Standardization of LRN Methods for Agent Identification

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Introduction

- The Laboratory Response Network.
- Development of methods for agent identification.
- Standardized review process for LRN methods.
- Future prospects.

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Operational Suppositions

- System must be flexible in order to respond to both overt and covert events as well as integrate with law enforcement.
- Frontline response begins at the local level.
- Laboratory-based biodetection must be rapid to support timely public health decision making and consequence mitigation.
- Testing algorithms and reagents must be standardized for interoperability and consequence management.
- Leverage existing Public Health infrastructure and the strength of collaborative partnerships.
- Infrastructure investments should have dual use.

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Public Health Laboratories in the U.S. in 1997

- 56 State and territorial public health labs
- 85 Branch public health labs
- 6,000 employees
- 20 million specimens in 1997
- $300 million annual budget
- Several labs threatened with closure
- Very few labs doing PCR; those that do, primarily use commercial test kits

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Public Health Laboratory Capacity in the U.S., 1998

PCR	Do already	Can do
Bacillus anthracis	0	3
Brucella species	0	3
Francisella tularensis	0	2
Yersinia pestis	0	4

Lack of trained staff, 50%; Lack of facilities, 34%; Lack of reagents, 82%; Lack of equipment, 66%

Data based on 38 respondents to a 1998 APHL Bioterrorism Survey evaluating public health laboratory capacity

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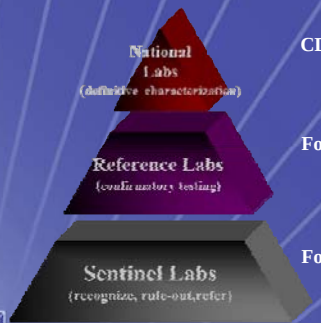
Current LRN Membership



CDC and USAMRIID

Formerly Level B/C

Formerly Level A

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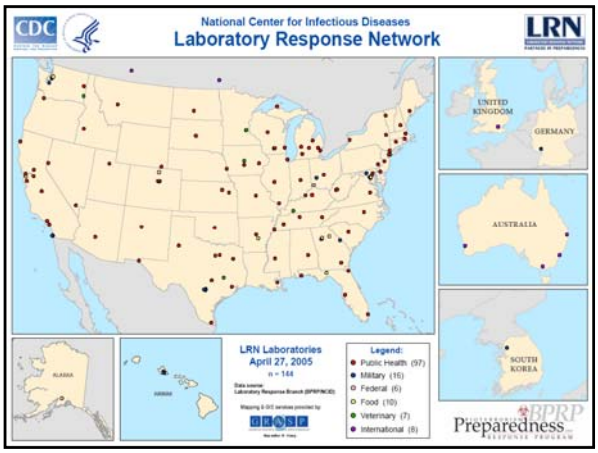

LRN Membership Services Provided to All Reference Labs

- Help Desk and Lab Qualification Support
- Agent- and Platform-Specific Protocols
- Same Reagents & Controls Supplied to all LRN Labs
- Secure On-line Lab Referral Directory
- Secure Website Communications
- Secure Electronic Lab Results Reporting
- Training & Technology Transfer
- Proficiency Testing
- Appropriate Vaccinations for Lab Workers

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Time to Results

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CDC and DOE use an assay panel to provide high confidence detection for bacteria

- Assays for three to six highly discriminating DNA signatures are used for identification of target
- Detection targets include:
  - ✓ Genomic
  - ✓ Plasmid
  - ✓ Known virulence genes
  - ✓ Antibiotic resistance (future)

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Identification of Potential Signatures

- **Whole genome approach**
  - Generates thousands of signatures using comparative genomic sequence analysis
  - High-throughput batch processing facilitates in-depth screening capability
- **Standard approach**
  - Generates a few signatures based on knowledge of biology of organism
  - Screening usually constrained to a small number of samples

This effort exploits infrastructure and expertise developed at the National Labs for the Human Genome Project

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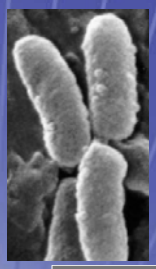
All assays are screened against DNAs that may be present in samples



- Diverse collections of target;
- Closely related microbes;
- Soil and air samples from around the country;
- Organisms causing similar clinical symptoms;
- Microbes representative of diversity found in nature and
- Animal and insect DNAs.

✓ Screening is conducted with wet chemistry and computational databases

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Assays are defined by their input samples, extraction method, detection process and detection instrumentation

Defining the entire assay process ensures robust performance in diverse laboratory settings

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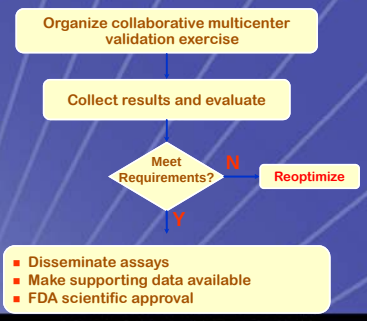
Assays are validated through a multicenter collaborative study



- Disseminate assays
- Make supporting data available
- FDA scientific approval

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Current Real-time PCR Assays

- *Bacillus anthracis*
- *Yersinia pestis*
- *Francisella tularensis*
- *Brucella* species
- *Burkholderia mallei*
- *B. pseudomallei*
- Ebola virus*
- Marburg virus*
- Variola major
- Vaccinia virus
- Varicella zoster
- Orthopox viruses
- SARS
- *Coxiella burnetii*
- Ricin toxin DNA
- Influenza A H5-Asian

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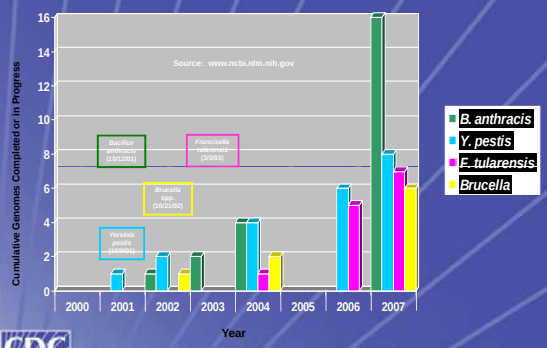
Introduction of LRN RT-PCR Assays



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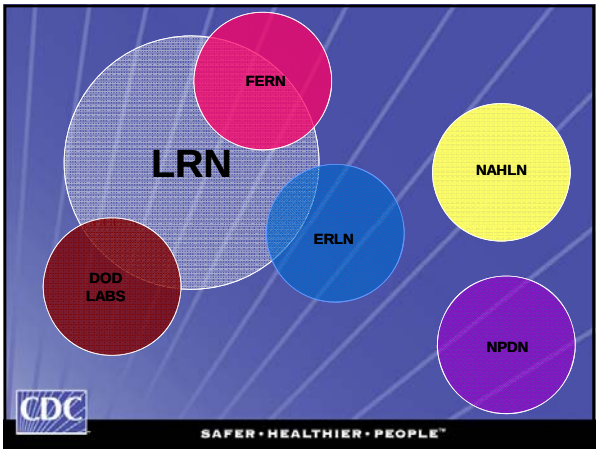

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How are LRN Assays Used?

- Agent identification in clinical specimens.
- Culture confirmation.
- Agent identification in environmental samples (powder, food, water, air).
- Determining the extent of environmental contamination.
- Post-remediation.
- Microbial forensics.

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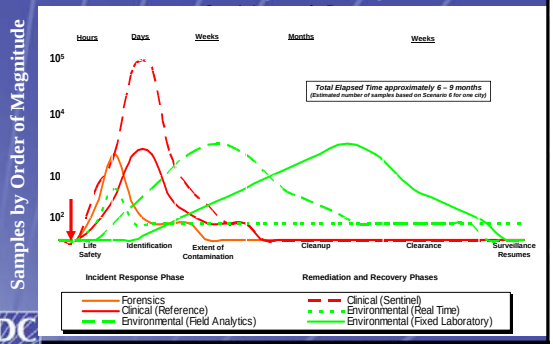
Anatomy of a Response
Sampling for Testing



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Standardized Review Process is a Strategic Necessity

- Ensures a coordinated public health response when LRN assays identify a potential threat.
- Employs a standard process for the evaluation of both CDC and external agency assays.
- Provides a standardized review process for food, air, and water sample tests that are not covered by FDA regulations.
- Prepares LRN distributed assays for FDA compliance, if required.

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Standardized Review Process

- Documented process with written forms and procedures
- Technical Review Committee oversight

DESIGN **Form A** → Continue development → DEVELOPMENT **Form B** → Performance studies → PERFORMANCE **Form C** → Multicenter evaluation → FINAL RELEASE **Form D**

LRN Assays that produce consistent and accurate results to aid in a coordinated response to threat agents or emerging diseases

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Design Review

- Detects potential terrorism agent or emerging infectious disease
- Appropriate technology for use in LRN labs
- Acceptable specimen types
- Test results valuable to response effort

Design and operability are applicable to the LRN.

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Development Review

- Acceptable intended use & appropriate detection level
- Acceptable sensitivity and specificity for specimen type
- Result achieved in time to impact response
- Draft test procedure, production documents and QC test

Adequate data, procedures and specifications to demonstrate potential value to response effort; continue performance evaluation

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Performance Review

- Reproducible results by different technicians
- Reproducible results with different specimen types
- Reproducible results with different instrumentation, (if applicable)
- Quality controls available & Multicenter eval plan

Assay meets performance specifications and is ready for multicenter evaluation to determine performance by user groups

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Evaluates Reagent Production

- Written and approved specifications, production procedures and QC tests
- Product stability for determination of proper expiration and storage requirements.
- Production at CDC or external source
- Quantity to be produced and maintained

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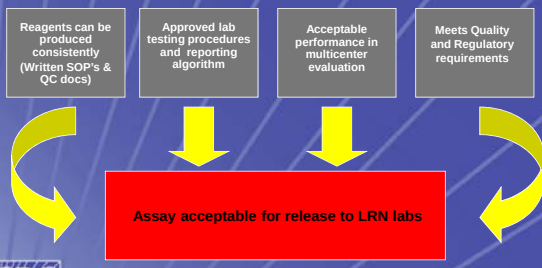
Final Review



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Review Process is a Regulatory Necessity

Bioshield legislation for emergency use

FDA *in vitro* diagnostic regulations

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Test Performance

- Stakeholder Panel on Agent Detection Assays (SPADA)
  - Establish standards and testing methodology to evaluate assays that detect *B. anthracis*, *Y. pestis*, and *F. tularensis.*
- BioNet Assay Equivalence and Interoperability Study
  - To establish assay performance equivalency between tests deployed by CDC and DOD in civilian and/or military environmental monitoring programs.

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