Responding to Ebola and Managing Patient Care

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No Disclosures to Reveal

Ebola today what will be the emerging pathogen tomorrow?
Objectives

• Historical perspectives
  • How NPHL became involved in the care of EVD patients

• Response of the laboratory to provide testing
  – Safety considerations

• What we learned
Response to the Ebola Epidemic

• **Patient #1**
  - Admit Dt  9/5/14
  - Discharge Dt  9/25/14
  - Cured

• **Patient #2**
  - Admit Dt  10/6/14
  - Discharge Dt  10/21/14
  - Cured

• **Patient #3**
  - Admit Dt  11/15/14
  - Discharge Dt  11/17/14
  - Died

• **Patients #4-#9**
  - Admit Dt  1/4/15
  - Discharge Dt  3/20/14
  - All high-risk PUIs
  - Two admitted to the unit
  - None had Ebola virus
Background History

• NPHL was designated as the lab to test specimens for the BCU
  • BSL-3 lab
  • Connection with CDC
  • Part of LRN

• MUA provisions
  • iSTAT, electrolytes
  • Malaria smears
  • Transport of specimens to the CDC
Prior to 1st Patient

- Lab test menu needed to expand
  - Additional POC tests
    - Liver function
    - Coagulation
  - Menu of other tests to care for a critically ill patient

- Identify commercial carrier to transport specimens off-site
  - Assumed the CDC would be involved

- Molecular testing capabilities
  - Monitor known positive patient

- Work force training
  - Not all laboratorians can work in BSL-3
During 1st Patient

• Defined an essential test list
• On-site laboratory developed
  • Decreased TAT
  • Decreased specimen handling
  • Provided interaction with care team
  • Laboratory became operational upon receipt of 2nd patient
### Table 1

**Essential and Supplemental Tests Used for the Support of a Patient Infected With Ebola Virus**

<table>
<thead>
<tr>
<th>Test</th>
<th>Laboratory Location</th>
<th>Centrifugation Required</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Essential</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBC count with automated differential</td>
<td>Core</td>
<td>No</td>
</tr>
<tr>
<td>Basic metabolic panel</td>
<td>Core</td>
<td>Yes&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Comprehensive metabolic panel</td>
<td>Core</td>
<td>Yes&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ionized calcium&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BCU</td>
<td>No</td>
</tr>
<tr>
<td>Standard calcium</td>
<td>Core</td>
<td>Yes&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Cortisol</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Troponin</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Blood gases&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BCU</td>
<td>No</td>
</tr>
<tr>
<td>Lactate</td>
<td>Core</td>
<td>Yes&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protamine&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BCU</td>
<td>No</td>
</tr>
<tr>
<td>Partial thromboplastin time&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BCU</td>
<td>No</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Core</td>
<td>No</td>
</tr>
<tr>
<td>Blood typing&lt;sup&gt;f,g&lt;/sup&gt;</td>
<td>BCU</td>
<td>No</td>
</tr>
<tr>
<td>Culture procedures&lt;sup&gt;h&lt;/sup&gt;</td>
<td>NPHL&lt;sup&gt;i&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td>Molecular assay</td>
<td>NPHL&lt;sup&gt;i&lt;/sup&gt;</td>
<td>No</td>
</tr>
<tr>
<td><strong>Supplemental</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manual differential</td>
<td>Core</td>
<td>No</td>
</tr>
<tr>
<td>Lipase</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Amylase</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Creatine kinase total</td>
<td>Core</td>
<td>Yes</td>
</tr>
<tr>
<td>Malaria smear&lt;sup&gt;k&lt;/sup&gt;</td>
<td>Core</td>
<td>No</td>
</tr>
<tr>
<td>HIV screen</td>
<td>Core</td>
<td>No</td>
</tr>
</tbody>
</table>

Be flexible!

Iwen, PC, et al. 2015. Safety considerations in the laboratory testing of specimens suspected or known to contain Ebola virus (editorial). Amer J Clin Path, 143: 4-5
# Specimen Collection Guidance

<table>
<thead>
<tr>
<th>Test</th>
<th>Order Code</th>
<th>Tube type</th>
<th>performed at (Instrument)</th>
<th>Centrifugation (NPHL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase</td>
<td>AMY</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
</tr>
<tr>
<td>B12 level</td>
<td>VB12</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
</tr>
<tr>
<td>Blood culture</td>
<td>BLDCU</td>
<td>Plastic Aerobic Bactec bottle</td>
<td>NPHL lab</td>
<td>No</td>
</tr>
<tr>
<td>Blood Gas arterial</td>
<td>POC113</td>
<td>Heparinized blood gas syringe</td>
<td>BCU lab (iStat)</td>
<td>No</td>
</tr>
<tr>
<td>Blood Gas venous</td>
<td>POC114</td>
<td>4.5 ml green top PST</td>
<td>BCU lab (iStat)</td>
<td>No</td>
</tr>
<tr>
<td>Blood type</td>
<td>ABORH</td>
<td>3 ml lavender top</td>
<td>BCU Lab (slide forward type)</td>
<td>No</td>
</tr>
<tr>
<td>Basic metabolic panel</td>
<td>BMET</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>CBC with automated diff</td>
<td>CBCP</td>
<td>3 ml lavender top</td>
<td>Hospital Core Lab (Sysmex)</td>
<td>No</td>
</tr>
<tr>
<td>CBC with manual diff</td>
<td>CBCM</td>
<td>3 ml lavender top</td>
<td>Hospital Core Lab (Sysmex)</td>
<td>No</td>
</tr>
<tr>
<td>Comp metabolic panel</td>
<td>CMET</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Cortisol</td>
<td>CORTS</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Creatine kinase Total</td>
<td>CK</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>C-Reactive protein</td>
<td>CRP</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab</td>
<td>Yes</td>
</tr>
<tr>
<td>DIC screen (see note below)</td>
<td></td>
<td>3 ml lavender top</td>
<td>Hospital Core Lab (Sysmex)</td>
<td>No</td>
</tr>
</tbody>
</table>

**NOTE:** Lab will provide platelet count and examination of peripheral smear for schistocytes to be used in conjunction with coag results from BCU lab.

<table>
<thead>
<tr>
<th>Drug Study experimental</th>
<th>No test code</th>
<th>Tube type</th>
<th>performed at (Instrument)</th>
<th>Centrifugation (NPHL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe/Ferritin/TIBC</td>
<td>TIBC</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
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<tr>
<td>Folate level</td>
<td>VFOL</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
</tr>
<tr>
<td>Sputum Culture</td>
<td>HPT</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>HIV</td>
<td>SUD</td>
<td>5 ml red top</td>
<td>BCU Lab (iStat)</td>
<td>No</td>
</tr>
<tr>
<td>Ionized Ca(STAT CHEM8+)</td>
<td></td>
<td>4.5 ml green top PST</td>
<td>BCU Lab or NPHL Lab</td>
<td>Yes</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>LA</td>
<td>5 ml grey top</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Lipase</td>
<td>LIPA</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
</tr>
<tr>
<td>Magnesium</td>
<td>MG</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Malaria</td>
<td>MALP</td>
<td>3 ml lavender top</td>
<td>Hospital Core lab</td>
<td>Yes</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>PO4</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Prealbumin</td>
<td>PAB</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core Lab</td>
<td>Yes</td>
</tr>
<tr>
<td>PT/PTT</td>
<td>Coagulation Panel</td>
<td>1.8 ml or 2.7 ml blue top</td>
<td>BCU lab (Hemochron)</td>
<td>No</td>
</tr>
<tr>
<td>Reticulocyte Count</td>
<td>RETCT</td>
<td>3 ml lavender</td>
<td>Hospital Core lab (DXI)</td>
<td>No</td>
</tr>
<tr>
<td>Sputum Culture</td>
<td>SPUCU</td>
<td>Not applicable</td>
<td>NPHL lab</td>
<td>No</td>
</tr>
<tr>
<td>Standard Ca++</td>
<td>CA</td>
<td>5 ml gold top SST tube, or 4.5 ml green top PST</td>
<td>Hospital Core lab (DXC)</td>
<td>Yes</td>
</tr>
<tr>
<td>Troponin</td>
<td>TROP</td>
<td>6 ml green top PST</td>
<td>Hospital Core lab (DXI)</td>
<td>Yes</td>
</tr>
<tr>
<td>Urine Culture</td>
<td>URNCU</td>
<td>Not applicable</td>
<td>NPHL lab</td>
<td>No</td>
</tr>
<tr>
<td>Urine electrolytes</td>
<td>UNA, UKS,UCLS</td>
<td>BD Urinalysis Plus conical tube</td>
<td>NPHL lab</td>
<td>No</td>
</tr>
</tbody>
</table>

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*SST Gold Top, Serum Separator Tube; Green PST Top, Lithium heparin Plasma Separator Tube; Lavender Top, EDTA anticoagulant; Grey Top, fluoride oxalate; Blue, sodium citrate*

*updated 11-14-14*
BCU Laboratory

Mobile laboratory unit
Major Learning Lessons

• On-site laboratory optimized specimen testing

• Laboratory test menu needed to be flexible

• Not all tests could be performed safely

• Communication was essential
  • Physicians
  • Critical care
  • Infectious diseases
  • Other medical staff
  • Laboratorians

• Laboratory policies/procedures needed to be fluid

• Required an expanded pool of trained laboratorians
Risk of Handling EV-Infected Specimens

- High viral loads in symptomatic patient
  - >100,000,000 pfu/ml
- Infectious dose
  - <10 viable viral particles
- Micro-droplets of blood could easily contain enough virus to cause infection

Figure 1. Ebola virus RNA copy levels in sera over time from 45 Ebola Virus Disease (EVD) patients (27 fatal, 18 non-fatal)\textsuperscript{14}

CDC document, Review of Human-to-Human Transmission of Ebola Virus
Safety Considerations

Interim CDC Recommendations

• “….specimens from (a) PUI for EVD (can safely be handled) by following blood borne and body fluid precautions.”

• “Perform a risk assessment to determine the potential for sprays, splashes, or aerosols generated from lab procedures.”

29CFR Part 1910 No. 1030, OSHA Bloodborne pathogens
“Based on the (risk) assessment, a plan to mitigate the identified risk should be implemented using engineering controls, administrative controls (include work practices) and use of appropriate PPE.”

CDC. 2015. Guidance for U.S. Laboratories for Managing and Testing Routine Clinical Specimens When There is a Concern About Ebola Virus Disease
Results of Our Risk Assessment

- Chemistry automated analyzer
  - Initial centrifugation did not use sealed rotors
- Coagulation automated analyzer
  - Required open tube testing
- Blood Bank
  - Cross matching required open tube centrifugation
- Biosafety cabinets were not universally available

Comment: Not all laboratory sections were able to safely handle specimens from a patient with the potential to have Ebola virus.
Core Laboratory
Automated Chemistry Analyzer

Beckman Coulter
DxC880i
How will Ebola-virus infected specimens be transported to the CDC?

• CDC provided molecular and serological screening to monitor patient progress.

• DOT classified as Category A infectious substances
  • Shipper trained and certified to be a Category A shipper
  • Courier must meet certain certification requirements to ship Category A specimens

• Major commercial couriers would not ship
  • CDC described this as a “nightmare”
Transportation of EV-infected Specimens
Select Agent Issue

CDC Guidance: Compliance with Select Agent Regulations for Labs Handling Specimens Containing Ebola Virus

Are specimens collected from a patient suspected to have Ebola infection covered by the select agent regulations?

No, specimens would not be subject to the Federal select agent regulation until identified as containing Ebola virus by viral isolation.

How do select agent regulations apply to specimens that have tested positive by molecular methods?

The select agent regulations would not apply until the specimen that has tested presumptively positive using molecular methods has been proven to contain live-infectious Ebola virus by virus isolation.

Any specimens that are confirmed by virus isolation to contain live-infectious Ebola virus must be reported to DSAT immediately by telephone (404-718-2000), Email (Irsat@cdc.gov), or FAX (404-718-2096) and be followed up with APHIS/CDC Form 4 within seven days of the initial report.
What level of risk were we willing to accept?

- Knew that specimens contained Ebola virus
- Our “line in the sand”
  - No open-tubed processing or centrifugation would occur outside BSL-3 containment.
    - BCU laboratory
      - Centrifugation
      - POC testing
    - NPHL BSL-3 laboratory
      - Molecular assay
      - Microbiology testing e.g. blood cultures
      - Archive specimens
    - Hospital Core laboratory
      - Closed tubed testing

EUA-Approved PCR Assays

- EZ1 Real-Time RT-PCR Assay (DOD) 8/5/14 m10/10/14
  - Triazole inactivated and not inactivated whole blood and plasma
- Ebola Virus NP Real-Time RT-PCR Assay (CDC) 10/10/14 m3/2/15
  - Whole blood, serum, plasma, and urine (when tested with the other specimens)
- Ebola Virus NP-40 Real-Time RT-PCR Assay (CDC) 10/10/14 m3/2/15
- FilmArray BioThreat-E Test (BioFire Defense, LLC) 10/25/14
  - Whole blood and urine (when tested with blood)
- FilmArray NGDS BT-E Test (BioFire Defense, LLC) 10/25/14 m8/2/15
  - US Department of Defense-specified laboratories
- RealStar Ebolavirus RT-PCR Kit (altona Diagnostics-GmbH) 11/26/14
  - Tests for all 5 species of Ebola virus
- LightMix Ebola Zaire rRT-PCR (Roche Molecular Systems) 12/23/14
  - Whole blood
- Xpert Ebola Assay (Cepheid) 3/23/15
TABLE 3. Comparison of the FilmArray® BioThreat panel and the CDC qRT-PCR assay results for the detection of *Zaire ebolavirus* from whole blood, plasma and urine.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th>Specimen</th>
<th># Specimens</th>
<th>Result</th>
<th>Percent Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>qRT-PCR</td>
<td>FilmArray\textsuperscript{®}</td>
<td>RT+/FA+</td>
<td>RT-/FA-</td>
</tr>
<tr>
<td>Whole Blood</td>
<td>Whole Blood</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Plasma</td>
<td>Whole Blood</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Urine</td>
<td>Urine</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: RT, qRT-PCR; FA, FilmArray\textsuperscript{®}

\textsuperscript{a}Testing was performed using both the FilmArray\textsuperscript{®} BioThreat panel and the CDC real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assay.

\textsuperscript{b}qRT-PCR equivocal results were not included in this table or analysis.

TR Southern, LD Racsa, U Stroher, CG Albarifio, CE Hill, CS Kraft, CN Murphy, AK Mehta, JB Varkey, VL Herrera, AR Sambol, JC Ritchie, EL Ryan, GM Lyon III, PD Fey, SH Hinrichs, BS Bibner, KP Brantly, PC Iwen, and EM Burd. (pending review at the CDC)
Abstracts

• P-06
Herrera V, et al. Experience of the NPHL in response to the Ebola virus public health emergency

• P-07
Stiles K, et al. Transportation of Ebola virus-infected specimens: a PHL’s experience
Acknowledgements

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TEM-kidney  Lung  Hepatocytes

SR Zaki/C Goldsmith (CDC); SH Hinrichs/P Iwen (UNMC)