



The Pathologic Perspective on Ebola Virus Disease

James C Ritchie, Ph.D.

On behalf of the

Serious Communicable Diseases Unit

Emory University Hospital

DISCLOSURE STATEMENT

Speaker: **James C. Ritchie, Ph.D.**

Dr. Ritchie has disclosed the following financial relationships. Any real or apparent conflicts of interest related to the content of this presentation have been resolved.

Research / Educational Grants

BeckmanCoulter, Inc

Roche Diagnostics

T2 Biosciences

Chromsystems

BioRad

Sebia



The Cases at Emory

- July 29th , we were informed that two humanitarians from the US had become infected with EBOV in Liberia
- During the first week of August, both were transferred by air ambulance to our hospital, 3 days apart
 - 33 yo male physician, day 11 of illness at arrival
 - 59 yo female missionary, day 15 of illness at arrival

AACC in Chicago July 27 - 7/31/14





Clinical Pearls

- There are no proven therapeutics
 - No clear idea of the availability of any experimental agents when we started
 - Limited safety or efficacy data in humans
 - Great support and advice from CDC, FDA, and medical and scientific colleagues throughout the world
- With the help of the CDC, we monitored EBOV viral loads in blood by PCR
 - Progressive declines in viral loads that correlated with improvements in clinical condition
 - Had very low level of nucleic acid detection for several days despite resolution of symptoms



Clinical Pearls

- Our patients had marked electrolyte abnormalities and nutritional deficiency
 - Laboratory testing for chemistries was critical for patient specific care
 - Hypokalemia, hypocalcemia and hyponatremia to varying degrees
 - Required both intravenous and oral replacement
 - Both required significant potassium replacement
 - Used oral supplements including nutritional drinks high in easily absorbed proteins, minerals and vitamins



Clinical Pearls

- The ability to provide high-level nursing care and supportive care made a significant impact
 - 24/7 one-on-one nurses allowed for rapid response to changes and adjustment of care
 - Ability to support patients in nutrition, physical therapy, and self care
 - Emotional support
- Enabling safe interactions between family and patients was also beneficial
 - Glass window and intercom/phone system



Serious Communicable Disease Unit





SCDU CLINICAL LABORATORY

- Established in 2003 under contract with CDC
- Directed by Dr. C. Fantz until 2013
- Originally intended to treat CDC personnel only
- Provided 24/7 on-call coverage by a physician, nurse, and medical technologist
- Staff trained every six months
- Activated twice in 12 years



Clinical Laboratory: Operations

- The SCDU had previously established a small point of care lab within the anteroom
- However after the unit was activated, we realized it would become too crowded
 - Commandeered an office adjacent to anteroom
 - Facilities and Engineering built a complete point of care lab facility in **less than 72 hours**
- **CDC: A dedicated lab is NOT required to work with blood from patients with Ebola**

Laboratory Test Support for Ebola Patients Within a High-Containment Facility

Charles E. Hill, MD, PhD,¹ Eileen M. Burd, PhD,¹ Colleen S. Kraft, MD,¹ Emily L. Ryan, PhD,¹ Alexander Duncan, MD,¹ Anne M. Winkler, MD,¹ John C. Cardella,² James C. Ritchie, PhD,¹ Tristram G. Parslow, MD, PhD^{1}*

Lab Med Summer 2014 45:e109-e111

- Chemistry analyzer (Abaxis Piccolo Xpress)
- Arterial blood-gas analyzer (GEM Premier 4000)
- Automated urinalysis analyzer (CLINITEK)
- Coagulation analyzer (INR only) (CoaguChek)
- Hematology analyzer (poch 100i)
- Malaria POC device (Alere BinaxNOW)

• Advantages

- Less fear among hospital and lab staff
- Less impact on staffing and patient care
- Immediate results (really STAT)

• Disadvantages

- Limited test panel
- If machine goes down, no back up
- Costs of maintaining equipment rarely used



**Patient Testing Request Form –
Isolation Laboratory**

Patient Identification (Name, ID#, Stamp Plate)

Indicate the testing desired below. Deliver to Isolation Laboratory staff as soon as possible or page POCC on call at 63495. Laboratory staff will notify nursing when SCU lab is up and ready for sample collection.

Date and Time testing desired: Date: ____/____/____ Time: ____:____

Requested by: _____

Contact Number to call any critical results: SCU Ante-Room (404-712-7265) Other _____

Hematology: CBC (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT)
(lavender/EDTA)

Chemistry: Comprehensive Metabolic Profile
(green/heparin) (NA, K+, CO2, CL-, GLU, CA, BUN, CREAT, ALP, ALT, AST, TBILI, ALB, TP)

Magnesium LD (Lactate Dehydrogenase)

GGT Amylase Lactate Phosphorous

Blood Gas: Venous (pH, pCO2, pO2, Na+, K+, Cl-, Ca++, Glu, Lac, Hct, tHb, O2Hb,
(green/heparin) Arterial COHb, Methb, BE)

Urinalysis: Urinalysis
(yellow/plain)

PCR: GI Panel Sample Type Stool (Cary-Blair Medium)

BT Panel Sample Type(s) Blood (EDTA) Urine
 Stool (Cary-Blair medium)
 Semen

Respiratory Panel Sample Type Nasopharyngeal Swab (viral transport medium)

Parasitology: Malaria
(lavender/EDTA)

POCT: INR Glucose

Additional Sample Needs: (list information of what/how many additional sample types and tubes are needed for sendout/CDC) _____

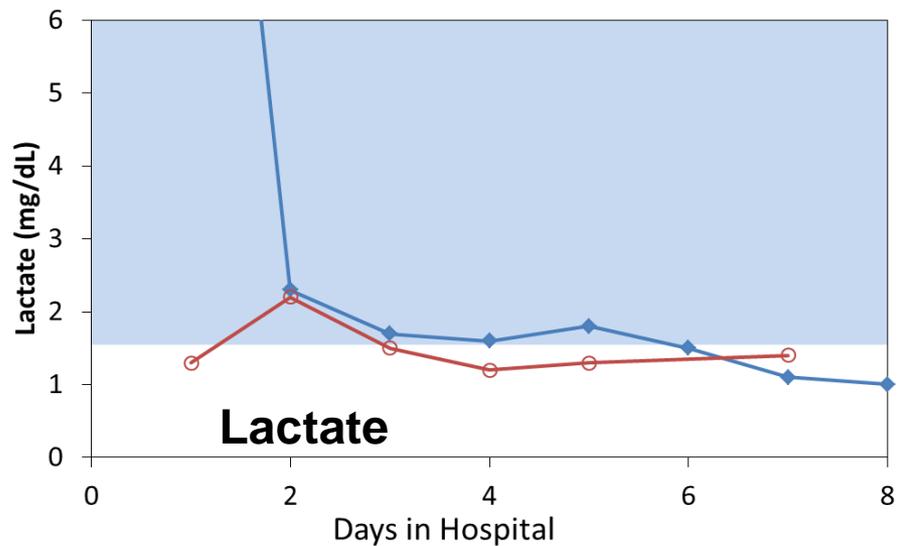
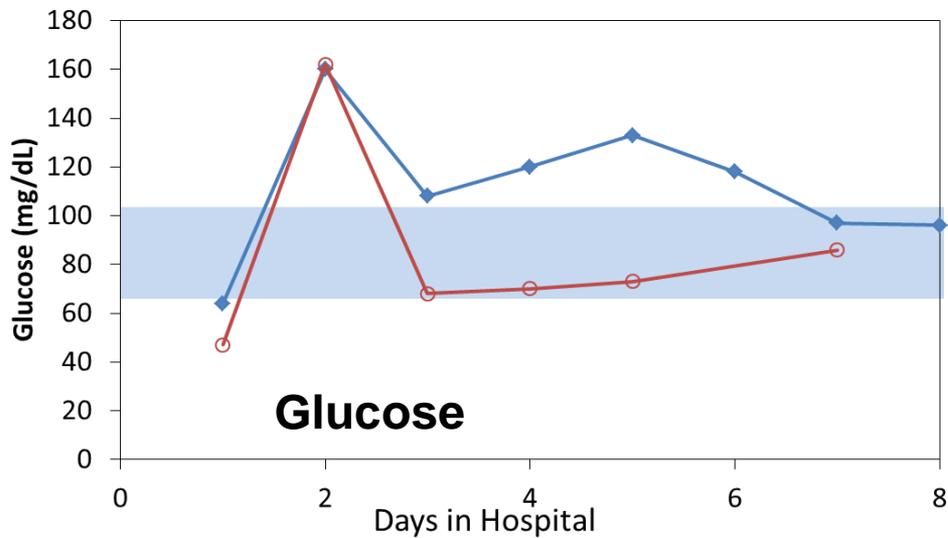
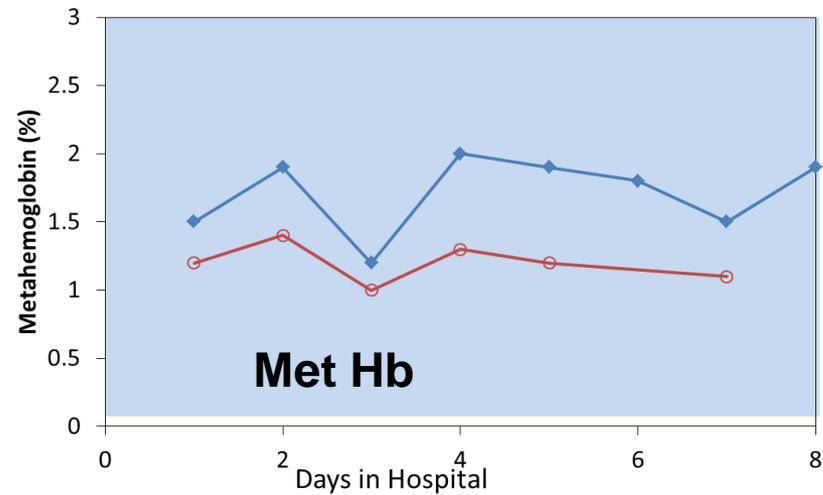
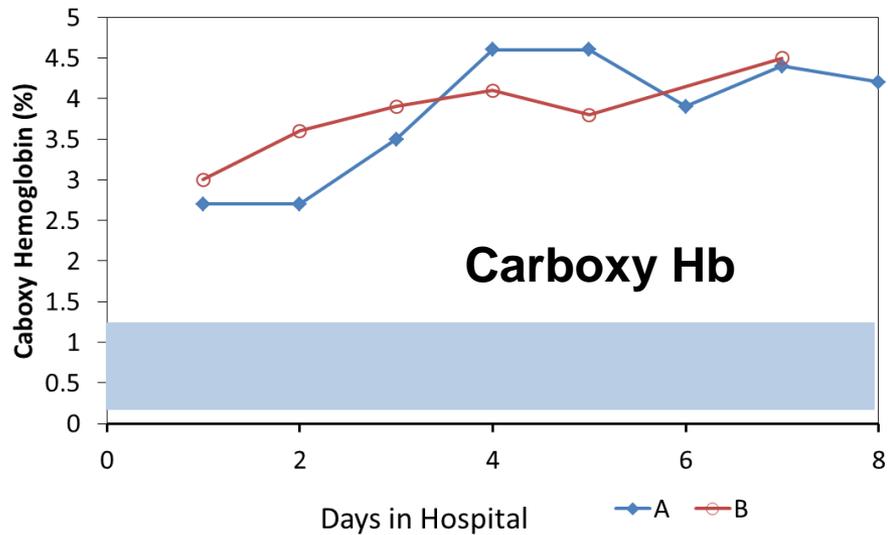
Laboratory Address and CLIA Director:

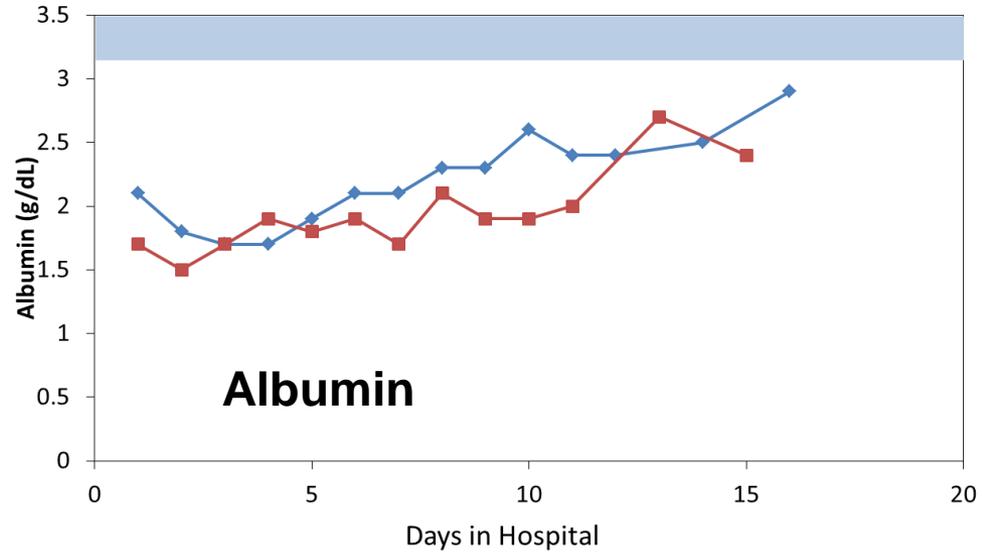
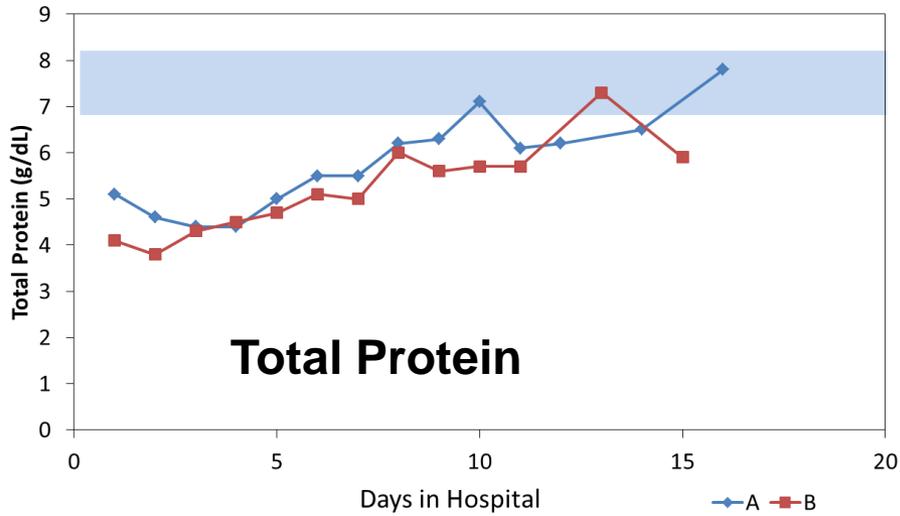
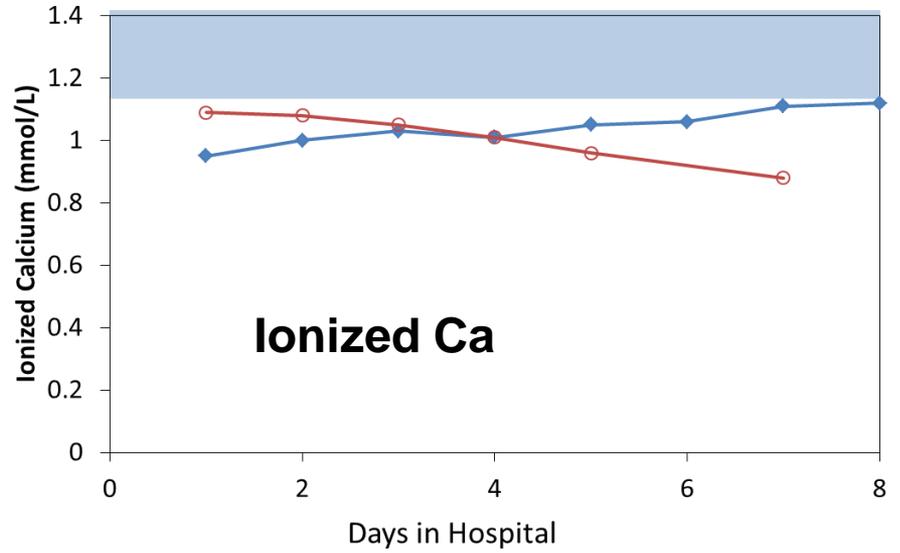
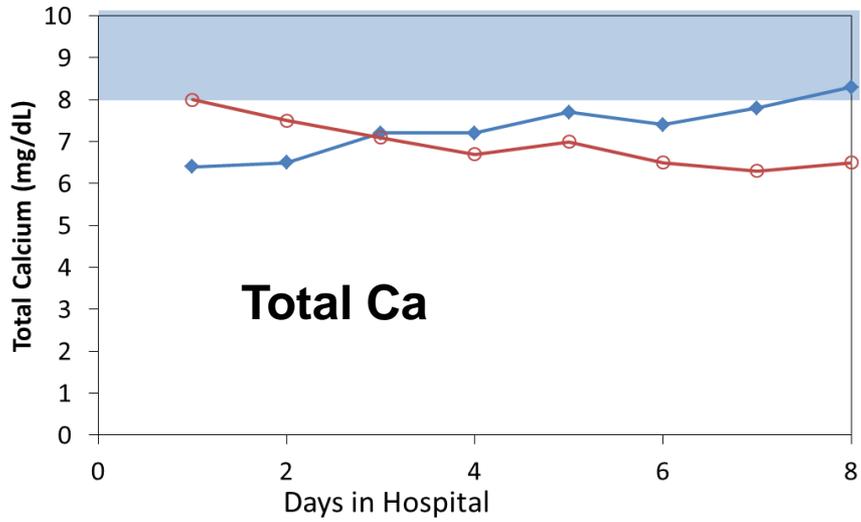
Emory Medical Laboratory at Emory University Hospital, 1364 Clifton Rd NE, Atlanta, GA 30322; James C. ~~Wicks, PhD~~ – Laboratory Director

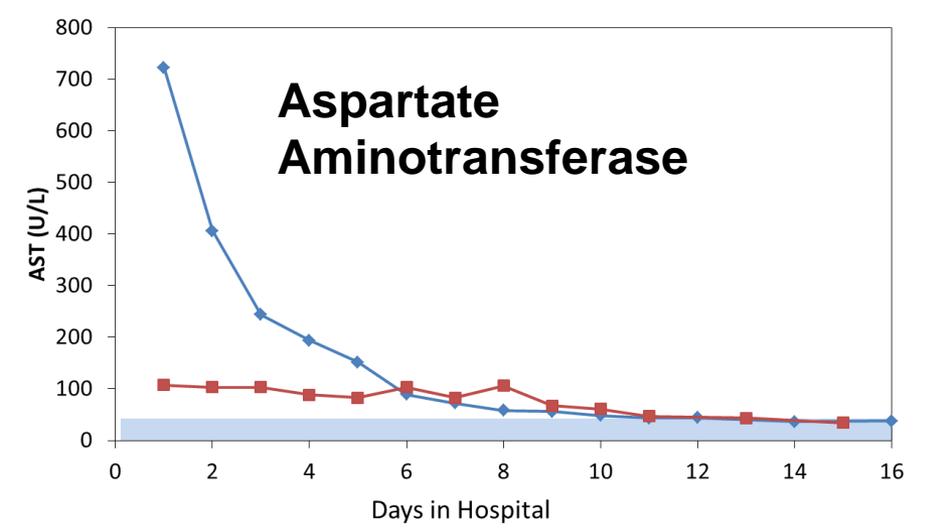
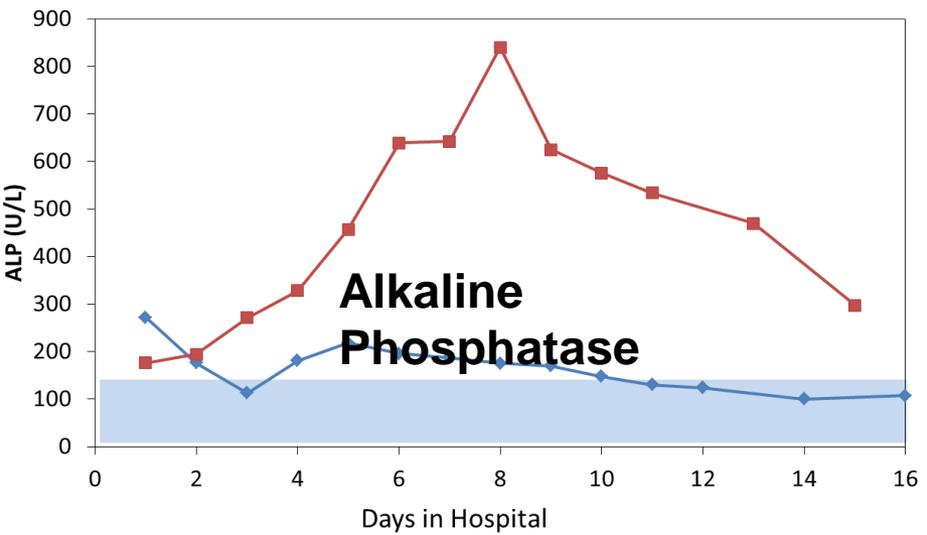
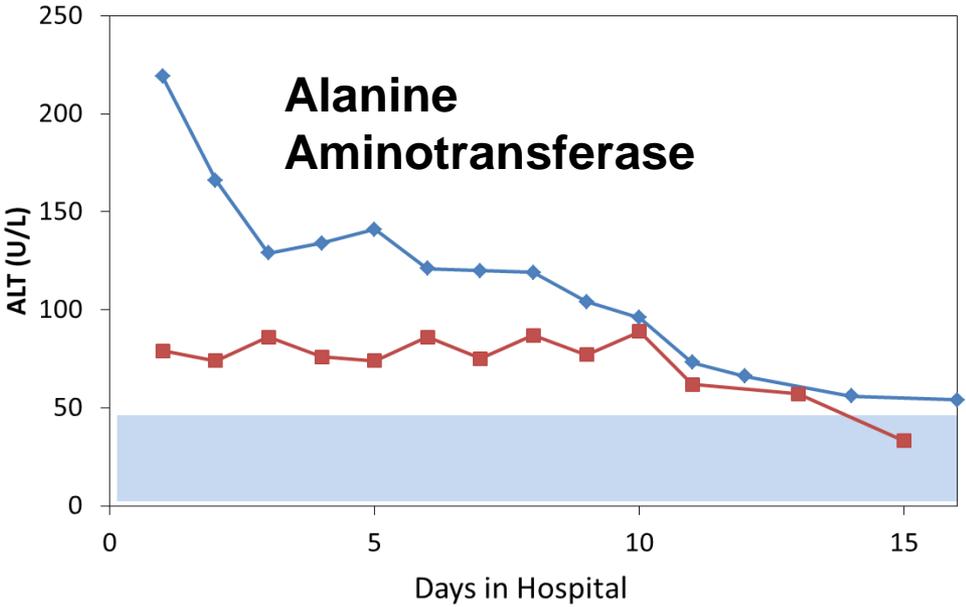
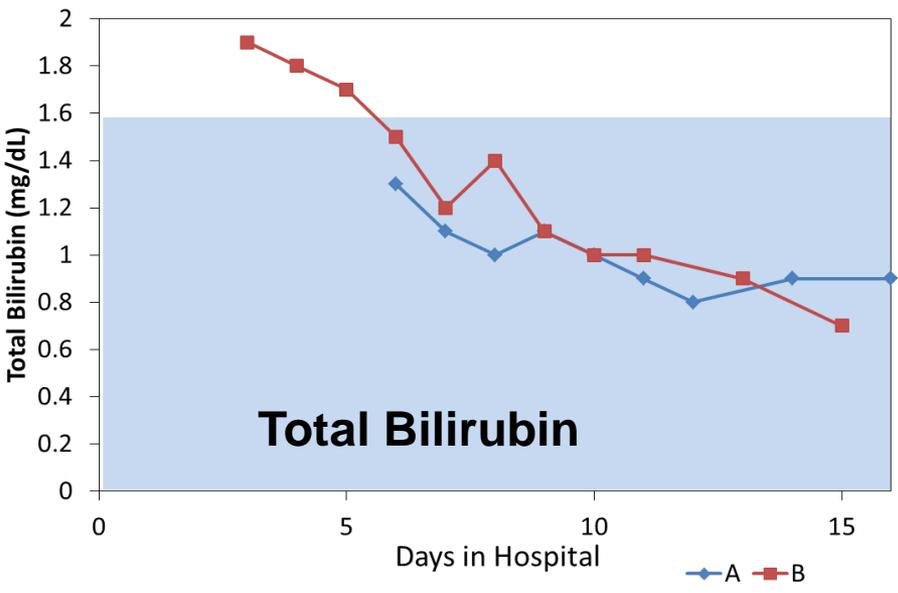
VER912.14

Our First Two Patients:

- Patients were admitted 10 days (A) and 12 days (B) after symptoms.
- At admission blood gases and comprehensive chemistry panel were run.
- Menu expanded to meet the needs of each patient
- Testing done from once per day to up to Q6 hrs depending on patient care needs









Clinical Laboratory: The Impact of Electrolytes

- Our patients had MARKED electrolyte abnormalities and nutritional deficiencies
 - Hypokalemia, hypocalcemia and hyponatremia
 - Required both intravenous and oral replacement
 - Both required **significant** potassium replacement
 - Laboratory testing for chemistries was **critical** to provide supportive care
 - Used oral nutritional supplements including nutritional drinks high in easily absorbed proteins, minerals and vitamins

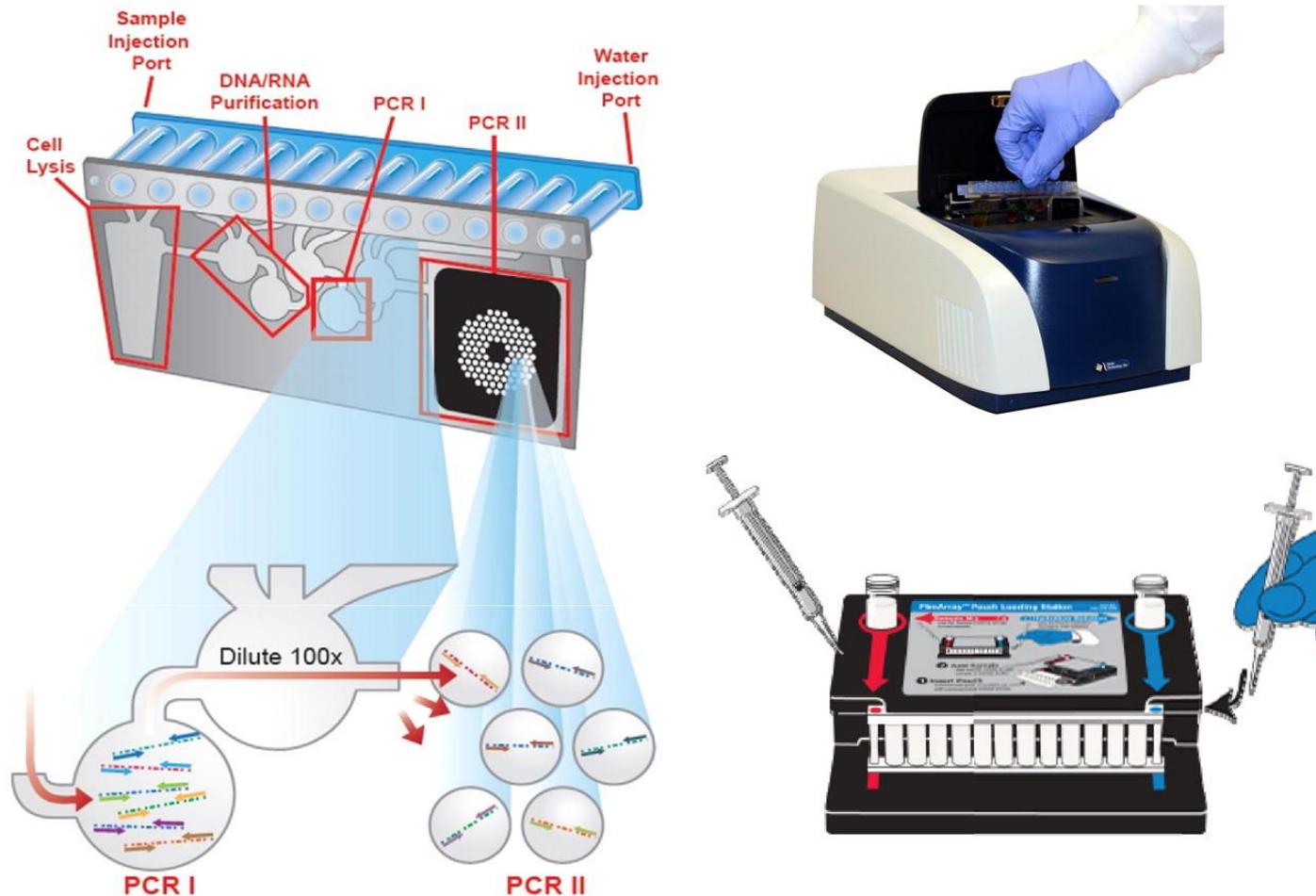


Clinical Laboratory: FilmArray[®] BioThreat Panel

- Fully automated PCR technology, combining nested and multiplex PCR with internal controls followed by melt curve analysis
- Samples are injected directly into a reaction pouch without nucleic acid extraction and the process is complete in one hour

The dried reagents in the FilmArray RP pouch are reconstituted by the addition of 1 ml distilled water to the blue port (lower right of diagram), and the diluted sample is injected into the port shown in red.

The FilmArray Pouch



Rand K H et al. J. Clin. Microbiol. 2011;49:2449-2453

Journal of Clinical Microbiology

Detected: Ebola Zaire	Controls: Passed
Equivocal: None	

Result Summary	
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Not Detected	<i>Bacillus anthracis</i>
Not Detected	<i>Brucella melitensis</i>
Not Detected	<i>Burkholderia mallei/pseudomallei</i>
Not Detected	<i>Clostridium botulinum</i>
Not Detected	<i>Coxiella burnetii</i>
Not Detected	<i>Francisella tularensis</i>
Not Detected	<i>Ricinus communis</i>
Not Detected	<i>Rickettsia prowazekii</i>
Not Detected	<i>Staphylococcal enterotoxin gene</i>
Not Detected	<i>Yersinia pestis</i>
✓ Detected	Ebola Zaire
Not Detected	Marburg virus
Not Detected	Orthopox genus virus
Not Detected	Variola virus
Not Detected	EEE virus
Not Detected	VEE virus
Not Detected	WEE virus

Run Details	
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Pouch: BioThreat Panel v2.4	Protocol: BT Blood v2.0
Run Status: Completed	Operator: Karen Jenkins (MPLJKM)
Serial No.: 01532571	Instrument: ITI FA "FA2702"
Lot No.: 140522C	



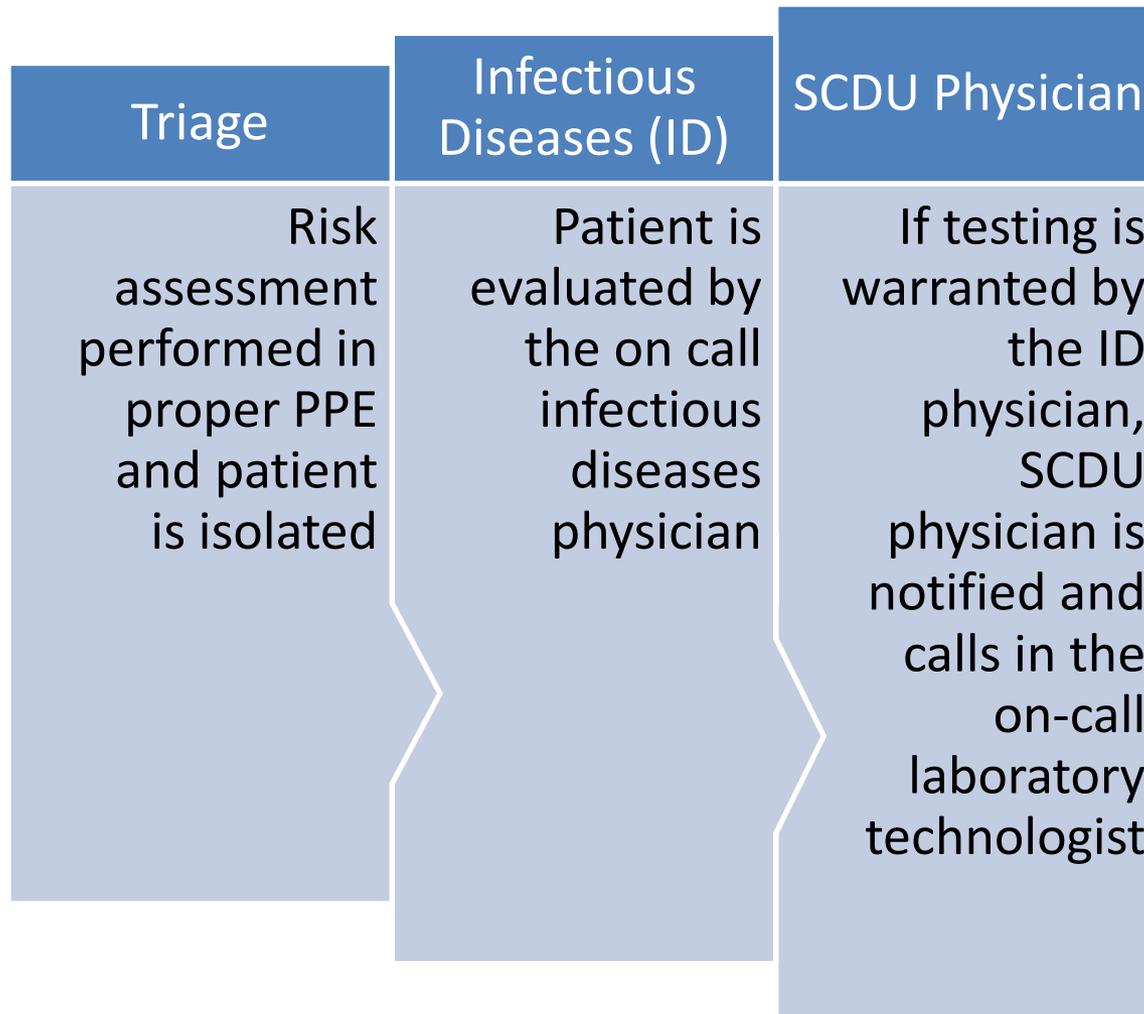
Clinical Laboratory: Pre-analytical considerations



- Found that the Ebola virus result in the plasma component cleared before the whole blood component
- Ebola virus, like other filoviruses, preferentially infects certain cell types, including blood monocytes, and this discrepancy in whole blood may be due to cell associated viral nucleic acids in these blood cells



Clinical Laboratory: “Rule out Ebola” testing





Clinical Laboratory: Interfering Substances

Hospital Day	Sample Time	Hemolysis	Lipemia	Icterus
1	14:31	0	0	0
2	14:47	0	0	0
3	9:06	0	1+	0
4	9:42	2+	1+	0
5	9:40	2+	2+	0
6	9:58	2+	1+	0
7	9:25	3+	3+	0
8	9:44	3+	3+	0
9	11:07	3+	3+	0
10	7:14	3+	3+	0
11	22:13	3+	3+	0
12	22:30	3+	3+	0
13	0:12	3+	3+	0
14	6:42	3+	3+	0
Scale:	0 = Clear			
	1+ = slight			
	2+ = moderate			
	3+ = gross			

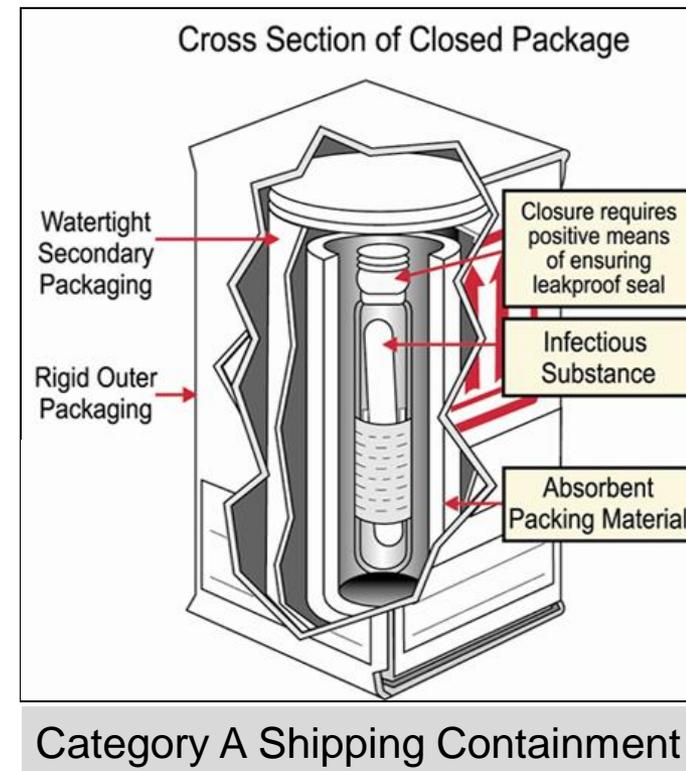


Introduction of TPN



Clinical Laboratory: Shipping

- Limited staff trained in Category A shipping
 - Not part of the unit team
 - Reluctant to come to unit
 - Emory U. safety officers trained members of our team
- Commercial couriers, even those certified in Category A shipping, refused to pick up anything from Emory destined for CDC





Clinical Laboratory: Reporting

- Division of Select Agents and Toxins (DSAT) is located in CDC's Office of Public Health Preparedness and Response (OPHPR)
- Upon viral isolation, the corresponding clinical samples also become select agents
- Documentation for all positive samples as well as their destruction

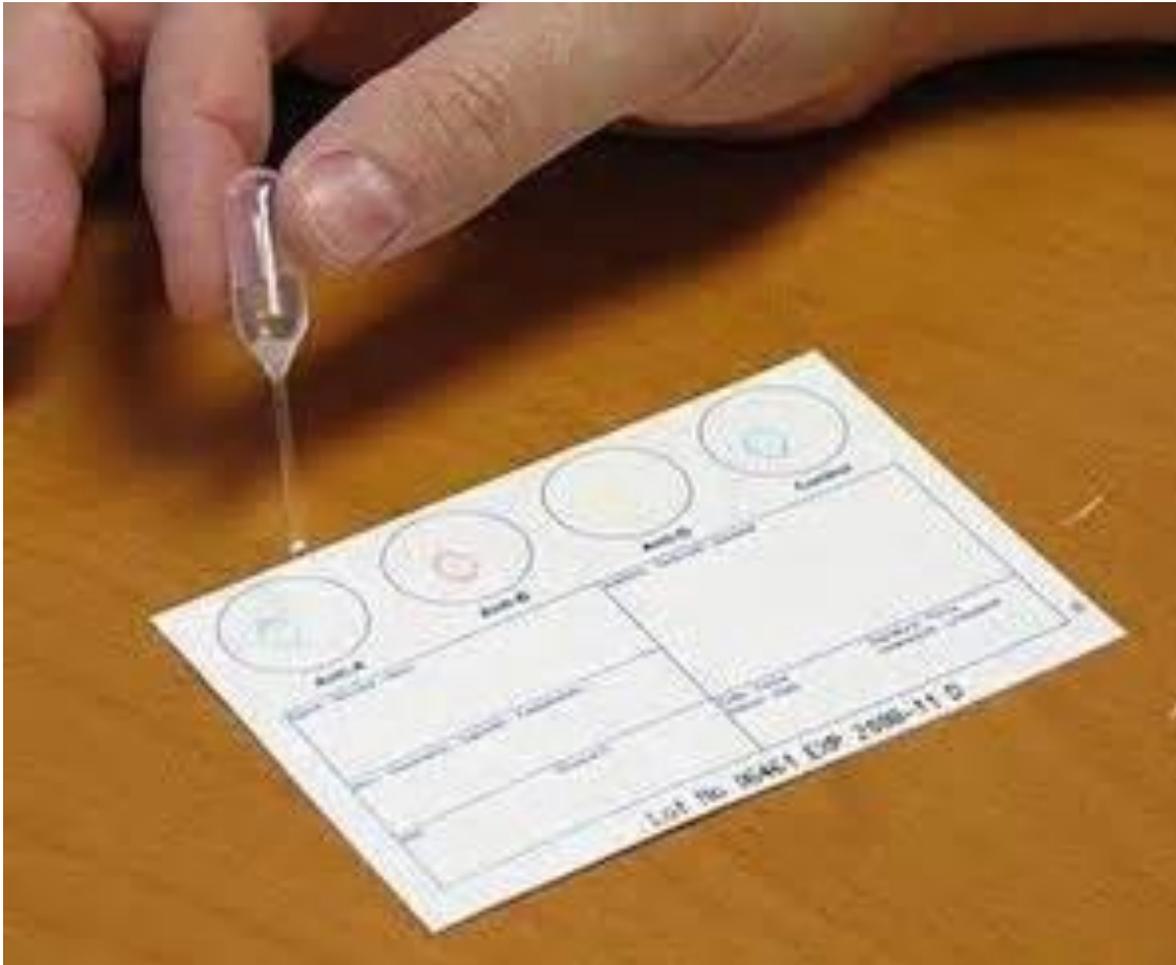




COAGULATION/TRANSFUSION



Transfusion Medicine: Blood Typing



<http://www.eldoncard.com/>



Transfusion Medicine: Convalescent Plasma

- Nebraska patient received convalescent plasma from recovered patient
- Underwent plasmapheresis (with albumin replacement) at their center of 1 L of plasma, and given in 2 aliquots 24 hours apart
- By definition, are ineligible donors, and this requires eIND from FDA



Coagulation: Strategies

- Can only test INR and platelet count
- Strategies
 - Supportive RBC transfusion (O negative uncrossmatched)
 - Aggressive platelet transfusion ($>50,000$)
 - Supportive coagulation factor replacement with FFP



MICROBIOLOGY



Microbiology: CDC guidelines

Blood Cultures:

Perform only if required and minimize blood draws for blood cultures. Use plastic bottles if available.

Once received in the laboratory, all specimens should be opened as described above in General Considerations: E. Wipe the outside of the bottles with bleach and inspect for any signs of breakage and positivity before loading onto the blood culture instrument or placing into an incubator for manual incubation. If the blood culture bottles are flagged as positive, or if they show any sign of positivity upon visual inspection, unload the bottles from the instrument or remove from the incubator, place the bottle(s) into a double-bag that contains absorbent pads soaked with bleach, place in a biohazard rigid plastic impervious container and process as described above in General Considerations: E.

1. Prepare slides for Gram stain examination and allow them to dry.
2. Fix the blood smear in methanol for 30 minutes, followed by dry heat at 95°C for 1 hour to inactivate the specimen. Perform testing of the gram stain QC smear in this same manner.
3. The smears can then be stained and read as usual.

Do not perform any direct testing on positive blood cultures.

Inoculate plates as per protocol based on Gram stain result.

1. Use shrink seal (Parafilm® or other suitable plate wrap) on all sub-cultured plates, place plates in a biohazard baggie and incubate in the AFB suite (if available) in the 35°C CO₂ incubator.
2. Examine plates for growth twice per day.
3. Perform all spot testing and inoculations of appropriate ID/AST systems from isolated colonies. AS AN OPTION: If any growth occurs, subculture the organism (as described above in General Considerations: E.) onto fresh plates and incubate overnight. Work only from the sub-cultured plates to minimize risk of contact with blood from the patient.



Organisms Detected: Applicable Antimicrobial Resistance Genes:	1030 None	Controls:	3:26 PM Passed
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Result Summary - Interpretations

Antimicrobial Resistance Genes

<input type="checkbox"/> N/A	KPC (carbapenem-resistance gene)
<input type="checkbox"/> N/A	<i>mecA</i> (methicillin-resistance gene)
<input type="checkbox"/> N/A	<i>vanA/B</i> (vancomycin-resistance genes)

⚠ NOTE: Antimicrobial resistance can occur via multiple mechanisms. A Not Detected result for the FilmArray antimicrobial resistance gene assays does not indicate antimicrobial susceptibility. Subculturing is required for species identification and susceptibility testing of isolates.

Gram Positive Bacteria

Not Detected	<i>Enterococcus</i>
Not Detected	<i>Listeria monocytogenes</i>
Not Detected	<i>Staphylococcus</i>
Not Detected	<i>Staphylococcus aureus</i>
Not Detected	<i>Streptococcus</i>
Not Detected	<i>Streptococcus agalactiae</i> (Group B)
Not Detected	<i>Streptococcus pneumoniae</i>
Not Detected	<i>Streptococcus pyogenes</i> (Group A)

Gram Negative Bacteria

Not Detected	<i>Acinetobacter baumannii</i>
Not Detected	<i>Enterobacteriaceae</i>
Not Detected	<i>Enterobacter cloacae</i> complex
Not Detected	<i>Escherichia coli</i>
Not Detected	<i>Klebsiella oxytoca</i>
Not Detected	<i>Klebsiella pneumoniae</i>
Not Detected	<i>Proteus</i>
Not Detected	<i>Serratia marcescens</i>
Not Detected	<i>Haemophilus influenzae</i>
Not Detected	<i>Neisseria meningitidis</i>
Not Detected	<i>Pseudomonas aeruginosa</i>

Yeast

Not Detected	<i>Candida albicans</i>
Not Detected	<i>Candida glabrata</i>
Not Detected	<i>Candida krusei</i>
Not Detected	<i>Candida parapsilosis</i>
Not Detected	<i>Candida tropicalis</i>

Run Details

Pouch:	BCID Panel v2.0	Protocol:	BC v2.1
Run Status:	Completed	Operator:	brenda eaves (mpllbg)
Serial No.:	00825295	Instrument:	ITI FA "FA2702"
Lot No.:	136313		



OTHER CONSIDERATIONS



Autopsy considerations

- Would contact and have CDC Pathology Branch perform the autopsy in the room of the Unit



Operations:

Staff & Environmental Safety

- Single patient room with a private bathroom
 - Maintain a log of persons entering the patient's room
- Dedicated medical equipment
 - Disposable, when possible
- Personal Protective Equipment (PPE)
 - All persons entering the patient room should wear at least:
 - Gloves
 - Gown (fluid resistant or impermeable)
 - Eye protection (goggles or face shield)
 - Facemask
 - Additional PPE might be required in certain situations depending on patient and environment, including but not limited to:
 - Double gloving
 - Disposable shoe covers
 - Leg coverings



Operations: Waste Management

- Patient 1: Admitted for 19 days
- Patient 2: Admitted for 14 days

- Autoclaved **350** bags of regulated medical waste
 - ***Total weight: 3,058 lbs***

- Packaged **218** boxes of regulated medical waste

- 6 shipments of regulated medical waste were transported for incineration



Operations: Staff & Environmental Safety

- Team meeting everyday to review plans and protocols
 - Open forum for questions and concerns from any team member
- Only approved personnel allowed in isolation area
 - Log of everyone who entered room
- All personnel required to enter twice daily temperature and symptom review into a online registry
 - 21 day after last shift in the unit

Direct Healthcare Provider (including Lab Personnel)

Symptom Questionnaire (Ebola Virus)

* Required

Employee Name: *

Employee ID #

Date: *

Month Day 2014

Time: *

Hr : Min AM

Cell Phone Number

Best Contact #

1) Temperature *

in degrees F

2) Nausea/Vomiting *

Yes

No

2.1) If yes, please explain:

3) Diarrhea: *

Yes

No

3.1) If yes, please explain:

4) Headache: *

Yes

No



Internal Communications

- Open forums for all physicians and staff
 - Twice a day for 3 consecutive days
 - Every other day for 1st week
- Hospital administration, nursing administration and hospital epidemiology leaders available for Q & A.
- Key leaders rounded on the floors to answer questions from staff AND patients
- Patient education video

Teamwork

- When you work in the regular lab what another tech does isn't important to you.
- “In the SCDU you have to worry what the other person does before you, what the person does after you and what you are going to do, because one mistake can kill us all”
- That is how the **Team** began to think of its self as a **Family**

Family Rules

1. I will follow all SOP's
2. I will ensure that others follow all SOP's
3. I will report any new signs and symptoms
4. I will report any change in medical condition
5. I will report any incidents



Our Team

• Emory Nursing

- Toni Ash
- Chris Barnes
- Jason Calhoun
- Lauren Chapman
- Tracey Daye
- Haley Durr
- Shunasee Evans
- Janice Gentry
- Jan Ginnane
- Susan Grant
- Chris Haynes
- Carolyn Hill
- Dustin Hillis
- Crystal Johnson
- Jessica Loomis
- Josia Mamora
- Laura Mitchell
- Susan Mitchell
- Jill Morgan
- Nancy Osakwe
- Jacqueline Owen
- Sarah Piazza
- Kristina Shirley
- Jodi Siddens
- Carrie Silas
- Jason Slabach
- Elaina Tirador
- Donnette Todd
- Sharon Vanairsdale
- **Pharmacy**
- Amir Emamifar
- Susan Rogers

• Health & Safety

- George Golston
- Sean Kaufman
- Patricia Olinger
- Sean Olinger
- Kalpana Rengarajan
- Scott Thomaston

• Infection Control

- Connie Bryant
- Betsy Hackman
- Regina Howard
- Marolyn Jones

• Environmental Services

- Jeff Broughton
- Brian Frisle
- Robert Jackson
- Jerry Lewis

• Emory Medical Labs

- Jim Ritchie
- Anne Winkler
- Nicole Brammer
- Juli Buchanan
- Eileen Burd, PhD
- John Cardella
- Brenda Eaves
- Crystal Evans
- Charles Hill, MD, PhD
- Krista Hostetler
- Karen Jenkins
- Maureen Lindsey
- Jordan Magee
- Randall Powers
- Emily Ryan, PhD
- **Occupational Health**
- Emily Beck
- Paula Desroches



Our Team

• Administration

- Robert Bachman
- Bill Bornstein
- John Fox
- Bryce Gartland
- Anne Adams
- Dee Cantrell
- Mary Beth Allen
- Nancye Feistritz
- Jen Goodman
- Ira Horowitz
- Chad Ritenour

• Media Relations

- Vince Dollard
- Nancy Seideman
- Holly Korschun
- Janet Christenbury

• Pastoral Care

- Robin Brown-Haithco
- Miranda Lynn Gartin
- Erica Gerald-Washington
- Rhonda James-Jones
- Donald Miller
- Dan Stark

• Supplies/Logistics

- Gentrice McGee
- Porcia Jones

• EUH Security

- Linda Scott-Harris
- James Cain
- Roderick Davis
- Tyrone Johnson
- Tyrone Pickett
- Anthony Shaw
- Tenina Truesdale

• Emory Critical Care

- Jen Schuck
- Jim Blum
- Matthew Klopman
- Ricky Matkins
- Kathy Schwock
- Francis Wolf
- Kathy Stack
- Joel Zivot
- Laureen Hill
- Cathy Meechan
- Paul Meechan
- Jon Sevranski
- Seth Walker

• Emergency Medicine

- Alex Isakov
- Sam Shartar

• Emory Infectious Diseases

- **Bruce Ribner**
- **Sonia Bell**
- G Marshall Lyon
- Aneesh Mehta
- Colleen Kraft
- Jay Varkey
- Vince Marconi
- Mark Mulligan
- Carlos Del Rio
- Phyllis Kozarsky
- Rachel Friedman
- Monica Farley
- David Stephens



Thank You to Our Colleagues

- Centers for Disease Control and Prevention (CDC)
 - **Timothy Uyeki**
 - **Anita McElroy**
 - **David Kuhar**
 - Ute Stroehler
 - Christina Spiropoulou
 - Jonathan Towner
 - Stuart Nichol
 - Shelley Campbell
 - Aridh Gibbons
 - Deborah Cannon
 - Paul Meechan
 - Viral Special Pathogens Branch
- National Microbiology Laboratory of the Public Health Agency of Canada
 - Dr. Gary Kobinger
- Food & Drug Administration (FDA)
 - Dr. Debra Birnkrant
 - Dr. Robert Kosko
 - Division of Antiviral Products
- SIM and Samaritan's Purse
 - Dr. Lance Plyler
 - Dr. John Fankhauser
 - Dr. Deborah Eisenhut
 - Medical team at the ELWA hospital Liberia
- CBR International Corp
 - Dr. Miles Brennan
 - Dr. Jeanne Novak



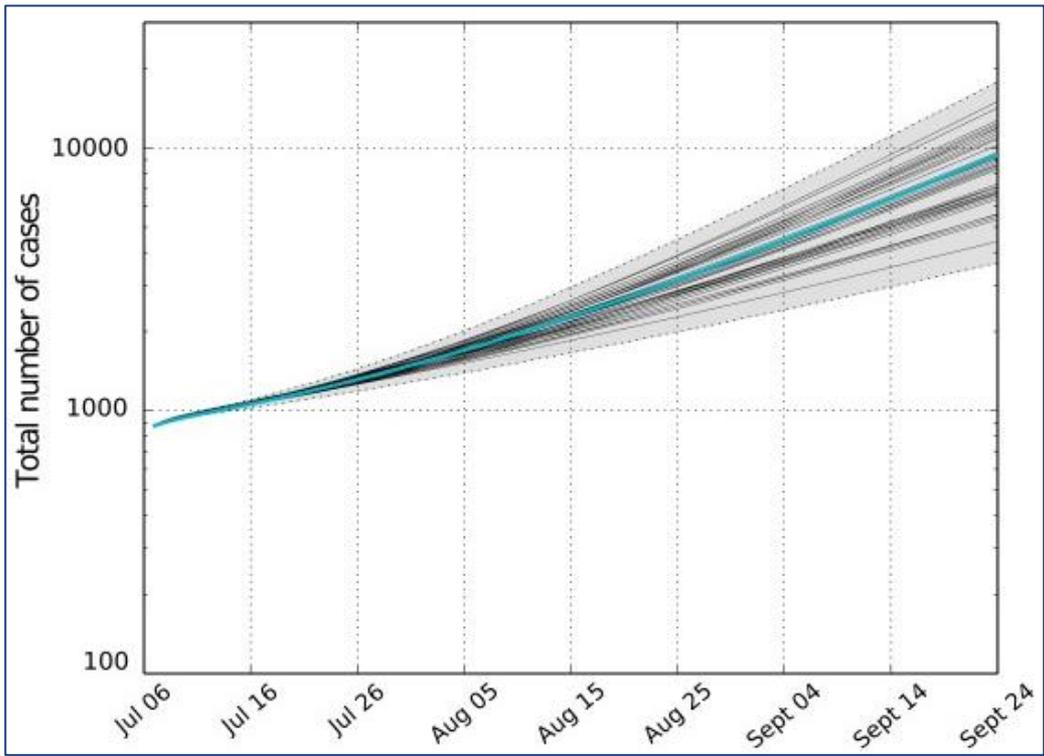
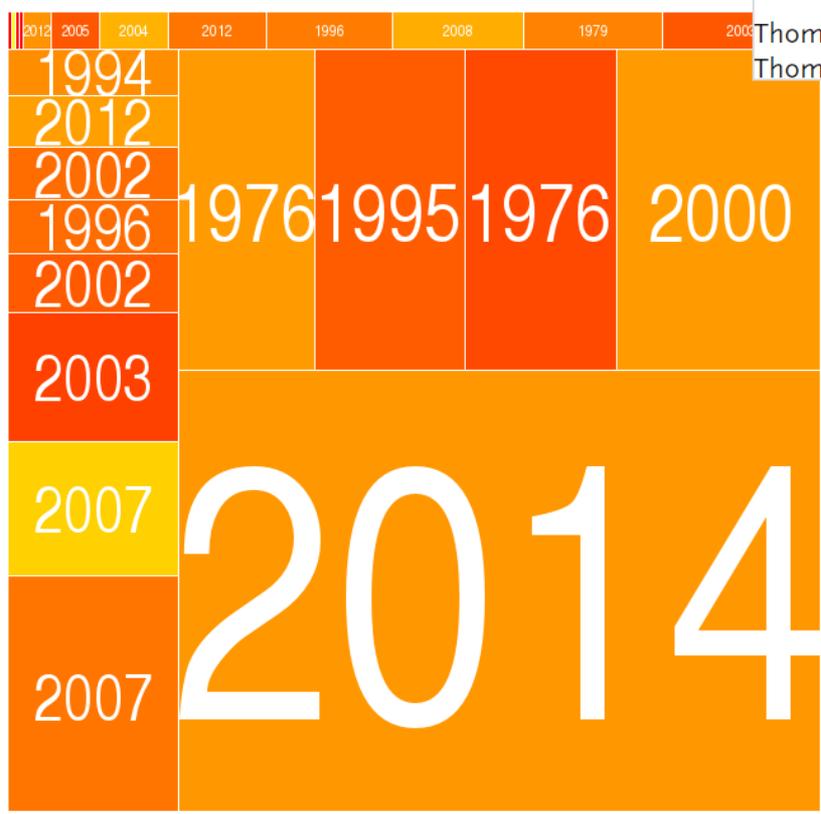
The Bigger Picture – much more than the patients at Emory

Ebola Virus Disease in West Africa —

No Early End to the Outbreak
Margaret Chan, M.D.

Ebola 2014 — New Challenges, New Global Response and Responsibility

Thomas R. Frieden, M.D., M.P.H., Inger Damon, M.D., Ph.D., Beth P. Bell, M.D., M.P.H., Thomas Kenyon, M.D., M.P.H., and Stuart Nichol, Ph.D.



<http://onforb.es/Y3YjoG>

A. Vespignani et al. Modeling projection of cases if spread continues at current rates. <http://news.sciencemag.org/health/2014/08/disease-modelers-project-rapidly-rising-toll-ebola>



Our Patients and Their Families



Their message: Help is needed in West Africa

