

Laboratory Overview on diagnostics

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I have no relationship with any of the manufacturers mentioned in the presentation.

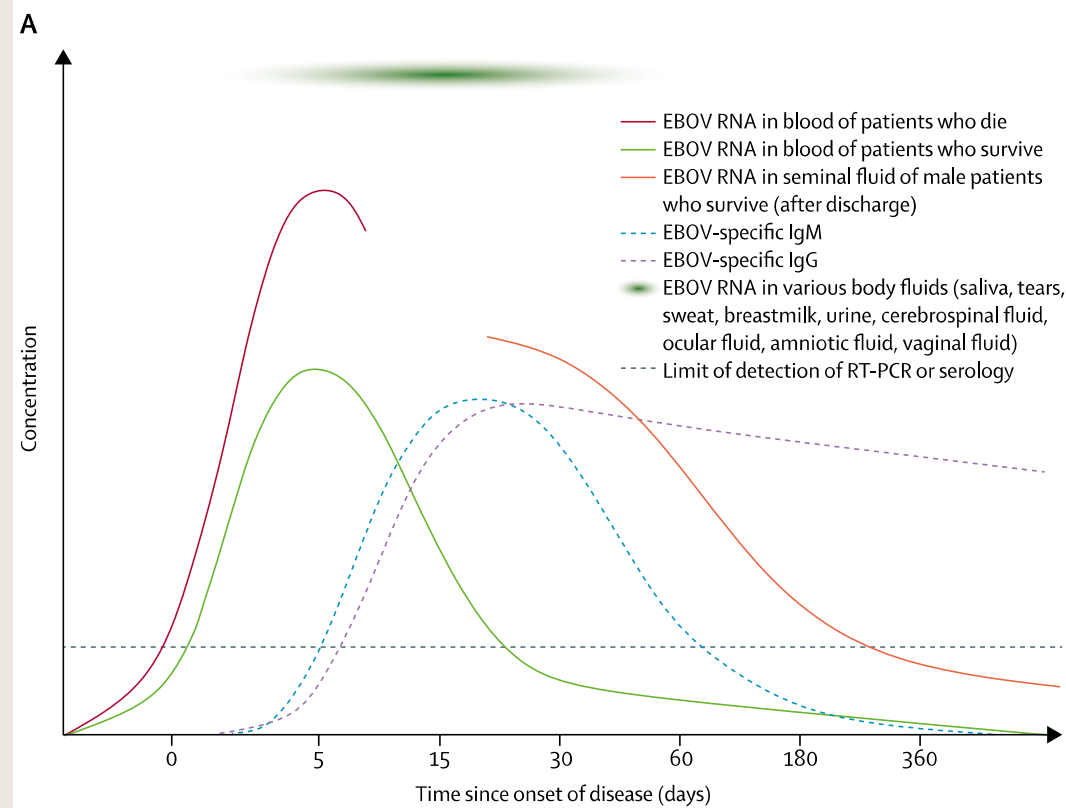


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Clinical virology of filovirus disease

- Patients present at a hospital 3-6 days after onset of disease → **Virus load is detectable by RT-PCR in blood of the vast majority of cases**
- A negative RT-PCR early after onset does not exclude infection → **repeat testing for a period of 72 hours if the clinical suspicion persists (contacts)**
- Five days after onset of symptoms, viremia reaches a maximum that is 10-100 fold higher in fatal cases compared to survivors
- Viremia declines in survivors below the limit of detection of RT-PCR, i.e. approximately 1,000 virus RNA copies/ml of blood, about 2-3 weeks after disease onset

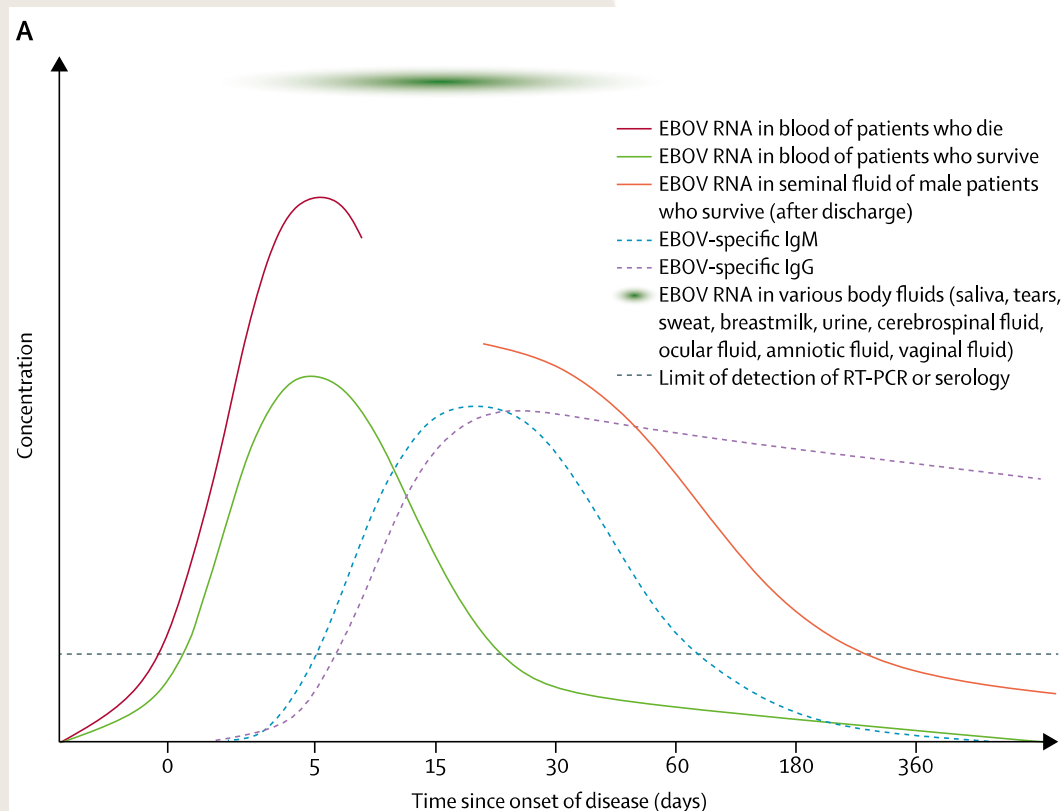


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Filovirus persistence and types of specimens

- Virus RNA can be detected in various body fluids besides blood, including **saliva, tears, sweat, breast milk, urine, cerebrospinal fluid, ocular fluid, amniotic fluid, vaginal fluid, and seminal fluid** → alternative types of specimens for RT-PCR testing
- Seminal fluid of male survivors remains positive in RT-PCR and infectious for months to years → **sexual transmission**.
- Women may transmit virus in milk to their babies via breast-feeding.
- Consequences of persistence are clinical sequelae, disease re-activation, long-term virus shedding and transmission → **New clusters or epidemics (Guinea 2021)**

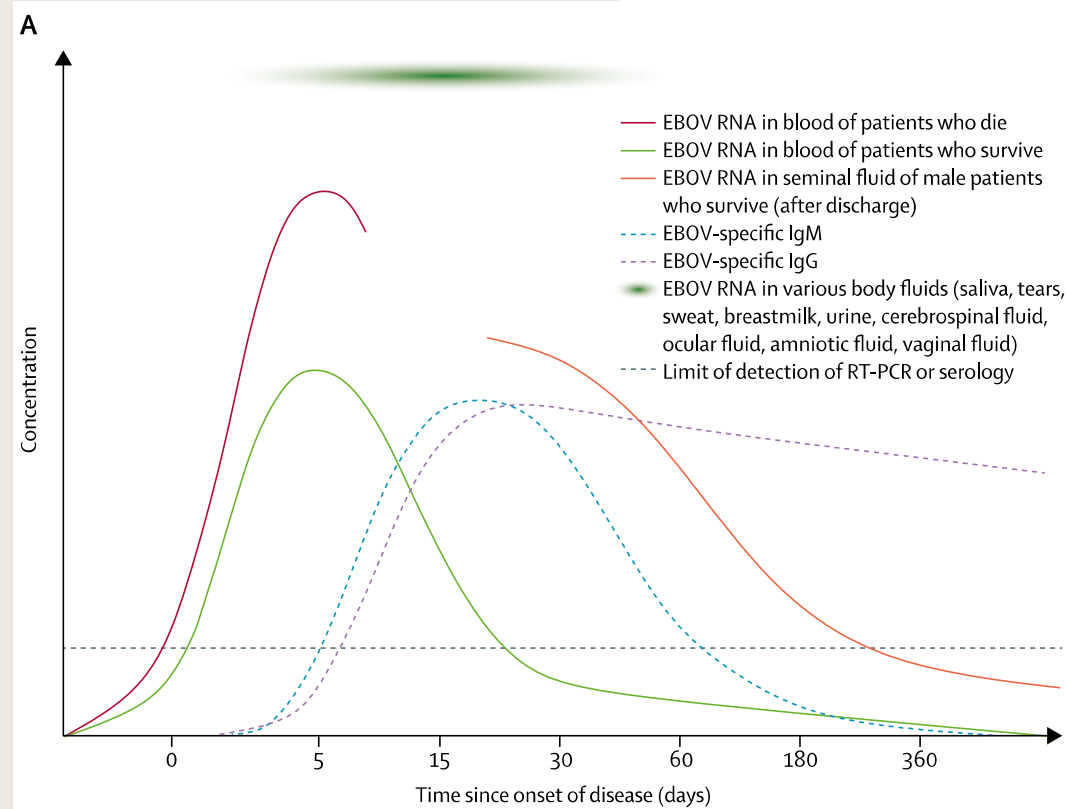


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Filovirus serology

- IgG and IgM develop in survivors, but not in all fatal cases → **serology is not suitable for diagnosis of acute disease**
- Serology may be used to diagnose pauci- or asymptomatic infections, which are characterized by low viremia (below detection limit of standard PCR) and development of IgG and IgM about 3 weeks days after inoculation.



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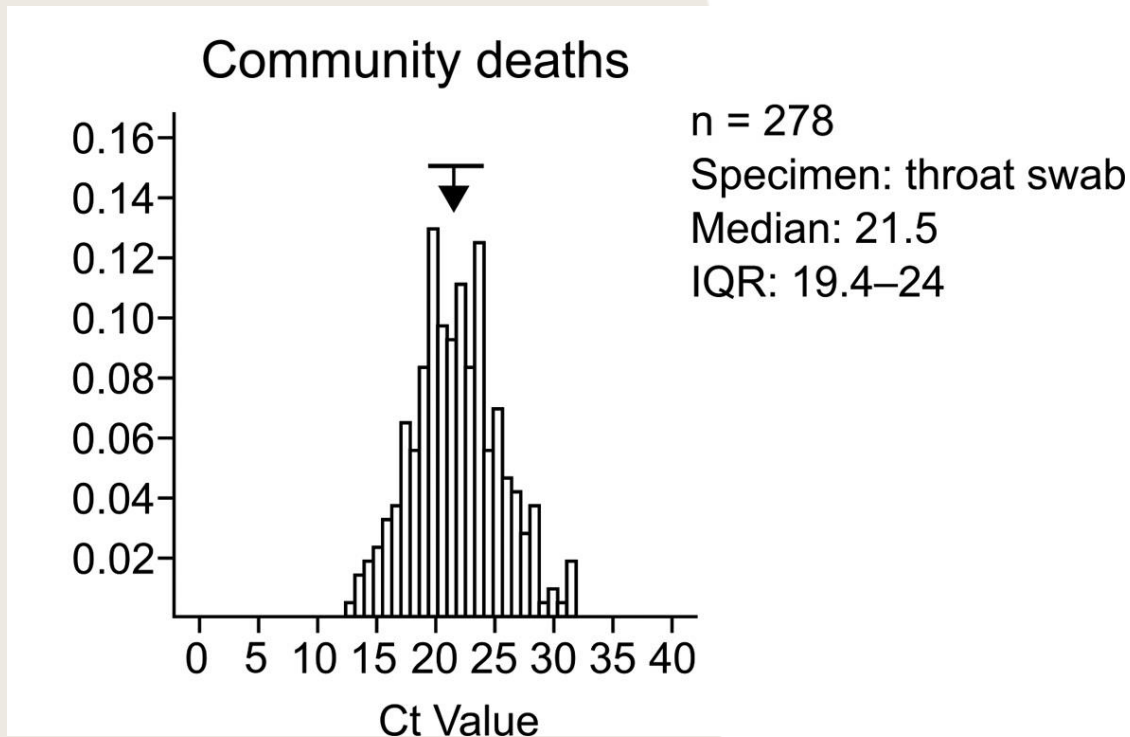
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Postmortem diagnosis

Postmortem EVD diagnosis for ill persons, who died in the community, is

- RT-PCR or
- OraQuick Ebola RDT

→ on an oral swab



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Detection technologies

- Real-time RT-PCR assays are now state-of-the-art in filovirus diagnostics
- Commercially available and come with industry-standard features such as internal controls to monitor RNA extraction and reaction efficacy or lyophilized reagents.
 - Open-platform PCR test kits
 - Closed-platform cartridge-based nucleic-acid amplification tests
- Semi-quantitative readout in terms of cycle threshold (Ct) values, which inversely correlate with virus RNA concentration

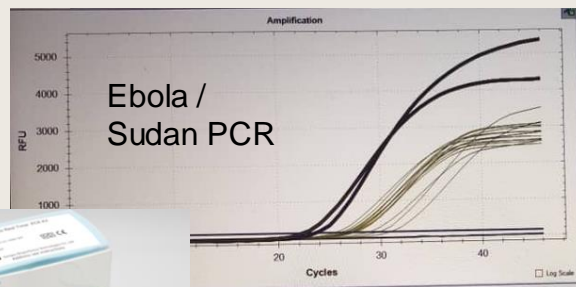
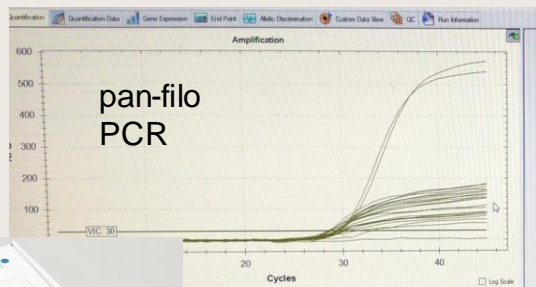


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Open-platform PCR test kits

- Broadly-reactive pan-filo PCR assays able to detect all known human-pathogenic filovirus species → **surveillance / outbreak detection**
- Species-specific assays are more sensitive than assays reactive at virus family level → **preferable in a confirmed outbreak**
- Time from sample reception to diagnosis about 4-6 hours.
- Requires substantial laboratory infrastructure, logistics, and a pool of well-trained experts



Link	Link	Neg
Link	NTC	Pos
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Closed-platform cartridge-based nucleic-acid amplification tests

- Automated commercial real-time RT-PCR tests, for example Xpert® Ebola (Cepheid) system
 - Integrate RNA extraction, amplification, and detection.
 - The Xpert® Ebola system has proven useful for near-patient diagnostics in hospital settings and may be operated by trained local staff.
 - Delivers results within 2 hours, shows high sensitivity, and besides blood, can process other body fluids such as seminal fluid
 - Not yet available for Marburg and other Ebolavirus species
 - Alternative: BioFire® Global Fever Special Pathogens Panel (Ebola and Marburgvirus species)
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- Disadvantage: high price



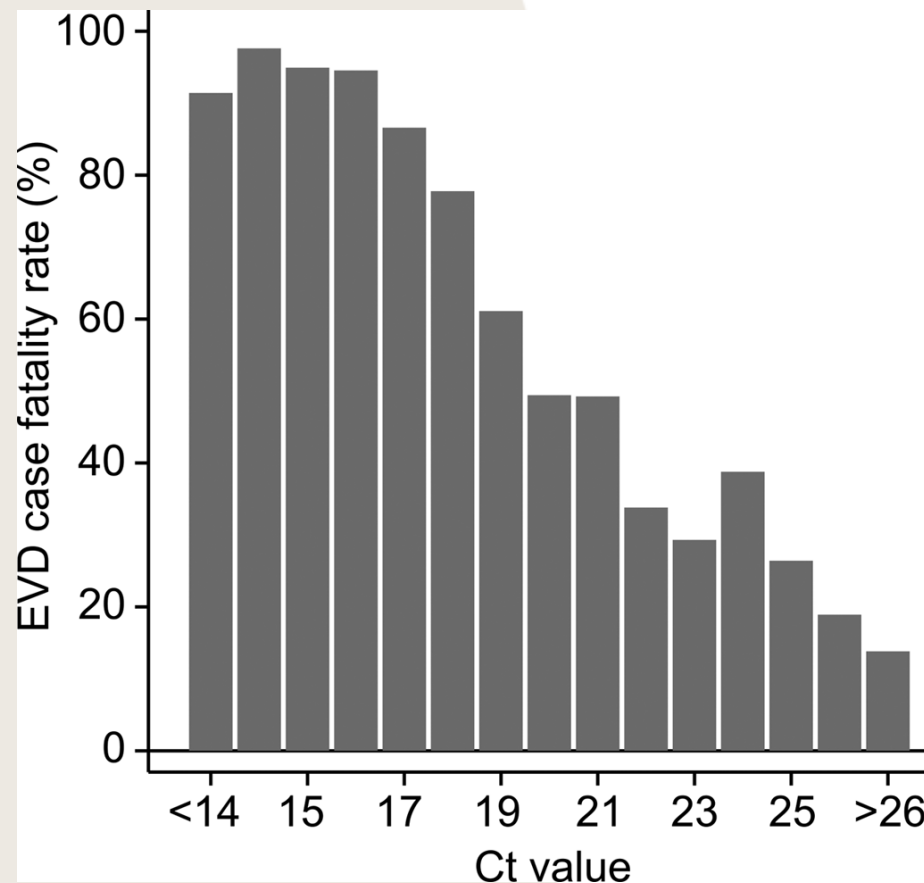
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Ct value as prognostic factor

Ebola virus load on admission is an important prognostic marker → the higher the virus load (i.e. the lower the Ct value), the poorer the outcome

Option to stratify patients in clinical trials by Ct value as marker of virus load and severity



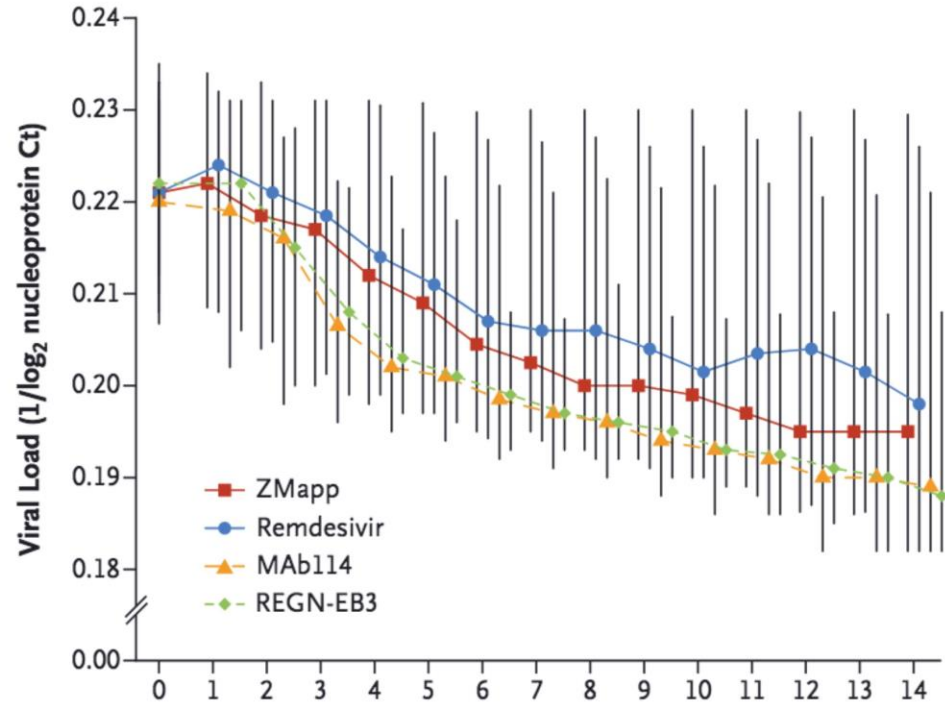
Ct to measure virological response

Ct kinetics might be used as an endpoint for virus directed therapies.

However:

- Ct value is influenced by virus variability → to consider when pooling data from various outbreaks
- Risk of partial PCR inhibition from tissue damage in patients with fulminant Ebola virus disease (+5 Ct units)
- Polymerase inhibitors may act by decreasing the ratio between infectious and non-infectious particles (mutagenesis) → Ct does not reflect this mode of action

Viral Load According to Day



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Rapid diagnostic tests (RDT)

- Lateral flow immunoassays for detection of virus antigen
- Commercially available for Ebola virus, experimental for Ebola Sudan and Marburg virus
- Delivers results within minutes and may be used bed-side in the primary health care setting
- Sensitivity 60-90% and specificity 95-99% compared to PCR → detection of cases with high virus load and high transmission potential
- Due to high specificity, RDT could be considered as first line test in clinical trials to shorten time between presentation and inclusion (plus confirmatory PCR)



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Ebola outbreak detection and response since 2013

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Ebolavirus Outbreaks Since 2013

Year	Outbreak	Days from Onset of Symptoms of First Identified Probable Case to Diagnostic Confirmation of Outbreak	Days from Confirmation to Start of Outbreak Response Vaccination	Days from Index Case Onset of Symptoms to End of Outbreak	Number of Ebolavirus Vaccine Doses Administered	Number of Cases*	Number of Deaths
2013-2016	West Africa	110	366	920	16,000	28,610	11,308
2014	DRC Equateur	29	N/A	117	N/A	69	49
2017	DRC Bas Uele	19	N/A	71	N/A	8	4
2018	DRC Equateur	33	14	111	3,481	54	33
2018-2020	DRC North Kivu	93	7	787	345,000	3,470	2,287
2020	DRC Equateur	13	5	184	40,870	130	55
2021	Guinea	30	9	155	10,612	23	12
2021	DRC North Kivu	12	9	98	1,898	12	6
2021	DRC North Kivu	33	5	102	2,026	11	9
2022	DRC Equateur	18	4	90	2104	5	5
2022	DRC North Kivu	23	10	66	550	1	1
2022	Uganda	42	N/A**	156	N/A**	164	77

DRC = Democratic Republic of the Congo

*Number of cases includes suspected, probable, and confirmed cases for 2014-2016 West Africa outbreak and probable and confirmed cases for all other outbreaks.

**2022 Uganda outbreak involved *Sudan ebolavirus*, against which the Merck VSV-ZEBOV and the Jansen Ad26.ZEBOV/MVA-BN-Filo vaccines used in one or more other outbreak responses are ineffective. All other ebolavirus outbreaks since 2013 have involved *Zaire ebolavirus*.

The best outbreak is a prevented outbreak

In 2023, 23 African countries have Ebola Virus diagnostic capacity, compared to five in 2010

(WHO, Lancet Microbe, 2023)

Laboratory infrastructure for surveillance AND outbreak response in affected countries – elements of success

1. **Central laboratories** with high-end capabilities including sequencing
2. **Decentralized laboratories** in high-risk areas for surveillance and outbreak response / trial support
 - Open-platform PCR test kits and/or
 - Closed-platform cartridge-based nucleic-acid amplification tests
 - Challenges:
 - Supply chain
 - Training and SOP implementation
 - Funding
3. **Mobile laboratory units** operated by Central laboratories to be deployed to treatment centers / entry points etc. for rapid on-site EVD confirmation and management of patients and contacts (versatile use, Covid) → Rapid response mobile Laboratory (RRML) initiative of WHO

9 x EAC Mobile Laboratories
1 x Nigeria (since 2014)



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Thank you for your attention



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17 were included in the qualitative review and nine were included in a meta-analysis. Prevalence of coinfection was between 19% and 72%. One study reported significantly lower coagulatory response biomarkers in coinfecting cases but no difference in inflammatory markers. Case fatality rates were similar between EBOV(+)/PI (+) and EBOV(+)/PI(-) cases (62.8%, 95% CI 49.3–74.6 and 56.7%, 95% CI 53.2–60.1, respectively), and there was no significant difference in risk of mortality (RR 1.09, 95% CI 0.90–1.31)



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Classical methods for detection of filoviruses

- Virus isolation in cell culture, usually in Vero cells,
- Electron microscopy
- Immunofluorescence assay (IFA) and ELISA using virus-infected cells as antigen for IgM and IgG
- Requires biosafety level (BSL)-4 facilities



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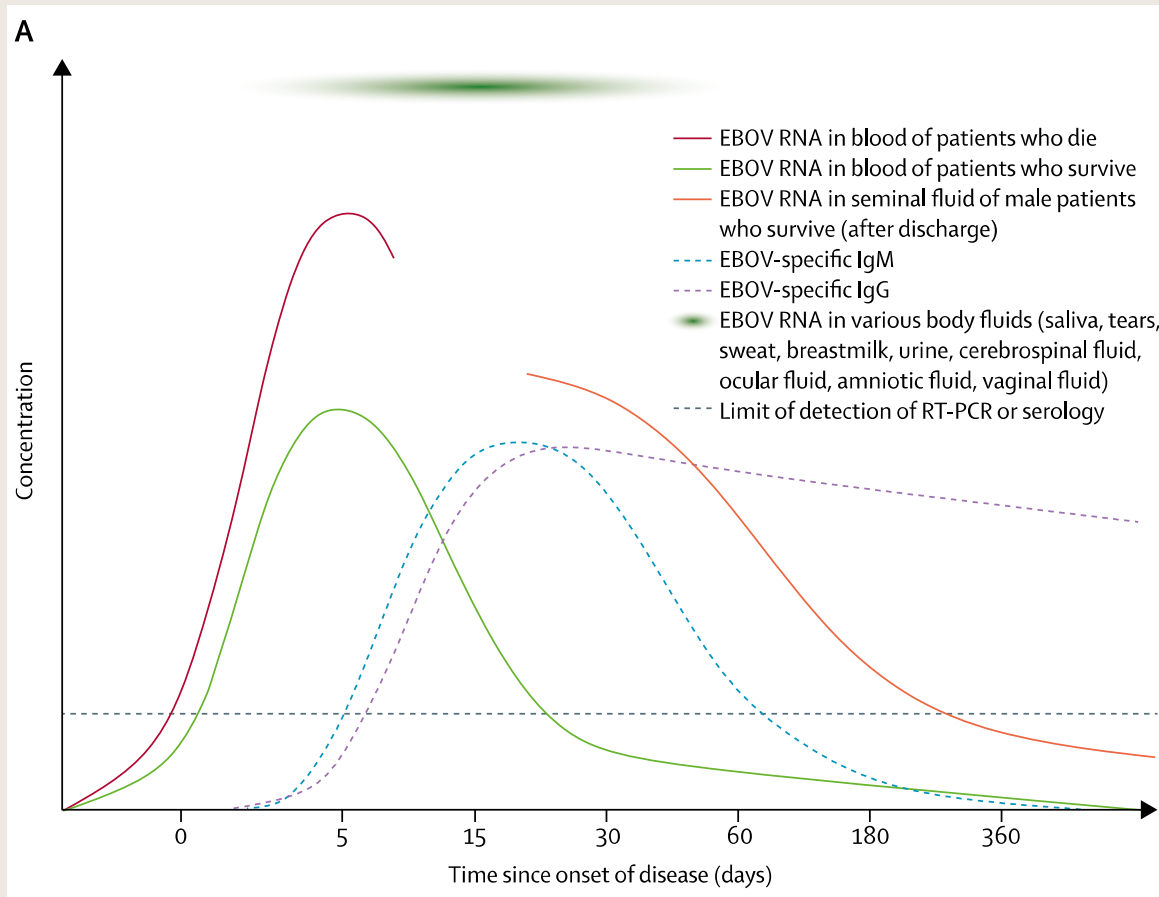
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Ebola outbreak detection and response since 2013

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Open-platform PCR test kits have regularly allowed confirmation of Ebola virus outbreaks within a day or less of samples from suspected cases arriving at diagnostic laboratories and confirmation of cases throughout outbreaks, whereas closed-platform cartridge-based nucleic-acid amplification tests have provided valuable surge capacity during confirmed EVD outbreaks.

