Ebola Epidemic in the World and Iran- Requirements of Laboratory Diagnosis

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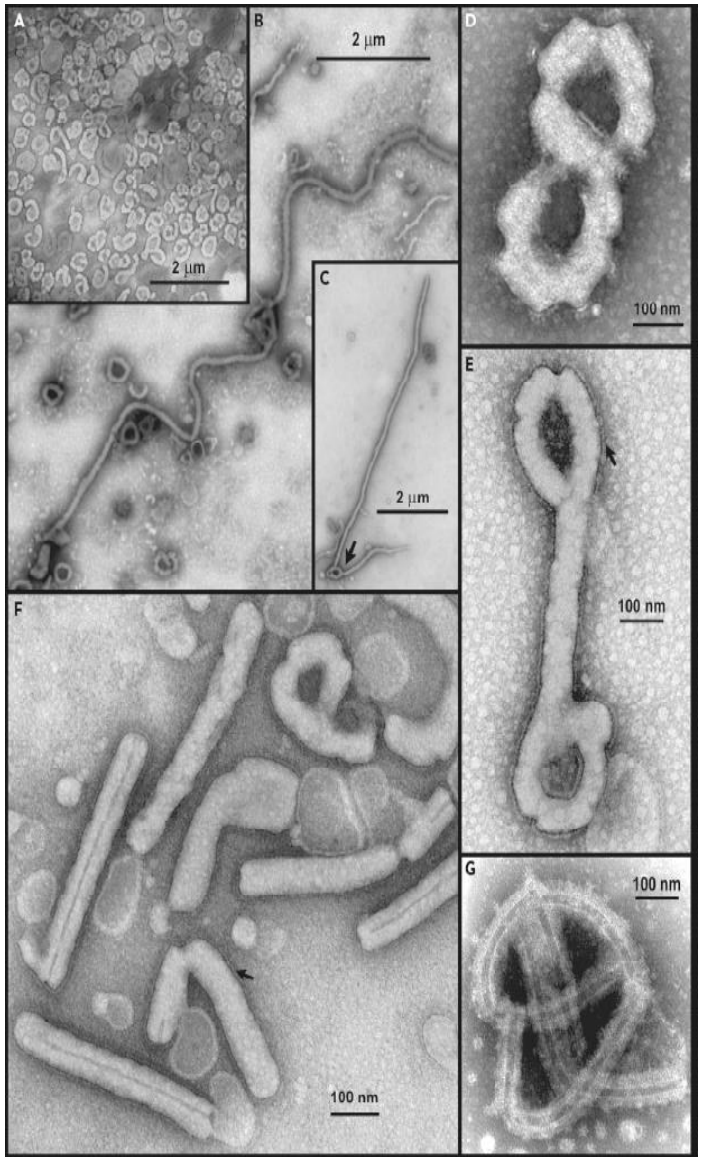
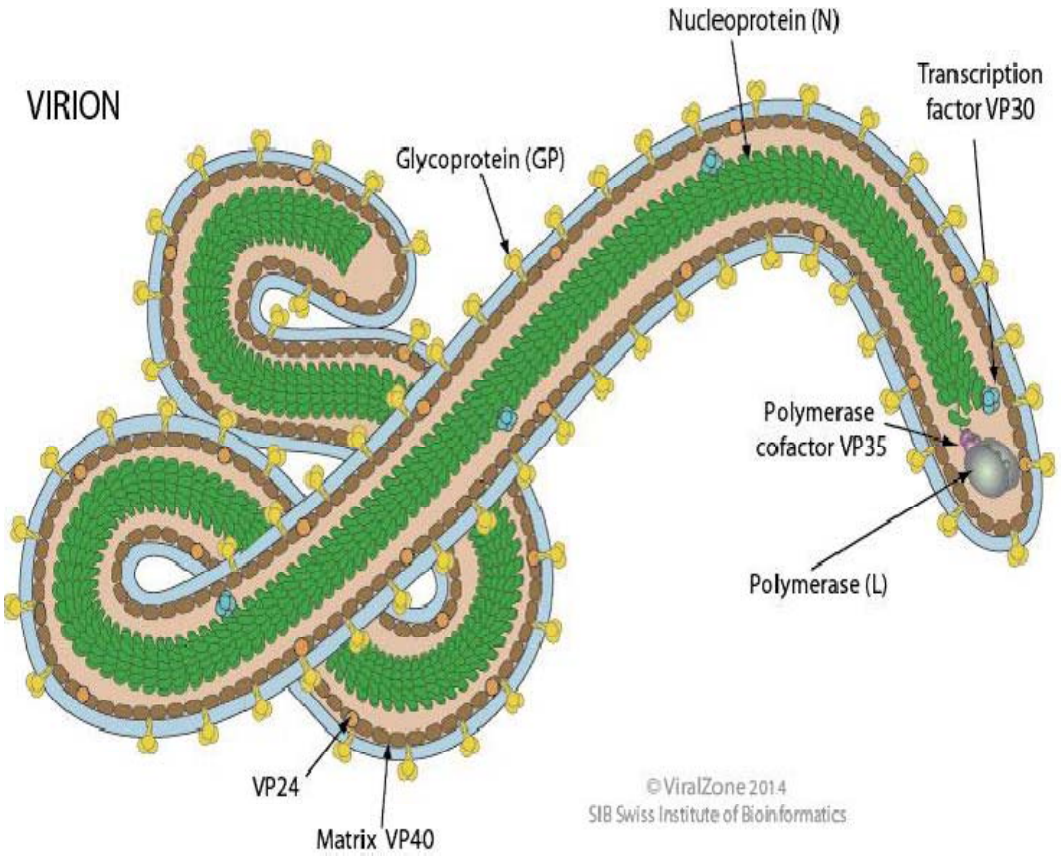
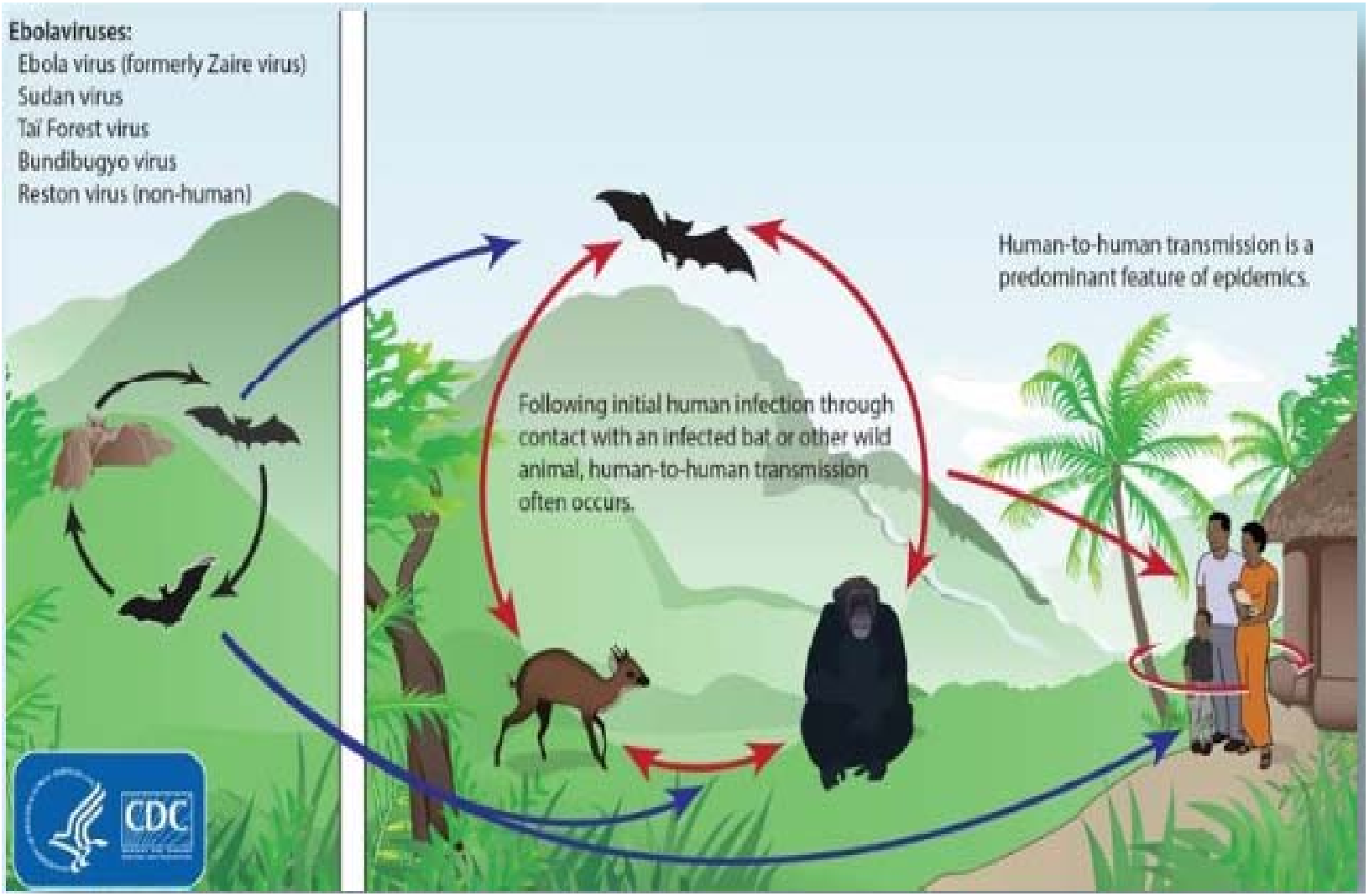
Morphostructure of ebolavirus particles

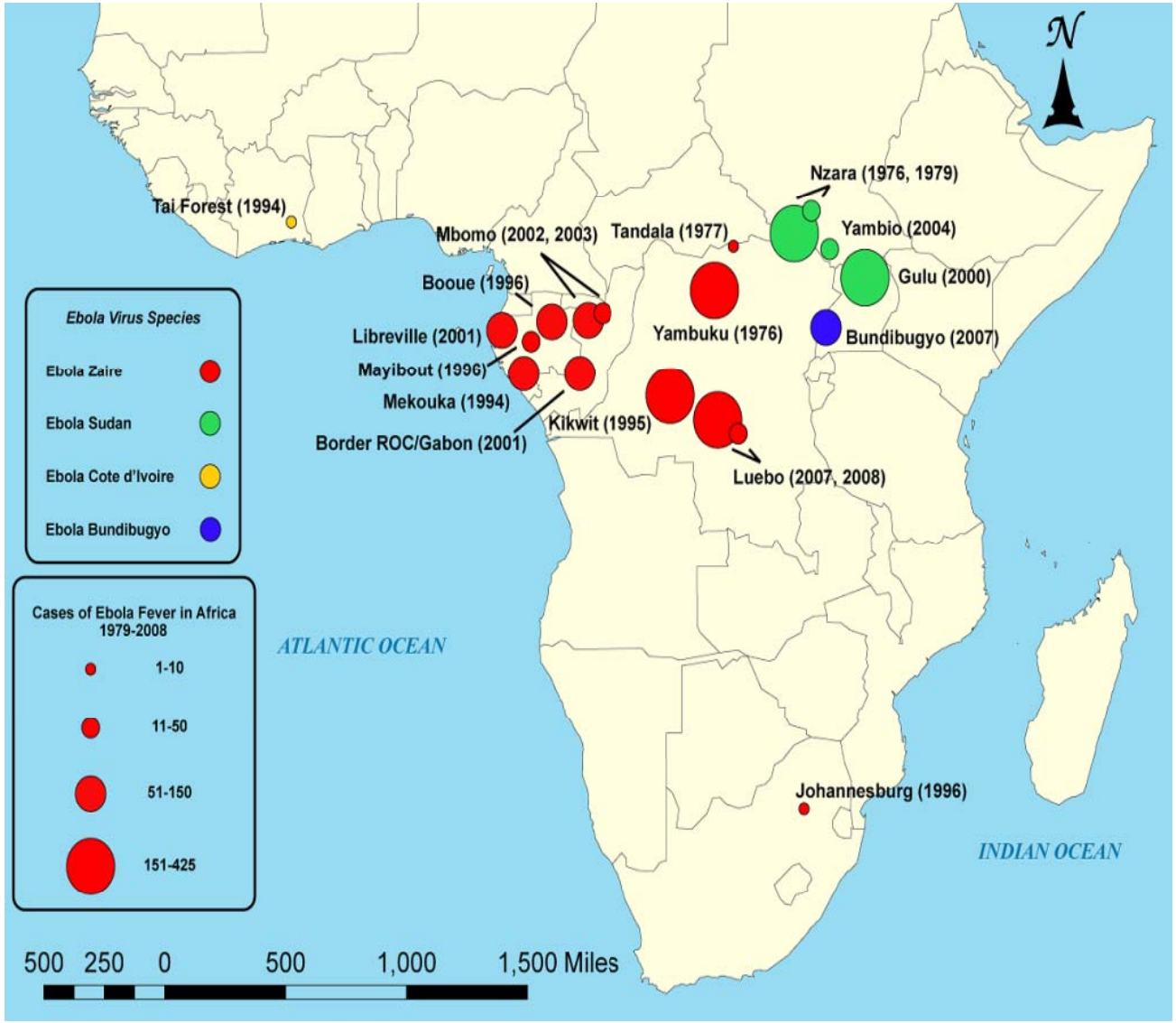




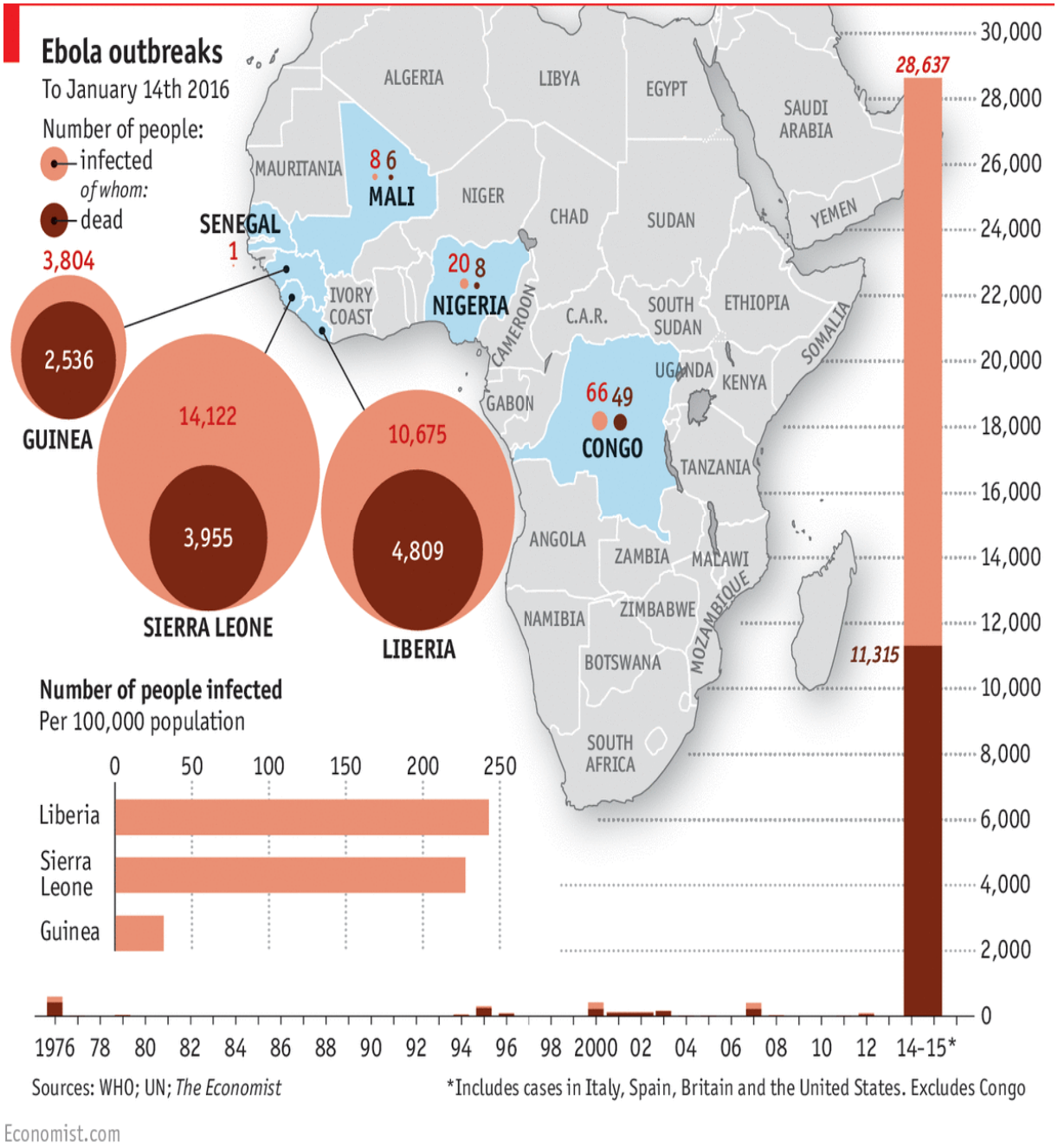
Ebola Virus


Ebolaviruses:
Ebola virus (formerly Zaire virus)
Sudan virus
Taï Forest virus
Bundibugyo virus
Reston virus (non-human)
Following initial human infection through contact with an infected bat or other wild animal, human-to-human transmission often occurs.
Human-to-human transmission is a predominant feature of epidemics.
CDC

Ebola Outbreaks before 2014



Ebola Outbreaks



Ebola outbreak control

- Case management
- Surveillance and contact tracing
- Good laboratory service
- Safe burials
- Social mobilization

The origins of the 2014 Ebola virus epidemics

- **February-March 2014** : mysterious disease with fever, diarrhea, vomiting, hemorrhages and high fatality rate in Guinean forest
- **March 10** : Hospitals and Public Health Services in Guéckédou and Macenta alert the Ministry of Health
- **March 12-14** : « MSF » Europe sends a team (arrival on 18 March) & alerts the WHOCC for Viral Haemorrhagic Fever (Pasteur-Paris, BNI-Hamburg)

The origins of the Ebola virus epidemics

- **19 March 2014**: WHOCC-VHF in Pasteur, Paris (CIBU) receives the suspected samples (from Ginea) and transfers them to the National Reference Centre (NRC) for VHF in Pasteur-Inserm, Lyon.
- **March 21** : the NRC-VHF in Pasteur-Inserm, Lyon detects by PCR Filovirus L genes in suspected sera.
- **March 22-23**… : the CIBU in Pasteur-Paris is sequencing the PCR amplicons : a subtype of Ebola virus (strain Zaire)

The origins of the Ebola virus epidemics

- **From March 23** : a team of the IP Dakar is starting diagnosis laboratory in Conakry
- **April 3-16** : a team of CIBU in Pasteur-Paris is reinforcing the IP-Dakar team in Conakry.
- **From April** : Institut Pasteur is sending experts to help Guinean Health Authorities to control the epidemics
- **From April**: Pasteur-Lyon is contributing to the EU mobile lab in Guéckedou.

Assessing the risks of any emerging infectious diseases

- The route of transmission
- The location of the virus in the body during infection
- How much infectious virus is present
- How common the infection is
- How pathogenic the agent is
- What clinical activities are performed on infected or potentially infected patients.

Risk Assessment of EVD

Not airborn
Low dose for infection
No need to culture
Not the only hazard

HIGH-RISK EXPOSURE

Percutaneous (e.g., needle stick) or mucous membrane contact with blood or body fluids from an Ebola patient

OR

Direct skin contact with, or exposure to blood or body fluids of, an Ebola patient

OR

Processing blood or body fluids from an Ebola patient without appropriate personal protective equipment (PPE) or biosafety precautions

OR

Direct contact with a dead body (including during funeral rites) in a country with wide-spread Ebola transmission** without appropriate PPE

LOW-RISK EXPOSURE

Household members of an Ebola patient and others who had brief direct contact (e.g., shaking hands) with an Ebola patient without appropriate PPE

OR

Healthcare personnel in facilities with confirmed or probable Ebola patients who have been in the care area for a prolonged period of time while not wearing recommended PPE

NO KNOWN EXPOSURE

Residence in or travel to a country with wide-spread Ebola transmission** without HIGH- or LOW-risk exposure

The laboratory context

- What tests should be performed?
- Who collects the specimens?
- How is the specimen transported?
- How are patients at-risk or infected with Ebola virus identified?
- How is the laboratory notified of patients and of specimens from these patients?
- How are lab personnel who handle specimens tracked and assessed for potential exposure?
- What PPE will be used for each exposure?
- How will PPE competency be established and maintained?
- How will the sample be handled once in the laboratory?
- How will spills be managed?

The POC perspective

- POC testing potentially avoids:
  - Specimen transport
  - Centrifugation
  - Aliquoting
  - Reducing the risks of droplet formation or aerosolization.
- Patient samples are contained within the patient's isolation room or suite, reducing the number of people exposed.

Detection Methods

- Antibody-capture enzyme-linked immunosorbent assay (ELISA)
- Antigen-capture detection tests
- Serum neutralization test
- Reverse transcriptase polymerase chain reaction (RT-PCR) assay
- Electron microscopy
- Virus isolation by cell culture.

CPE

- BSC
- Centrifuge
- Pipette Aid
- Autoclave

PPE for Lab staff

Overshoes

Gloves (2x)

FFP2 mask

Safety glasses

Coverall with hood

Disposable gown

Head cover

Institut Pasteur

Institut Pasteur lab in Macenta

Glove box (inactivation)

Automated extractor

Institut Pasteur lab in Macenta

Preparation of the PCR mix

Detection Methods

In-house design

- CDC Ebola Virus NP Real-time RT-PCR Assay / VP40 Real-time RT-PCR Assay (FDA approved)
- Sanchez et al, adapted to qRT-PCR by Drosten et al [T]
- Department of Defense EZ1 Real-time RT-PCR Assay (FDA approved)

Detection Methods

Commercial kit – Cross platform

- Roche TIB MolBiol LightMix® Ebola Zaire rRT-PCR Test (FDA approved, CE-IVD) [T] **LoD: 4781 PFU/mL**
- Altona Diagnostics RealStar® Ebolavirus RT-PCR Kit 1.0 (FDA approved, CE-IVD) [T] → Reference kit **LoD: 3 copies/uL**
- Bioneer *AccuPower*® EBOV Real-Time RT-PCR (CE) **LoD: ~ 50 copies/test**
- Bioneer *AccuPower*® EBOV Quantitative RT-PCR Kit (CE)

Detection Methods

Commercial kit – Dedicated platform

- BioFire Defense LLC FilmArray Biothreat-E test / NGDS BT-E Assay (FDA approved)

- Cepheid GeneXpert Xpert® Ebola Assay (FDA approved)

- Progenie RealCycler EBOL T

Sample Inactivation

For Molecular Testing

- Lysis buffer containing guanidine salts
- TRIZOL
- Heating at 60 C for 1 hour.
- Heating does not significantly affect the estimates of electrolytes, as well as urea, creatinine, bilirubin, glucose, and C-reactive protein.
- Are not suitable for enzymes like Alkp, transaminases and antibodies!

Sample Inactivation

- Serum samples for **ELISA** based determinations can be inactivated with final concentrations of 0.2% of sodium dodecyl sulphate (SDS) / 0.1% Tween 20 and heat treatment at 60 C 15 min.
- The blood smears should initially be fixed by 5 min in methanol and subsequently 15 min in 10% buffered formalin, followed by 3 washings with distilled water pH 7.0 before carrying out the staining. Or, fixation with methanol can be extended to 30 min, followed by dry heat (95 C) during 1 hour.
- The treatment of serum or other organic fluids with 10 ml of 10% Triton X-100 by ml of liquid during 1 hour is also recommended to reduce viral titers.

Samples should be inactivated before storage

Disinfection

- The holders of the centrifuge (buckets) and rotors should be sterilized in autoclave or by immersion in 1% glutaraldehyde (in a sealed container) during 10 min.
- Any automated equipment should be decontaminated with 0.5% hypochlorite (repeated cycles of cleaning).
- Any reusable PPE should initially be washed with water/detergent solution and subsequently soaked in 0.5% hypochlorite solution (minimum 30 min; leaving it overnight is highly recommended) for decontamination.

Thank you for Attention!