

Validation of testing algorithms and collection kits for orthopox testing utilizing Laboratory Response Network (LRN) methods

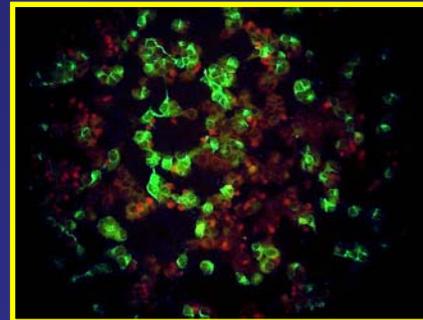
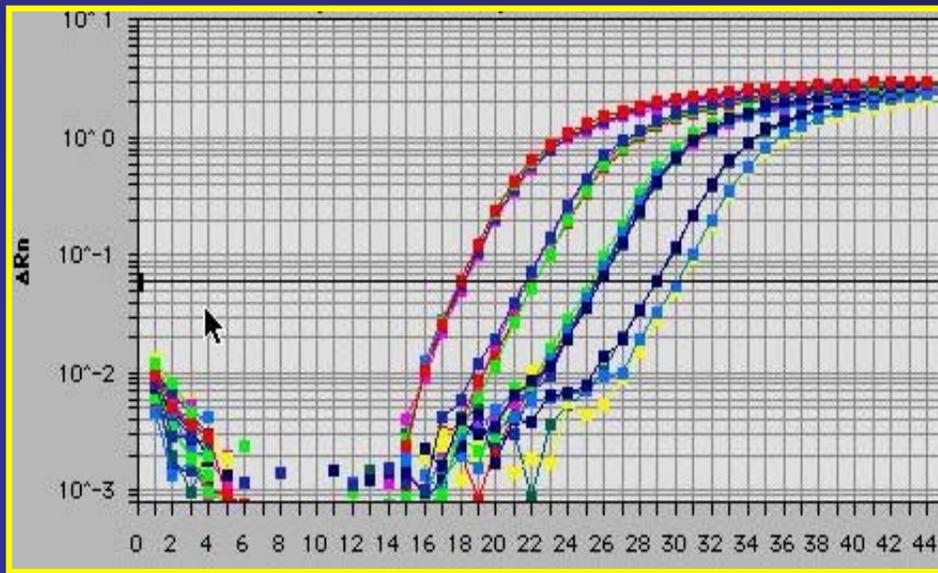
Vaccination Campaign 2003

- Risk of Smallpox as a BT agent “increased” pre-Iraq war.
- Available vaccine from original stockpile.
- First stage of vaccination campaign began January 2003



Tasks for Biodefense Lab

- Rapid response clinical vaccinia Rule-In
- Rapid response clinical variola Rule-Out
- Analysis of environmental unknowns



Vaccinia virus Rule-In

- Live virus vaccine.
- Wadsworth Center testing required for adverse event monitoring, transmission, and release of VIG or Cidofovir.
- Requires rapid turn-around (<24h).
- Requires enhanced containment measures.

Preparation of Protocols and Submission Guidelines

Guidelines for Laboratory Testing at the NYSDOH Wadsworth Center Biodefense Laboratory for:

1. Vaccination-associated adverse events (VAE) monitoring and confirmation during the smallpox vaccination program
2. Evaluation of patients with fever and an acute, generalized vesicular or pustular rash (i.e., suspect smallpox).

- Developed based on CDC guidelines
- Collaboration between Biodefense Laboratory, Clinical Virology Laboratory, NYS LRN, and the NYSDOH Div. of Epidemiology

Lab Preparation

- Assays:
 - LRN: real-time PCR, EM
 - WC: real-time PCR, EM, culture (non-variola only), DFA in development, multiplex rtPCR in development
- Staffing (24/7):
 - Current team (4 people) vaccinated.
 - Additional 6-8 vaccinated staff to perform primary testing and support.
 - Separate BSL-3 lab for Orthopoxvirus testing.

Lab Readiness

- Collection supply list and kits
 - Collection materials
 - Packaging/transport materials (labels, bags, shipping container).
 - Forms (test requisition form).
 - Protocols (specimen collection, packaging, transport).



Orthopox Collection Kits

- Purpose: Used to collect clinical specimens from VAE patients or from moderate to high risk smallpox cases.
- Contents:
 - Viral Culturette Swabs
 - Microscope Slides
 - Screw-capped Tubes
 - IATA Certified Shippers, Containers & Gel Packs
 - Collection Guidance Document
 - Collection Supply List

Orthopox Accessory Kits

- Purpose: To facilitate safe collection of specimens at non-clinical sites and enhance specimen quality.
- Contents:
 - Absorbent Underpads
 - Biohazard Bags
 - Safety Scalpels
 - Alcohol Wipes
 - Alcohol Gel Hand Cleanser
 - Ruler
 - Sharps Disposal Container

Distribution of Collection Kits

- Distributed to Regional Epidemiology offices.
- Distributed to Regional Resource Centers.
- Distributed to all local health depts.
- Surplus kits assembled and ready to go in storage.
- Protocols and guidelines were distributed to all sentinel hospitals.
- Collection kits, accessory kits, protocols were distributed through Div. of Epidemiology

Testing Algorithm

- Screening
 - Perform clinical evaluation (rash algorithm, history form).
 - Contact County HD and Local Epidemiologist.
 - Submission
 - Contact State Epidemiology.
 - Determine method of transport to the laboratory.
 - Collect samples (scabs, slides, wet swabs [SOPs in kit]).
 - Package samples [SOPs in kit].
 - Contact WC lab (518-474-4177 or 518-465-9720) with ETA.
 - Complete poxvirus testing submission form [included in kit].
- Lab and Epi BT coordinators exchange info.

Vaccinia Adverse Events

- Vaccinated:
 - 825 NYS (excluding NYC)
 - 39,608 USA
- Adverse Events:
 - 30 NYS
 - 3 patient specimens sent to the laboratory

BDL-03-01

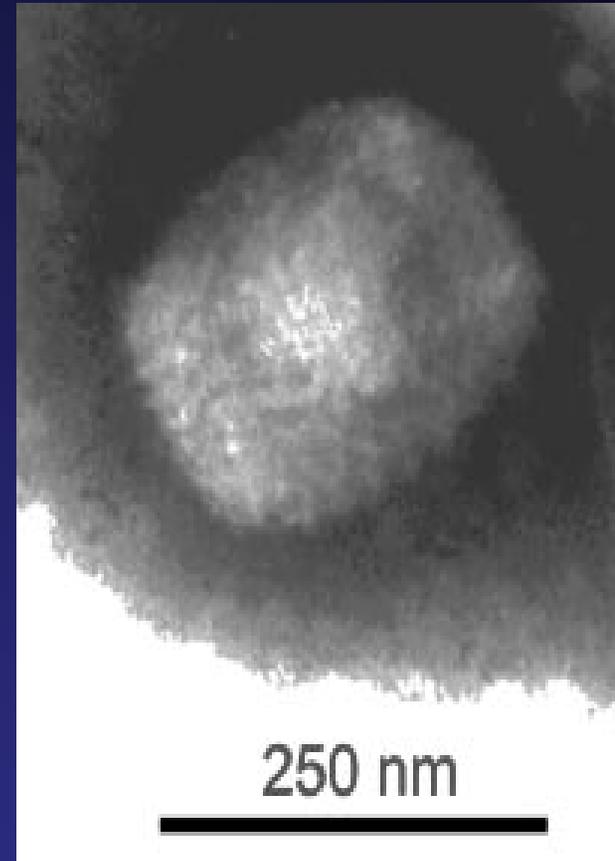
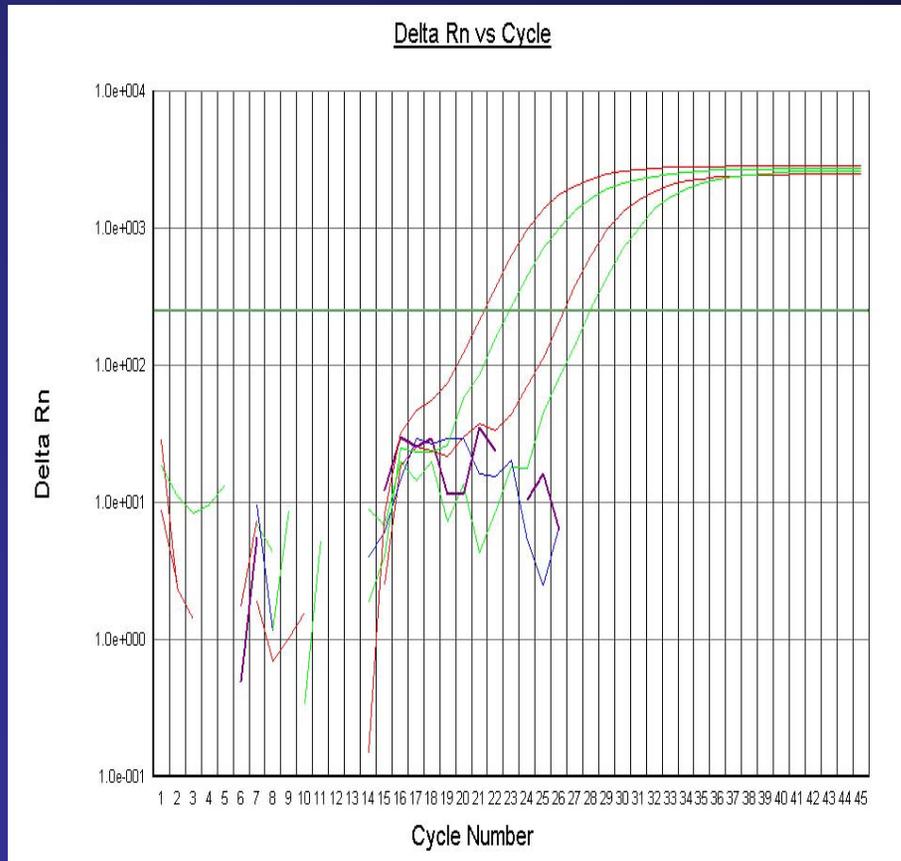
- 34 year old female vaccination volunteer
- Distribution of small lesions over body
- Team deployed with collection kit
- Samples put through the Adverse Event Algorithm



LRN Sample Preparation

- Extraction for PCR from Touch Preps
 - ~2 hours using BioRad Aquapure Extraction Kit
 - ~2 hours to run rt-PCR on ABI 7000
 - Tissue Culture and EM from Cultures
 - No extraction time
 - Grid Prep for EM ~1.5hours
 - Tissue Culture results can take days to weeks
- Scabs and blood are other potential sample sources

BDL-03-01 Results



**Result: Laboratory- Confirmed Generalized
Vaccinia in 6 hours**

Published in JCM 42:1373-1375 (2004)

BDL-03-02

- Not a Vaccination Volunteer
- Recent travel to Argentina
- Atypical rash
- Negative for Orthopox, Vaccinia and VZV by rt-PCR and EM
- Low priority of case and high probability of topical skin infection – no further testing required



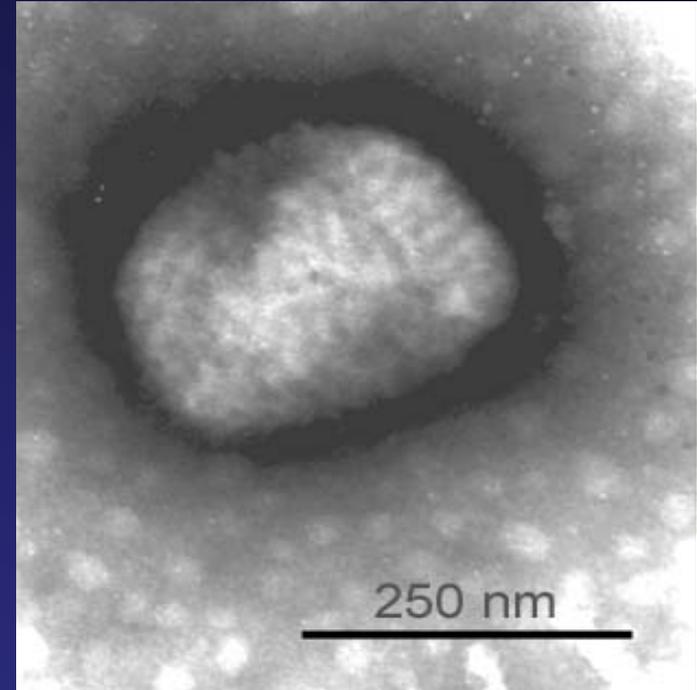
Aug. 2003-Mystery Virus

“Expect the Unexpected”

- Female-mid 20's presented at a clinic with small bump 10-12 days after sexual contact.
- Urine culture and sensitivity performed and swab taken.
- Vaginal swab was negative for Herpes but did produce viral response (CPE).
- Specimen sent to Clinical Virology Laboratory, Wadsworth Center for identification.
- The viral isolate tested negative for HSV 1 and 2 by both PCR and ELISA, negative for enterovirus by PCR and IFA, negative for adenovirus by IFA, and negative for VZV by PCR. The viral isolate was also hem adsorption negative ruling out orthomyxo- and paramyxoviruses. Viral isolate in cell culture was then sent to Biodefense Laboratory

Mystery Virus

- Cell supernatant sent to BD Lab
- **rtPCR Positive for Vaccinia & Orthopox**
- Negative for Variola, Monkeypox & Rabies
- EM confirms Orthopox infection



Further investigation reveals sexual contact with a recent military vaccinee

“Pox in the News”



© Marshfield Clinic

Preparedness for Monkeypox in NYS

- In place:
 - Collection protocols & kits for vaccination-associated adverse event testing distributed through-out state
 - Collection protocols have been validated by real-world positives
 - LRN real-time PCR (rtPCR) assays for VZV & pan-Orthopox available and validated.
 - In-house rtPCR assays specific for variola (smallpox), vaccinia, pan-orthopox & VZV viruses available and 'validated'.

Prairie Dogs in NYS



- 13 euthanized
- Necropsies done at NYS Wadsworth Laboratory
- Organs collected:
 - Spleen, Heart, Liver
 - Salivary glands,
 - Cervical lymph nodes
 - & Epiglottis
- Organs from each prairie dog pooled & ground with a mortar and pestle
- DNA extraction using BioRad Aquapure tissue extraction kit

Result: Negative for Orthopoxviruses

Human Monkeypox (July 2003)

- Two patients suspected of contact with infected prairie dogs:
 - Patient 1: Blood only
 - Patient 2: Blood, roof of lesion & touch prep



Negative Result for both patients

Conclusions

- **BT Preparedness does work to enhance public health for non-BT events!**
- Critical to have collaboration of lab and epidemiology personnel!
- Having carefully thought-out and proven algorithms and protocols yield higher quality specimens and aids in confirmation of epidemiological findings
- Can use past preparedness plans for responding to unanticipated events IF flexibility built into algorithms and SOPs.

Acknowledgements

- NYS LRN
- NYSDOH Division of Epidemiology
- Wadsworth Center Clinical Virology Laboratory
- Wadsworth Center Rabies Laboratory
- NYS Biodefense Laboratory
- Electron Microscopy Core (Bill Samsonoff)