid Analyzer, developed by LLNL, Cepheid, and ETG, Inc.

- Challenge remains in automating sample preparation and analysis
  - Pathogen cells or spores must be ruptured to liberate the DNA/RNA
  - DNA/RNA must be separated from protein debris/environmental impurities
Overview of Sample Preparation

1) Sample Collection

2) Concentration

3) Purification

4) Chemical Amplification

5) Signal Analysis and Readout
Add water & magnetic beads to soil sample
Stir well to allow adherence to beads
Recover target & beads with magnet
Purify with SNAP, then PCR
DNA Purification:
Simple Nucleic Acid Prep (SNAP)

- Chemically treated paper is the key component of SNAP
- Lyses cells, binds PCR-assay inhibitors, and purifies DNA
- **Advantages:**
  - Fast and easy (1/5th the time of other published protocols)
  - Water is only added reagent (no phenol, chloroform, or alcohol)
  - Lightweight, compact, enables archiving
  - On-site fixation: preserves DNA & kills pathogenic organisms

1. Apply sample
2. Discard paper with PCR inhibitors
3. Desiccate, then soak in H₂O
4. Retain H₂O + DNA, then PCR amplify
Lincoln Interim Nucleic-acid Kit (LINK)  
(Developed in response to October 2001 events)

• LINK as a solution:
  – Incorporates SNAP paper but in a more user-friendly format
  – Faster processing than basic SNAP
  – Easier to sample, handle, and process
  – Enables on-site fixation
  – Outside can be decontaminated
  – 6 minute processing time
  – Single-step processing
  – Results equal to or better than basic SNAP
How to Use LINK

1) Apply sample
   Sit for 5 minutes

2) Process in **one step**

3) Remove DNA
   **Total time ~6 minutes!**
LINK Cartridge Works with Varied Samples

- LINK detection from:
  - **Portal Shield** air-to-liquid samples seeded with vegetative bacteria
  - Untreated domestic sewage (Boston) seeded with vegetative bacteria
  - Paper, envelopes, skin seeded with bacterial spores
  - Air impaction with dry bacterial spores

![Graph showing detection of LINK in various samples](image)
What About DNA Microarrays?

- DNA Microarray: Any 2D or 3D substrate having many (~ $10^2$-$10^5$) different nucleic-acid capture sites (probes)
- Can identify both strain and drug resistance of pathogens
- Can offer highly multiplexed assay capability

Current method:

Sample → 1-4 tests

DNA Microarray:

Sample → 1000’s of tests
Pathogen Identification via DNA Microarray

- Detect small amounts (<100 copies per ml) of pathogen-specific nucleic acids in environmental sample

- Arrays might provide log orders more information than current PCR-based approaches (e.g. TaqMan)

- **Challenges for diagnostic applications:**
  - Never demonstrated for environmental (or clinical) samples
  - Amplification may be necessary before micro-array assay
  - Sample preparation required (as in PCR techniques)
Assay Times for Current and Emerging PCR/DNA Systems

< 1 Hour
- Motorola
- Nanogen
- Host Genotype

1 – 2 Hours
- Cepheid PCR
- Roche PCR
- Host Genotyping
- 10’s expressed RNAs
- 10’s pathogen genes

2-4 Hours
- MICROARRAYS
- Expression Profiles
- Host Genotyping
- 100’s Pathogen genes (*)

12+ Hours
- MICROARRAYS
- Expression Profiles
- Host Genotyping
- 1000’s Pathogen genes (#)

(*) w/ PCR
( #) w/ culture

Cepheid GeneXpert
Summary

- Environmental sample analysis parallels methodology developed for clinical sampling
  - Immunoassays for rapid estimate of exposure (not yet CDC authorized)
  - PCR techniques being deployed in some laboratories to provide strain specificity and drug resistance
  - Culture still used to provide “gold standard” for pathogen ID

- New technology developments could greatly increase the speed, sensitivity, and multiplicity of environmental assays
  - Protein microarrays could offer highly multiplexed, rapid ID capability on collected samples
  - DNA microarrays could offer hundreds to thousands of pathogen tests on single-chip format, provided sample preparation can be made compatible
Improving Public Health Measures: Advances in Risk Analysis

Kimberly M. Thompson, Sc.D.
Harvard School of Public Health
Boston, Massachusetts
Where are we?

- The Age of Ignorance (no understanding of science, no control, all R no B)
  - Cotton Mather on colonial times: “A dead child was a sight no more surprising than a broken pitcher”
- The Age of Discovery (revolution in science, ability to understand and control disease, take R to get B)
- The Age of Miracles (idea of the magic pill or magic bullet, science can cure any problem, pursue B with abandon)
- The Age of Risk Management (science is critical, but we have to make good choices to avoid overkill, balancing R and B)
What does this mean for public health? Longer lives...
Great progress – a few examples

- Diagnosis of disease based on gross physical characteristics --> laboratory analyses of body fluids and genetic testing and interventions that save lives
- Sulfanilamide --> numerous antibiotics
- Focus on feeding and milk composition for infants --> pasteurization, refrigeration, infant formulas, dehydration treatments, and improvements in medical care
Great progress

- The iron lung and deformities associated with polio --> immunizations for polio and many other diseases and eradication of small pox
Public health improvements

• Are we winning the war with germs?
  – Certainly doing better with respect to health outcomes (e.g., saving lives once lost to some infections, and reducing the severity and spread of infections)
  – Public perception now that infectious disease is not as much of a problem (immunization)

• Wait
  – BIG issues remain with antibiotic resistance/“Superbugs”/new diseases
  – Prevailing assumption that releases of organisms would be unintentional (i.e., we’re fighting nature)
  – Infectious disease still a leading cause of death
Context

- Given the background of ID, what does BT preparedness look like and how does it fit in with basic public health?
- What tools can help us understand the risks and measure the impacts of interventions?
- How will we know that a BT preparedness program works?
- What decisions get made about characterizing the different agents?
Human health risk continuum

Source → Transport and transformation → Accumulation in environment → Human contact: exposure → Potential dose to body

Health effects ← Early disease expression ← Biologically-effective dose ← Internal dose

Elimination, accumulation, transformation ← Bioavailability

Lioy, ES&T, 1990
The need for risk and decision analyses

- Risk analysis and decision analysis are used to integrate information and sift it down into a usable form.
- Used support many actions:
  - Initiating regulatory activity or treatments
  - Setting protective standards
  - Selecting products, technologies, or substances
  - Siting hazardous facilities, isolation choices
  - Cleaning up or control of contaminated areas
  - Initiating research and establishing priorities
  - Others....
- Key component of decision (but not only)
Decision tools

- Risk analysis
- Benefit-cost analysis
- Cost-effectiveness analysis
- Decision analysis
- Comparative risk analysis

All share common elements to some degree, but differences do matter
Variability vs. uncertainty

- Variability - heterogeneity or diversity in a well-characterized population which is usually not reducible through further measurement or study.
- Uncertainty - ignorance about a poorly characterized phenomenon that is sometimes reducible through further measurement or study.
- Variability and Uncertainty = f(decision context)
  - NRC (1994): “Uncertainty forces decision makers to judge how probable it is that risks will be overestimated or underestimated for every member of the exposed population, whereas variability forces them to cope with the certainty that different individuals will be subjected to risks both above and below any reference point one chooses.”
Risk estimates do matter

- Example 1 – uncertainty about the effectiveness of airbags in motor vehicles
- Example 2 – variability in the mortality risk to people on the ground from crashing airplanes

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Cost-effectiveness analysis

• One of many tools
• Growing role in medical decision making
• Panel on Cost-effectiveness in Health and Medicine
  – Total costs/Total effectiveness (Incremental ratio)
  – Recommended methods (QALYs, 3% discount rate, societal perspective)
• Typical CEA ignores uncertainty, variability, time, preferences and other attributes, troubles with zeros, criteria for “acceptability”
Why care about dynamic nature?

- Optimal strategies change with time
- Dynamics may be very important to model to characterize the benefits of herd immunity
- Times of major shifts (e.g., perceptions of risk and benefits change going from wild type cases to vaccine-associated cases, with eradication risk shifts to polio in bio warfare)
- When we assess the CE ratio may matter in terms of policy
Changing CE model components

- Most vaccine CEA’s assume constant probabilities of getting infection (for both vaccinated and unvaccinated children) – may not capture big herd immunity effects (e.g. mass vaccination reduces risks for unvaccinated as well as vaccinated people)

- Other time-dependent factors:
  - Costs (For single vaccine and program, do these go up, down, or stay the same over time?)
  - Preferences and values
  - Societal dynamics (urbanization, more women working so staying home has greater opportunity costs)
  - Technology
Do these matter?

- Consider a case study on polio
  - Long history
  - … but not too long
  - Numerous interventions
  - Near eradication
  - Good time to remind people
  - Story of many successes
  - Could make the transition from ID to possible BW agent if public health community successful
Project: Background

- Herd immunity effects following polio vaccination.
- E.g. mass vaccination of 95% of infants will reduce the probability for unvaccinated persons as well.
- Other time-dependent factors:
  - price of vaccine
  - with discounting of health and dollars: \( \rightarrow \) point of time of disease is important
  - demography, technology, etc.
Retrospective Polio CEA Model(1)

- Ideally, we have for all vaccine programs:
  - Cost(t) = (V(t)tg(t)vc(t) + (D(t)-D^0(t)) H(t)) e^{rt}
  - Effectiveness(t) = (D^0(t)-D(t)) Q(t)

vc(t) = vaccine coverage (as function of time)
V(t) = vaccine costs per completed vaccine schedule
tg(t) = target group
r = interest to year 2000 dollars
D(t) = disease burden (incidence) under mass vaccination
D^0(t) = incidence in absence of immunization program
H(t), Q(t) = health costs resp. QALYs lost per disease case
Retrospective Polio CEA Model(2)

- **Cumulative cost-effectiveness ratio:**

  \[ CCE(t) = \frac{\text{integrated discounted costs until } t}{\text{integrated discounted health gains until } t} \]

- **Cost-effectiveness ratio:**

  \[ CE = CCE(T_{end}) \quad \text{Suggested } T_{end} : 2015 \]
Retrospective Polio CEA Model(3)

- The disease incidence with or without immunization program can be calculated with a transmission model -> requiring assumptions about transmission, and data

- For every variable except incidence, real historic data will be used.
Concept of Transmission Models: SIR Models

- $S(t) =$ number of *susceptibles*: those individuals that could get infection
- $I(t) =$ number of *infecteds*: those that are infectious: they can contaminate susceptibles
- $R(t) =$ number of *removeds*: those that are immune to infection (*recovereds, resistants*)
- Transition rates between S, I, R -> differential eqns.
- $\lambda (t) = \beta I(t)$ = force of infection = per susceptible rate of infection, $\beta$ is the *transmission coefficient*
Transmission model (1)

\[ \lambda_i(t) = \beta_i^*(I_1(t)+I_2(t)+\ldots+I_6(t)) \]

\[ \beta_i = \text{transmission coefficient for } i\text{-th age group} \]
Example Results

Vaccine 1, paralytic polio incidence with static (green) and dynamic (red) transmission model and without vaccine (blue):
Insights

• Risk analysis and decision analysis tools have evolved to the point where they are helpful in characterizing and understanding the trade-offs associated with tough choices.
• Must consider the dynamics of the disease to accurately quantify the health benefits.
• Complex problem – analysis is needed
  – No zero risk
  – Real trade-offs
Microarrays

New England Bioterrorism Preparedness Workshop

Dr. David Walt

Tufts University

4 April 2002
Imaging Fiber

Individual Cladded Optical Fibers

Silica Jacket

TUFTS
Optical Imaging Fiber Before and After Tapering

Individual Core Diameter ~ 2.6 μm

Individual Core Diameter ~ 0.85 μm
AFM of a Chemically-Etched 1000-?m Diameter Imaging Fiber

Well Profiles

SEM of a Chemically-Etched 1000-? m Diameter Imaging Fiber

Microspheres in Microwells

A B C

Sensor stock

Randomly distributed Addressable High-density Sensor Array*

Microwells on etched face

Distal face of imaging fiber

CCD chip

*Michael et al. 1998 Anal. Chem. 70: 1242-1248
DNA Array Principle
Instrumentation: Modified Fluorescence Microscope

- 75 W Xenon Arc Lamp
- Excitation Filter Wheel and Shutter Control
- Z Positioner
- X-Y Positioner
- Imaging Fiber
- 10X
- 20X
- Dichroic Housing
- CCD Camera
- Emission Filter Wheel and Shutter Control
- G3 Macintosh w/ IP LAB

Diagram shows the components of the modified fluorescence microscope, including the light source, positioning devices, and imaging systems.
Sequences of 25 Probes used together in a Microsphere Array

1) ?-glo (segment of human ?-globin)26
TCA ACT TCA TCC ACG TTC ACC

2) IFNG (interferon gamma 1)26
IFNG TGG GTT CTC TTG GCT GTT ACT

3) IL2 (interleukin-2)26
TA CAA GAA TCC CAA ACT CAC CAG

4) IL4 (interleukin-4)26
CC AAC TGC TTC CCC CTC TGT

5) IL6 (interleukin-6)26
GT TGG GTC AGG GGT GGT TAT T

6) K-ras WT27
GGA GCT GGT GGC GTA TAC GCC ACC AGC TCC

7) H-ras WT27
CCG GCG GTG T ACA CCG CCG G

8) CFTR (cystic fibrosis exon 11)13
CAT TAT ACT TGT AGA G

9) R553X (cystic fibrosis exon 10)13
TGT AGA ATT ATC TTC GAA GAT GTT AAA GTA TAG AGG

10) PAN13216 (human peripheral lymphocyte)
CCT CTA TAC TTT AAC GTC AAG

11) Schena-216
AAG TTT AAC CTA TAC CCT GTC

12) Hakala-120
CCT ATG ATG AAT ATA G

13) Hakala-220
AAT ATG ATA ATG GCC T

14) complement to probe 1
TG AAC GTG GAT GAA GTT G

15) complement to probe 2
AG TAA CAG CCA AGA GAA CCC AAA

16) complement to probe 3
CT GGT GAG TTT GGG ATT CTT GTA

17) complement to probe 4
AC AGA GGG GGA AGC AGT TGG

18) complement to probe 5
AA TAA CCA CCC CTG ACC CAA C

19) complement to probe 6
TAC GCC ACC AGC TCC

20) complement to probe 7
ACA CCG CCG G

21) complement to probe 8
CTC TAC AAG TAT AAT G

22) complement to probe 9
GAA GAT GTT AAA GTA TAG AGG

23) complement to probe 10
CTA GAC GTT AAA GTA TAG AGG

24) complement to probe 12
CTA TAT TCA TCA TAG G

25) complement to probe 13
AGG CCA TTA TCA TAT T
E. coli Allelic Discrimination

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= probe 1 signal  
= no signal

*ycgW locus is 77 nucleotides long
Microsphere Functionalization

Microsphere Encoding dyes

DNA coupling

DNA1

DNA2

DNA3

DNA4
**E. coli** Genomic Discrimination Flowchart

1. NO SIGNAL
2. TACTCCCCCC
3. TACTCAACCCCC

1. NO SIGNAL
2. TTTTTTTGAGGGG
3. AAAGCTCGCACT

**ycgW #1**
1 probe
2 groups

**b2345**
2 probes
3 groups

**Pool of 36 strains with 29 total differences**

3. 7-(20), 10, 16, 27, 37, 43

**serW locus**

1-2

1-2, 4, 13, 23, 24, 30, 34, 54, 55, 56

1-2, 4, 13, 23, 24, 30, 55, 56

34, 54

4, 13, 23, 24, 30, 55, 56

**total 4 probes 3 known strains**

1. 1-2, 4, 13, 23, 24, 30, 34, 54, 55, 56
2. 3, 6, 8, 9-12, 11, 14, 15, 21, 22-53, 38, 48, 52
3. 7-(20), 10, 16, 27, 37, 43
4. 34, 54
total 13 probes classifying 27 strain clusters
### E. coli Genomic Pattern Response

<table>
<thead>
<tr>
<th>No.</th>
<th>strain</th>
<th>ycgW</th>
<th>srW</th>
<th>osmB</th>
<th>yaiN</th>
<th>ykgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>O26:H-</td>
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<td>3</td>
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<td>6</td>
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</table>

[Blue] = signal response  [Yellow] = no signal
### E. coli Genomic Pattern Response

<table>
<thead>
<tr>
<th>No.</th>
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<th>serW</th>
<th>galS</th>
<th>Osmb</th>
<th>yKgE</th>
<th>ycgW#3</th>
<th>serW#2</th>
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</tr>
</tbody>
</table>

**Legend:**
- Blue = signal response
- Yellow = no signal
Bead Encoding

Sequential Decoding
Decoding 16 Probes

Hyb #   1 2 3 4

Sequence

Hyb. #1  Hyb. #2  Hyb. #3  Hyb. #4

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>green</td>
<td>green</td>
<td>red</td>
</tr>
<tr>
<td>red</td>
<td>green</td>
<td>green</td>
</tr>
<tr>
<td>red</td>
<td>red</td>
<td>red</td>
</tr>
</tbody>
</table>

Hyb. #1: green, green, red, green
Hyb. #2: red, green, green, red
Hyb. #3: red, red, red, green
Hyb. #4: red, red, red, green
Decoding is Exponential

\[ x \text{ labels} \times z \text{ steps} = x^z \text{ codes} \]

- 2 Dyes ^ 4 Steps = 16 Codes
- 4 Dyes ^ 6 Steps = 4,096 Codes
Four-Color Decoding

~13,000 Wells, 16 Probe Sequences

D.R. Walt, Science, 2000
13K Fiber Bundle

Fiducial fibers
57K Fiber Bundle
Array of Arrays™
Scalability of Technology

Unique Experiments
(with ~20-fold redundancy)
## Bead Summing

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Hybridization Time: No Summing</th>
<th>Hybridization Time: 100 Bead Summed</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 pM</td>
<td>10 minutes</td>
<td>4 minutes</td>
</tr>
<tr>
<td>10 pM</td>
<td>30 minutes</td>
<td>7 minutes</td>
</tr>
<tr>
<td>100 fM</td>
<td>4 hours</td>
<td>20 minutes</td>
</tr>
<tr>
<td>10 fM</td>
<td>17 hours</td>
<td>30 minutes</td>
</tr>
</tbody>
</table>
### Size and Concentration

<table>
<thead>
<tr>
<th>Volume</th>
<th>Concentration (L)</th>
<th>1 μM</th>
<th>1 nM</th>
<th>1 pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1 mm)$^3$</td>
<td>??? L</td>
<td>10$^{-6}$ L</td>
<td>6x10$^{11}$</td>
<td>6x10$^8$</td>
</tr>
<tr>
<td>(100 μm)$^3$</td>
<td>1 nL</td>
<td>10$^{-9}$ L</td>
<td>6x10$^8$</td>
<td>6x10$^5$</td>
</tr>
<tr>
<td>(10 μm)$^3$</td>
<td>1 pL</td>
<td>10$^{-12}$ L</td>
<td>6x10$^5$</td>
<td>6x10$^2$</td>
</tr>
<tr>
<td>(1 μm)$^3$</td>
<td>1 fL</td>
<td>10$^{-15}$ L</td>
<td>6x10$^2$</td>
<td>6x10$^{-1}$</td>
</tr>
<tr>
<td>(0.1 μm)$^3$</td>
<td>1 aL</td>
<td>10$^{-18}$ L</td>
<td>6x10$^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>
Probe and Target Sequences for DNA Microarray Detection Limits

Probe

IL2 (interleukin-2)  5'-TA-CAA-GAA-TCC-CAA-ACT-CAC-CAG-3'
IL6 (interleukin-6)  5'-GT-TGG-GTC-AGG-GGT-GGT-TAT-T-3'
F508C               5'-TAG-GAA-ACA-CCA-CAG-ATG-ATA-3'

Target

IL2 (interleukin-2)  5'-CT-GGT-GAG-TTT-GGG-ATT-CTT-GTA-3'
IL6 (interleukin-6)  5'-AA-TAA-CCA-CCC-CTG-ACC-CAA-C-3'
F508C               5'-TA-TCA-TCT-GTG-GTG-TTT-CCT-A-3'
DNA Minimum Hybridization Time with ICCD Camera

<table>
<thead>
<tr>
<th>DNA Concentration</th>
<th>Hybridization Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 pM</td>
<td>10</td>
</tr>
<tr>
<td>100 fM</td>
<td>20</td>
</tr>
<tr>
<td>10 fM</td>
<td>30</td>
</tr>
<tr>
<td>1 fM</td>
<td>60</td>
</tr>
</tbody>
</table>
Multiple beads provides a signal averaging benefit.

1000 target molecules
10 beads

=100 target molecules/bead

Fewer beads provide more target molecule numbers per bead.

1000 target molecules
4 beads

=250 target molecules/bead

S/N increases by $\sqrt{n}$
Multiplexed Array Sensitivity and Selectivity with 1 fM IL2 Target Solutions

IL2 Target - 1 fM concentration - 12 hour hybridization time

<table>
<thead>
<tr>
<th>Target/Probe</th>
<th>Mean background ± s.d</th>
<th>Hybridization ± s.d.</th>
<th>Signal ± s.d</th>
</tr>
</thead>
<tbody>
<tr>
<td>F508 F508C</td>
<td>530.43 ± 1.8</td>
<td>550.17 ± 7.5</td>
<td>{19.74} ± 7.7</td>
</tr>
<tr>
<td>IL2</td>
<td>563.99 ± 7.7</td>
<td>677.08 ± 8.1</td>
<td>113.09 ± 11</td>
</tr>
<tr>
<td>IL6</td>
<td>445.99 ± 3.9</td>
<td>449.16 ± 1.4</td>
<td>{3.17} ± 4.1</td>
</tr>
<tr>
<td>IL2 F508C</td>
<td>439.64 ± 3.5</td>
<td>443.34 ± 5.6</td>
<td>{3.70} ± 6.6</td>
</tr>
<tr>
<td>IL2</td>
<td>432.52 ± 5.6</td>
<td>503.31 ± 6.6</td>
<td>70.79 ± 8.7</td>
</tr>
<tr>
<td>IL6</td>
<td>431.11 ± 2.1</td>
<td>432.13 ± 2.8</td>
<td>{1.02} ± 3.5</td>
</tr>
<tr>
<td>IL6 F508C</td>
<td>454.84 ± 3.6</td>
<td>465.82 ± 1.4</td>
<td>{10.98} ± 3.8</td>
</tr>
<tr>
<td>IL2</td>
<td>429.42 ± 0.92</td>
<td>517.38 ± 2.6</td>
<td>87.96 ± 2.8</td>
</tr>
<tr>
<td>IL6</td>
<td>459.81 ± 3.0</td>
<td>467.82 ± 5.3</td>
<td>{8.01} ± 6.1</td>
</tr>
</tbody>
</table>
### Microsphere Array Sensitivity and Selectivity with 100 aM IL2 Target Solutions

**IL2 Target - 100 aM concentration - 12 hour hybridization time**

<table>
<thead>
<tr>
<th>Probe/Target</th>
<th>Mean background ± s.d</th>
<th>Hybridization ± s.d.</th>
<th>Signal ± s.d</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2 F508C</td>
<td>386.97 ± 3.2</td>
<td>387.98 ± 1.4</td>
<td>{1.01} ± 3.5</td>
</tr>
<tr>
<td>IL2</td>
<td>378.55 ± 2.3</td>
<td>394.00 ± 3.7</td>
<td>15.32 ± 4.4</td>
</tr>
<tr>
<td>IL6</td>
<td>382.80 ± 6.3</td>
<td>393.81 ± 6.1</td>
<td>{11.01} ± 7.1</td>
</tr>
<tr>
<td>IL2 F508C</td>
<td>268.66 ± 2.3</td>
<td>274.22 ± 8.5</td>
<td>{5.56} ± 8.8</td>
</tr>
<tr>
<td>IL2</td>
<td>297.73 ± 2.3</td>
<td>310.02 ± 2.3</td>
<td>12.29 ± 3.2</td>
</tr>
<tr>
<td>IL6</td>
<td>247.59 ± 2.7</td>
<td>248.70 ± 6.9</td>
<td>{1.11} ± 7.4</td>
</tr>
<tr>
<td>IL2 F508C</td>
<td>410.73 ± 2.6</td>
<td>413.63 ± 2.6</td>
<td>{2.90} ± 2.9</td>
</tr>
<tr>
<td>IL2</td>
<td>410.69 ± 2.7</td>
<td>455.26 ± 6.5</td>
<td>44.57 ± 7.0</td>
</tr>
<tr>
<td>IL6</td>
<td>390.24 ± 7.4</td>
<td>392.88 ± 2.8</td>
<td>{2.64} ± 7.9</td>
</tr>
</tbody>
</table>
SEM of a Microwell Array

7 \mu m well diameter

~3 \mu m well depth

~90 fL well volume
Single NIH 3T3 Mouse Fibroblast Cell in a Fiber-optic Microwell
Single Yeast (*Saccharomyces cerevisiae*) Cells Array

White light

Calcofluor White 360/440
SEM images of Single Yeast Cells on the Microwells array
SEM images of Single Yeast Cells on the Microwells array
SEM images of Single Yeast Cells on the Microwells array
Encoded Yeast Cells on the Fiber Array

- ConA-Alexf633
- ConA-Oregon514
- ConA-Texas Red
- ConA-Alexf660
- Calcofluor White
pH Measurement of Single Yeast Cells Microenvironment in the Array

- Yellow: Concanavalin A-FITC
- Red: Concanavalin A-FITC + Concanavalin A-Alexa fluor 660
- Green: Concanavalin A-FITC + Concanavalin A-Texas Red
Smarter Sensors - Anticipatory

Is it bad?
What does it resemble?
What will it do?

e.g. GI, neurotoxic, etc.
“common virulence mechanisms”

surrogates
Sensor Design

A) Lock-and-key Sensor

B) Cross-reactive Sensor
Solvatochromic Effect
Nile Red
Role of Polymer Polarity

poly(acrylic acid), **PAA**

poly(N-vinyl pyrrolidone), **PVP**

acryloxypropylmethyl-cyclosiloxane, **CPS2067**

diethoxymethylsilyl-modified polybutadiene, **PS078.8**

![Graph showing normalized intensity vs. wavelength for different polymers](chart.png)

Decreasing polarity
CCD-based imaging system

- 75 W Xe lamp
- excitation filter wheel
- dichroic wheel
- objectives
- optical fiber
- microscope stage
- coarse/fine focus
- CRI tunable filter
- emission filter wheel
- SensiCam CCD
The Olfactometer — J. Kauer

To Vacuum

Air

Manostat Needle Valve

Gilmont Flowmeter

Teflon tubing

Solvent

Solenoid

Push-button controller

Fiber
Sensor Array Response to Benzene Vapor Pulse

Temporal Plots from 19-Fiber Sensor Array Response to Benzene Vapor Pulse
### Classification Results
Learning Vector Quantization Approach

<table>
<thead>
<tr>
<th>True Identity</th>
<th>Network Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Acetone</td>
<td>1</td>
</tr>
<tr>
<td>2) Butyl Acetate</td>
<td>24</td>
</tr>
<tr>
<td>3) Beauty</td>
<td>3</td>
</tr>
<tr>
<td>4) Camphor</td>
<td>19</td>
</tr>
<tr>
<td>5) Carvone -</td>
<td>1</td>
</tr>
<tr>
<td>6) Carvone +</td>
<td>1</td>
</tr>
<tr>
<td>7) Chloroform</td>
<td>4</td>
</tr>
<tr>
<td>8) Dichloroethane</td>
<td>18</td>
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<tr>
<td>9) DMSO</td>
<td>2</td>
</tr>
<tr>
<td>10) Drakkar Noir</td>
<td>21</td>
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<tr>
<td>11) Water</td>
<td>1</td>
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<tr>
<td>12) Heptane</td>
<td>21</td>
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<tr>
<td>13) Isopropanol</td>
<td>23</td>
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<td>14) Indole</td>
<td>1</td>
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<tr>
<td>15) Mercaptoethanol</td>
<td>17</td>
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<tr>
<td>16) Methanol</td>
<td>23</td>
</tr>
<tr>
<td>17) Propanol</td>
<td>2</td>
</tr>
<tr>
<td>18) Propionic Acid</td>
<td>20</td>
</tr>
<tr>
<td>19) Pseudoexplosive</td>
<td>2</td>
</tr>
<tr>
<td>20) Toluene</td>
<td>2</td>
</tr>
</tbody>
</table>
NOTE: the Sensor Array is a ‘Self-Encoding’ Bead Array (SEBA). Billions of Sensors are Fabricated at Once.

Hollow Poly(benzyl methacrylate) Spheres

3.5 h polymerization

6.5 h polymerization

14h polymerization

Nile Red/PolyMethylStyrene Beads in Wells:
Response of 40 beads to methanol pulse

Nile Red-soaked poly(87% methyl styrene, 13% DVB) beads, 3.2µm

bright = wells with beads
dark  = empty wells
Sensor Registration Problem

Randomly Ordered 5 Bead Array

Response of Bead Array to Methanol

Decoded 5 Bead Array
5 Sensor Types with 4 Analytes

50% Saturated Acetone

50% Saturated Toluene

50% Saturated Ethanol

50% Saturated Methanol
TIME (s) vs. FLUORESCENCE RESPONSE:
250 INDIVIDUAL Bead Sensors

~23 ppb 2,4-DNT

~80 ppb 1,3-DNB

8% saturated TNT vapor Strips (~0.4 ppb)

Signal/Noise Improvement:
Average of 1000 Sensors

3.25 s vapor exposure

Signal Summing

Individual Response of 5 Bead Sensors

Summed Responses of 5 Random Groups of 40 Beads

Summing improves signal-to-noise ratio.
Analytes for Two Class Problem

• Pure Analytes
  – Acetone
  – Benzene
  – Chloroform
  – Ethanol
  – Ethyl Acetate
  – Heptane
  – Methanol
  – Toluene
  – 1,3-Dinitrobenzene
  – 4-Nitrotoluene

• Binary Mixtures
  – Ethyl Acetate/Heptane
  – Methanol/Benzene
  – 4-NT/Benzene
  – 4-NT/Heptane
  – 4-NT/Methanol
  – 1,3-DNB/Ethyl Acetate
  – 1,3-DNB/Heptane
Concentrations of Analytes

Table 1: The concentration of the pure analytes ±15%.
The concentrations were calculated based on literature values for analyte vapor pressures.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Vapor Pressure @25 °C (mmHg)</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>2.31E+02</td>
<td>7.6E+04</td>
</tr>
<tr>
<td>Benzene</td>
<td>9.53E+01</td>
<td>3.1E+04</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1.97E+02</td>
<td>6.5E+04</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5.90E+01</td>
<td>1.9E+04</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>9.45E+01</td>
<td>3.1E+04</td>
</tr>
<tr>
<td>Heptane</td>
<td>4.57E+01</td>
<td>1.5E+04</td>
</tr>
<tr>
<td>Methanol</td>
<td>1.27E+02</td>
<td>4.2E+04</td>
</tr>
<tr>
<td>Toluene</td>
<td>2.84E+01</td>
<td>9.4E+03</td>
</tr>
<tr>
<td>1,3-Dinitrobenzene</td>
<td>9.00E-04</td>
<td>6.0E-01</td>
</tr>
<tr>
<td>4-Nitrotoluene</td>
<td>1.64E-01</td>
<td>1.1E+02</td>
</tr>
</tbody>
</table>

Table 2: The concentration of the binary mixtures ±15%.

<table>
<thead>
<tr>
<th>Analyte 1</th>
<th>Analyte 2</th>
<th>Concentration analyte1 (ppm)</th>
<th>Concentration analyte2 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>Methanol</td>
<td>3.1E+04</td>
<td>4.2E+04</td>
</tr>
<tr>
<td>Benzene</td>
<td>4-Nitrotoluene</td>
<td>3.1E+04</td>
<td>5.5E+01</td>
</tr>
<tr>
<td>Benzene</td>
<td>4-Nitrotoluene</td>
<td>3.1E+04</td>
<td>1.1E+02</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>Heptane</td>
<td>3.1E+04</td>
<td>1.5E+04</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>1,3-Dinitrobenzene</td>
<td>3.1E+04</td>
<td>3.0E-01</td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>1,3-Dinitrobenzene</td>
<td>3.1E+04</td>
<td>6.0E-01</td>
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<tr>
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<td>1,3-Dinitrobenzene</td>
<td>1.5E+04</td>
<td>6.0E-01</td>
</tr>
<tr>
<td>Heptane</td>
<td>4-Nitrotoluene</td>
<td>1.5E+04</td>
<td>1.1E+02</td>
</tr>
<tr>
<td>Methanol</td>
<td>4-Nitrotoluene</td>
<td>4.2E+04</td>
<td>5.5E+01</td>
</tr>
<tr>
<td>Methanol</td>
<td>4-Nitrotoluene</td>
<td>4.2E+04</td>
<td>1.1E+02</td>
</tr>
</tbody>
</table>
Reproducible Responses from Training to Testing array
First Testing Array (1 Month)

- 98.2% Correct

- 4-Nitrotoluene
- 1,3-Dinitrotoluene
- Volatile Organic
- False Negative
Second Test Array (7 months)

93.8 % Correct

GWMW(2,2) vs. GWMW(1,1)

- 4-Nitrotoluene
- 1,3-dinitrotoluene
- Volatile Organics
- False Positive
- False Negative
## Live/Dead Bacteria Discrimination

### Calculated Identity

<table>
<thead>
<tr>
<th>Actual Identity</th>
<th>Live B10</th>
<th>Live B4</th>
<th>Live B5</th>
<th>Live B8</th>
<th>Live B9</th>
<th>Dead B10</th>
<th>Dead B4</th>
<th>Dead B5</th>
<th>Dead B8</th>
<th>Dead B9</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live B10</td>
<td>4</td>
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<td>Live B4</td>
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<td>Live B5</td>
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<td>5</td>
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<td>0</td>
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</tr>
<tr>
<td>Live B8</td>
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<td>4</td>
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<td>0</td>
<td>1</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Live B9</td>
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<td>5</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>Dead B10</td>
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<tr>
<td>Dead B4</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dead B5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dead B8</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dead B9</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

85% Correct, 87% Variance (7PCs)

- **B10**: *Acinetobacterium*
- **B4**: *M. luteus*
- **B5**: *E. coli*
- **B8**: *Salmonella*
- **B9**: *Klebsiella pneumoniae*
Acknowledgements

ONR
DARPA
DOE
NIH
NSF

Walt Group

Myoyong Lee
Karri Michael
Paul Pantano
Caroline Schauer
Jenny Tam
Keith Albert
Todd Dickinson
Jane Ferguson
Michael Fleming
Jason Epstein
Ferenc Szurdoki
Frank Steemers
Shannon Stitzel
Laura Taylor
Tarun Mandal

Lawrence Livermore
Fred Milanovich
Pennsylvania State University
Peter C. Jurs

Tufts University School of Medicine
John S. Kauer
Joel White

Johns Hopkins University
Lenore Cowen

Illumina
Mark Chee
Kevin Gunderson
Laboratory Response Network

Ralph Timperi
Massachusetts Department of Public Health, and Association of Public Health Laboratories (www.aphl.org)
Laboratory Response Network
For Bioterrorism

Level A Lab
Use Class II Biosafety Cabinet

Level B Lab
BSL-2 facility + BSL-3 Safety Practices

Level C Lab
BSL-3
C - Molecular assays, reference capacity

Level D Lab
BSL-4
D - Highest level characterization (CDC, USAMRID)

A - Rule-out and forward organisms
B - Limited confirmation and Transport

Diagram: Pyramid structure with levels labeled as per the text above.
CDC BT Rapid Response and Advanced Technology Lab

- BSL -3
- Agent Identification and Specimen Triage
- Refer to and Assist Specialty Lab Confirmation
- Evaluate Rapid Detection Technology
- Rapid Response Team
LRN Capacity

Specimen Collection and Transport
- Appropriate specimens
- Forensic issues and chain of custody
- Timely transport & testing safety

Capacity to Diagnose
- Surveillance
- Rapid screens - People/environment
- Definitive and trusted testing
- Secure, reliable means of electronic communication
- The right answer, to the right persons at the right time
LRN: Work-in-progress

• State and large city / county public health laboratories - secure internet website (reagents, protocols, capacity locator)
• Training and proficiency on ‘highest priority agents’
• Conventional and rapid methods
• Validation of methods
• ‘Surge capacity’
LRN: Growing capacity

• Clinical microbiology laboratories collaboration—standard protocols, rule-out testing for clinical specimens, (future) definitive identification of agents
• Building a secure system for electronic laboratory reporting of test results— the technology is not the problem
• Surge capacity—build, protect, access
• Technology and reagents to more laboratories—capacity to validate and accept
Laboratory and Testing Issues

• Surveillance- Numbers of ill persons, general syndromes, laboratory-based species and DNA characteristics
• Field testing- First responders, environmental, risk characterization
• Laboratory diagnosis of human and animal illnesses- coordination and communication
• 24/7 available and accessible capacity
Human Arbovirus Cases, MA
Timelines: Onset to Diagnosis

- Onset to Health Care
- Health Care to Notification
- Notification to Diagnosis

WNV #1

WNV #2

WNV #3

EEE

DAYS

0 7 14 21 28 35 42
Impact of Surveillance on Survivability ( Anthrax )

- **Phase I**: Initial Symptoms
- **Phase II**: Acute Illness

**Surveillance**

**Gain of 2 days**

1. **Effective Treatment Period**
2. **Incubation Period (Hours)**

**Anthrax Attack**
- Non Communicable Victims Directly Exposed
- Fatalities With Traditional Public Health Response
- Fatalities With Early Warning and an Informed Public Health Response

**Number Dead**

- (Linear) $10^5$

**Animal or Human Indicators**

**Time (days)**
FRAMEWORK FOR AN INTEGRATED SLIS

Internal Client Access / Bureau of Communicable Diseases
Browser Inquiry & Data Extraction

SLI NETWORK

Interface Engine

HL7 Registration & Orders
HL7 Results & Reports
E-mail & Faxed Reports
Publish to Web site

Instruments

Instrument Pre-Processors

Integrated SLIS

Results & QC Data
Orders

Instrumentation Pre-Processors

Test Requester

E-mail & Faxed Reports

Blood Lead Results
Registration & Orders
Results & Reports

Public Website

E-mail & Faxed Reports

Order Entry

Browser Inquiry

E-LexNet, PulseNet

INTERNET

Hospitals (B&W), Reference Labs CDC Atlanta, Puerto Rico
CDC Colorado, Channing), & other Clients

CDC Reporting

Standard Report Formats

Internally Client Access / Bureau of Communicable Diseases
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INTERNET

Hospitals (B&W), Reference Labs CDC Atlanta, Puerto Rico
CDC Colorado, Channing), & other Clients

CDC Reporting

Standard Report Formats
Anthrax Sent By Mail
September / October 2001
<table>
<thead>
<tr>
<th>Description</th>
<th>Number Submitted</th>
<th>Risk / Testing Priority</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. Mail contaminated with anthrax</td>
<td>0</td>
<td>Low to high / High</td>
</tr>
<tr>
<td>U.S. Mail with a suspicious powder (not anthrax or other pathogen) with or without a threat letter</td>
<td>52</td>
<td>None / High</td>
</tr>
<tr>
<td>Powders, particulate matter and various liquid or solid material on surfaces of floors, walls, furniture, clothing, appliances or food</td>
<td>~800</td>
<td>None / Low</td>
</tr>
<tr>
<td>Clothing, household items, business products, etc. without evidence of powder, particulate matter, etc.</td>
<td>~1800</td>
<td>None / None</td>
</tr>
</tbody>
</table>
LRN validated methods and reagents available

- *Bacillus anthracis*: C, PCR, TRF
- *Brucella sp.*: C
- *Francisella tularensis*: C, TRF
- *Yersinia pestis*: C, PCR, TRF
- *Clostridium botulinum*: C

Conventional, polymerase chain reaction, time resolved fluorescence
Methods in development

- Ricin: TRF
- *Brucella* sp.: PCR, TRF
- *Francisella tularensis*: PCR
- *Staph.* enterotoxin B: TRF
- *Burkholderia mallei*: PCR
- *Burkholderia pseudomallei*: PCR
- *Coxiella burnetii*: TRF
- *Clostridium botulinum*: EIA, TRF

- Validation in progress
- Validation by summer
- Validation by late summer
- EDA not estimated
- Fall/Winter 2002
- Fall/Winter 2002
- EDA not estimated
- 2004
Testing Methods - Environmental

• 1- Gross examination- (environmental samples only)
• 2- Microscopic examination for bacteria and spores
• 3- DNA test methods
• 4- Culture (growth of bacteria on artificial media)

• Most samples tested by methods 1, 4
• U.S. Mail and similar items tested by methods 1, 2, 4 and possibly 3
• Some items with no apparent contamination, no risk indicators tested by method 1 only
Targeting Immunity to Biothreats

David Scadden
Massachusetts General Hospital
Harvard Medical School
Cellular immunity and HIV disease

Immune control of HIV infection is possible without anti-retroviral therapy
Evidence for CTL control of HIV

- Negative correlation between CTL and viral load by more sensitive assays (Ogg et al)
- Increase in SIV viremia with CD8 cell depletion (Schmitz et al; Jin et al)
- Association between appearance of CTL and decline in viremia in acute infection (Koup et al; Borrow et al)
Optimal CTL function depends on virus-specific T helper cells.

Class II

TCR

CD4

Antigen Presenting Cell

CD4+ Th Cell

Lymphokine Secretion
HIV-specific CD4+ T cell responses are associated with control of HIV

Rosenberg et al. Science 1997; 278, 1447
HIV specific helper T cell function

Rosenberg et al. Nature 2000; 407, 523
HIV specific helper T cell function

Low in chronically infected patients

Rosenberg et al. Nature 2000; 407, 523
HIV specific helper T cell function

Very low in acutely infected patients

Rosenberg et al. Nature 2000; 407, 523
Hi
Controls
Chronic treated
Acute untreated
Acute treated
LTNP

May be preserved by early treatment of acute infection

Rosenberg et al. Nature 2000; 407, 523
Treatment of acute HIV-1 infection results in augmentation of T helper cell responses

- Stimulation Index
- Weeks on Treatment
- Graph showing increase in T helper cell responses over weeks on treatment
Preserved HIV specific T cell helper function is associated with control of HIV without HAART.
Structured treatment interruptions (STI) in acute HIV infection may result in immunologic control of viremia.
CD4ζ-modified T-cell survival and gene expression in peripheral blood mononuclear cells (PBMCs)

Mitsuyasu et al, Blood 2000; 96:785
Persistence of cells with chimeric TCR DNA

![Graph showing the persistence of CD4ζ cells with chimeric TCR DNA over time. The x-axis represents Time (weeks) from -2 to 24, and the y-axis represents CD4ζ (log copies/10⁶ cells) ranging from 0 to 4. The graph includes arrows indicating the time points for assessment.]
Persistence of cells with chimeric TCR DNA

![Graph showing the persistence of CD4 and CD8 cells with chimeric TCR DNA over time. The graph plots CD4+ and CD8+ cells in log copies/10^6 cells against time in weeks. Arrows indicate specific time points.]
Persistence of cells expressing chimeric TCR RNA

The graph shows the persistence of CD4+ T cells expressing chimeric TCR RNA over time (weeks), with the x-axis representing time in weeks and the y-axis representing the CD4+ RNA level in log copies/10^6 cells.
CD4+ T cell counts after cell infusions

Gene modified cells

Unmodified cells
Plasma viral load over time

Cumulative Viral Load Rebound (# Patients)

- Pre-Infusion
- 4 Weeks
- 8 Weeks
- 12 Weeks
- 16 Weeks
- 20 Weeks
- 24 Weeks

Gene-modified

Unmodified

Time (Weeks)

P=0.07

P=0.19

P=0.70