A need for improved sample inactivation and extraction methods to support systems biology analysis of biological threat organisms

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Ultra-high Pressure in Bioscience **21 May 2010** **Harvard, Cambridge, MA**

Required Statements

Disclaimer

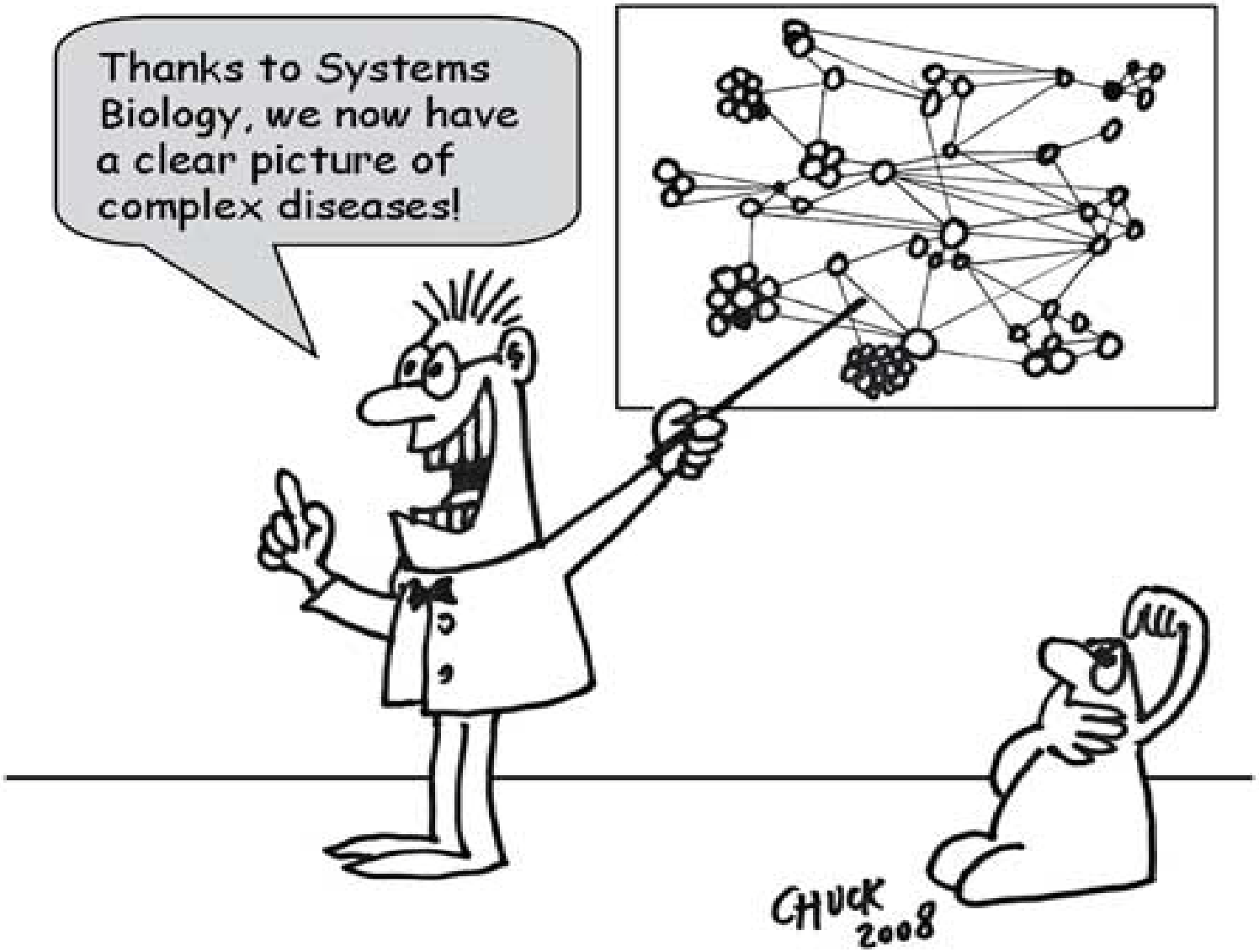
Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Funding Acknowledgement

The research described herein was sponsored by the Department of Defense Medical Biological Defense Research Program, U.S. Army Medical Research and Materiel Command with support from the National Institute of Allergy and Infectious Diseases.

Mission

USAMRIID conducts basic and applied research on biological threats resulting in medical solutions to protect the warfighter



nature publishing group language editing
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Molecular Systems Biology **2008 impact factor 12.243** from Journal Citation Reports, Thomson 2009

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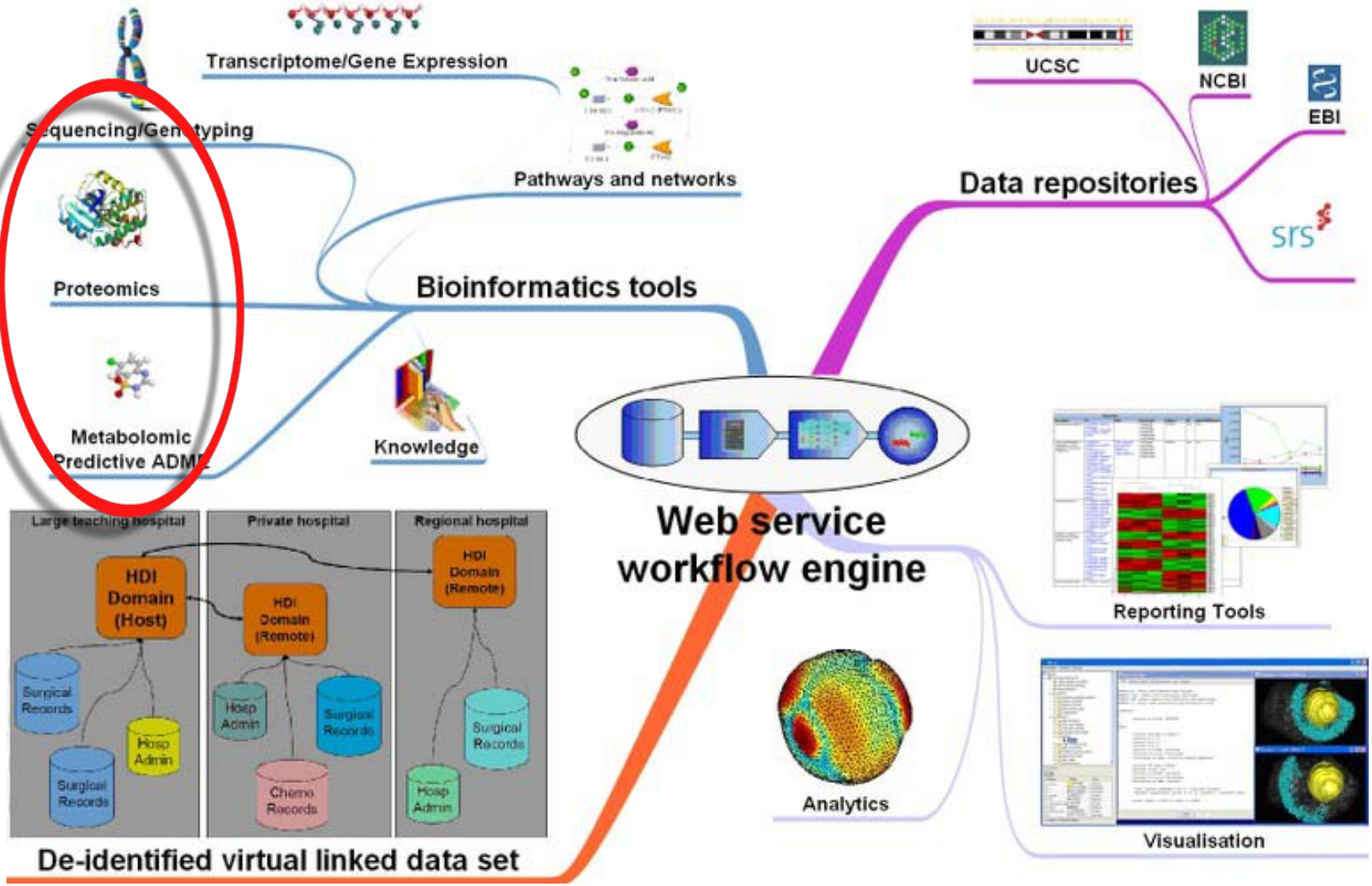
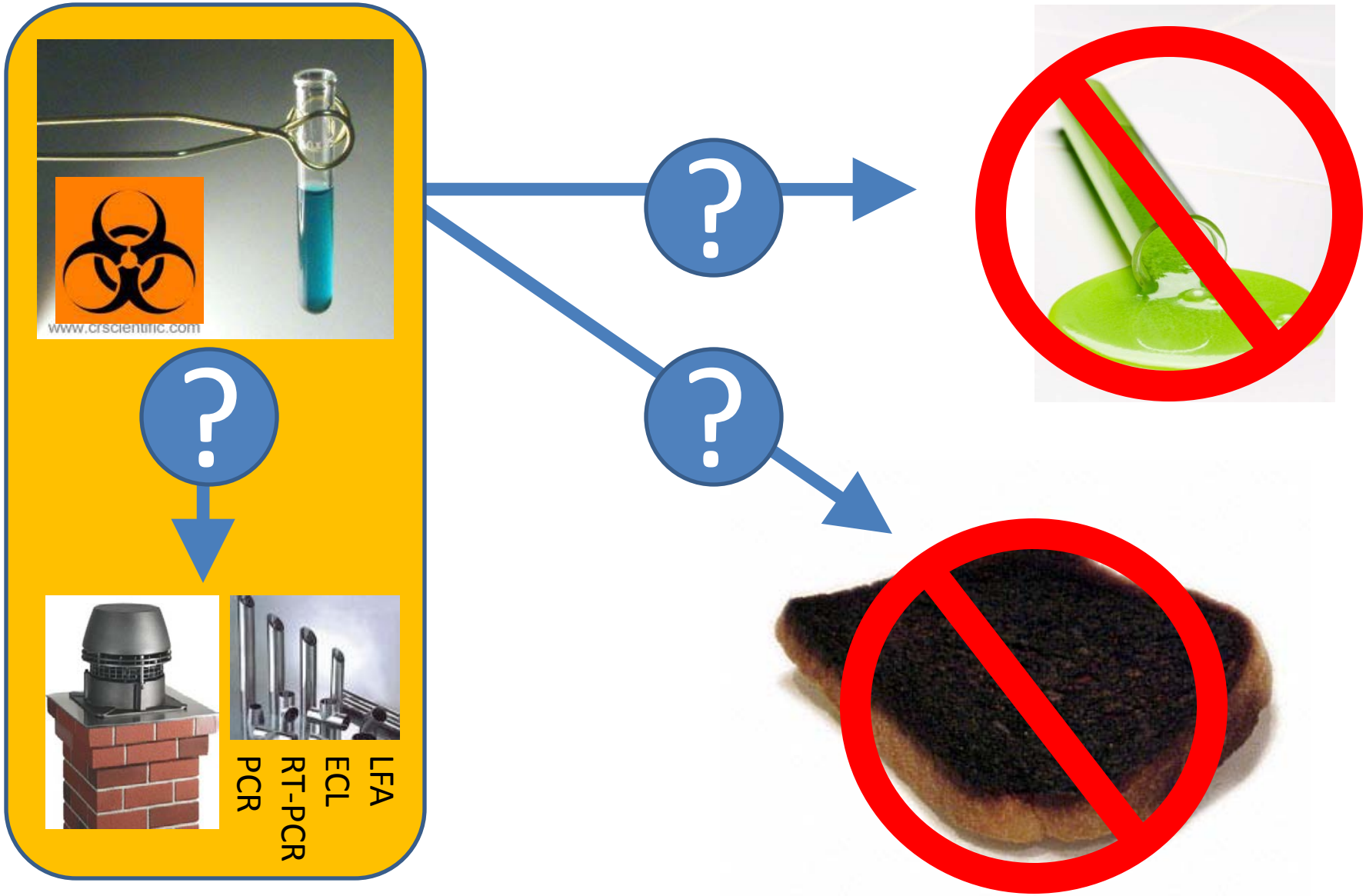
利用你的机遇
Aumenta tus posibilidades

Systems Biology Workflow



The Situation



BSL-3/BSL-4

Narrow scope of methods:

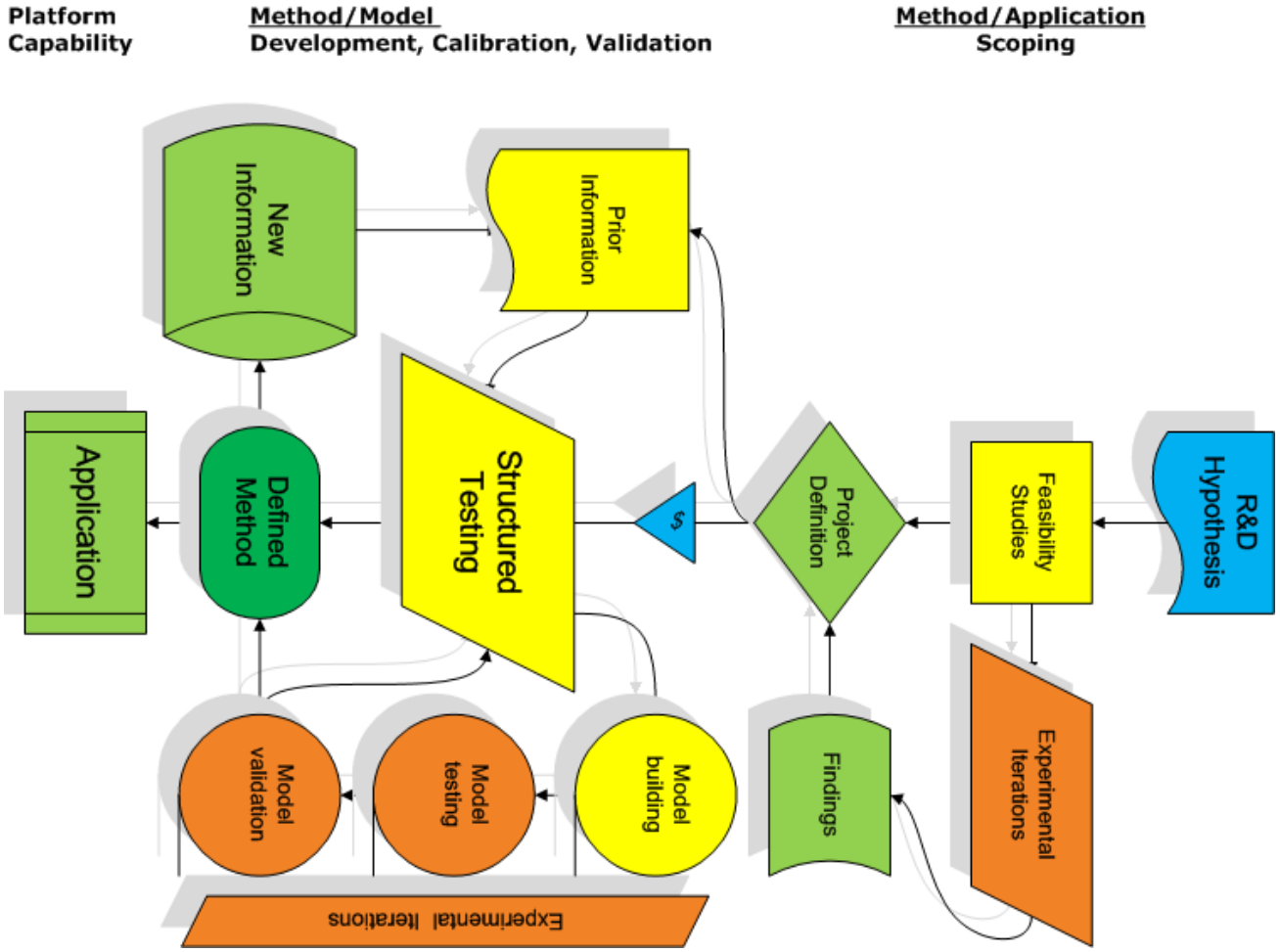
Animal studies
Histology
Telemetry
Microscopy
Nucleic acid analysis
Simplified protein assays

***NOT* tandem mass spectrometry**

BSL-2

Wide scope of methods:

Highly sensitive instrumentation
LC-MS/MS
NMR, Etc…….


Platform
Capability
Method/Model
Development, Calibration, Validation
Method/Application
Scoping
New Information
Prior Information
Application
Defined Method
Structured Testing
$
Project Definition
Feasibility Studies
R&D Hypothesis
Model validation
Model testing
Model building
Findings
Experimental Iterations
Experimental Iterations

* Traditional BTA decontamination impedes systems biomolecular analysis platform development
R&D Hypothesis
Feasibility Studies
Experimental Iterations
Project Definition
Findings
BIOHAZARD
Method/Application Scoping
Method/Model Development, Calibration, Validation
Prior Information
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Model building
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Model validation
Experimental Iterations
New Information
Defined Method
Application
Platform Capability
ACCESS DENIED
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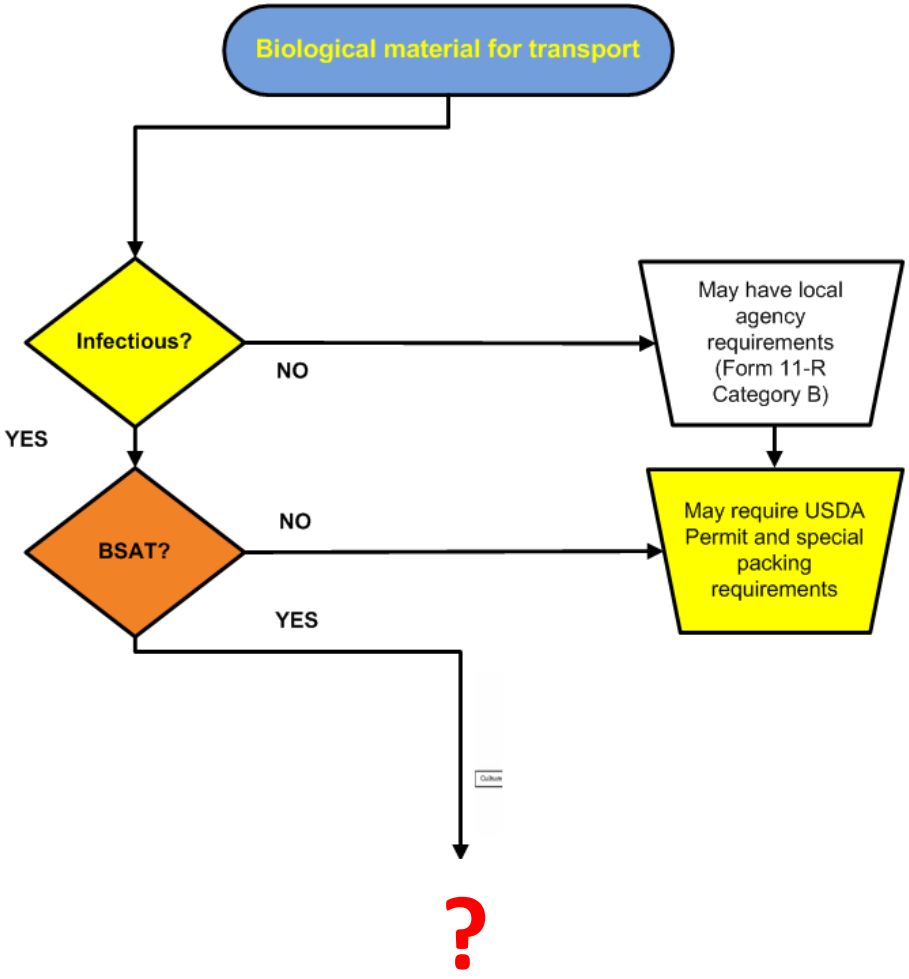
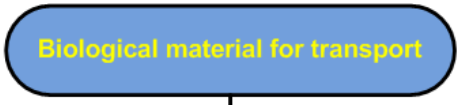


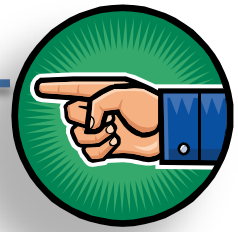
Transport Concerns

Regulations
Authorizations
Permits
Labels
Cold Chain Custody

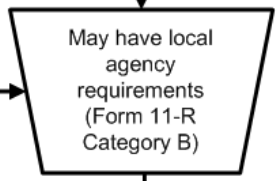


NO

YES



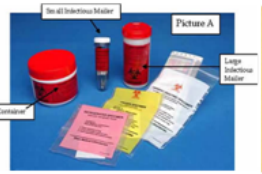
NO



YES

NO

YES



- Recipient Institution/Agency must have CDC Registration for Agent
- Recipient PI must have CDC Registration for Agent
- Recipient requires CDC or USDA permit (APHIS/CDC Form 2), and
- If not federal agency or contractor, then approval from ASTD-CBN
- If recipient is a military installation or its contractor, an active Personnel Reliability Program is mandatory (AR-50-1)
- Internal Chain of Custody forms for the BSAT
- USDA Permit may be needed prior to shipping
- Special Packing requirements (needs certified packer)
- Special Shipping (FedEx White glove service)

http://www.selectagents.gov/TransferForm.html

Hypothesis

New approach will expand capability and save cost/time

Methods to Inactivate BSAT/IAT

Inactivation Method	Molecular Analysis	Comments
Autoclave	Partial	PCR; Physical hazard, facilities, instrument & training requirements
γ-irradiation	Partial	Antigen detection; Physical hazard, facilities, instrument & training requirements
Formaldehyde	No	Antigen detection; Chemical hazard, Facility & training requirements
UV-irradiation	No	Poor inactivation; instrument & training requirements
Ethylene oxide	No	Chemical hazard, facilities, instrument & training requirements
*TriZol™/$CHCl_3$	Partial	Nucleic acids; chemical hazard
Bead Beater	Partial	Unsafe, known breaches
Filtration	Yes	Unsafe, documented incidences
PCT	Yes	Safe, Under development
FMT	Yes	Safe, Under development

*TriZol extraction contains guanidinium thiocyanate, phenol and chloroform. Positive strand virus (e.g., VEE) requires additional care and treatment due to infectivity of RNA.

Pressure Cycling Technology (PCT)

Focused Microwave Technology (FMT)

***Disclaimer: This is not an official endorsement.**

- ***Bacillus anthracis* Sterne spores:**
- ~7 log decrease in survival 45k psi, 50°C, ProteoSolve (0.5 ml volume, 6.87 x 10^8/ml, 38% = 2.6 x 10^7 plated)
- PCT-assisted extraction & trypsinolysis (20 min trypsin digest vs 18 hr standard)

VEE TC83 virus:

- At 37°C, PCT with ProteoSolve inactivates TC83 at concentrations as high as 4.4x10^6 PFU/mL

- ***Bacillus anthracis* Sterne spores:**
- ~4 log decrease in survival after 125 W
- FMT-assisted trypsinolysis (20 min trypsin digest vs 18 hr standard)

BSL-3/4

BSAT/IAT Sample

TriZol or Buffer AVL

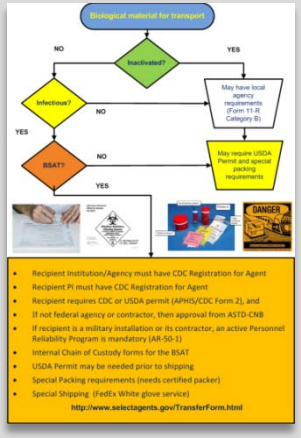
Legacy Method Inactivation

Confirmation

- RT-PCR
- ECL
- LFA
- Culture

Safety Testing

BSL-2



Regulated Transport, Archival & Processing

BSL-3/4

Legacy Method Inactivation

Safety Testing

Limited Biomolecular Analysis

- ~~LC-MS/MS~~
- ECL
- RT-PCR
- PCR
- Microarray

BSL-3/4

1 ml Reagent A

BSAT/IAT Sample

TriZol or Buffer AVL

Confirmation

- **RT-PCR**
- **ECL**
- **LFA**
- **Culture**

PCT or FMT

Safety Testing

BSL-2

Safe Transport, Archival & Processing

1:1 Reagent B, Phase separation

Biomolecules Intact & Separated

DNA
RNA
$C(H_2O)_n$
Lipids
Proteins

Storage (RT)

Biomolecular Analysis

- LC-MS/MS
- ECL
- RT-PCR
- PCR
- Microarray

Conclusion

- “Unified” means for sample inactivation
- Bacteria, spores, virus, vectors, matrix
- Preservation of protein covalent structure
- Enable proteomics & systems biology
- Leverage limited sample for multiplex analysis
- Simplify transport & archival
- Toward standardization of methods & findings

Acknowledgements

Loreen Lofts

Chris Cote

Anthony Bassett

Jeffrey Enama

Vanessa Melanson

Stephen Little

DTRA and NIAID