

2015 APHL™ ANNUAL MEETING

and ninth government environmental laboratory conference

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^a

Test	Laboratory Location^b	Centrifugation Required^c
Essential		
CBC count with automated differential	Core	No
Basic metabolic panel	Core	Yes ^d
Magnesium	Core	Yes
Comprehensive metabolic panel	Core	Yes ^d
Ionized calcium ^e	BCU	No
Standard calcium	Core	Yes ^d
Phosphorous	Core	Yes
Cortisol	Core	Yes
Troponin	Core	Yes
Blood gases ^e	BCU	No
Lactate	Core	Yes ^d
Protime ^e	BCU	No
Partial thromboplastin time ^e	BCU	No
Platelet count	Core	No
Blood typing ^{f,g}	BCU	No
Culture procedures ^h	NPHL ⁱ	No
Molecular assay ^j	NPHL ⁱ	No
Supplemental		
Manual differential	Core	No
Lipase	Core	Yes
Amylase	Core	Yes
Creatine kinase total	Core	Yes
Malaria smear ^k	Core	No
HIV screen	Core	No

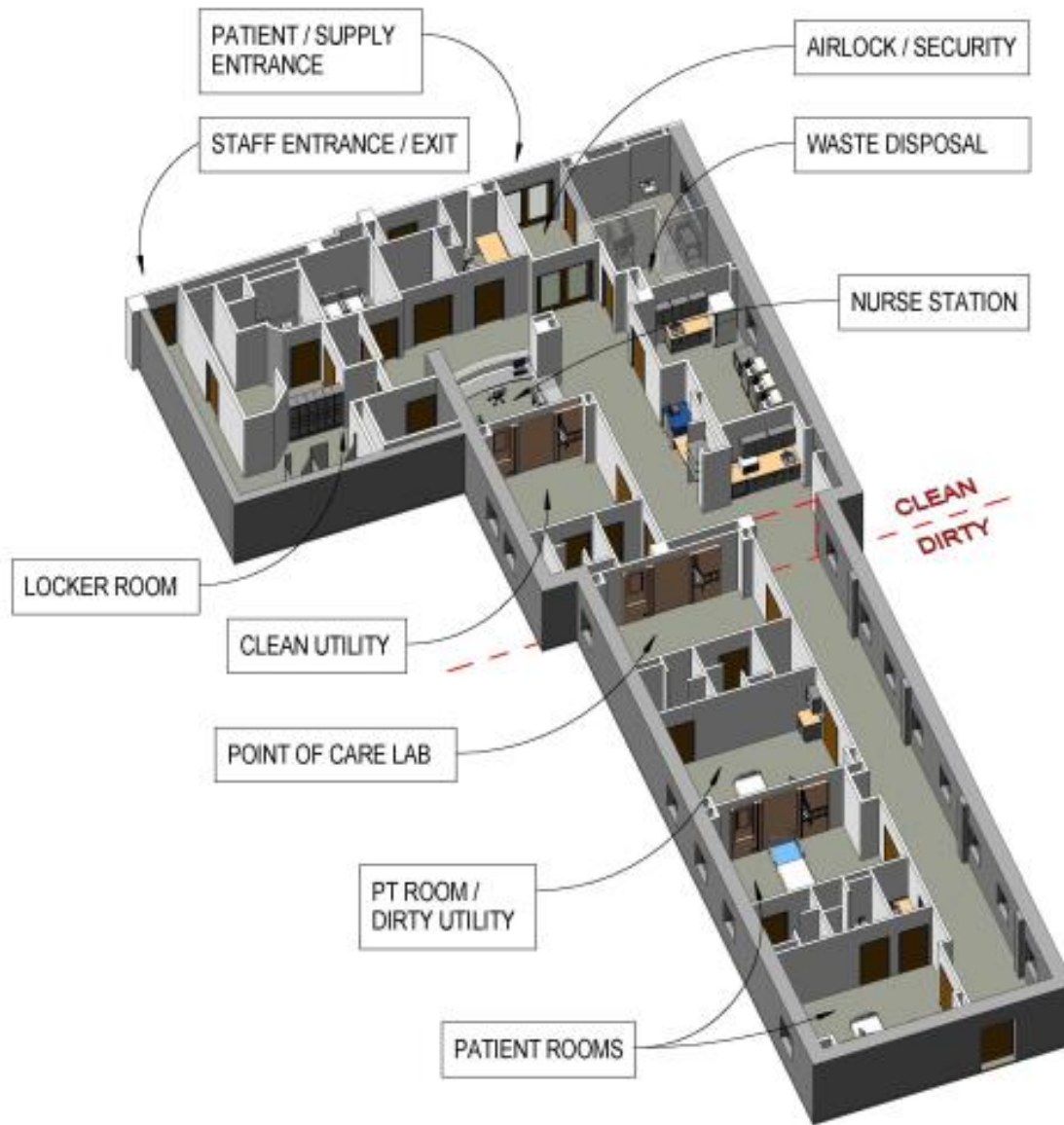
Be flexible!

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

Test	Order Code	Tube type	performed at (Instrument)	Centrifugation (NPHL)
Amylase	AMY	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
B12 level	VB12	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
Blood culture	BLDCU	Plastic Aerobic Bactec bottle	NPHL lab	No
Blood Gas arterial	POC113	Heparinized blood gas syringe	BCU lab (iStat)	No
Blood Gas venous	POC114	4.5 ml green top PST	BCU lab (iStat)	No
Blood type	ABORH	3 ml lavender top	BCU Lab (slide forward type)	No
Basic metabolic panel	BMET	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXC)	Yes
CBC with automated diff	CBCP	3 ml lavender top	Hospital Core Lab (Sysmex)	No
CBC with manual diff	CBCM	3 ml lavender top	Hospital Core lab (DXI)	No
Comp metabolic panel	CMET	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXC)	Yes
Cortisol	CORTS	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
Creatine kinase Total	CK	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
C-Reactive protein	CRP	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core Lab	Yes
DIC screen (see note below)		3 ml lavender top	Hospital Core Lab (Sysmex)	No
NOTE: Lab will provide platelet count and examination of peripheral smear for schistocytes to be used in conjunction with coag results from BCU lab				
Drug Study experimental	No test code	5ml lavender (Qty 1) top OR 3ml lavender top (Qty 2)	BCU Lab or NPHL Lab	Yes
Fe/Ferritin/TIBC	TIBC	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
Folate level	VFOL	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core Lab	Yes
Sputum Culture	HPT	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
HIV	SUD	5 ml red top		No
Ionized Ca(STAT CHEM8+)		4.5 ml green top PST	BCU lab (iStat)	No
Lactic Acid	LA	5 ml grey top	Hospital Core lab (DXC)	Yes
Lipase	LIPA	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXI)	Yes
Magnesium	MG	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXC)	Yes
Malaria	MALP	3 ml lavender top	Hospital Core lab	BCU lab will prepare smears
Phosphorus	PO4	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXC)	Yes
Prealbumin	PAB	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core Lab	Yes
PT/PTT	Coagulation Panel	1.8 ml or 2.7 ml blue top	BCU lab (Hemochron)	No
Reticulocyte Count	RETCT	3 ml lavender	Hospital Core lab (DXI)	No
Sputum Culture	SPUCU	Not applicable	NPHL lab	No
Standard Ca++	CA	5 ml gold top SST tube, or 4.5 ml green top PST	Hospital Core lab (DXC)	Yes
Troponin	TROP	6 ml green top PST	Hospital Core lab (DXI)	Yes
Urine Culture	URNCU	Not applicable	NPHL lab	No
Urine electrolytes	UNA, UKS,UCLS	BD Urinalysis Plus conical tube	NPHL lab	No



3D Illustration

Bio Containment Unit

LEO A DALY

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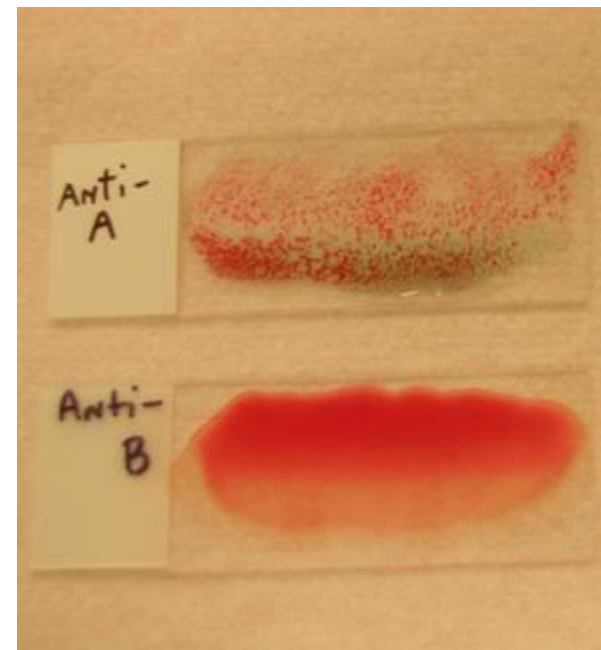
BCU Laboratory



Mobile laboratory unit

Major Learning Lessons

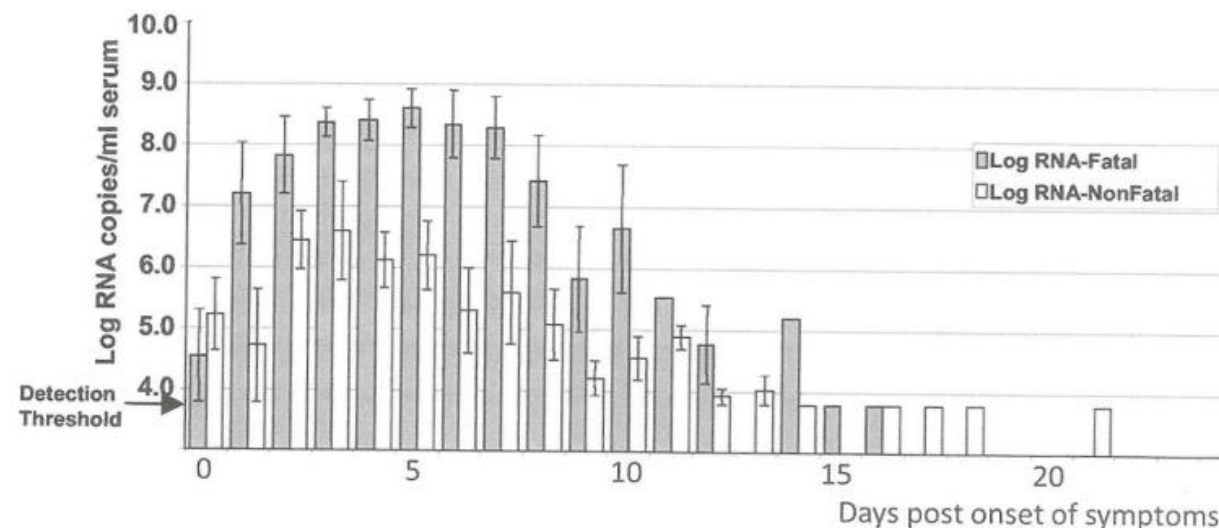
- On-site laboratory optimized specimen testing
- Laboratory test menu needed to be flexible
- Not all tests could be performed safely
- Communication was essential
 - Physicians
 - Critical care
 - Infectious diseases
 - Other medical staff
 - Laboratorians
- Laboratory policies/procedures needed to 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)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.”

Most Recent CDC Guidance

30Jan2015

“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



INVOICE

REMITTANCE ADVICE
PLEASE MAIL PAYMENT TO:

WORLD COURIER INC.
P.O. BOX 842325
BOSTON, MA 02284-2325

313 FOURTH AVE., NEW HYDE PARK, NY 11040-5541
PHONE 516 354-2600
TOLL FREE 800-223-4461
FAX # 516 354-2879
EIN # 13-2682321

QUESTIONS ON THIS INVOICE?
PLEASE CALL 800-223-4461

INCLUDE TOP PORTION WITH YOUR REMITTANCE

DATE 10/01/14	INVOICE 063737
UNIVERSITY OF NEBRASKA ATTN: ELLEN DEYSEN 586805 NEBRASKA MEDICAL CTR OMAHA, NEBRASKA 68198	ACCOUNT# 11519 INVOICE AMT 1,900.80 BALANCE DUE: 1,900.80 PAGE 1

SHIPPER	CONSIGNEE	DESCRIPTION	TOTAL
09/22/14 HMD#: 101186591 Caller: Karen Stiles Ref: 3353013476	Nebraska Public Health / Centers for Disease Karen Stiles/Peter I / ATTN STAT LAB: USP 560 South 45th Street / 1600 Clifton Road DRC #2 Loading Dock OMAHA NE / ATLANTA GA United States / United States Signed: Latagna Green	PCS 1 WGT 24.0 BASE RATE 240.00 ORIG- CHICAGO 1,334.00 DANGEROUS GOODS, DTD 75.00 AIRLINE SECURITY/ FUEL 251.80	USD 1,900.80

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Select Agent Issue

Monday, September 08, 2014

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



9052

'Select agents' are those pathogens that have been deemed to pose a significant biological hazard, and among those, **Tier 1 select agents are considered** 'biological agents and toxins present the greatest risk of deliberate misuse with significant potential for mass casualties or devastating effect to the economy, critical infrastructure, or public confidence, and pose a severe threat to public health and safety.'

Tier 1 Select Agents and Toxins		
HHS Agents and Toxins	Overlap Agents	USDA Agents
Botulinum neurotoxins Botulinum neurotoxin producing species of Clostridium Ebola virus Francisella tularensis Marburg virus Variola major virus (Smallpox virus) Variola minor virus (Alastrim) Yersinia pestis	Bacillus anthracis Burkholderia mallei Burkholderia pseudomallei	Foot And Mouth Disease virus Rinderpest virus

As you might have already guessed, the various species of Ebolavirus are Tier 1 select agents, which makes everything contaminated (*by blood, sweat, feces, urine, etc.*) by an Ebola infected patient potentially a select agent – and subject to rigorous safety regulations – as well

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.

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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

Iwen, PC, et al. 2015. An integrated approach to laboratory testing for patients with Ebola virus disease (Special Rpt). Lab. Med. 45: e146-51.

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.^{a,b}

Specimen		# Specimens Evaluated	Result				Percent Agreement
qRT-PCR	FilmArray®		RT+/FA+	RT-/FA-	RT+/FA-	RT-/FA+	
Whole Blood	Whole Blood	22	17	4	0	1	95.4%
Plasma	Whole Blood	15	8	5	0	2	86.7%
Urine	Urine	9	5	3	0	1	88.9%

Abbreviations: RT, qRT-PCR; FA, FilmArray®

^aTesting was performed using both the FilmArray® BioThreat panel and the CDC real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assay.

^bqRT-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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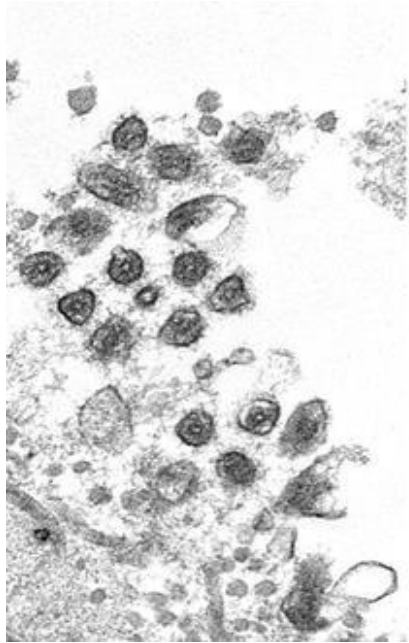
David Moran

Caitlin Murphy, PhD

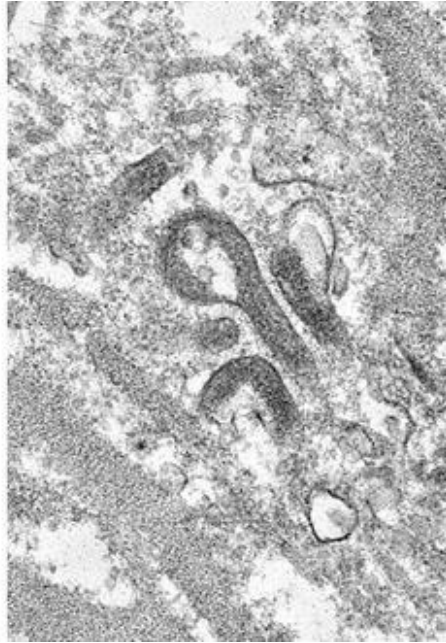
Robbie Southern, PhD

Steve Hinrichs, MD

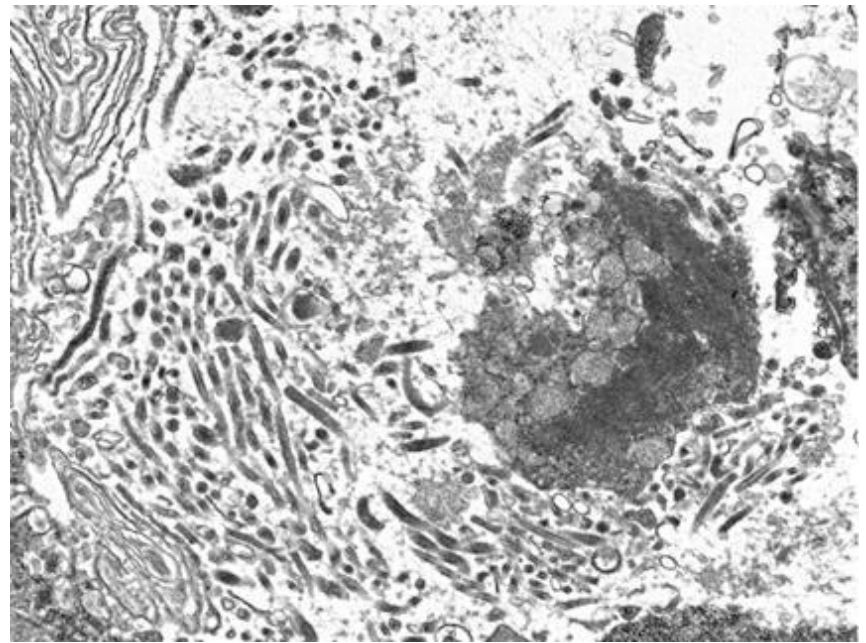
Bin Li



TEM-kidney



Lung



Hepatocytes

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