



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
<i>Bacillus anthracis</i>	0	3
<i>Brucella species</i>	0	3
<i>Francisella tularensis</i>	0	2
<i>Yersinia pestis</i>	0	4

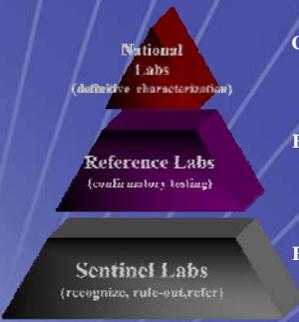
Lack of trained staff, 50%; Lack of facilities, 34%; Lack of reagents, 82%; Lack of equipment, 66%

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



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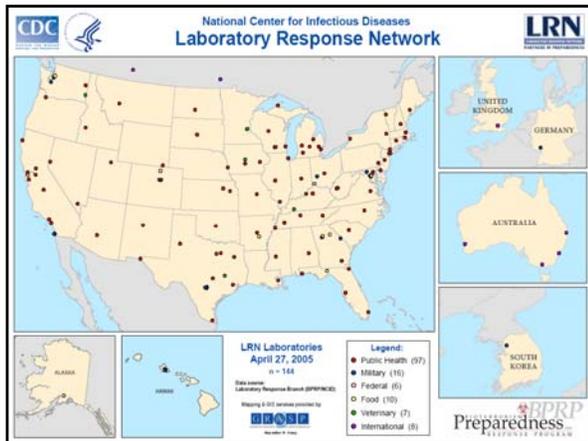
Current LRN Membership



- National Labs (definitive characterization) - CDC and USAMRIID
- Reference Labs (confirmatory testing) - Formerly Level B/C
- Sentinel Labs (recognize, rule-out, refer) - Formerly Level A



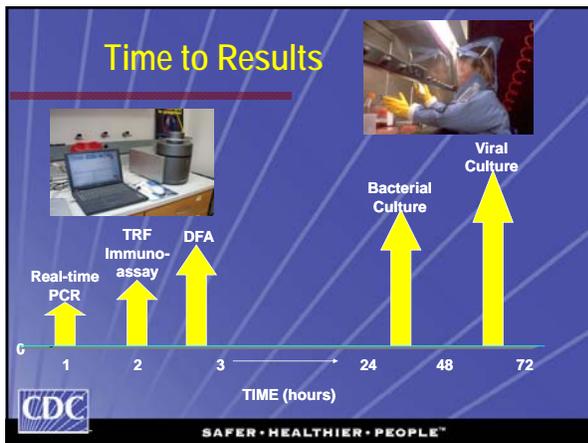
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LRN Membership Services Provided to All Reference Labs

- 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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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

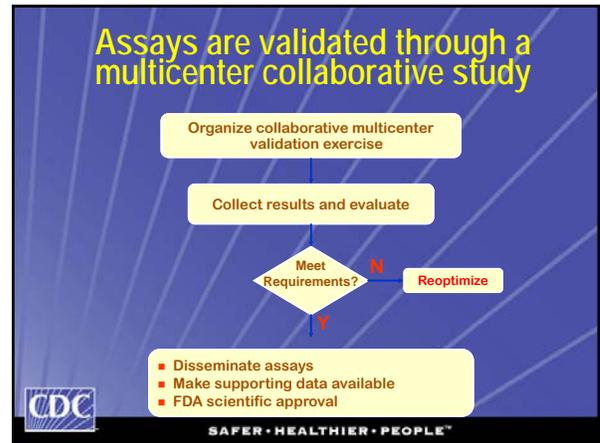
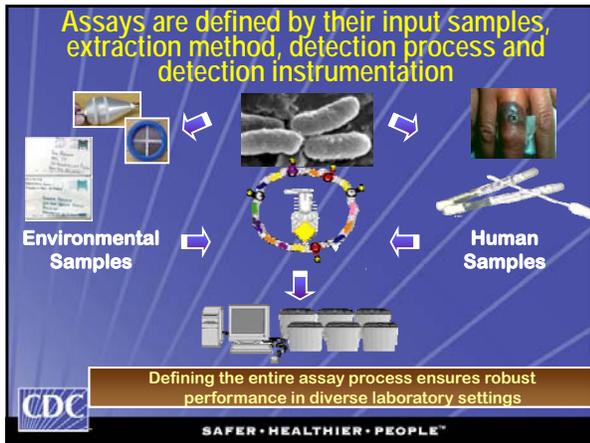
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All assays are screened against DNAs that may be present in samples

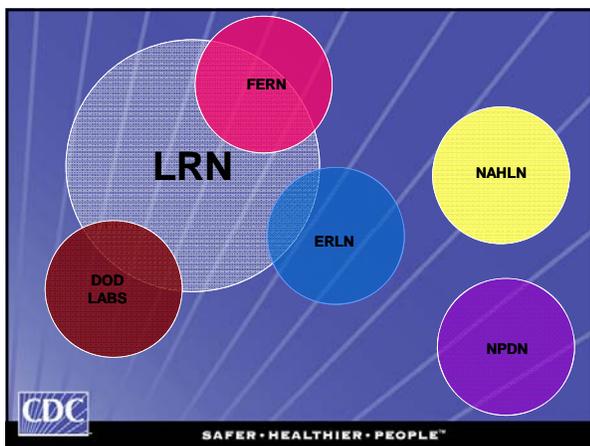
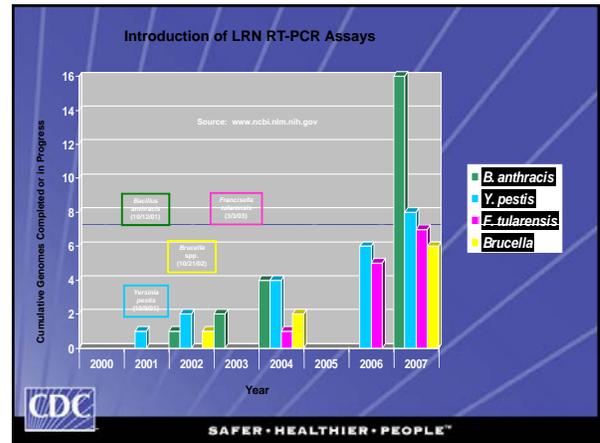
- Diverse collections of target;
- Closely related microbes;
- Soil and air samples from around the country;
- Organisms causing similar clinical symptoms;
- Microbes representative of diversity found in nature and
- Animal and insect DNAs

Screening is conducted with wet chemistry and computational databases

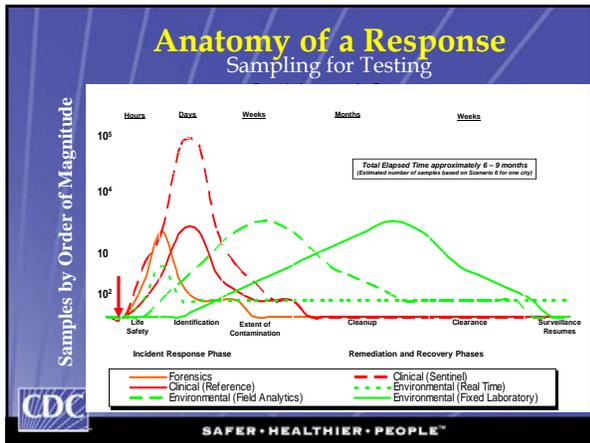
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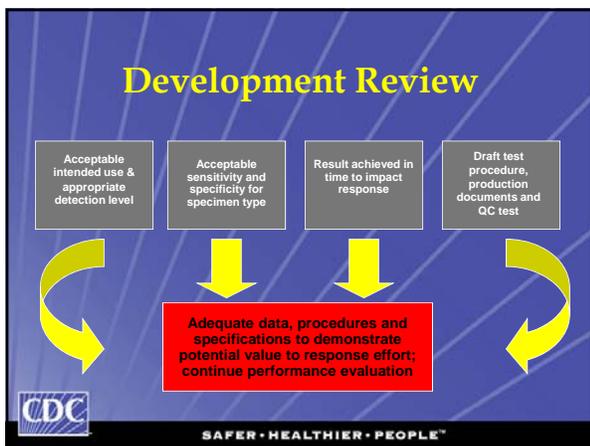
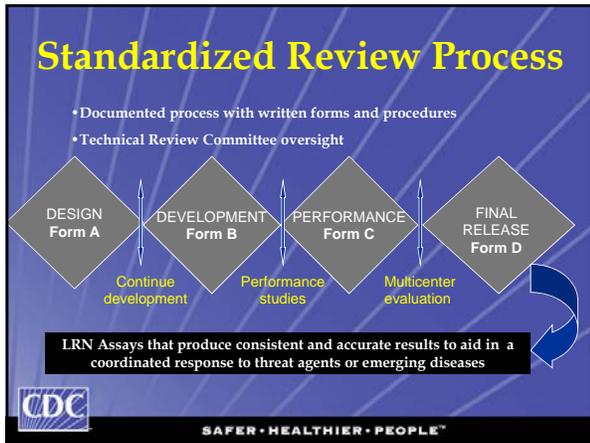
- ### Current Real-time PCR Assays
- *Bacillus anthracis*
 - *Yersinia pestis*
 - *Francisella tularensis*
 - *Brucella* species
 - *Burkholderia mallei*
 - *B. pseudomallei*
 - Ebola virus*
 - Marburg virus*
 - Variola major
 - Vaccinia virus
 - Varicella zoster
 - Orthopox viruses
 - SARS
 - *Coxiella burnetii*
 - Ricin toxin DNA
 - Influenza A H5-Asian
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- ### 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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- ### 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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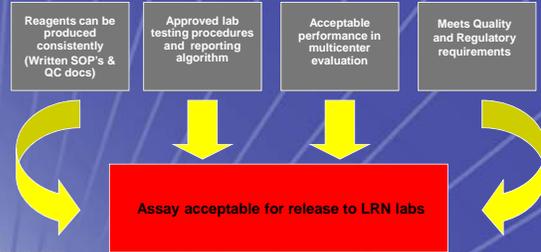
Evaluates Reagent Production

- 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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