Faculty of Pharmaceutical, Biomedical and Veterinary Sciences

Department of Biomedical Sciences

Implementation and evaluation of new tools for Ebola virus disease response during outbreaks in eastern Democratic Republic of the Congo: from rapid diagnostic tests to genomic sequencing

Implementatie en evaluatie van nieuwe instrumenten voor de bestrijding van de ebolavirusziekte tijdens uitbraken in het oosten van de Democratische Republiek Congo: van snelle diagnosetests tot genomische sequencing

Thesis submitted to obtain the degree of Doctor in
Biomedical Sciences at the University of Antwerp

Daniel MUKADI-BAMULEKA

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Promoters:

Prof. Dr. Kevin K. Ariën

Prof. Dr. Johan van Griensven

Co-Promoter:

Prof. Dr. Steve Ahuka-Mundeke

Dissertation submitted to obtain the degree of Doctor in Biomedical Sciences at the University of Antwerp, Belgium to be defended by

Daniel MUKADI-BAMULEKA

Promoters:

- **Prof. Dr. Kevin K. Ariën**
  Department of Biomedical Sciences, University of Antwerp, Belgium
  Department of Biomedical Sciences, Institute of Tropical Medicine, Belgium

- **Prof. Dr. Johan van Griensven**
  Department of Clinical Sciences, Institute of Tropical Medicine, Belgium

Co-Promoter:

- **Prof. Dr. Steve Ahuka-Mundeke**
  Department of Virology, Institut National de Recherche Biomedical, DRC
  Department of Medical Biology, University of Kinshasa, DRC

Members of the Individual Doctoral Committee, University of Antwerp

- **Prof. Dr. Wim Vanden Berghe (Chair)**
  Protein chemistry, proteomics and epigenetic signaling (PPES), Belgium

- **Prof. Dr. Peter Delputte**
  Department of Biomedical Sciences, University of Antwerp, Belgium

External members of the PhD Jury

- **Em. Prof. Dr. Greet Leven**
  Vaccin & Infectious diseases Institute, University of Antwerp/UZA

- **Prof. Dr. Rosanna Peeling**
  London School of Hygiene and Tropical Medicine, England

- **Prof. Dr. Jean-Marie Kayembe-Ntumba**
  University Hospital of Kinshasa, Faculty of Medicine, DRC

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List of Abbreviations

- ASSURED: Affordable, Specificity, Sensitivity, User friendly, Robustness, Equipment-free, Delivered to those in need
- BDBV: Bundibugyo ebolavirus
- BOMV: Bombali virus
- BSL-3: Bio Safety Level-3
- BSL-4: Bio Safety Level-4
- CFR: Case Fatality Rate
- ChAd: Chimpanzee Adenovirus
- Ct-values: Cycle threshold values
- DNA: Deoxyribonucleic Acid
- DPP: Dual Path Platform
- DRC: Democratic Republic of Congo
- dsRNA : double strand Ribonucleic Acid
- EBOV: Zaïre ebolavirus
- EDTA: Ethylen Diamine Tetraacetic Acid or edetic acid
- ELISA: Enzyme-linked immunosorbent assay
- ETC: Ebola Treatment Center
- EUA: Emergency Use Authorization
- EVD: Ebola virus disease
- FANG: Filovirus Animal Non-Clinical Group
- GP : Glycoprotein
- HIV: Human Immunodeficiency Virus
- HMBS: human hydroxymethylbilane synthase gene
- HRP2: Histidin Rich Protein 2

- HUJV: Huángjiāo virus
- HZ : Health Zone
- IF: Immunofluorescence
- IgG: immunoglobulin G
- IgM: immunoglobulin M
- IQR : Interquartile range
- INRB: Institut National de Recherche Biomédicale
- KDa: Kilo Dalton
- Ebola L gene : Ebola virus polymerase
- LCD: Liquid Crystal Display
- LFI: Lateral Flow Immunoassay
- LLOV: Lloviu virus
- mAb : monoclonal antibody
- Mab114: Monoclonal Antibody 114
- MARV: Marburg virus
- MCM: medical countermeasures
- MEURI: Monitored Emergency Use of Unregistered Investigational interventions
- µL : Microliter
- MLAV: Mengla virus
- MONUSCO : Mission de l'Organisation des Nations Unies pour la stabilisation en République démocratique du Congo
- mRNA: Messenger Ribonucleic Acid
- MVA-BN® -Filo: Modified Vaccinia Virus Ankara, Bavarian

Nordic Filo

- Ng: Nanogramme
- NGS: Next Generation Sequencing
- NHP: Non-Human Primate
- NP: Nucleoprotein
- PALM: Pamoja Tulinde Maisha
- PBMC: Peripheral Blood Mononuclear Cells
- PCR: Polymerase Chain Reaction
- pLDH: Parasite lactate dehydrogenase
- POC: Point-of-care
- PPE: Personal Protective Equipment
- rAd26 ZEBOV-GP: recombinant Adenovirus type 26 vector Zaïre ebolavirus glycoprotein
- RAVV: Ravn virus
- RdRp: RNA-dependent RNA-polymerase
- RDT: Rapid Diagnostic Test
- REGN-EB3: Regeneron
- RESTV: Reston ebolavirus
- RNA: Ribonucleic acid
- RT-PCR: Reverse Transcriptase Polymerase Chain Reaction
- RT-qPCR: Reverse Transcriptase quantitative polymerase Chain Reaction
- Rt RT-PCR: real time Reverse Transcriptase Polymerase Chain Reaction

- rVSV-ZEBOV-GP: recombinant Vesicular Stomatitis Virus Zaïre ebolavirus glycoprotein vaccine
- SAC: Sample Adequacy Control
- SDB: Safe and Dignified Burials
- sGP: secreted/soluble Glycoprotein
- ssGP : small soluble glycoprotein
- SPC: Sample Processing Control
- SR: Sample Reagent
- SUDV: Sudan ebolavirus
- TAFV: Taï Forest ebolavirus
- TPP : Target Product Profile
- VP40 : Virion protein 40
- VP24 : Virion protein 24
- VP35 : Virion protein 35
- VP30 : Virion Protein 30
- VSV: Vesicular Stomatitis Virus
- WFP: World Food Program
- WHO: World Health Organization
- US CDC: United States Centers for Diseases Control and prevention
- US FDA: United States Food and Drug Administration
- XILV: Xīlǎng virus

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Summary

The Democratic Republic of the Congo has faced fifteen Ebola virus disease (EVD) outbreaks, among which the 2018-2020 was the most widespread and deadliest so far. During that outbreak associated with chronic insecurity, community mistrust and resistance, thirteen field laboratories were deployed to support the EVD diagnosis with the GeneXpert®. As this latter cannot be used everywhere in remote areas due to its additional requirements, rapid diagnostic tests (RDTs) were proposed as an alternative tool to support quick decision-making at the point-of-care. However, questions regarding RDTs diagnostic performance and usability were raised due to their disparate performances.

In the first part of this PhD thesis, we showed how decentralized and strategically positioned diagnostic laboratories quickly helped to mitigate the risk of Ebola virus spread through rapid, efficient, accurate and well-structured response. Quick hand over of competences and capacities to local teams led to successful management of further health emergencies (EVD flare-ups and Covid-19 pandemic), as those laboratories had dedicated equipment and well-trained local personnel. Sequencing data guided public health decision-making, helped understanding the outbreak dynamics, at risk populations and exposed health zones.

In the second part of this PhD, QuickNavi™ RDT had high specificity and quite good sensitivity than OraQuick® and Coris® tests in outbreaks conditions. QuickNavi-Ebola™ was less impressive compared to previous studies; OraQuick® test was almost in line with previous findings, although it performed better for the middle and lower Ct-values in laboratory conditions. None of the four Ebola RDTs evaluated throughout our studies, achieved the desired (sensitivity >98%, specificity >99%) or acceptable (sensitivity >95%, specificity >99%) levels of performance as stated by the WHO Target Product Profile for EBOV tests. However, respective specificities of the QuickNavi™ (>99%) and OraQuick® Ebola (98%) in most our studies were close to the acceptable level of performance (>99%).

Based on overall performances, QuickNavi™ and Oraquick® Ebola RDTs were proposed as a screening panel at the point-of-care to triage and isolate

suspect-cases waiting for the RT-qPCR results. RDTs results expectancy will be done separately i.e. individuals with at least one positive RDT isolated in the high-risk area and those with negative RDTs results in low-risk area.

For postmortem surveillance, OraQuick® RDT effectively complemented the response efforts, improved the community engagement, and decreased the number of systematic safe and dignified burials (SDBs) in corpses with non-reactive test. Trust towards postmortem testing led families to voluntarily request for SDBs despite OraQuick® non-reactive results.

Key-Words: ***Ebola, EVD, DRC, GeneXpert, RDT, laboratory, sensitivity, specificity***

Samenvatting

De Democratische Republiek Congo werd geconfronteerd met vijftien uitbraken van de ebolavirusziekte (EVD), waarvan de uitbraak van 2018-2020 de meest wijdverspreide en dodelijkste tot nu toe was. Tijdens die uitbraak, die gepaard ging met chronische onveiligheid, wantrouwen van de gemeenschap en weerstand, werden dertien veldlaboratoria ingezet ter ondersteuning van de EVD-diagnose met de GeneXpert®. Aangezien deze laatste wegens de bijkomende vereisten niet overal in afgelegen gebieden kan worden gebruikt, werden snellediagnosetests (RDT's) voorgesteld als alternatief instrument ter ondersteuning van snelle besluitvorming op de plaats van zorg. Er werden echter vragen gesteld bij de diagnostische prestaties en bruikbaarheid van RDTs vanwege hun gemengde prestaties.

In het eerste deel van dit proefschrift toonden we aan hoe gedecentraliseerde en strategisch geplaatste diagnostische laboratoria snel het risico van de verspreiding van het ebolavirus konden beperken door een snelle, efficiënte, accurate en goed gestructureerde reactie. De snelle overdracht van bevoegdheden en capaciteiten aan lokale teams leidde tot een succesvol beheer van verdere gezondheidscrises (EVD-opflakkeringen en Covid-19-pandemie), aangezien die laboratoria over specifieke apparatuur en goed opgeleid lokaal personeel beschikten. Sequencinggegevens gaven richting aan de besluitvorming op het gebied van volksgezondheid en hielpen inzicht te krijgen in de dynamiek van de uitbraak, risicopopulaties en blootgestelde gezondheidszones.

In het tweede deel van dit doctoraat had QuickNavi™ RDT een hoge specificiteit en een vrij goede gevoeligheid dan OraQuick® en Coris® tests in uitbraakomstandigheden. QuickNavi-Ebola™ was minder indrukwekkend in vergelijking met eerdere studies; de OraQuick® test kwam bijna overeen met eerdere bevindingen, hoewel hij beter presteerde voor de middelste en lagere Ct-waarden in laboratoriumomstandigheden. Geen van de vier Ebola RDTs die in onze studies werden geëvalueerd, bereikte de gewenste (gevoeligheid >98%, specificiteit >99%) of aanvaardbare (gevoeligheid >95%, specificiteit

>99%) prestatieniveaus zoals vermeld in het WHO Target Product Profile voor EBOV-tests. De respectieve specificiteiten van QuickNavi™ (>99%) en OraQuick® Ebola (98%) lagen in de meeste van onze studies echter dicht bij het aanvaardbare prestatieniveau (>99%).

Gebaseerd op de algemene prestaties, werden QuickNavi™ en Oraquick® Ebola RDTs voorgesteld als een screening panel op de point-of-care voor triage en isolatie van verdachte gevallen in afwachting van de RT-qPCR resultaten. De verwachting van de RDTs resultaten zal afzonderlijk worden gedaan, d.w.z. personen met ten minste één positieve RDT geïsoleerd in het hoogrisicogebied en personen met negatieve RDTs resultaten in het laagrisicogebied.

Voor postmortale surveillance vulde OraQuick® RDT de respons effectief aan, verbeterde de betrokkenheid van de gemeenschap en verminderde het aantal systematische veilige en waardige begrafenissen (SDB's) bij lijken met een niet-reactieve test. Vertrouwen in postmortale testen leidde ertoe dat families vrijwillig om SDB's vroegen ondanks OraQuick® niet-reactieve resultaten.

Trefwoorden: Ebola, EVD, DRC, GeneXpert, RDT, laboratorium, gevoeligheid, specificiteit

Part 1: General Introduction

Chapter 1

Ebola virus

1.1. Ebola virus: discovery and history

Ebolaviruses belong to the genus *Ebolavirus*, family of *Filoviridae*, order of *Mononegavirales*. These viruses have a 19 kilobase genome consisting of a single strand of RNA with negative polarity (Baseler et al, Malvy et al, Stein). The virus nameis derived from the Ebola River in the northern part of the Democratic Republic of Congo (formerly Zaire), where the second cluster of hemorrhagic fever cases was recorded. The first reported outbreak started in Nzara and Maridi in 1976, two towns inSouth-Sudan, where 150 of 284 victims died, with a case fatality rate (CFR) of 53%. The outbreak in Nzara is thought to have originated among workers from a cotton factory. It was reported that insectivorous bats were present in the roof space of the factory. The second outbreak occurred in Yambuku, Democratic Republic of the Congo(DRC), near the borders with Sudan and the Central African Republic. In this outbreak,318 persons were infected, 284 died giving an 89% mortality rate. The first person whodeveloped the disease was a 44-year-old school-teacher who presented to the Yambuku Mission Hospital on 26 September 1976 with fever and received parenteral chloroquine for suspected malaria. His fever subsided but subsequently reappeared together with other signs and symptoms, including gastrointestinal bleeding. The patient was admitted on 5 September, died on 8 September 1976 and is considered the index case of the outbreak. While travelling during the preceding weeks, he had purchased and eaten antelope meat. Almost all patients who subsequently developedEbola hemorrhagic fever during this outbreak either received injections at the same hospital or were close contacts of patients who received injections. At the Yambuku General Hospital, it was reported that five syringes and needles were distributed every morning, and were usually rinsed in a pan of warm water between their uses on different patients and sometimes, at the end of the day, they were boiled. Eventually, 55 of the 250 neighboring villages in the area, all within 120 km from Yambuku, reported patients. From these independent outbreaks, two distinct viruses, Zaïre Ebolavirus (EBOV) and Sudan Ebola virus (SUDV) were identified (Pourrut et al, WHO 1978,Tyagi et al, Muyembe-Tamfum et al, Zawilińska et al, Furuyama et al, Malvy et al). The name of the family *Filoviridae* comes from the Latin word “filum” or thread, becausethe virion shape resembles a twisted thread

when viewed under an electron microscope. The *Filoviridae* are divided into three genera: *Ebolavirus, Marburgvirus*, and *Cuevavirus.* The genus *Ebolavirus* contains five species with the taxonomic designations: Bundibugyo ebolavirus (BDBV), Reston ebolavirus (RESTV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), and Zaire ebolavirus (EBOV). Hence, the disease caused by Bundibugyo virus is designated Bundibugyo virus disease, by Sudan virus as Sudan virus disease, and by Ebola virus as Ebola virus disease (Baseler et al, Kuhn et al, Malvy et al). This taxonomy, revised in 2011, is emphasized because nearly identical terms have different meanings: *Ebolavirus* and *Zaire ebolavirus* refer to taxonomic classifications, whereas Ebola virus is a virus (Bukreyev et al). Ebola virus genus can cause severe hemorrhagic fever in humans and Non-Human Primates (NHPs). EBOV, SUDV, and BDBV have been responsible for numerous small and limited outbreaks in central Africa. Overall case fatality rate (CFR) has been 25% for Bundibugyo virus disease, 50% for Sudan virus disease, and80% for Ebola virus disease and combined CFR ranged from 30% to 90% (Del Rio etal, CDC 1976, Malvy et al). However, the 2013–2016 EBOV epidemic in west Africa, which originated in Guinea and spread to Liberia and Sierra Leone, resulted in 11,323 fatalities among at least 28,646 cases (Coltart et al, WHO 2016, Baseler et al, Burk etal). In 1994, Tai Forest Ebolavirus was isolated in the Ivory Coast from an ethnologistmaking the autopsy of chimpanzee from the Tai National Park reserve. Because it hasbeen described only in a single nonfatal human case, therefore was assumed to infectmainly chimpanzees. BDBV appeared in 2007 from human isolates collected during an outbreak in Uganda. It cannot be differentiated from other ebolaviruses by clinical symptoms (Towner et al). Further, the Reston ebolavirus (RESTV) originates from thePhilippines. It was first detected in Reston (Virginia, USA) in macaques imported from the Philippines and housed at a quarantine facility. This species caused, hemorrhagicfever with high mortality in Non-Human Primates (NHP). In the Philippines, it was alsofound in pigs, usually co-infected with porcine respiratory and reproductive virus, but the actual pathogenic potential of RESTV in pigs remains unclear. It is known to be non-pathogenic to humans, although the presence of specific antibodies in humans suggests that humans can be

infected upon exposure to infected animals (Zawilińskaet al). Recently, a new ebolavirus, Bombali virus (BOMV) was discovered in bats in Sierra Leone and Kenya. While BOMV has the potential to infect human cells, there iscurrently no evidence that the virus causes disease. Similarly, Mengla virus (MLAV) was identified in fruit bats in China. It is not known whether these viruses cause diseasein humans or NHPs (Goldstein et al, Forbes et al, Yang et al). Virus genomes from more than 5% of all recorded cases have been sequenced, which has allowed the spread of the disease to be reconstructed across country borders and the molecular clock of Ebola virus in the human host to be estimated at $1.2x10^{-3}$ substitutions per site per year (Holmes et al, Dudas et al, Hoenen et al). This evolutionary rate overlapswith that of other RNA viruses. Few adaptive mutations, notably an alanine to valine exchange at glycoprotein position 82, have been selected in the West Africa outbreakstrain because they enhance virus entry into human cells (Diehl et al, Urbanowicz et al). In addition, polymorphism in residue 544 has been identified as enhancing infection by decreasing the threshold for activation of membrane fusion activity triggered by host factors cathepsin B and Niemann-Pick C1 (EBOV-Makona, EBOV-Mayinga, EBOV Gabon 1994, EBOV Kikwit 1995). However, no evidence has shown that the presenceof these mutations or the accumulation of neutral substitutions measurably changed the clinical presentations, disease severity, or transmissibility of the virus (Wang et al,Marzi et al). In contrast, the genus *Marburgvirus* contains only one known species, *Marburg marburgvirus*, consisting of *Marburg virus* (MARV) and *Ravn virus*. Likewise, the genus *Cuevavirus* has one species with one known virus named *Lloviu virus*. Similar to BOMV and MLAV, only sequence information is available, and no virus has been isolated from infected bat samples in Europe (Negredo et al, Kemenesi et al).

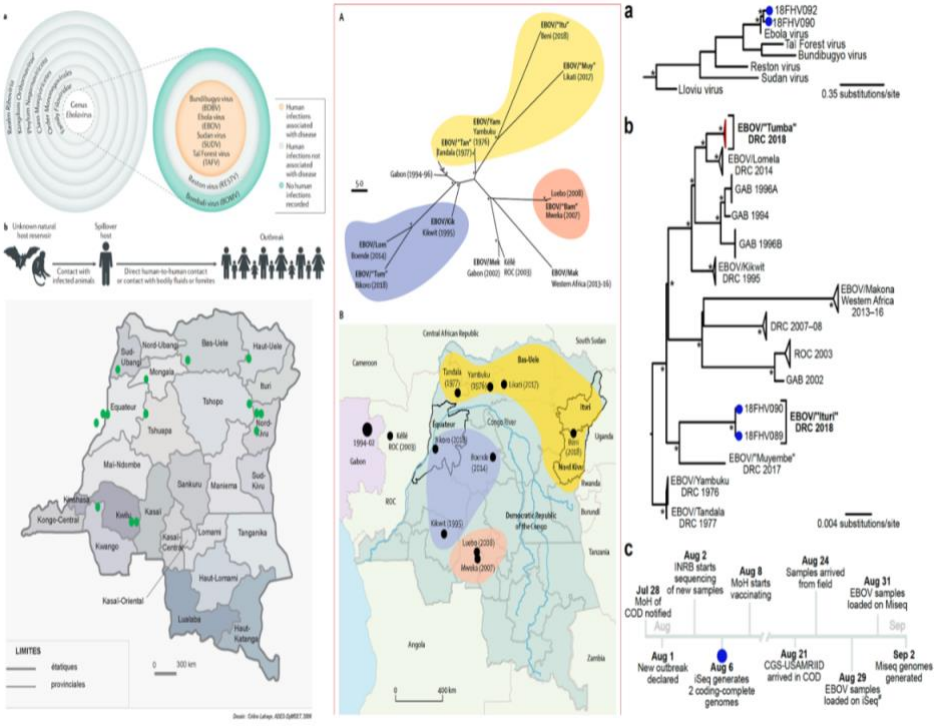


Figure 1. Filovirus taxonomy, EVD outbreaks' map and EBOV genetic diversity

a. Taxonomy of the genus Ebolavirus. Thus far, five ebolaviruses have been associated with human infections, and four of them have been identified as pathogens

b. DRC has faced fifteen EVD outbreaks of which 14 were caused by EBOV species and one with BDBV species (Isiro, 2014).

1.2. Ebola virus: Transmission and epidemiology

Most outbreaks can be traced back to a single spillover introduction of EBOV into the human population from an unknown reservoir (animal) by unknown means (e.g. hunting, direct contact with infected live or dead animals, consuming of bush meat). Subsequently, the virus is transmitted by direct contact with a sick person (particularlyin the late stages of infection, when viral loads are the highest), contaminated objectsused by the patient (fomites) and human corpses. Infection of healthcare workers or those taking care of sick persons, in the absence of appropriate personal protective equipment (PPE), and during the funeral ritual are an important element in theepidemiological chain. Although rare, sexual transmission during convalescence stage was proven or strongly suspected during the West African and DRC outbreaks, with virus persisting in immune privileged sites such as testis. In the hospital environment,infection through aerosols is also possible (e.g. during intubation, bronchoscopy), although there is no clear evidence that airborne spread of EBOV from person to person occurs. In an animal model it was confirmed that in the case of needle-stick injury the risk of infection and disease progression is significantly greater than after administration of a similar dose by aerosol. It is also known that infectious EBOV has been recovered from breast milk, saliva, urine, semen, cerebrospinal fluid, and aqueous humor, in addition to blood and blood derivatives, and detected in amniotic fluid, tears, skin swabs and stool by reverse transcription RT-PCR (Jacob et al, Zawilińska et al, Bausch et al, Deen et al, Kreuels et al, Moreau et al, Vetter et al).

Additionally, the natural reservoir of the virus is thought to be infected fruit bats, whoseRNA sequences of filoviruses are characterized by very high genetic diversity. Phylogenetic analyses of Marburg virus RNA sequences derived from both, people andbats, suggest that the virus spread from bats might generate an epidemic in humans. In addition, macaques, chimpanzees, antelopes, rodents and other so far unidentifiedspecies may represent a significant source of infection for humans (Kuhn at al, Zawilińska et al).

1.3. Virion structure and replication

1.3.1. Virion structure

Ebolaviruses (family: *Filoviridae*) are non-segmented, negative sense, single-strandedRNA viruses. The virion has a uniform diameter of about 80 nm, and a length of 970 to1200 nm. When propagated in cell cultures it is characterized by a significant pleomorphism and its length can then increase up to 14,000 nm. The virion core contains one molecule of linear, non-segmented, single-stranded, negative-sense RNA. The RNA is helically wound and complexed with the nucleoprotein (NP), virion protein 35 (VP35), virion protein 30 (VP30), and RNA-dependent RNA-polymerase (RdRP) derived from the large-gene (L). The helical nucleocapsid is surrounded by anouter envelope with anchored specific glycoprotein (GP) spikes, of about 10 nm length.These glycoproteins play a key role in the pathogenesis, due to the role in virus entry and its immunogenicity. Glycoproteins are targets for the immune cells, and thus are taken into account in the development of vaccines. Viral matrix protein, VP40 and VP24, are located between the nucleocapsid and the outer envelope (derived from the host cytoplasmic membrane). The viral genome, approximately 19 kilobase in length, is the longest among all viruses belonging to the order *Mononegavirales* (single- stranded, negative-sense RNA). Sequentially arranged genes encode seven structuralproteins, respectively 3'-nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), soluble glycoprotein (sGP), protein VP30, minor matrix protein (VP24),RNA-dependent RNA polymerase (L). The 3' terminus of viral genome is not polyadenylated and the 5' end is not capped. The leader and trailer at the 3'- and 5'- ends are non-transcribed regions, but they carry important signals to control transcription, replication, and packaging of the viral genomes into new capsids.Sequences encoding the viral structural proteins contain open reading frames and are flanked by the non-translated intergenic regions.

The GP gene is 681 amino acids, 450kDa structural transmembrane protein. It transcription results in three different mRNAs leading to the expression of full-length GP (cleaved by furin into GP1 and GP2), sGP (delta peptide cleaved off by furin), andssGP. The Glycoprotein contributes to the pathogenesis of Ebola virus by causing cytotoxicity in cells and damaging endothelial cells. GP has a

trimeric crystal structureand consists of a highly glycosylated region known as the mucin domain, which induces cell cytotoxicity in infected host cells. The GP forms spike-like structures on the viral surface and helps in attachment and entry of the virus. The GP is expressed in large amounts in infected cells and also circulates in the blood, making it an attractive target for Ebola antigen screening procedures, as it can be screened from the blood ofinfected patients. The small glycoprotein is not a part of the virion, but is excreted fromthe infected cell in large quantities. It may play a role in confounding the immune system to prevent the marshaling of an effective immune response.

The virion protein 40 (VP40) is a 37 kDa and 326 amino acid, peripheral matrix protein.This protein is transported to the plasma membrane by exploiting the retrograde late endosomal pathway. It may occur in hexamer and octamer forms in infected cells, binding to the inner surface of the plasma membrane and helping to form new virus particles. Abundantly present in the cytoplasm of infected cells, VP40 supports in membrane association, capsid assembly, and budding of the virus. Due to its abundance in the infected host cells, it is a good candidate for developing antigen detection assays.

Virion Protein 24 (VP24) is a matrix protein that is 251 amino acids long, with a molecular weight of 28 kDa (56). It remains in inclusion bodies in infected cells, interacting with NP for its localization, and helping in the packaging of the viral RNA genome. As well, VP24 has been reported to interfere with the interferon signaling pathway. VP24 is a minor matrix protein, and it is associated with lipid membranes. Therefore, the protein is not a suitable diagnostic target.

Nucleoprotein (NP) is 739 amino acid long (83.3kDa), sialylated, O-glycosylated structural protein. Along with RNA-dependent RNA polymerase and the viral RNA, NPforms a nucleoprotein complex, helping in the transcription and replication of RNA. TheNP has ten linear B-cell epitopes, ten antigenic sites, and five surface accessible epitopes, predicted as a conserved region among EBOV species. Among these regions, GEQYQQLR has been reported to have immunogenic and antigenic properties, making NP a suitable target for antigen detection.

VP35 consists of approximately 340 amino acids and its molecular weight is 37

kDa. Containing a C-terminal dsRNA binding domain and acting as a cofactor of RNA- dependent RNA polymerase, VP35 plays important roles in transcription, replication, and assembly of the virus. It also has been reported to mask viral RNA from host innateimmune system VP30 consists of approximately 288 amino acids and its molecular weight is 33 kDa. Itis a part of the viral RNA transcription complex, facilitating mRNA transcription. VP30 contains a zinc finger between amino acids 68 and 95. These zinc finger amino acids are necessary for transcription. In inclusion bodies, VP30 becomes phosphorylated, and in the cytoplasm of infected cells, it takes a non-phosphorylated form. Phosphorylation of VP30 inhibits transcription of the Ebola virus gene.

Viral Polymerase (L Protein) consists of 2,212 amino acids and its molecular weight is253 kDa. It acts as an RdRp and has three domains: the RNA binding element, a phosphodiester bond formation domain, and a purine ribonucleotide triphosphate- binding domain. This is among the most conserved proteins in the order *Mononegavirales* and helps in transcription as well as replication (Singh et al, Zawilińska et al, Goldstein et al).

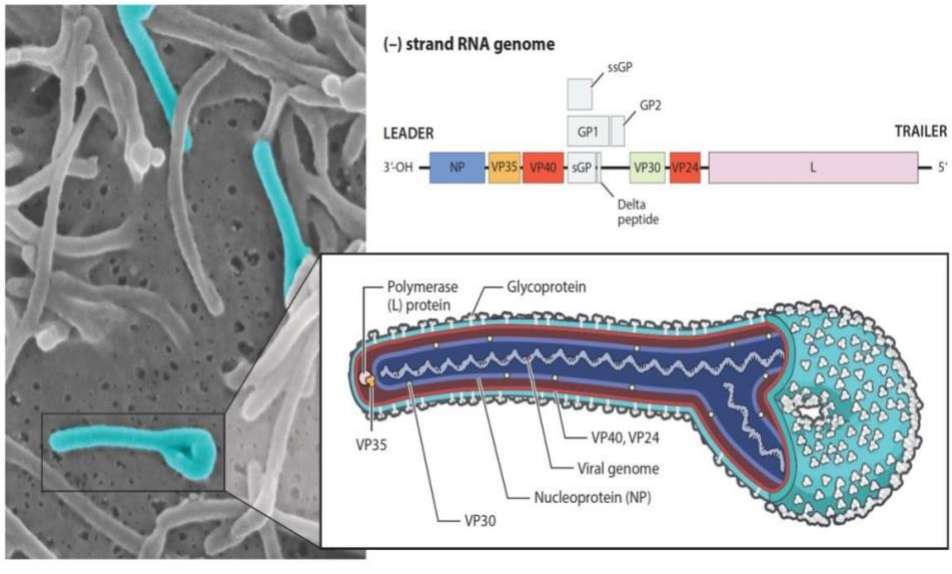


Figure 2. Electron micrograph and schematic of the EBOV particle and genome.

EBOV particles (blue) on the surface of an infected cell are shown. Abbreviations: EBOV, Ebola virus; GP, glycoprotein; sGP, soluble glycoprotein; ssGP, small soluble glycoprotein; VP, virion protein.

1.3.2. Virus replication

The first step in viral replication is attachment to the host cell membrane and penetration into the cell. This process is not completely understood, but it is known thatglycoprotein (GP) spikes are involved in entry of virions into the host cell and are used in the mechanisms similar to macropinocytosis (Aleksandrowicz et al). The other proposed mechanisms of cell entry include: clathrin-mediated endocytosis or glycoprotein-facilitated receptor binding. The glycoprotein is post-transcriptionally cut into GP1 and GP2 proteins. The GP1 takes part in the attachment of the virus particleto the cell membrane, while the GP2 participates in viral fusion with the cell membrane.Viral membrane fuses with cell vesicle membrane to allow the release of the nucleocapsid into the cytoplasm. It is believed that the further steps of replication occur in the cytoplasm, analogously to paramyxoviruses and rhabdoviruses. Encapsidated, genomic RNA is used then as a template for transcription into seven polyadenylated, monocistronic mRNAs and translated by the cellular translation machinery into individual viral proteins. Transcription is regulated by conserved transcription start andstop signals located at the viral gene borders. The gene start signals are parts of RNAsecondary structures, and it has been proposed that VP30 binds to the RNA at the firstgene start signal to initiate transcription. In addition, VP30 was shown to be importantfor reinitiation of transcription of subsequent genes (Biedenkopf et al). VP30 activity is regulated via its phosphorylation state: phosphorylation of VP30 inhibits viral transcription while viral replication is increased. Because of its essential function in these processes, VP30 is a potential interesting candidate as antiviral therapy target (Ascenzi et al). Subsequently, when a positive-sense full-length genome is replicated,it is concomitantly encapsidated by newly synthesized NP molecules. Other structuralnucleocapsid proteins (polymerase cofactor — VP35, and the viral RNA polymerase L) participate in the synthesis of the viral genome. The presence of matrix VP24 together with NP and VP35 is required for assembly of viral nucleocapsids, and silencing of VP24 expression prevents the release of viruses. Moreover, in the VP24- deficient viral particles VP30 transcription and translation are diminished. Further, themost abundantly expressed matrix protein VP40 plays an important role in the formation of new virus particles, and

is associated with the endosomal pathway and virus budding from the cell (Stahelin). The mechanisms of this process are not fully understood but it is known that mutations in the sequences encoding the VP40 leads to inhibition of virus release from the infected cell (Zawilińska et al).

1.4. Democratic Republic of the Congo as the epicenter of Ebola outbreaks

Democratic Republic of the Congo (DRC) is the second largest country on the African continent, with a population ofover 70 million and is the hub of Africa's most crucial continental crossroad, being bordered by Rwanda, Uganda, Burundi and Tanzania to the east, Central African Republic and South Sudan to the north, Angola and Zambia to the south, and the Republic of the Congo in the west. Formally established as a Belgian colony in 1908, DR Congo became independent in 1960, became Zaire in 1972, and became the DRCin 1997. DR Congo has more than 200 ethnic groups and different languages, including French, Lingala, Kiswahili, Tshiluba and Kikongo as National languages (Guetiya- Wadoum et al, DRC's constitution 2015). Since the discovery of the virus in 1976, theDRC has faced fifteen EVD outbreaks (table 1), among which eight occurred in the lastsix years (as of 23 August 2022). The tenth outbreak (in 2018-2020) was the longest with 22 months, the most widespread with 29 health zones affected in three provinces,and caused the most fatalities (2287 overall deaths) recorded to date in DRC (Mukadiet al, Aruna et al, Christie et al, WHO, Mbala-Kingebeni et al, Guetiya-Wadoum et al).

Table 1: DRC EVD outbreaks (Time, place, cases, deaths, case fatality rate, strains)

Period	Place	Cases	Deaths	Case Fatality rate (%)	Ebola Virus involved
1976	Yambuku/Mongala	318	280	88	Ebolavirus
1977	Tandala/South-Oubangi	1	1	100	Ebolavirus
1995	Kikwit/Kwilu	315	254	81	Ebolavirus
2007	Mweka/Kasaï	264	187	71	Ebolavirus
2008- 2009	Luebo/Kasaï	32	14	45	Ebolavirus
2012	Isiro/Haut-Uele	57	29	51	Bundibugyo
2014	Djera/Tshuapa	66	49	74	Ebolavirus
May-July 2018	Bikoro/Equateur	54	33	61	Ebolavirus
Aug-June 2020	North-Kivu, Ituri & South-Kivu	3470	2287	66	Ebolavirus
June-Sept 2020	Equateur	130	55	42,3	Ebolavirus
Feb-May 2021	Butembo/North-Kivu	12	6	50	Ebolavirus
Oct-Dec 2021	Beni/North-Kivu	8	6	75	Ebolavirus
April to July 2022	Mbandaka/Equateur	4	4	100	Ebolavirus
August to September 2022	Beni/North-Kivu	1	1	100	Ebolavirus

Unlike previous outbreaks in the DRC, security challenges in North Kivu and Ituri Provinces hampered response efforts, making it difficult to identify and follow-up contacts of people infected with Ebola during the tenth Ebola outbreak. Thus, the scenario of Ebola spreading to the nine countries sharing borders with DRC was factualand scary as the life of about 290 million African citizens - representing 23% of Africa'stotal population - was at risk (table 2). In the eventuality that this spread happened, it would have led to an unprecedented major humanitarian crisis with a critical socio- economic impact on Africa. The high traffic at the DRC borders and insecurity constituted some drivers of spillover (Mukadi et al, Aruna et al, Christie et al, WHO, Mbala-Kingebeni et al, Guetiya-Wadoum et al).

Table 2 Distribution of the population in countries neighboring the DRC

Region	SDG[a] Region	Sub-Region	Country	Country Code	Population[b]
Africa	Sub-Saharan Africa	Middle Africa	The Democratic Republic of the Congo	180	86,791
			Angola	24	31,825
			Congo	178	5381
			The Central African Republic	140	4745
		Eastern Africa	United Republic of Tanzania	834	58,005
			Uganda	800	44,270
			Zambia	894	17,861
			Rwanda	646	12,627
			Burundi	108	11,531
			South Sudan	728	11,062
Total population at risk (≈23%)					**284,098**
Total population of Africa					**1308,064**

Notes: [a]SDG, Sustainable Development Goal; [b]Total population, both sexes combined, as of July 1, 2021 (thousands); data from United Nations, Department of Economic and Social Affairs, Population Division. World population prospects 2019, Online edition; 2019. Available from: https://population.un.org/wpp/Download/Standard/Population/.[7]

1.5. Ebola virus disease: surveillance, vaccination, treatment & prevention

The conflict in DRC has resulted in a health-system collapse and created a humanitarian disaster (Zarocostas). An estimated 5.4 million excess deaths occurred from 1997 to 2004, with fewer than 10% attributable to violence and the rest to preventable and treatable medical conditions such as malaria, diarrhea, pneumonia and malnutrition (Coghlan et al). In eastern Congo, the prevalence of rape and other sexual violence is documented as among the highest in the world (Brown, Guetiya- Wadoum et al).

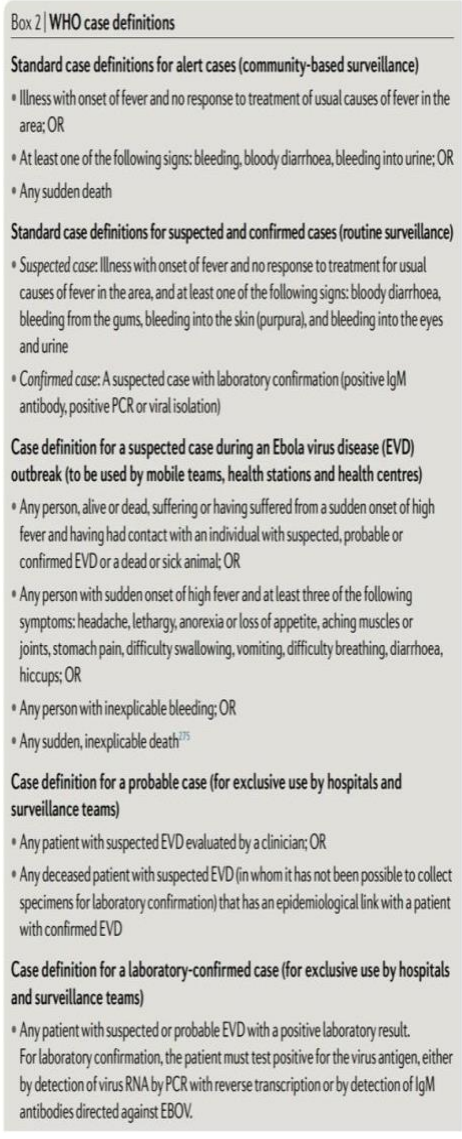
1.5.1. Surveillance

To control Ebola disease outbreaks, it is essential to stop transmission and interrupt spread of disease in the most affected populations. To date, this control has been achieved through a multidisciplinary, holistic approach including early case identification, rapid isolation, and clinical management of patients with Ebola virus disease; safe and dignified burial practices; health promotion and community engagement; support to health structures; and transversal coordination (Malvy et al, Jacob et al, Lindblade et al, Okware et al, Caleo et al, Mukadi et al).

1.5.1.1. Case identification

Early case identification is important to prevent outbreaks spreading, which would require reinforcement of surveillance systems for early identification of suspect cases in at-risk areas. These surveillance systems should be complemented with the establishment of national laboratory capacity able to rapidly and reliably detect *ebolaviruses* and other hemorrhagic fever viruses using state-of-the-art technologies (Shoemaker et al, Jacob et al). The WHO has developed EVD standard case definitions for alert, suspected, probable and confirmed cases in the context of routineand community-based surveillance; whereas the US Centers for Disease Control andPrevention (CDC) created a case definition for persons under EVD investigation (Figure 3: Box 2 and Box 3). These standard case definitions are utilized by public health authorities to optimize surveillance and notification of EVD, particularly before an outbreak has been identified. As increasing numbers of patients with possible EVD present to health facilities at the beginning of an outbreak, case definitions are refined from standard public health case definitions to reflect clinical and epidemiological features associated with a particular outbreak context. A robust case definition and accurate confirmatory testing are key to ensuring that individuals with suspected EBOVinfection are efficiently identified and, upon admission to an Ebola Treatment Center (ETC), isolated for confirmation of diagnosis and treatment. Despite refinement, case definitions are rarely 100% sensitive or specific, and attempts to optimize one come atthe expense of the other. A case definition with a low sensitivity will mislabel true EBOV-positive individuals as EBOV-negative, leading to an increased risk of discharge of EBOV-infected individuals back to the community, where EBOV transmission can be reinitiated. Particularly in a setting with a low community incidence of EVD, the sensitivity of the case definition should be maximized. By contrast, a case definition with a low specificity might result in misclassification of true EBOV-negative individuals as EBOV-positive. Such individuals might be admitted to an ETC with suspected EVD,placing them at increased risk of EBOV exposure and nosocomial infection, especiallywhen the probability that other patients with suspected EVD might be EBOV-positive is high. Thus, in a community with a high incidence of EVD, increased specificity in EVD case

definition may be crucial. Given these considerations, currently no EVD case definition is globally applied. Indeed, the EVD case definition can be reiterated during the course of an outbreak as the outbreak evolved from a high incidence to a low incidence (Jacob et al).



Box 2 | **WHO case definitions**

Standard case definitions for alert cases (community-based surveillance)

- Illness with onset of fever and no response to treatment of usual causes of fever in the area; OR
- At least one of the following signs: bleeding, bloody diarrhoea, bleeding into urine; OR
- Any sudden death

Standard case definitions for suspected and confirmed cases (routine surveillance)

- *Suspected case:* Illness with onset of fever and no response to treatment for usual causes of fever in the area, and at least one of the following signs: bloody diarrhoea, bleeding from the gums, bleeding into the skin (purpura), and bleeding into the eyes and urine
- *Confirmed case:* A suspected case with laboratory confirmation (positive IgM antibody, positive PCR or viral isolation)

Case definition for a suspected case during an Ebola virus disease (EVD) outbreak (to be used by mobile teams, health stations and health centres)

- Any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with an individual with suspected, probable or confirmed EVD or a dead or sick animal; OR
- Any person with sudden onset of high fever and at least three of the following symptoms: headache, lethargy, anorexia or loss of appetite, aching muscles or joints, stomach pain, difficulty swallowing, vomiting, difficulty breathing, diarrhoea, hiccups; OR
- Any person with inexplicable bleeding; OR
- Any sudden, inexplicable death[275]

Case definition for a probable case (for exclusive use by hospitals and surveillance teams)

- Any patient with suspected EVD evaluated by a clinician; OR
- Any deceased patient with suspected EVD (in whom it has not been possible to collect specimens for laboratory confirmation) that has an epidemiological link with a patient with confirmed EVD

Case definition for a laboratory-confirmed case (for exclusive use by hospitals and surveillance teams)

- Any patient with suspected or probable EVD with a positive laboratory result. For laboratory confirmation, the patient must test positive for the virus antigen, either by detection of virus RNA by PCR with reverse transcription or by detection of IgM antibodies directed against EBOV.

Box 3 | **CDC definition for a person under investigation for EVD**

- Individual with both "1. Elevated body temperature or subjective fever or symptoms, including severe headache, fatigue, muscle pain, vomiting, diarrhoea, abdominal pain or unexplained hemorrhage; **AND** 2. An epidemiologic risk factor within the 21 days before the onset of symptoms"[276].
- Such risk factors include direct contact with "blood or bodily fluids (urine, saliva, sweat, feces, vomit, breast milk, and semen) of a person who is sick with or has died from [...] (EVD)", "objects (such as clothes, bedding, needles and medical equipment) contaminated with bodily fluids from a person who is sick with or has died from EVD", "infected fruit bats or nonhuman primates (such as apes and monkeys)", and "semen from a man who recovered from EVD (through oral, vaginal, or anal sex)"[277].

Figure 3: Box 2: WHO case definitions

Box 3: CDC definition for a person underinvestigation for EVD).

1.5.1.2. Rapid isolation and testing

Rapid isolation of Ebola disease cases is crucial in order to achieve outbreak control. One of the most effective ways to achieve rapid isolation of the disease is through provision of care in appropriate settings, such as ETCs and laboratory testing. Isolationand high-quality care need to be provided with dignity, respect, and compassion to beaccepted by affected communities. As long as their safety is guaranteed, affected families should be informed about and, as much as possible, involved in the care of their close ones, and visits by family members should be encouraged. ETCs should beclose to the affected communities and operate with a transparent policy (including open communication with the affected communities about medical activities that occur in the ETC and possibility for families to visit their affected family members). Recently, individual treatment units (such as biosecure emergency rooms) have been developed during the 2018 epidemics in the DRC, with the aim of ameliorating adaptability and facilitating access for medical staff and families. Anecdotal evidence suggests that isolation and elementary care of Ebola disease patients within their homes or communities could be an alternative to care in ETCs, notably when isolation is not accepted by the patient or the community. Importantly, patient screening time should be minimized to limit exposure of uninfected individuals,including ETC staff, to potentially infected individuals. Within an ETC, patients with suspected EBOV infection may be further separated, based on the probability of EBOV infection or the risk of infectiousness, to avoid nosocomial infection within the ETC. The corresponding jargon for ETC wards or sections of wards to reflect levels of risk (of having EVD, and therefore infectiousness) has varied across ETCs and has included descriptions such as ‘suspect vs probable’ or ‘wet vs dry’ (Lindblade et al, Caleo et al, Tiffany et al, Funk S et al, Gray et al, Okware et al, Jacob et al).

1.5.1.3. Contact tracing

Contact tracing (identification, listing, and following up of contacts of patients with Ebola disease) contributes to the control of outbreaks, although people's dignity and privacy need to be respected. Prerequisites for effective contact tracing include the availability of sufficient skilled human resources and logistical support, including mobiletelephones and motorbikes or cars, which might be challenging in the low-resource settings in which Ebola disease outbreaks occur. However, effective coordination andcommunity involvement might allow effective contact tracing even when resources are restricted. Safe burial practices also contribute to effective control of Ebola disease outbreaks (Okware et al, Saurabh S & Prateek S, Ajelli et al, Jacob et al).

1.5.1.4. Respect of beliefs & societal leadership

Permitting attendance and allowing time for traditional ceremonies, while taking all necessary safety precautions, can greatly increase the community's acceptance and compliance with safe burial activities (Caleo et al). Cultural practices and beliefs mightcreate barriers to the perception and management of patients with symptoms of Eboladisease, resulting in delayed referral of infected individuals for care. In many places where Ebola disease occurs, the disease remains unknown and can often be seen asa consequence of witchcraft. Health promotion activities are crucial to obtain the acceptance of the different intervention control activities by the communities. These health promotion activities should preferably be done by people who had been exposedto Ebola disease and be based on a dialogue with the community, rather than an exercise in top-down information They should aim to address the concerns and experiences of the affected communities. The communities, represented by local trusted leaders should be encouraged to get involved in the different outbreak control interventions (eg, through participation in outbreak intervention teams, such as local religious leaders being part of burial teams). Traditional healers and religious leaders should have a central role in vaccination programs, not just for their own safety, but also to set an example within their communities, and should receive health education to enhance their potential to recognize cases of Ebola disease and react appropriately. Survivors can be crucial witnesses to increase community acceptability, eg, by testifying about

the care and testing given. Since high case fatality has discouraged people from seeking care, the availability of dedicated ETCs close to the affected regions might help to increase acceptance (Gray et al, Okware et al, Jacob et al). During DRC 2018-2020 EVD post-epidemic period, postmortem surveillance with OraQuick Ebola Rapid Diagnostic Tests (RDTs) complemented outbreak-response efforts, improving community trust, and decreasing the number of mandatory safe anddignified burials (SDBs). In this case, families voluntarily requested SDB despite nonreactive Ebola RDT contributing to community engagement (Mukadi et al).

1.5.2. Ebola Vaccines

The West-Africa EVD outbreak accelerated efforts to develop antiviral strategies, andsome experimental therapeutic and vaccine candidates which were evaluated in clinical trials (Coltart et al). Furthermore, the two preclinically most-promising vaccine candidates, Vesicular Stomatitis Virus (VSV)-EBOV and Chimpanzee Adenovirus (chAd)-EBOV, were deployed toward the end of the epidemic in phase 3 clinical trialswith the hope of interrupting human transmission chains using a ring-vaccination approach (Suder et al, Venkatraman et al). As the EBOV glycoprotein is the major viralimmunogen, all candidate vaccines in advanced development are designed to stimulate a host immune response against this protein, among others (Kuhn et al june 2019, Kuhn et al may 2019, Furuyama et al). During the 2018-2020 DRC EVD outbreak and onwards, vaccines and therapeutics were available at the beginning of the epidemic, allowing their use at a larger scale as the outbreak lasted 22 months and affected 29 health zones across 3 affected provinces in the east of the DRC (Jacob et al).

1.5.2.1. rVSV-ZEBOV-GP (Ervebo®)

The rVSV-ZEBOV-GP (Ervebo®) vaccine is a live attenuated recombinant of the Vesicular Stomatitis Virus (rVSV) strain Indiana, genetically engineered to replace theVSV glycoprotein, with the EBOV glycoprotein from the Kikwit 1995 virus (DRC). Vaccination induces replication of viral particles similar to VSV but expressing the EBOV surface GP which is responsible for receptor binding and membrane fusion between the virus and host target cells. The rVSV-ZEBOV-

GP (Ervebo®) induces the production of functional antibodies, including neutralizing antibodies (Garbutt et al, Watanabe et al).

The vaccine was developed by the Public Health Agency of Canada, licensed to BioProtection Systems (NewLink Genetics), and sublicensed to Merck. In October 2019, the European Medicines Agency granted the rVSV-ZEBOV-GP candidate vaccine conditional marketing authorization. Soon afterwards in November and December 2019, the European Commission and US Food and Drug Administration (US FDA) announced approval for the same vaccine for prevention of EVD. With full approval from the European Commission, the vaccine is cleared for use in the countries that are part of the European Union. As of December 2019, candidate vaccines have also been licensed in China and Russia (European Commission). Owingto the success of the Ebola ça Suffit! phase III ring vaccination trial in Guinea, the rVSV-ZEBOV-GP was actively administered to help contain the EVD epidemics in DRC (2018-2022) and Guinea (2021). Using a ring vaccination strategy, whereby contacts of infected individuals (primary ring) and contacts of those contacts (secondary ring) are vaccinated. These advances will undoubtedly facilitate production, stockpiling and wider distribution of vaccines to health-care workers and other at-risk individuals (Henao-Restrepo et al 2017, Henao-Restrepo et al 2015, WHO 2020, Regules et al, Jacob et al). The WHO reported an estimated vaccine efficacy of 97.5% (95% CI 95.8–98.5%). However, determination of true vaccine efficacy is impossible in the absence of a placebo-controlled group. Notably, a model of the EBOV infection risk during the 2018 EVD outbreak in Equateur Province in the DRC found that the introduction of ringvaccination with rVSV-ZEBOV-GP vaccine resulted in a decrease of 70.4% in the at-risk geographical area and 70.1% of the level of EBOV infection risk. However, if ring vaccination is delayed by as little as one week, the size of this effect is considerably diminished (Wells et al).

1.5.2.2. rAd26 ZEBOV-GP (Zabdeno®) & MVA-BN-Filo vaccines (MvaBea®)

In May 2019, the WHO-convened Strategic Advisory Group of Experts (SAGE) issuedrecommendations regarding vaccination strategies that included the use of a second vaccine. In August 2019, a clinical trial evaluating the safety and

immunogenicity of the rAd26 ZEBOV-GP (Zabdeno®) & MVA-BN®-Filo (MvaBea®) vaccines among health- care workers was initiated in Uganda (LSHTM 2019). In light of the risk of delayed ringvaccination with the rVSV-ZEBOV-GP vaccine owing to violence, a pre-exposure vaccination strategy using the rAd26 ZEBOV-GP & MVA-BN-Filo vaccine was also introduced in Goma (east of the DRC) from 14 November 2019to December 2020. This pre-exposure vaccine complemented the ring vaccination efforts with the rVSV-ZEBOV-GP vaccine (WHO 2020, Jacob et al). The vaccination regimen comprised two vaccine candidates, both given as a 0.5 mL intramuscular injection in the upper section of the deltoid muscle or in the thigh in the case of youngchildren:

1) rAd26.ZEBOV.GP: a monovalent vaccine expressing the full-length GP from EBOVMayinga, that is produced in the human cell line PER.C6, $5x10^{10}$ viral particles and given at day zero.
2) MVA-mBN226B, or MVA-BN-Filo: a multivalent vaccine expressing the GP of EBOV (100% homologous with the GP expressed by Ad26.ZEBOV), SUDV and Marburg Musoke viruses; and the nucleoprotein of TAFV, $1x10^{8}$ infectious units given at day 56 (−14day/+28 day).

This regimen has been evaluated for immunogenicity and safety in 11 clinical trials in the United Kingdom (UK), USA and East and West Africa, including previously EVD- affected countries.

When administered in a 0, 56-day schedule in phase 2 and 3 studies, geometric meanconcentrations of immunoglobulin G (IgG) binding antibody to the EBOV GP measuredby the Filovirus Animal Non-Clinical Group (FANG) ELISA 21 days post dose-2 were 3810–11 790 EU/mL in healthy participants. Unblinded safety data from 2390 adults showed only mild-to-moderate adverse events of short duration with no sequelae. No safety concerns were raised in HIV-infected individuals. In 649 children, the vaccine was highly immunogenic and had a similar safety profile as adults, with no SuspectedUnexpected Serious Adverse Reactions. The European Commission granted marketing authorization for Ad26. ZEBOV & MVA-BN-Filo on 1 July 2020 (Milligan et al, Mutua et al, Pollard et al, Ishola et al, Barry et al, Afolabi et al, Watson-Jones D et al)

1.5.3. Ebola therapeutics

1.5.3.1. Supportive care

Without a specific treatment, management of patients with Ebola virus disease consistsof the provision of supportive and, as required and when possible, critical care, on thebasis of the pathophysiological similarities between, on the one hand, Ebola virus disease and, on the other hand, septic and possible hypovolemic shock (Malvy et al, Sprecher et al, Bray et al, Lamontagne et al). In addition, symptomatic and empirical treatment for concurrent infections is provided, tailored to the needs of individual patients. Adequate prevention or correction of fluid losses, by providing oral or parenteral fluids, is standard practice in the critically ill, even if there is little evidence supporting its clinical utility in the case of Ebola virus disease. Intravenous fluids are usually required when oral fluid intake is not possible, including crystalloid in sufficientquantities to achieve hemodynamic stability (Sprecher et al, Rhodes et al, Lamontagneet al). Additionally, possible complications can occur requiring oxygen supplementation, mechanical ventilation, or kidney replacement therapy. In recent outbreaks, the installation of point of care for biochemistry testing has facilitated the correction of electrolyte and other biological imbalances (Leligdowicz et al, van Griensven et al, Sprecher et al, Mulangu et al, Malvy et al).

1.5.3.2. Specific therapeutics

Early 2018, the WHO led a panel of experts to evaluate the latest (human and animal) efficacy data on available therapeutics to inform the Monitored Emergency Use of Unregistered Investigational Interventions (MEURI), an ethical framework to guide compassionate access to investigational therapeutics during an EVD outbreak and asa bridge to a clinical trial. In the same period, the Pamoja Tulinde Maisha (PALM) group set up a phase II/III controlled trial to evaluate the efficacy of four investigational therapeutics [ZMapp (control arm, Mapp Biopharmaceutical Inc.), Remdesivir (nucleoside analog, Gilead Sciences,Inc.), mAb114 (a single monoclonal antibody derived from a Congolese human survivorof the 1995 Kikwit EVD outbreak, Ridgepack Biotherapeutics) and REGN-EB3 (a cocktail of three murine-derived but fully human monoclonal antibodies, Regeneron Pharmaceuticals)] for the treatment

of EVD in four trial sites in Nord-Kivu Province, eastern DRC. Patientsreceiving optimized supportive care were randomly assigned in a 1:1:1:1 ratio to intravenous administration of these four therapeutics with a primary end point of day 28 lethality. At day 28, mAb114 (Ebanga®) and REGN-EB3 (Inmazeb®) were significantly more effective thanZMapp and Remdesivir in treating EVD cases after an interim analysis performed by the Data Safety Monitoring Board for the PALM study in August 2019 (Mulangu et al, Malvy et al, Tshiani Mbaya et al, Qiu et al, Davey et al, Gaudinski et al, Corti et al, Sivapalasingam et al, Pascal et al, Warren et al, NIAID 2019, Wec et al, Misasi et al).

Although the efficacy results of mAb114 and REGN-EB3 were noticeable, 35.1% (61/174) and 33.5% (52/155) of the participants who received respectively mAb114 and REGN-EB3 died. The mortality was even higher, around 69.9% (51/73) and 63.6% (42/66) for mAb114 and REGN-EB3 respectively in the subset of participants presenting with high viral load (Ct ≤22) at baseline. Causes explaining this residual mortality may be related to the virus, the host, and even the intervention itself (TshianiMbaya et al, Malvy et al, Jacob et al).

Table 3 Brief comparison of mAb114 and REGN-EB3 characteristics

Features and properties	MAb114	REGN-EB3
Presentation	- Single mAb	- Cocktail of 3 mAbs (REGN3470, REGN3471, and REGN3479 in a ratio of 1:1:1)
Origin	- Derived from memory B cells from a survivor of the 1995 EBOV outbreak in Kikwit, Democratic Republic of the Congo, approximately 11 years after infection	- Immunization VelocImmune mice with DNA encoding Ebola virus glycoprotein and purified EBOVGP followed by cloning the human variable regions onto human constant regions, leading to a fully human antibody
Targeted epitope	- glycan cap and the core domain of the glycoprotein sub-unit 1 (GP1)	- REGN 3479: conserved GP2 fusion loop. - REGN 3471: outer glycan cap - REGN 3470: GP1 Head
Mechanism of action	- Neutralization - Antibody-dependent cell cytotoxicity	- Antibody-dependent cell cytotoxicity, - phagocyte stimulants, - virus internalization inhibitors
Advantages	- Single Antibody - Resisting at low pH environment (GP rearrangement with cathepsin); - Binding in a highly conserved region reduces the risk of escape mutants; - Single-shot option with short infusion time (30' to 1h); - Good half-life - Highly stable - Easy to manufacture with large scale production	- Cocktail mAbs - Targeting several different epitopes (reduce the selection of resistant virus) - Single shot - Good half-life
Dosing	- 50 mg/kg	- 150mg/kg

Table 4 EVD therapeutics evaluated in the PALM study

Agent name	Agent design, origin and key biology	Previous study design	Previous study results	PALM design (NCT03719586)	PALM key results	Notes
mAb114	Monoclonal anti-EBOV-$GP_{1,2}$ IgG1 antibody derived from a human survivor of the 1995 Kikwit outbreak	Phase I dose escalation study (NCT03478891)[183]	Well tolerated, no infusion reactions and mild systemic symptoms in 4/18 patients (22%) in all dose groups; linear pharmacokinetics, $t_{1/2}$: 24.2 days; no development of anti-mAb114 antibody	Dosing 50 mg per kg IV infusion over 30 min compared with ZMapp control[126]	61/174 patients (35.1%) died compared with 84/169 (49.7%) in the ZMapp control group, absolute difference 14.6% (95% CI −25.2 to −1.7; $P = 0.007$). Median time to first negative result 16 days compared with 27 days in the control group	Superior efficacy
REGN-EB3	Cocktail of three fully human anti-EBOV-$GP_{1,2}$ IgG1 antibodies in 1:1:1 ratio; the antibodies have three different epitope targets but can bind simultaneously; initially derived from VelocImmune humanized mice then fully humanized	Phase Ia randomized double-blinded, placebo-controlled dose escalation study (NCT002777151)	Well tolerated, no deaths, serious adverse events or adverse events leading to discontinuation; mild treatment-emergent adverse events included headache (33%, mild–moderate) and, less common, nausea, chills and polyuria; linear pharmacokinetics, $t_{1/2}$: 21.7–27.3 days for all monoclonal antibodies; no development of anti-REG-EB3 antibody	Dosing 150 mg per kg IV infusion over 2 (adults) to 4 (paediatric population) hours compared with ZMapp control	52/155 patients (33.5%) died vs 79/154 patients (51.3%) in the comparable ZMapp control group[a], absolute difference 17.8% (95% CI −28.9 to −2.9; $P = 0.002$); median time to first negative result 15 days compared with 27 days in the control group	Superior efficacy
Remdesivir (also known as GS-5734)	Prodrug of adenosine nucleoside analogue that is metabolized intracellularly to adenosine triphosphate analogue and inhibits viral polymerase	Case reports of compassionate use in neonate with acute EVD a survivor of EVD with meningo-encephalitis[226,253]	To be reported	Dosing: for body weight ≥40 kg, 200 mg IV loading dose then 100 mg IV per day for 10–14 days; for body weight <40 kg, 5 mg per kg loading dose IV then 2.5 mg per kg IV for 10–14 days; infusion over 30 min	93/175 patients (53.1%) died vs 84/169 patients (49.7%) in the comparable ZMapp control group, absolute difference 3.4 % (95% CI −7.2 to 14.0; non-significant); time to first negative result >28 days[b]	Equivalent efficacy; does not require refrigeration; broad activity against other filoviruses; requires monitoring of AST and/or ALT
ZMapp	Cocktail of three monoclonal anti-EBOV-$GP_{1,2}$ antibodies; chimeric, initially derived from *Nicotiana* spp. tobacco plants	Phase II/III randomized controlled trial of ZMapp plus oSOC versus oSOC control group[182] (NCT02363322); no phase I study; compassionate use case reports[199]	91.2% posterior probability that ZMapp plus oSOC was superior to oSOC alone; failed to meet predefined threshold of >97.5%; infusion reactions required slowing administration	Dosing: 50 mg per kg IV on days 0, 3 and 6, infused per protocol over 4–6 hours	See above for comparison with other agents. 84/169 patients (49.7%) died overall; time to first negative result 27 days	ZMapp served as the control group comparator for the other agents in the PALM trial

ALT, alanine aminotransferase; AST, aspartate aminotransferase; EBOV, Ebola virus; EVD, Ebola virus disease; IV, intravenous; oSOC, optimized standard of care. [a]This subgroup was created to accomodate the delayed introduction of the REGN-EB3 randomization arm to the PALM trial. [b]Patients who died were considered not to have cleared the infection; this assumption explains the >27 days time to first negative.

1.5.4. Prevention

The overall strategy for mitigating the spread of an ongoing EVD outbreak is to interruptcommunity and nosocomial transmission of EBOV from patients to susceptible individuals. Effectively achieving this outcome depends upon the quality of measures in place; ideally, interruption of the chain of transmission in the community can be achieved by anthropological and sociological measures; isolating suspected individuals or EVD confirmed-cases (which includes contact tracing and following-up over 21 days); and treatment in an ETC or holding centre. The crucial importance of contact tracing is illustrated by the backdrop of the tenth EVD outbreak in the DRC, where a longstanding conflict has impeded maximal tracing of contacts of EVD, and violent incursions of armed groups in outbreak areas are associated with increase in estimated EBOV transmission rates (Jacob et al, Wannier et al). In a mathematical model estimating changes in EBOV transmissionin 12 districts in Sierra Leone from June 2014 to February 2015, introduction of additional treatment beds within the area to isolate patients with suspected or confirmed EVD would have theoretically averted ~56,000 new EVD cases (Kucharski et al). The risk of nosocomial transmission can be reduced by isolation of EVD suspected or confirmed cases, the use of appropriate PPE, strategies for donning and doffing PPE and strict adherence to infection prevention and control practices. Such practices include the provision of dedicated or disposable patient care equipment, safe injection practices, hand hygiene and attention to environmental infection control. The provision of guidelines for discharge criteria is an important aspect of clinical care to avoid subsequent transmission events in the community. During the 2013–2016 West Africa EVD outbreak, the WHO recommended that patients diagnosed with EVD can be considered for discharge from health-carefacilities if ≥3 days have elapsed since resolution of clinical signs, if they show appreciable improvement in clinical condition, if they are able to perform activities of daily living and if a blood sample is negative for EBOV RNA (detected with RT-PCR tests) from the third day of the patient becoming asymptomatic. Patients with unresolved signs and symptoms should be discharged after two negative blood test results (48 hours apart), and in these patients an alternative diagnosis should be sought that may explain the

lack of clinical improvement (WHO 2016).

1.6. Laboratory diagnostic methods

Since the Ebola virus has been classified by the CDC as a pathogen of category A, thecategory that includes the most dangerous pathogens causing diseases with high morbidity and mortality, viral diagnosis should be performed only in specialized laboratories with the highest level of biosafety, i.e. Bio Safety Level-4 (BSL-4). There are several methods currently available for the diagnosis of EBOV infection, but all have limitations, warranting more research in this area. Due to a relatively short incubation period of the infection and non-specific symptoms, a diagnosis should be fast and accurate. An ideal Ebola virus diagnostic method must satisfy the globally recommended features of an ideal diagnostic test, which follows the ASSURED principles (The acronym stands for A- affordable, S- specificity, S-sensitivity, U- User friendly, R- robust, E-equipment free and D-delivered to those in need). Currently, realtime Reverse Transcriptase Polymerase Chain Reaction (rt RT-PCR) is considered asthe most sensitive method to detect the number of viral copies in patient specimens even after 48 hours post onset. RT-PCR amplifies viral RNA and the targeted genes. Most often,targeted genes are NP, GP, L and VP40. But there are still other methods of virus identification (Singh et al, Zawilińska et al, Broadhurst et al).

1.6.1. Immunological assays

Immunoglobulin G (IgG) and Immunoglobulin M (IgM) develop in survivors, but not in all fatal cases, thus, diagnosis of Ebola virus disease using serology is only possible in a fraction of symptomatic patients and, for an unambiguous diagnosis, requires seroconversion or a substantial increase in antibody titer in paired serum samples (Malvy et al, Towner et al, Ksiazek et al, Baize et al). However, serology is the methodof choice to diagnose pauci symptomatic or asymptomatic Ebola virus infections, whichare characterized by extremely low viraemia and development of IgG and IgM about 3weeks after infection (Nkuba et al, Leroy et al, Glynn et al).

1.6.1.1. ELISA based assay for EBOV detection

The enzyme-linked immunosorbent assay (ELISA) may be used to detect both

antibodies as well as virus-specific antigens. Assays for the detection of antibodies areless useful because the patient often dies before the formation of a specific antibodies.Therefore, they are carried out mainly for epidemiological purpose, for patients who survived this terrible disease. Positive results obtained by the ELISA can be confirmedby Western blot. Sometimes only IgM antibodies are detectable in specimen of sick person. They appear after 2 days of symptoms onset and may last for 30–168 days. Incontrast, IgG antibodies usually are detected between day 6 and 18 post onset of illness and persist for years. The antibody profile of the sera is significantly different inpatients with lethal disease as compared to those that survived. This difference can serve as a prognostic marker for the management of the patient. It has been shown that deceased patients show a much lower or even absent antibody response compared with survivors (Kaushik et al). In 1998, Prehaud et al purposed a NP and GP Gabon (94 strain) based method to detect EBOV infection in humans. GP and NPwere synthesized in Sf9 cells and expressed as recombinant proteins to develop an ELISA for the detection of IgG antibodies in convalescent human sera from Gabon andZaire. However, this assay showed issues with false results. Thus, developing a selective method to detect EBOV at clinical level was in demand (Zawilińska et al, Broadhurst et al, Prehaud et al). A sandwich ELISA system based on EBOV antigen- detection using a monoclonal antibody (MAb) prepared via immunization of NP (Zaïre subtype) was developed. The utilized Mab was also found reacting with a corresponding region of NP derived from the Reston and Sudan subtypes. Researchers claimed that this system can be used to detect Ebola virus at very low level (Niikura et al., 2001). One proposed ELISA and RT-PCR based detection of Ebolavirus in oral fluid during the EVD outbreak 2003 in the Republic of Congo. They used serum and oral fluid specimens of 24 Ebola virus infected patients along with 10 normal patients and confirmed EVD. In this study, authors failed to detect IgG against Ebola in oral fluid sample of patients whose serum samples were seropositive. Besides this,patients with positive serum, RT-PCR results were also found positive in oral fluid specimens (Formenty et al.,2006). An ELISA assay was developed by Nakayama et al. to detect filovirus species-specific antibodies. The secreted form of the transmembrane GPs of five

different EBOV species was used as an antigen for the detection filovirus species specific antibodies. An assay was performed on antisera collected from mice immunized with virus-like particles and from humans and non- human primates infected with virus. A little cross-reactivity of IgG antibodies was observed in most of the mouse antisera. Results suggest that virus specific IgM antibodies were specifically detected in acute infected patients and that the method could be of use for Ebola diagnostics and management (Nakayama et al). Lucht proposed the need of a Mab to develop an Ebola diagnostic ELISA kit to analyze Zairestrain-EBOV. Researchers produced a specific Mab from mice immunized with inactivated viral particles and directed against viral structural protein VP40. The efficiency of developed Mab was tested using ELISA-based antigen capture protocol. This assay exhibited detection of VP40 in all virus-infected samples. Later, the same research group developed an immune-filtration based assay to detect VP40 for rapid diagnostic of EBOV infection. This was an antigen capture based approach to detect Ebola virus in chemically inactivated clinical samples. This assay can be acknowledged as the first-generation on-site tool to detect Ebola virus within 30min (Lucht et al, Singhet al, Zawilińska et al, Broadhurst et al, Malvy et al).

1.6.1.2. Immunofluorescence based assay for EBOV detection

Saijo et al developed an immunofluorescent (IF) method to detect IgG of EBOV usingHeLa cells expressed a Baculovirus-delivered recombinant EBOV NP. This method isvery sensitive for Ebola detection and an alternate of highly complicated procedures performed only under BSL-4 facilities (Saijo et al). Ikegami et al also promoted IF assayto detect Reston subtype EBOV subtype using HeLa cell expressed by related EBOVNP. The authors performed IF detection of selected virus specific IgG in both hyperimmune rabbits' sera and monkey, and showed that the assay is more sensitivefor Reston subtype than Zaire subtype Ebolavirus samples (Ikegami et al).

1.6.2. Cell culture

Virus isolation in cell cultures is one of the very sensitive methods. Acute phase patientsera or postmortem tissue samples may be appropriate material for the virus isolation.Ebola virus is able to replicate in numerous cell lines and virus

growth can be detectedby cytopathic effect. Vero or Vero E6 African Green monkey kidney cells have been commonly used for this purpose. Propagated virus can be directly visualized by electron microscopy or indirectly visualized by immunofluorescence microscopy within 1 to 5 days of inoculation. While detection of Ebola virus by these methods is definitive,these methods require biosafety level 4 (BSL-4) containment and are typically restricted to research and public health laboratories, and they are slower to provide a result compared to RT-PCR (Braodhurst et al).

1.6.3. Electron Microscopy

Electron microscopy has also been useful in identification and detection of viral pathogens. Due to the very characteristic shapes of virus particles this method is specific and rapid, but requires a large number of virus particles in sample, specific and expensive equipment and trained personnel. Hence, this method is not used for routine diagnostic purpose.

1.6.4. Immuno assays detecting viral antigens: rapid antigen tests

During initial infection with EBOV, patients have high amounts of viral antigens circulating in their blood and excreted in urine, so antigen detection procedures have become a crucial focus for early diagnosis. Viral antigen can be detected in body fluidsbeginning at 3-6 days post infection. Over the course of infection, antigen titer may either decrease to negligible (i.e. for most survivors) or increase until death. Several EBOV antigen detection kits based on different antigens of the Ebola virus have beendeveloped with varying specificity and sensitivity. VP40 is abundantly present in the virus infected cells and hence is a good target for diagnosis of EVD (Elliott et al). NP appears to be an ideal target antigen for immunochromatographic assays because of its abundance in filovirus particles, its strong antigenicity, and the presence of commonepitopes among ebolavirus species. The NP plays an important role in the replication of the viral genome and is essential for formation of the ribonucleocapsid (Yoshida et al, Niikura et al). Rapid Diagnostic Tests are easy-to-use and affordable assays whichcan detect viral antigens in the blood and other bodily fluids without requiring any power supply, cold chain, sophisticated equipment or highly trained-personnel (Shorten et al,DeMers et al). Towards the end of the 2013–

16 outbreak in west Africa, several RDTstargeting different structural proteins (NP, VP40, GP, or a combination) of the various strains of Ebola virus were evaluated. The OraQuick (Orasure Technologies), ReEBOV(Corgenix), SD Q Line Ebola (SD Biosensor), and the DPP Ebola Antigen System (Chembio Diagnostics) received emergency use authorizations from the US Food and Drug Administration (FDA) or WHO (Cnops et al, WHO, US FDA, Mukadi-Bamuleka etal, Perkins et al).

Table 5 Characteristics of Ebola RDTs and ASSURED criteria

Name of RDTs / **Characteristics & ASSURED criteria**	**QuickNavi -Ebola™**	**OraQuick® Ebola Rapid AntigenTest**	**Coris® EBOLA Ag K-SeT rapid test**	**STANDARD Q Ebola Zaire Ag**	**ReEBOV Antigen Rapid Test kit**	**Dual Path Platform (DPP)**
Manufacturer	Denka, Niigata Co. Ltd. Japan	OraSure Technologies , Inc. USA	Coris Bioconcept Gembloux, Belgium	SD Biosensor Inc., Republic of Korea	Corgenix, Inc, USA	Chembio, Medford, USA
EBOV Target(s)	NP	VP 40	VP40	GP, NP, VP40	VP40	VP40
Time to results (minutes)	10-15	30	10-15	20-30	10-15	10-15
Thermostability (temperature °C)	Room temp.	2-40	Room temp.	2-40	18-30	2-25
Affordability	Not on free sale	Not on free sale	Yes	Yes (through UNICEF)	Not available	Not available
Specificity	+++	+++	++	++	+	+
Sensitivity	++	++	+	+	+	+
User friendliness	+++	+	+++	+	+	++
Robustness	++	++	++	+	+	++
Equipment-free	+++	++	+++	+++	++	++

1.6.4.1. Dual Path Platform (DPP), Chembio, Medford, USA

The Dual Path Platform (DPP; Chembio, Medford, USA) technology, which consists ofan immunochromatographic test cartridge and a small, battery-operated reflectance reader. The tests are designed for use with EDTA whole blood (venous or capillary), plasma, or serum. The DPP Fever Panel Antigen System (the “Fever Panel Test”) is amultiplex assay detecting *Plasmodium falciparum* (HRP2), pan-Plasmodium (pLDH), and protein antigens specific for Lassa, Pan-Ebola (Zaïre, Sudan, Bundibugyo), Marburg, Dengue, Chikungunya, and Zika Viruses. The DPP Ebola Antigen System (the “Ebola Test”) tests for Ebola Zaire antigen, and the DPP Ebola-Malaria Antigen duplex system (the “Ebola-Malaria Test”) detects Ebola Zaire antigen, *Plasmodium falciparum* (HRP2), and pan-Plasmodium (pLDH). All tests are qualitative and detect the VP40 antigen, which is specific to Ebola. The DPP format uses a sample strip perpendicular to the test strip (in contrast to the classic lateral flow assay format), whichdelivers sample directly to the test line. Sample (50uL) and buffer are added to a well on the cassette, and migrate to the test site, where Ebola VP40 antigen is captured bythe VP40 antibodies on the test line. The buffer dilutes blue and green dye at the test site, thereby indicating that the sample and buffer have migrated properly. Running buffer is then added to the conjugate pad after a 5-min incubation period, solubilizing gold nanoparticles conjugated to mouse VP40 antibodies. The buffer front sends any antigen left on the membrane towards the test line for additional capture, and the gold conjugate binds and accumulates on the test line. Additional buffer follows and washesthe membrane for increased contrast and sensitivity. After 10–15 min, a reflectance reader is used to read the test results. This “Micro Reader” analyzes the reflectance oftest and control lines to detect VP40 antigen. It interprets the results using assay- specific cut-off values, and reports a reactive Ebola Virus Disease (EVD+), non- reactive (EVD-), or invalid (INV) result as text scrolling through a liquid crystal display (LCD) on the top of the instrument. The DPP tests and Micro Reader are illustrated inFigure 4. A separate limit of detection study using gamma irradiated serum samples estimated the limit of detection to be 150 ng/ml, corresponding to 7.5 ng/test. Chembio provided technical training and guidance on operating the tests, but was not involved in

the funding or execution of this study. As of September 2019, none of the DPP Eboladevices are yet available commercially (Moran et al, Chembio).

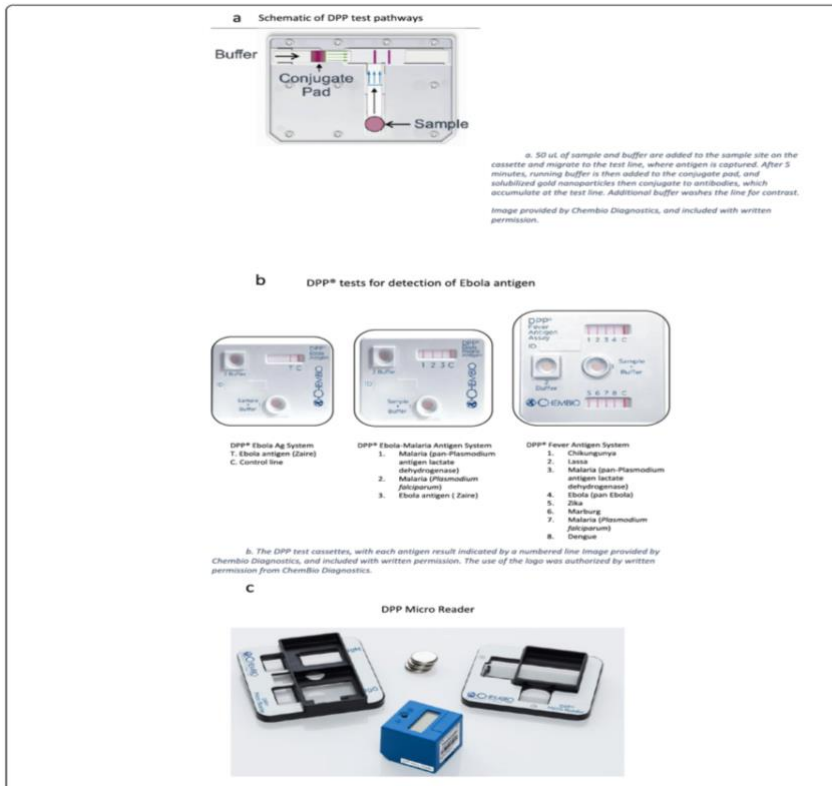


Figure 4 A Schematic of DPP test pathways

a. 50 uL of sample and buffer are added to the sample site on the cassette and migrateto the test line, where antigen is captured. After 5 min, running buffer is then added to the conjugate pad, and solubilized gold nanoparticles then conjugate to antibodies, which accumulate at the test line. Additional buffer washes the line for contrast. Image provided by Chembio Diagnostics, and included with written permission.

b. *The DPP test cassettes, with each antigen result indicated by a numbered line Image provided by Chembio Diagnostics, and included with written permission. The use of the logo was authorized by written permission from ChemBio Diagnostics.*

c. The DPP Micro Reader (middle) is positioned over the cassette holders (left, right) to read the lines of each test cassette. Text scans across the small screen on the MicroReader to display the results of each test line. The Micro Reader is battery-powered. The Micro Reader can also be connected to a laptop, making it easier to read multipleresults. Image provided by Chembio Diagnostics, and included with written permission.The use of the logo was authorized by written permission from ChemBio Diagnostics.

1.6.4.2. QuickNavi™-Ebola (Denka, Niigata, Japan)

QuickNavi™-Ebola is a lateral flow–based immunochromatography kit (85 mm x 21 mmx6.9 mm) for the direct detection of TAFV, BDBV and EBOV NP antigens. QuickNavi™-Ebola devices were produced by using mouse mAbs ZNP105-7 and ZNP108-2-5 specific to the ebolavirus NP. For each assay, 30 µL of serum/plasma or 10–20 µL of whole-blood samples can be used, followed by the addition of 2 drops (approximately 40 µL) of the sample buffer supplied together with the devices. Results are interpreted 10–15 min later. When a sample is added to the sample window of the kit, it migrates through the reagent pads via capillary action. One of the reagent pads is a conjugate pad on which latex conjugated with an anti–ebolavirus NP monoclonal antibody (mAb) has been dried. The ebolavirus NP antigens present in the sample bind to the latex-conjugated mAbs on the pad. Another ebolavirus NP-specific mAb is also immobilized on a nitrocellulose membrane at the test line position to capture the complexes of NP antigens and mAbs conjugated with latex. As the assay develops, those complexes deposit a visible blue line. QuickNavi™-Ebola has a shelf life of at least 24 months at room temperature (Yoshida et al, Makiala et al).

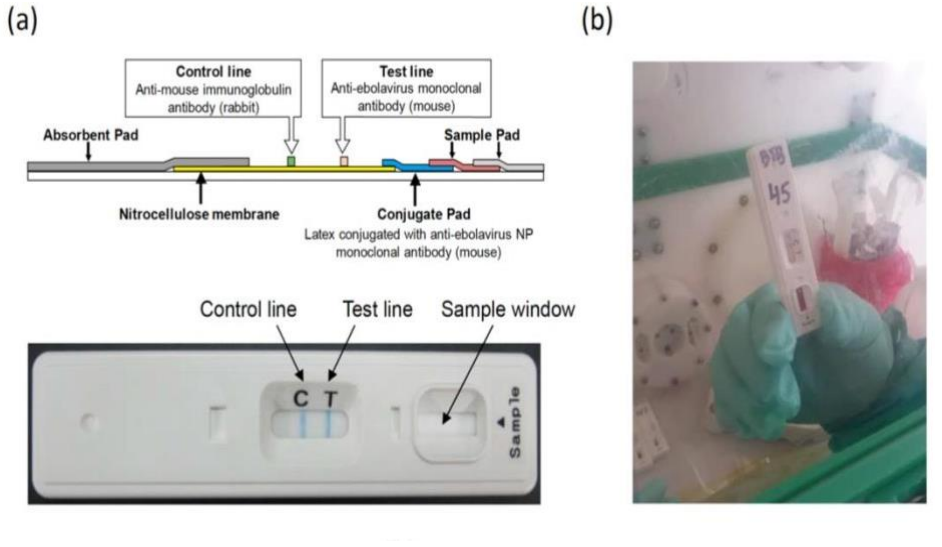


Figure 5 (a) Illustration of QuickNavi™-Ebola, (b) QuickNaviTM-Ebola used in the field lab, North-Kivu province

(a) Illustration of QuickNavi™-Ebola. A sample added to the sample window of the device migrates via capillary action. The ebolavirus NP antigens present in the sample bind to the latex-conjugated mAb on the conjugate pad. Another mAb is immobilized on a nitrocellulose membraneat the Test line position and captures the complexes of NPs and mAbs conjugated with latex. Those complexes deposit a visible blue line.

(b) QuickNavi™-Ebola used at a field laboratory inNorth-Kivu province.

1.6.4.3. ReEBOV Antigen Rapid Test kit (Corgenix, Inc, USA)

The ReEBOV Antigen Rapid Test kit (Corgenix, Broomfield, Colorado, USA) was the first Lateral Flow Immunoassay (LFI) for EVD to receive EUA status (both WHO and FDA). The FDA EUA allows for the testing of whole blood (collected by either fingerstickor venipuncture) or plasma, while the WHO EUA lists whole blood, plasma, and serumas acceptable specimen types. This test is a chromatographic dipstick immunoassay for detection of the Ebola virus VP40 matrix protein (EBOV, SUDV, BDBV) using affinity-purified caprine polyclonal antibodies (Autoimmune Technologies, New Orleans, Louisiana) specific for EBOV VP40 antigen. The immunochromatographic dipstick design incorporates a plasma separator sample pad to separate plasma fromwhole-blood specimens (obtained via capillaries or veins) or allow the use of processed plasma and serum. The test is operated by introduction of 30 µL of whole blood, plasma, or serum to the sample pad (when used at the bedside, a drop of

fingerstick blood is applied directly to the nitrocellulose test strip). Insertion of the dipstick into a culture tube containing 4 drops (approximately 200 µL) of sample buffer initiates the flow of sample and sample buffer. In the presence of EBOV antigen, VP40 is specifically absorbed by the colored nanoparticle reagent during flow through reagent pads. Capture of antigen nanoparticle complexes by the EBOV VP40–specific test lineresults in development of a faint pink-to-red signal that corresponds to the titer of EBOVVP40 antigen in the sample. Nanoparticles that are not complexed with VP40 antigenare captured by the control line, which indicates a valid result. Visual interpretation of the result is performed after incubation for 15–25 minutes. If present in the sample, VP40 is captured by gold-labeled anti-VP40 antibodies, forming immune complexes that are subsequently deposited along a stripe of anti-VP40 antibodies printed onto thedipstick at a specific location. The gold nanoparticles produce a pink-red line that can be visually interpreted in 15 to 25 minutes. No electronic equipment is needed to operate the test, though reagents do require refrigeration for storage (Broadhurst et al,Boisen et al). The assay is no longer commercially available.

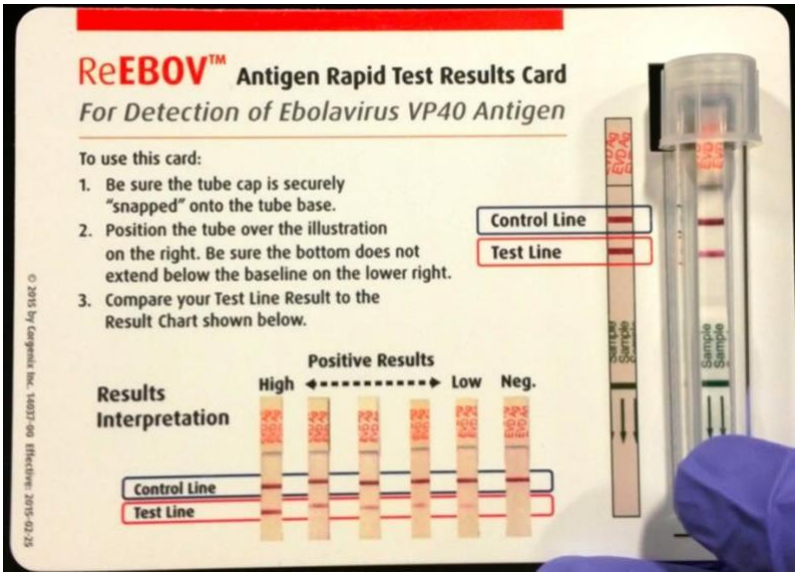


Figure 6 ReEBOV Antigen Rapid Test Kit results card.

The results card visual aid was developed in collaboration with the Food and Drug Administration and the World Health Organization to aid ReEBOV rapid diagnostic test operators in test results interpretation. Thecard provides a full-scale image of a developed rapid test and hole to align the capped tube. Correct alignment allows inexperienced operatorsto correctly identify the control and test line signals. A scale of test line signals aids in test interpretation.

1.6.4.4. OraQuick Ebola Rapid Antigen Test (OraSure Technologies, Bethlehem,PA, USA)

The OraQuick Ebola Rapid Antigen Test (OraSure Technologies, Inc., Bethlehem, PA, USA) is a chromatographic LFI (detection of VP40 matrix protein (EBOV, SUDV, and BDBV) that received FDA and WHO EUA status. The OraQuick Ebola Rapid Antigen Test is a single-use immunoassay which allows qualitative detection of Ebola antigensfrom the whole blood of patients or saliva of corpses in 30 minutes. In addition to whole blood (venipuncture or fingerstick specimens listed in the FDA EUA, the OraQuick RDTwas the first EVD diagnostic test to receive approval for use with cadaveric oral fluid (approved in EUAs from both the FDA and the WHO). For testing whole blood (either fingerstick or venous), the specimen is collected into a plastic micropipette provided inthe kit and applied to a sample port in an assay device containing the nitrocellulose test strip. Cadaveric oral fluid can be sampled directly by swabbing the oral mucosa with the flat pad at the end of the collection

device. Alternatively, fluid from an oral swab stored in viral transport medium can be collected into a provided micropipette and applied to the sample port. The sample is added to the OraQuick RDT device, then the device is inserted into a vial of developer solution to facilitate the capillary flow of the specimen into the device and onto an assay strip with a Test Zone and Control Zone. As the specimen flows through the device, Ebola antigens from the specimen are bound by Ebola antibody labeled gold colorimetric reagent. If Ebola antigens are present, the labeled complexes bind to the Test Zone resulting in a purple line, and if they are not present the Test Zone will remain colorless. The remaining colloidal gold continues to migrate and binds to the Control Zone resulting in a purple line to demonstrate there was adequate flow and the test was valid, regardless if the samplewas positive or negative for Ebola virus. Positive results may be interpreted as soon as lines are visible at the Test and Control Zones, however negative results must be read 30 minutes after inserting the device in the developer vial to allow adequate timefor migration of the sample. The intensity of the line color is not directly proportional to the amount of virus in the specimen; the test is interpreted as reactive or non-reactive.Kit components require storage at 2 to 30°C and can be used at 15 to 40°C (Broadhurst et al, VanSteelandt et al).

Figure 7 OraQuick rapid test device and buffer developer

1.6.4.5. SD Q Line Ebola Zaire Ag Test (SD Biosensor, Gyeonggi-do, SouthKorea)

SD Q Line Ebola Zaire Ag test (SD Biosensor, Inc.) is a chromatographic LFI that simultaneously detects NP, GP, and VP40 antigens of EBOV in whole blood, plasma, or serum. Gold-labeled mouse monoclonal antibodies form complexes with antigens present in the specimen and deposit along three antigen-specific test lines for visual detection. To perform the test, three drops of specimen are added to a sample port onthe assay device, using a provided disposable dropper; alternatively, 100µl of specimen can be added using a precision pipette. The test is visually read at 20 to 30 minute, with the appearance of any of the three test lines interpreted as positive (SD Q Line Ebola Zaire Ag test package insert R1-20150901.indd). Kit components can be stored between 1 and 40°C (Broadhurst et al).

1.6.5. Polymerase Chain Reaction (PCR)

1.6.5.1. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Real-time RT-PCR tests still be the cornerstone of the laboratory response during EVD outbreaks. During the acute phase of the disease and convalescence, viral RNA can be detected by RT-PCR in other bodily fluids, such as saliva, tears, sweat, breast milk,urine, cerebrospinal fluid, ocular fluid, amniotic fluid, vaginal fluid, and seminal fluid. Viral RNA can remain detectable in these fluids after the RT-PCR on blood becomes negative (Faye et al, Towner et al, de La Vega et al, Lanini et al, Mate et al). Diagnosisof post-mortem Ebola virus disease (eg, people who did not attend a hospital and died in the community) is best done by RT-PCR on an oral swab (Erickson et al, Kerber et al, Mukadi-Bamuleka et al, Malvy et al). Real-time RT-PCR assays utilizing fluorogenicprobes designed for the detection of Ebola virus were first developed and tested in EVD patient serum samples in the early 2000s. Results are expressed in Cycle threshold values (Ct-values) which is a proxy for viral load. Thus, viral load expressedin Ct-values can guide surveillance and patient's management during EVD outbreaks at the point of care. Lower Ct-values (higher viral RNA copy numbers) are associatedwith higher mortality (Towner et al). High viral loads (typically seen as Ct-values of <25)are associated with a poor prognosis in infected individuals (Schieffelin et al, de La Vega et al). Ct-

values greater than ~35 in the setting of convalescence is not associated with infectious virus and Ct-values ≤ 40 is typically used as the cut-off to designate a positive sample (Spengler et al). RT-PCR allowing the monitoring of clinical status of patients admitted in Ebola Treatment Units, persistence of EBOV in blood, semen or other body fluids of EVD survivors, and the reintegration EVD survivors to the community. Compared to conventional RT-PCR, real-time amplicon detection using sequence-specific probes offers greater specificity and more rapid results (typically 2 to 3 hours); however, limited data are available regarding the sensitivity and specificity of the various laboratory-developed and commercial Ebola virus real- time RT-PCR assays (Reusken et al). Only one study to date has compared the analytic characteristics of commercially available Ebola virus real-time RT-PCR assays, and it demonstrated up to 100-fold variations in the limits of detection and 1,000-fold variations in the lower limits of quantitation (Cherpillod et al). Despite its potential diagnostic advantages, RT-PCR methodology (both conventional and real- time approaches) requires significant laboratory infrastructure, electrical power, multiple temperature-sensitive reagents, the operation and maintenance of specialized equipment, and technical expertise in molecular biology, potentially complicating deployment in resource-limited settings (Broadhurst at al).

1.6.5.2. Conventional RT-PCR

Diagnostic RT-PCR tests for Ebola virus, developed by the CDC, were first evaluated on serum samples collected from acutely ill patients during the 1995 Kikwit outbreak. These assays used PCR to amplify the L, GP, and NP genes, followed by size-based amplicon detection via gel electrophoresis. An important advantage of this method was the simple, chemical inactivation of infectious virus during the initial steps of RNA extraction by using a chaotropic agent such as guanidine thiocyanate, allowing subsequent sample processing to be carried out on the benchtop. Conventional RT- PCR was found to be more sensitive than antibody and antigen detection ELISAs when evaluated over the complete course of symptomatic infection, and in 1999 the CDC recommended its use in conjunction with antigen detection ELISA testing for diagnosis of acute EVD (Sanchez et al). Importantly, early application of conventional RT-PCR

demonstrated that it also performed well for detection of virus in other body fluids, suchas saliva and seminal fluid. Indeed, RT-PCR for the NP gene was able to detect persistent viral RNA in multiple body fluids from convalescent patients for several weeks beyond the cessation of symptoms, and in a small study the yield of RT-PCR for the L and NP genes in saliva samples from acutely ill patients was consistent with that of serum RT-PCR testing. Furthermore, experience soon demonstrated that RT-PCR tests could be rapidly developed and adapted to newly identified viral strains, as exemplified during the first known outbreak of BDBV in 2007 (Rodriguez et al, Formenty et al, Towner et al, Broadhurst et al).

1.6.5.3. Point-of-Care molecular tests

1.6.5.3.1. GeneXpert® (Cepheid, Sunnyvale, CA, USA)

The Cepheid Xpert Ebola assay is a rapid, automated test for qualitative detection of the Ebola Zaire virus on the Cepheid GeneXpert Instrument System. The GeneXpert system allows automated extraction and quantitative real-time reverse-transcription (RT) PCR. The Xpert Ebola assay detects EBOV total nucleic acid, amplifying the GPand NP genes. Each test cartridge includes a sample adequacy control to ensure that human cells are present in the sample; a Cepheid internal control, an Armored RNA dry bead included in each cartridge to verify adequate lysing of the sample virus and monitor for the presence of inhibitors; and a probe check control to ensure bead rehydration, reaction-tube filling, probe integrity, and dye stability that occurs before RT-PCR. This test was granted an emergency use authorization from the US Food andDrug Administration on 23 March 2015. EBOV RNA detection in blood and semen has previously been validated using the Cepheid GeneXpert PCR platform. Sensitivity andrepeatability were verified by testing 40 EBOV-spiked positive and 20 negative donor samples for blood; 150 spiked samples and 25 negative samples tested for semen (Pinsky et al, Jansen van Vuren et al, Semper et al, Towner et al, Trombley et al).

Sample preparation

For sample preparation, 0.1 mL whole blood or other biological fluid (oral secretions, vaginal secretions, semen, breast milk etc) is transferred to the 2.5 mL Xpert® Ebola Sample Reagent (SR) bottle, which contains 4.5M guanidinium thiocyanate. SR inactivates EBOV within the recommended incubation time and temperature (20 minutes at ambient temperature), denatures and liquefies the sample, stabilizes the nucleic acid target, and creates the salt concentration needed for subsequent RNA purification. The SR buffer is transferred to the Xpert® Ebola Assay cartridge, where allsteps of sample preparation, reverse transcription and multiplex, fluorescent real-time PCR occur (Raja et al). All reagents required for these steps are included in the cartridge, either in liquid form or in lyophilized beads. Nucleic acids are isolated and purified, concentrated onto a small glass fiber column integrated into the base of the cartridge, and washed and eluted prior to combining with the RT-PCR reagents.

The Sample Processing Control (SPC), included in the cartridge, is automatically spiked into the sample during processing to monitor target recovery and inhibition. Once the sample is loaded, the cartridge is closed and remains closed for the remainder of the assay including disposal of the cartridge. This helps prevent cross-contamination of amplicons in the laboratory. The cartridge is loaded into the GeneXpert module where processing, detection, and fluorescent readout are performed automatically. Time to result is 100 minutes. Additionally, the GeneXpert® Ebola Assay system has remote monitoring and electronic reporting capability when the included computer has Internetaccess.

Primer and Probe Design

The primers and probes in the Xpert® Ebola Assay detect two conserved regions withinthe GP and the NP of the EBOV genome. The amplified target sequences are detected with TaqMan probes, each labeled with distinct proprietary fluorophores designed to operate with different detection channels of the optics of the GeneXpert system. Primers and probes for the EBOV targets are indicated in table 6. The Xpert® Ebola Assay cartridge also includes two

internal controls, the Sample Adequacy Control (SAC) and the SPC. The SAC detects a human genomic DNA target in the human hydroxymethylbilane synthase gene (HMBS). The proprietary SPC is an exogenous control that consists of armored RNA containing an artificial RNA sequence that doesnot exist in nature. The SPC amplicon shows no homology to the EBOV target sequences.

Test interpretation

The response (Ct-values) of both EBOV targets of the Xpert assay (NP & GP) is linearwith the log of input viral genome copies over 4–5 logs dilution. Test results are interpreted by the GeneXpert® Dx System from measured fluorescence signals basedon algorithms embedded within the assay software. Samples are considered positive if either target gene (GP or NP) was detected; the instrument identifies which targets are detected. The GeneXpert instrument uses the internal control and target gene Ct-values to determine whether the assay is valid and which of the targets (GP, NP, or both) is detected (Pinsky et al, Loftis et al).

Table 6 Primer and probe sequences for EBOV targets in the Xpert® Ebola Assay

Target	Primer/Probe	Sequence
GP	Forward primer	GGG CTG AAA ACT GCT ACA ATC TTG AAA TC
	Reverse primer	GGA AGC CCC GAA TCC CGT
	Probe	CCT GAC GGG AGT GAG TGT CTA CC
NP	Forward primer	GCT CCT TTC GCC CGA CTT TTG AA
	Reverse primer	CTG TGG CGA CTC CGA GTG CAA
	Probe	TGA GCA TGG TCT TTT CCC TCA AC
HMBS	Forward primer	CTG GCC TGC AGT TTG AAA TCA GTG
	Reverse primer	GCG GGA CGG GCT TTA GCT A
	Probe	TGG AAG CTA ATG GGA AGC CCA GTA CC

GP, Glycoprotien; NP, Nucleoprotein; HMBS, human hydroxymethilbilane synthase

1.6.5.3.2. Idylla™ Prototype Ebola Virus Test (Biocartis, Mechelen, Belgium)

The Idylla™ system is a miniaturized laboratory that integrates all sample-processingand RT-PCR analytical procedures and is composed of 3 physical components: aconsole, an instrument, and a disposable test-specific cartridge containing all requiredreagents to perform the test, including controls (Figure 8). The Idylla™ system providesa sample in–result out workflow that operates in fully automated and controlled fashion.The sample is loaded into the cartridge without requiring any sample-pretreatmentsteps. One sample loaded into the cartridge can be tested per instrument module. Upto eight separately and independently operating stackable modules can be used perconsole. A 200-µL whole-blood sample is directly dispensed into the sample-processing chamber of the cartridge. After sample lysis, the viral RNA is extracted onboard, using an extraction method adapted from previous publication (Boom et al, Cnops et al). Following extraction and purification, the eluate is then pumped towardthe PCR amplification and detection module within the cartridge, which containsprespotted RT-PCR reagents in 5 reaction chambers. The Idylla™ prototype Ebolavirus test detects EBOV and SUDV, as well as a sample processing control. Thesample processing control is an armored RNA with a sequence encoding part of thenucleocapsid protein of the negative-stranded RNA virus Phocine Distemper Virus(Asuragen, Austin, Texas) and is spotted in the sample-processing chamber of thecartridge. Besides the triplex PCR, the assay also contains an endogenous control,targeting a locus in the RPP30 gene of the human genome. For EBOV and SUDV, forward and reverse degenerated primers were used as described before to target a conserved region of 112 base pairs in the GP coding region of the EBOV genome together with one EBOV specific and one SUDV-specific probe that differ from each other by seven nucleotides. The fluorescently labeled detection probes were adapted (reverse complement) from the previous design (Gibb et al, Cnops et al). All primer and probe sequences used are presented in table 7. PCR curve interpretation and classification occurs automatically via a built-in decision algorithm. The test result is qualitative and has 3 possible outcomes: EBOV detected, EBOV not detected, or invalid. The EBOV-detected result is

reported if the sample is positive for EBOV or SUDV, even when the sample-processing control is not positive (which may exceptionally happen in case of a high viral load, owing to competitive inhibition). An invalid result is reported if the system is unable to detect the sample-processing control in the absence of EBOV and/or if no endogenous control is detected (Gibb et al, Cnopset al). The assay is no longer commercially available.

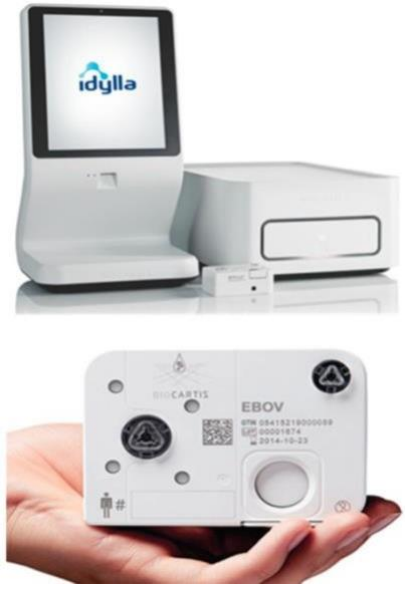


Figure 8 The Idylla™ molecular diagnostic platform

Table 7 Primers and Probes Used in the Idylla™ Prototype Ebola Virus Test

Primer Name	Position in Reference Sequence	NCBI/GenBank Accession Number	Oligonucleotide Sequence (5'–3')
5EBO-GP_1D (sense)	6348–6369	NC_002549	TGGGCTGAAAAYTGCTACAATC
3EBO-GP_1D (antisense)	6440–6459	NC_002549	CTTTGTGMACATASCGGCAC
EBO-GP_1DZ-FIBFQ (antisense)	6421–6402	NC_002549	CCGTCTGGCGCTGCTGGTAG (56-FAM/3IAbkFQ)
EBO-GP_1DS-TRIBRQ (antisense)	6402–6420	NC_006432	CATCCGGCGGTGGGGGTAA (5TexRd-XN/3IAbRQSP)
PDV FW20 (sense)	20430–20452	NC_028249	GCTGTCTGGGTATACTTCTGATG
PDV_Rev20 (antisense)	20 578–20 599	NC_028249	CCTCCCCATTTGTATCTGACTG
PDV_Prb20 (antisense)	20 496–20 519	NC_028249	TTGTCATGGTCCCCTTCCTGTGTC (5ATTO647/3IABRQS)
RNaseP63fw (sense)	41–58	U77665	CAGATTTGGACCTGCGAG
RNaseP63rev (antisense)	87–103	U77665	CGGCTGTCTCCACAAGT
RNaseP63Pr (antisense)	65–83	U77665	CTGACCTGAAGGCTCTGCG (5TexRd-XN/3IAbRQSP)

Abbreviation: NCBI, National Center for Biotechnology Information.

1.6.5.3.3. The BioFire FilmArray assays (Biomérieux, Lyon, France)

The BioFire Defense FilmArray assays (FilmArray Biothreat-E test and FilmArray NGDS BT-E assay) are automated real-time RT-PCR tests for detection of the EBOVL gene. The FilmArray Biothreat-E test has EUA status from the FDA, with approved use of whole blood and paired urine specimens, while the FilmArray NGDS BT-E test has FDA EUA status for use with whole blood, plasma, and serum specimens (US FDA). For both FilmArray assays, a pouch preloaded with lyophilized reagents for RNAextraction and real-time RT-PCR is rehydrated, followed by preparation of the patient sample in a sample injection vial containing sample lysis reagents. The contents of the sample injection vial are then loaded into the pouch and run on a single-assay FilmArray instrument. Per the package insert (BioThreat-E EUA IFU RFIT-PRT-0302-01, October 2014), sample preparation and loading of the pouch are to be performed in a biosafety cabinet. Kit components require storage at 15 to 25°C. The FilmArray Biothreat-E assay was utilized at an Ebola treatment center in Guinea on urine and saliva specimens collected from EVD patients. All urine and saliva specimens tested positive by the Biothreat-E test, demonstrating the utility of this platform in testing noninvasive specimen types near the point of patient care (Broadhurst et al).

1.6.6. Genomic sequencing

Viral sequencing tools hold the potential to benefit EVD diagnostic efforts on multiple fronts, including (i) de novo diagnosis in the setting of an emerging outbreak, (ii) identification of viral strains in an ongoing outbreak (new spillover events, reintroduction, relapse or reinfection), (iii) estimation of viral mutation rate, (iv) evaluation in silico and in real time of the impact of viral mutations on diagnostic measures and medical countermeasures (MCMs) such as vaccines and therapeutics prior to in vitro and in vivo testing, (v) characterization of intra-host responses to specific therapeutic interventions in real time, and (vi) characterization of EBOV populations and genetic drift. For example, accurate sequencing data from EBOV/"Itu"allowed for the rapid in silico assessment of the ability of the mAb114 (National Institutes of Health) and ZMapp (Mapp Biopharmaceutical) mAbs to bind to the receptor-binding domain of the EBOV spike glycoprotein GP predicting that these mAbs should be effective against circulating EBOV/"Itu". Using genomic approaches to understand how MCMs influence EBOV population dynamics and to identify mechanisms leading the mutant viruses to escape MCMs could augment future therapeutic/vaccines designs and filovirus-targeting strategies (Mbala-Kingebeni et al, Park et al, Sozhamannan et al, Baize et al, Gire et al; Korber et al, Kuhn et al, NCBI). However, the rapid prediction of response to outbreaks requires more detailed genomic information than virus consensus-genome sequencing. In this condition, metagenomic sequencing has become a powerful tool for identifying novel viruses and, crucially, for predicting pathogen emergence (Di Paola et al, Whitley, Palacios et al). Furthermore, field transcriptomics can improve our understanding of host responses to virus infection, help deciphering the differences between asymptomatic and symptomatic disease states while predicting whether patients with acute and chronic disease will survive (Liu et al). Functional genomics is becoming the tool of choice for the rapid characterization of patient-specific viruses that have not been isolated in culture or that cannot be equitably shared among laboratories across borders (McMullan et al). Genomics has clarified that highly divergent filoviruses, frequently with unknown pathogenic potential, are likely to be distributed widely over the African, Asian and European continents in highly diverse host

reservoirs. Thus, genomics may enable theprevention of future filovirus disease outbreaks by identifying filovirus natural hosts and by limiting host–human contacts as well as the initial introduction of filoviruses into the human population (Baize et al, Gire et al).

As an outbreak progresses and the sampling size increases, phylodynamic and spatiotemporal analyses reveal broader trends in the intra-outbreak evolutionary rate of the virus, its geographical migration and factors contributing to virus transmission, disease outcome and virus–host adaptation (Holmes et al, Blackley et al, de la Vega et al, Dudas et al, Meyers et al, Urbanowicz et al, Broadhurst et al; Carroll et al).

Nanopore sequencing technology (third-generation) offers two key advantages compared to standard Next Generation Sequencing (second-generation) methods, including longer sequencing reads and the ability to perform real-time sequence analysis concurrently with data acquisition (Baize et al, Broadhurst et al). The MinION® device (Oxford Nanopore Technologies, U.K.), a portable nanopore sequencer, has been evaluated as a diagnostic tool for outbreak responses. The MinION device showed a potential to sequence EBOV genomes from clinical specimens in a resource-limited setting such as Liberia and DRC (Hoenen et al, Mbala-Kingebeni et al, Greninger et al). Via an unbiased, metagenomic approach for pathogen identification on the MinION platform, EBOV was correctly identified in RNA extracted from whole- blood specimens collected during the 2014 DRC Ebola outbreak in under 6 hours (Greninger et al). The use of MinION markedly reduced the time required to obtain thegenome sequence from patient samples and enabled the reintroduction of EBOV into Guinea and Sierra Leone to be rapidly confirmed. Similarly, the EBOV variants causingthe 2018 Équateur Province EVD (EBOV/“Tum”) and the 2018-2020 Nord-Kivu/Sud- Kivu/Ituri provinces EVD outbreak caused by EBOV/“Itu” in the DRC were quickly identified by the use of MinION (Quick et al, Mbala-Kingebeni et al). Later on, field laboratories used the iSeq 100® (Illumina, San Diego, CA, USA), a portable bench-top sequencer with low error rates that can be transported in a suitcase, to obtain complete EBOV genome sequences to determine virus transmission in the Democratic Republicof the Congo (Mbala-

Kingebeni et al, Kinganda et al). During the 2014-2015 epidemic, next-generation sequencing (NGS) platforms enabled the characterization of large numbers of viral genomes in a relatively short time frame (days to weeks), and advances in portable sequencing tools have made it possible to acquire sequencing data acutely in field facilities (Baize et al, Broadhurst et al).

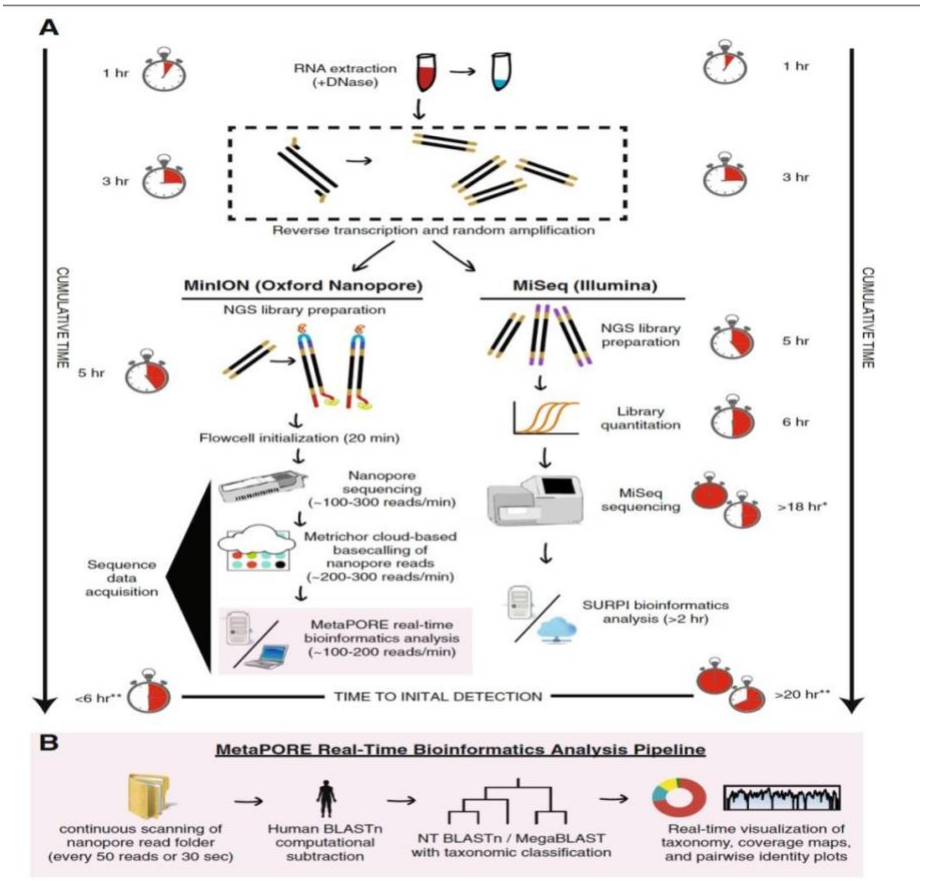


Figure 9 Metagenomic sequencing workflow for MinION nanopore sequencing compared to Illumina MiSeq sequencing.

a. Overall workflow

b. Steps in the MetaPORE real-time analysis pipeline. The turnaround time for sample-to-detection nanopore sequencing, defined here as the cumulative timetaken for nucleic acid extraction, reverse transcription, library preparation, sequencing, MetaPORE bioinformatics analysis, and pathogen detection, was under 6 hr, while Illumina sequencing took over 20 hr. The time differential is accounted for by increased times for library quantitation, sequencing, and bioinformatics analysis with the Illumina protocol. *Assumes a 12-hr 50-bp single-end MiSeq run of ~12–15 million reads, with 50 bp the minimum estimated read length needed for accurate pathogen identification. **Denotes estimated average SURPI bioinformatics analysis run length for MiSeq data. The stopwatch is depicted as a 12-hr clock

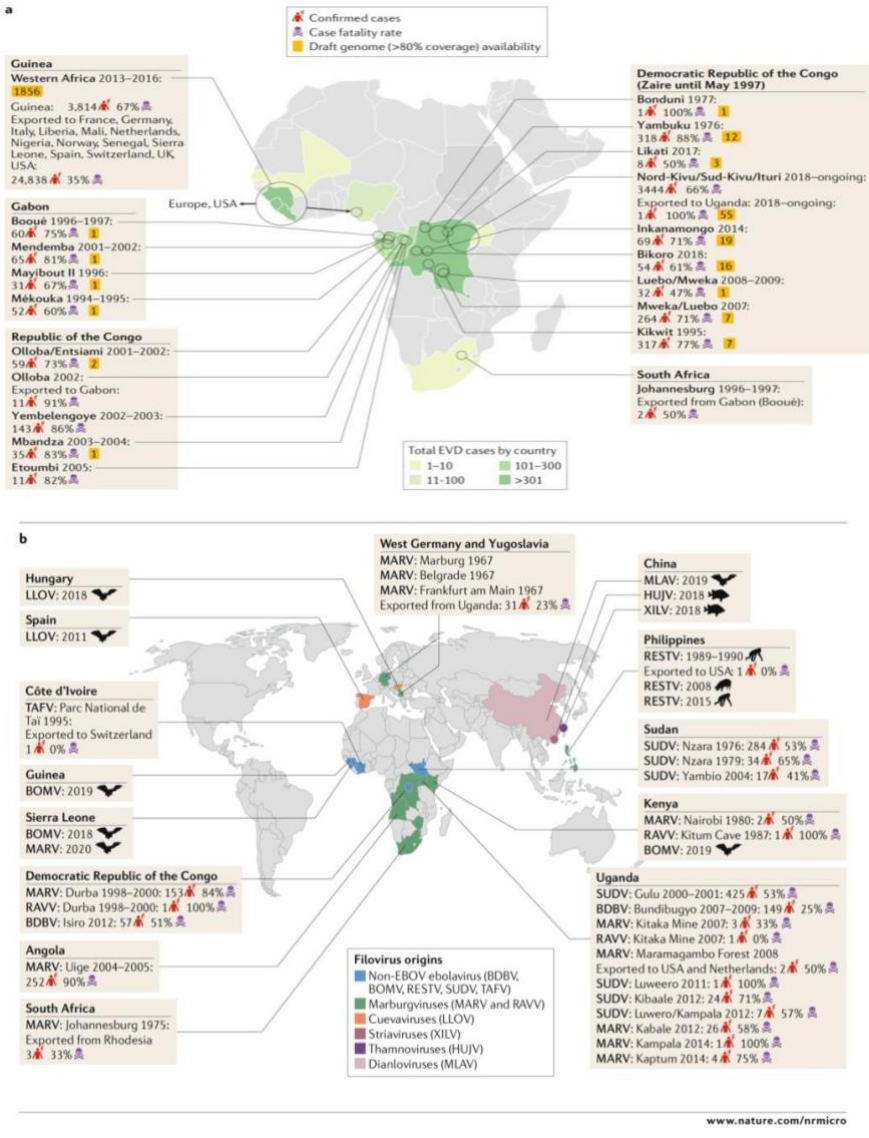


Figure 10 Geographical overview of filovirus discovery.

a | Outbreaks of Ebola virus disease (EVD) in Africa, including the number of confirmedcases, the case fatality rates and the number of publicly available Ebola virus (EBOV)draft genome sequences per outbreak, are depicted3. Circles represent the relative size (in terms of the number of cases) of the outbreaks. Documented accidental laboratory- acquired infections have been excluded from this figure.

b | Overview of global filovirus distribution, excluding EBOV. The place of isolation, known or suspected reservoir host and year of discovery are shown. The description of the distribution of non- EBOV filovirus disease outbreaks includes the total numberof confirmed cases and the case fatality rate. BDBV, Bundibugyo virus; BOMV, Bombali virus; HUJV, Huángjiāo virus; LLOV, Lloviu virus; MARV, Marburg virus; MLAV, Měnglà virus; RAVV, Ravn virus; RESTV, Reston virus; SUDV, Sudan virus; TAFV, Taï Forest virus; XILV, Xīlǎng virus.

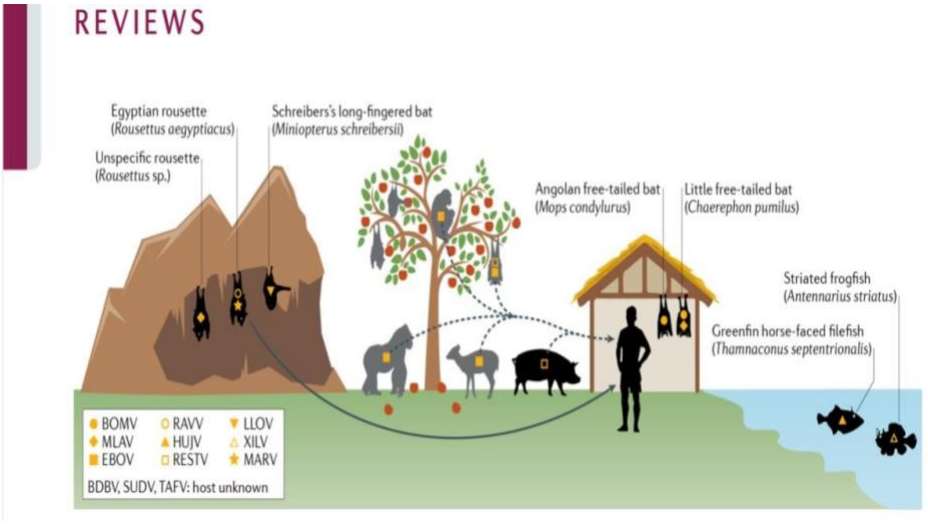


Figure 11 Filovirus host reservoirs.

Complete or coding- complete filovirus genome sequences have been obtained from cave-dwelling and house- dwelling bats and highly diverse fish on the African, Asian and European continents (see Fig. 1 for continental distribution). The pathogenic potential of most filoviruses remains unclear, as does the transmission route ofpathogenic filoviruses proven to infect humans and pigs or of pathogenic filoviruses suspected to infect chimpanzees, duikers and gorillas. Animals that have been provento be infected by filoviruses are indicated in black; grey animals are suspected but unproven reservoirs of the indicated viruses. Solid arrows indicate highly likely transmission routes; dashed arrows indicate hypothesized transmission routes. BDBV,Bundibugyo virus; BOMV, Bombali virus; EBOV, Ebola virus; HUJV, Huángjiāo virus;LLOV, Lloviu virus; MARV, Marburg virus; MLAV, Měnglà virus; RAVV, Ravn virus; RESTV, Reston virus; SUDV, Sudan virus; TAFV, Taï Forest virus; XILV, Xīlàng virus.

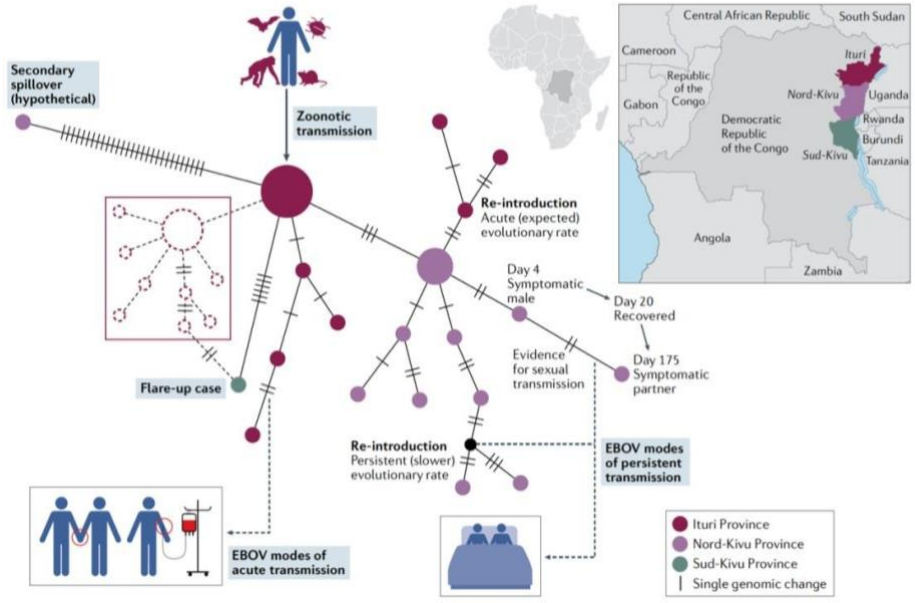


Figure 12 Ebola virus transmission

Following a zoonotic transmission event, sequencing the Ebola virus (EBOV) genomein real-time can help epidemiologists pinpoint the likely route of infection and of transmission during an outbreak of Ebola virus disease (EVD). A theoretical median- joining haplotype network of the Nord- Kivu/Sud- Kivu/Ituri Province EVD outbreak caused by EBOV/“Itu” in the Democratic Republic of the Congo (provinces are indicated on the map) provides different hypotheses that may explain person- to- person transmission events. A consistent number of genomic changes occurring in a specific window of time can indicate acute transmission events (that is, transmission via close contact with an individual with EVD, bottom left). The detection of transmission depends on diagnostics and sampling frequency. When sampling frequency is low, flare- up cases of EVD can appear with limited epidemiological dataand with an unexpectedly large number of differences to earlier sampled haplotypes, which may point to undetected transmission networks (middle left; indicated by dashedlines). When viral diversity cannot be explained by spillover and spatial–temporal estimations, a secondary spillover may be possible (top left). Persistent infections through sexual transmission present with low genetic diversity (that is, with a slow evolutionary rate) over periods (bottom right) that are much longer than expected for acute reintroduction at the expected evolutionary rate (top right). A similar analysis wasperformed during the first discovery of sexual transmission during the 2013–2016 Western African EVD outbreak 73, and a theoretical example is shown (bottom right). This example indicates the number of days after the initial presentation of symptoms at which an acutely infected male is sampled (day 4) and the day at which he recovers(day 20). On day 175, the sexual partner of this male becomes symptomatic owing to a very similar EBOV genotype, confirmed with epidemiological information and visualized using a median- joining haplotype network.

Chapter 2

Rationale & Research objectives

2.1. Rationale

Ebola virus disease (EVD) is a public health emergency with case fatality rate (CFR) ranging from 25 to 90 percent in untreated persons. Human infections associated witha disease are caused by Bundibugyo virus (BDBV), Sudan virus (SUDV), Taï Forest virus (TAFV) and Ebola virus (EBOV) species. This latter was involved in the occurrence of the two deadliest EVD outbreaks in Western Africa (in 2013-2016, with
> 11000 deaths) and in eastern Democratic Republic of the Congo (in 2018-2020, with 2287 deaths) (Jacob et al.; Mukadi et al, Sabue et al). Since the discovery of the virusin 1976, the DRC has faced fifteen EVD outbreaks (as shown in table 1), among whicheight occurred in the last six years (as of 7 January 2023). The tenth outbreak (in 2018-2020) was the longest with 22 months, the most widespread with 29 health zones affected in three provinces, and caused the most fatalities (2287 overall deaths) recorded in DRC to date (Mukadi et al, Aruna et al, Christie et al, Mbala-Kingebeni et al).

The general societal context of the tenth outbreak in eastern DRC was marked by morethan two decades of armed conflict and community mistrust, resistance and hostility commonly nourished by the messages of socio-political leaders, especially during the2018-2019 presidential election period. Distrust between the communities and response teams increased mostly around community deaths as the acceptance rate ofsystematic safe and dignified burials (SDBs) was very low. Indeed, the turn-around time for RT-qPCR results delivery was very long (4h to 72 h), whereas bereaved families needed to quickly proceed with traditional burials. Thus, all of the aforementioned situations led to the rejections of EVD response countermeasures. Inthis particular context, the Institut National de Recherche Biomédicale (INRB), which is the National Reference Public Health Laboratory, progressively deployed andstrategically positioned thirteen field laboratories across outbreak areas. These laboratories aimed to bring EVD diagnostic capacity closer to different epicenters of the outbreak (to shorten the turn-around time), to detect potential flare-ups from undetected transmission chains or recrudescence from EVD survivors and to provide real-time guidance for public health decision-making based on laboratory results (diagnosis and genomic sequencing) (Mukadi et al, Timothy

et al; Den Boon et al, Vetter et al, Sissoko et al; Diallo et al, Kinganda et al, Guetiya-Wadoum et al).

However, the implementation and management of those field laboratories in the aforementioned conditions were subjected to several challenges such as security threats, logistic concerns (cold chain, sample management, maintenance of equipment), technicalities, the management of laboratory types (diagnosis, genomic sequencing) and human resources. They also required specific efforts, stringent biosafety measures, updated and well-maintained procedures, and technical expertisetailored to function in a volatile environment (Mukadi et al).

During EVD outbreaks, identification and control of the disease relies on rapid and reliable diagnosis of cases based on laboratory testing, since the clinical definition is insufficiently accurate (Chua et al). In general, the diagnosis of EVD is based on the detection of viral ribonucleic acid (RNA) using the Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) technology. The Cepheid® GeneXpert Ebola assay is a rapid and accurate, efficient and fully automated RT-qPCR considered as the gold standard because of its high sensitivity and specificity with a very short turn- around time (results in <2 hours) (Dhillon et al, Chua et al, Makiala et al, Katawera et al, Fleck et al, Stone et al, Jean Louis et al, Jacob et al). The introduction of the GeneXpert® (Cepheid, Sunnyvale, CA, USA) in the management of EVD cases was a major step forward. Indeed, the GeneXpert® results supported the contacts tracing and vaccination, the decontamination of households and health facilities where EVD caseswere confirmed, the postmortem testing and clinical management of cases (triage, admission and discharge), the post-epidemic surveillance and Ebola survivor's biological follow-up (Makiala et al, Katawera et al, Couturier et al, Cnops et al, Perkinset al, Fleck et al, Wonderly et al, Shorten et al, Broadhurst et al, Jacobs et al, Moran etal, Jean Louis et al, VanSteelandt et al). Despite the high performance and advantages, the GeneXpert® assay has also shown some inconveniences such as the high cost, adequate infrastructure requirement, training of personnel, stable electricity supply and reagents procurement. These features hamper decentralization to the remote areas of

the country and the community. Indeed, the conditions needed for useof the GeneXpert® are often available only at the provincial level and rarely at lower levels of the health system where most of EVD outbreaks occur (Dhillon et al, Cnops et al, Mukadi-Bamuleka et al, Chua et al, Vansteelandt et al).

In these conditions, the World Health Organization (WHO) had published in 2014 a Target Product Profile (TPP) for Ebola diagnostic tests, stipulating a desired clinical sensitivity of at least 98% and a specificity of at least 99% (Cnops et al, Perkins et al). Thus, several rapid diagnostic tests (RDTs) detecting different viral antigens [nucleoprotein (NP), viral protein 40 (VP40), glycoprotein (GP)] in the blood and otherbodily fluids have been developed specifically to circumvent the logistical and organizational difficulties encountered with RT-qPCR at the point-of-care (POC) and inpoor-resource settings (Wonderly et al, Broadhurst et al, WHO, US FDA, Cnops et al,Perkins et al, Makiala et al, Katawera et al, Mukadi et al, Emperador et al). RDTs can be used as an alternative or complementary tool to the RT-qPCR to detect EVD in alive/suspect-cases at the point-of-care, or in deceased people in the community and health facilities. Since RDTs strongly reduce the turn-around time (results in 10-30 minutes), they can allow a quick decision-making on the case management and deathsscreening during or after EVD outbreaks. RDTs can also prevent nosocomial transmission by reducing the time spent by negative patients inside health facilities. Ina context of community resistance, RDTs can increase the access and acceptability ofEVD testing (Dhillon et al, Cnops et al, Mukadi-Bamuleka et al, Shorten et al, DeMerset al, Makiala et al, Couturier et al, Stone et al, VanSteelandt et al, Wonderly et al).

However, in all the studies conducted during outbreaks, none of the tests evaluated met the levels of both sensitivity and specificity as set out in the WHO ‘desired’ TPP (sensitivity >98%, specificity >99%) for Ebola diagnostic tests (Wonderly et al, Broadhurst et al, WHO, US FDA, Mukadi et al, Emperador et al). Therefore, questionswere raised regarding RDTs diagnostic performance and usability in outbreak conditions. Indeed, the decisions to authorize for their emergency use were mostly based on small-scale evaluations (ie, few clinical samples with generally high Cycle threshold values) that were done in ideal

laboratory conditions. Additionally, most previous studies included a range of different reference standard RT-qPCR tests which were less performant compared to the GeneXpert® which is the current gold standard for EVD diagnosis (Cnops et al, Wonderly et al, Colavita et al, Yoshida et al, Moran et al, Broadhurst et al, WHO, US FDA).

During the tenth DRC EVD outbreak, INRB field laboratories deployed across North- Kivu, Ituri and South-Kivu provinces tested > 200.000 diagnostic and follow-up samples with the GeneXpert® Ebola assay as per WHO recommendation. In parallel, several Ebola RDTs were run, when available, in some of these field laboratories. As data for their diagnostic accuracy were disparate, the RDTs results were documented in the laboratory registers and databases, but were not used for diagnostic decision and patient management [WHO, Mukadi et al. field performance]. INRB has stored forlong-term, the left-over samples from different EVD outbreaks, including those from theeast of the DRC, between 2018 and 2021. The many clinical samples that were tested across a wide range of Cycle threshold values (Ct-values), and simultaneously with theGeneXpert® Ebola assay and one or several RDTs, provide a unique opportunity to enhance the evidence based on the diagnostic accuracy of RDTs when used in field circumstances during an outbreak. Additionally, the availability of stored raw samples (Ebola positive & negative) from tenth outbreak and onwards within INRB biorepositoryoffers an opportunity to conduct a head-to-head comparison of different Ebola RDTs versus the GeneXpert® as the reference standard when using the same batch of samples.

Given the specific context of eastern DRC, the scale and mobility of the 2018-2020 EVD outbreak, field laboratories have been deployed 1) to bring diagnostic tools closerto the different epicenters of the outbreak, 2) to detect potential flare-ups, 3) to providea real-time guidance for public health decision-making through diagnosis and genomicsequencing. However, their implementation and daily management would require specific efforts, procedures and expertise to face a volatile and hostile environment. Additionally, the screening of alive or death EVD suspected-cases with at least one brand of RDTs compared to the reference-standard GeneXpert® could help to shapedout field and/or laboratory

performances based on archived, collected on-site or post-mortem samples tested either alone or in head-to-head comparison. Whereas RDT results can help with quickly and timely interventions in the management of EVD response.

2.2. Research objectives

2.2.1. General objective

The main objective of this PhD is to show how the implementation and management of field laboratories and genomic sequencing can guide and support public-health decision making throughout EVD outbreaks, and to improve the quality of EVDdiagnosis by the use of new tools (rapid diagnostic tests and RT-qPCR) at the point- of-care and in the community.

2.2.2. Specific objectives

1) To describe the deployment and management of field laboratories in terms of set- up, logistics, technicalities, human resources and security during the tenth EVD outbreak in Eastern DRC.
2) To provide an overview of an end-to-end genomic surveillance system, to describe the dynamics of the epidemic and to show how genomic sequencing guided publichealth decision making
3) To report on the diagnostic performance of the QuickNavi™-Ebola based on field data from the North-Kivu/ Ituri EVD outbreak.
4) To strengthen laboratory surveillance by quickly returning test results to families fortimely public health interventions and to improve community engagement and acceptance of safe and dignified burials.
5) To assess the field performance of three antigen detection RDTs performed on blood samples of people with suspected EVD compared with the gold standard GeneXpert® Ebola results
6) To estimate the performance characteristics of four Ebola RDTs against the GeneXpert® as the reference standard on a set of stored EVD positive and negativewhole blood samples from eastern DRC outbreaks.

2.3. Organization of the Thesis

Part I of this thesis describes the generalities of Ebola virus, pillars of outbreak response and diagnostic tools (**Chapter 1**); and presents the background, rationale and objectives of our research activities (**Chapter 2**).

Part II describes the implementation and management of Ebola field laboratories in a context of insecurity in the eastern DRC (**Chapter 3**). It also describes the management and outcomes of field genomic sequencing laboratory at the epicenter of an ongoing outbreak to guide public health decision-making (**Chapter 4**).

Part III reports the field performance of QuickNavi™-Ebola RDT compared to the reference-standard GeneXpert® (**Chapter 5**) during the tenth EVD outbreak. It shows how OraQuick Ebola RDT can improve surveillance activities, community engagementand acceptance of safe and dignified burials (**Chapter 6**). It also evaluates the retrospective performance of three Ebola rapid antigen tests on blood samples of alive suspected-cases versus the reference-standard GeneXpert results (**Chapter 7**). This part ends with a head-to-head comparison of four Ebola RDTs versus the GeneXpert®when used on a same set of stored samples (**Chapter 8**).

Part IV contains the general discussion and conclusions. This section summarizes themain findings of all our research activities and highlights the way forward for Ebola RDTs use in the field and the role of field laboratories deployed throughout EVD outbreaks.

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Part 2: Implementation and management of field laboratories using new tools for Ebola virus disease response in the eastern Democratic Republic of the Congo

Chapter 3

Efficiency of Field Laboratories for Ebola Virus Disease Outbreak during Chronic Insecurity, Eastern Democratic Republic of the Congo, 2018-2020

Efficiency of Field Laboratories for Ebola Virus Disease Outbreak during Chronic Insecurity, Eastern Democratic Republic of the Congo, 2018-2020

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Daniel Mukadi-Bamuleka[1,2,3], Fabrice Mambu-Mbika[1,2], Anja De Weggheleire[4], François Edidi-Atani[1,2], Junior Bulabula-Penge[1,2], Meris Matondo Kua Mfumu[1,2], Anaïs Legand[5], Antoine Nkuba-Ndaye[1,2], Yannick Tutu Tshia N'kasar[6], Placide Mbala- Kingebeni[1,2], John D. Klena[7], Joel M. Montgomery[7], Jean-Jacques Muyembe-Tamfum[1,2], Pierre Formenty[5], Johan van Griensven[4], Kevin K. Ariën[4,8], Steve Ahuka-Mundeke[1,2]

Author Affiliations: Institut National de Recherche Biomédicale, INRB, Kinshasa, Democratic Republic of the Congo (D. Mukadi-Bamuleka, F. Mambu-Mbika, F. Edidi-Atani, J. Bulabula-Penge, M. Matondo Kua Mfumu, A. Nkuba-Ndaye, P. Mbala-Kingebeni, JJ. Muyembe-Tamfum, S. Ahuka-Mundeke); Kinshasa Teaching School of Medicine, University of Kinshasa, Democratic Republic of the Congo (D. Mukadi-Bamuleka, F. Mambu-Mbika, F. Edidi-Atani, J. Bulabula-Penge, M. Matondo Kua Mfumu, A. Nkuba-Ndaye, P. Mbala-Kingebeni, JJ. Muyembe-Tamfum, S. Ahuka-Mundeke); Rodolphe Merieux INRB-Goma Laboratory, Goma, Democratic Republic of the Congo (D. Mukadi-Bamuleka); Institute of Tropical Medicine, Antwerp, Belgium (A. De Weggheleire, J. van Griensven, KK. Ariën); World Health Organization, Geneva, Switzerland (A. Legand, P. Formenty); Direction de la Surveillance Epidémiologique, Kinshasa, Democratic Republic of the Congo (Y. Tutu Tshia N'kasar); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (JD. Klena, JM. Montgomery); University of Antwerp, Antwerp, Belgium (KK. Ariën)

Address for correspondence: Daniel Mukadi-Bamuleka, Department of Virology, Institut National de Recherche Biomédicale (INRB), 5345, Avenue de la Démocratie (Ex Des Huileries), Kinshasa-Gombe, Democratic Republic of the Congo; email: drmukadi@gmail.com

Summary

During the 10th outbreak of Ebola virus disease in the Democratic Republic of the Congo, the Institut National de Recherche Biomédicale strategically positioned 13 decentralized field laboratories with dedicated equipment to quickly detect cases as the outbreak evolved. The laboratories were operated by national staff, who quickly handed over competencies and skills to local persons to successfully manage future outbreaks. Laboratories analyzed ≈230,000 Ebola diagnostic samples under stringent biosafety measures, documentation, and database management. Field laboratories diversified their activities (diagnosis, chemistry and hematology, survivor follow-up, and genomic sequencing) and shipped 127,993 samples from the field to a biorepository in Kinshasa under good conditions. Deploying decentralized and well-equipped laboratories run by local personnel in at-risk countries for Ebola virus disease outbreaks is an efficient response; all activities are quickly conducted in the field.

Key words: EVD, Ebola, laboratory, DRC, INRB, outbreak

I. Introduction

Since the discovery of Ebola virus in 1976, the Democratic Republic of the Congo (DRC) has faced 15 Ebola virus disease (EVD) outbreaks. The 10th out-break was the longest (August 1, 2018, through June 25, 2020) and the most widespread and caused the most fatalities recorded to date in DRC (1,2). That outbreak occurred in urban–rural areas with high population mobility, was aided by good road infra-structure, and was driven by economic reasons and humanitarian issues. The movement of the population probably contributed to virus spread and created challenges for controlling the outbreak, in particular for contact tracing and ring vaccination. Community distrust of response teams, resulting from ≈2 decades of armed conflict in eastern DRC and commonly encouraged by sociopolitical leaders' messaging, especially during the 2018–2019 election period (3), led to recurring misunderstandings and rejection of most countermeasures proposed to control the outbreak. In response to the scale and mobility of this out-break, the Institut National de Recherche Biomédicale (INRB) in Kinshasa, DRC, deployed 13 field laboratories and strategically positioned them across the outbreak areas (3). The laboratories were aimed at bringing diagnostic capacity closer to the outbreak epicenters, shortening turnaround times, and providing real-time guidance for newly available medical countermeasures. Most laboratories were provided with equipment and reagents to improve supportive care (3–5). Implementation and management required specific efforts, stringent biosafety measures, updated and well-maintained procedures, and technical expertise tailored to function in a volatile environment (3). We describe the deployment and management of field laboratories in terms of setup, logistics, technicalities, human resources, and security during the 10th EVD outbreak in eastern DRC.

II. Deployment and decommissioning of field laboratories

A field laboratory is a mobile diagnostic unit, set up for specific purposes and for a limited time of operation (6,7). Field laboratories can be located in buildings adapted to the purpose or in transient structures or mobile platforms. In past EVD out-breaks, field laboratories were often established and managed through bilateral agreements between the host country and international

entities. In DRC, a strong national EVD response and the expertise and availability of reverse transcription quantitative PCR diagnostic tools, GeneXpert Ebola assay (Cepheid, https://www.cepheid.com), ensured that the DRC INRB could coordinate and entirely manage laboratory response activities. Preparatory stages included strategic internal INRB meetings to discuss which type of laboratory to deploy.

During the 10th EVD outbreak, INRB deployed 3 types of laboratories: basic, standard, and advanced. The basic setup was focused on Ebola virus (EBOV) diagnosis, with a maximum of 2 GeneXpert instruments; biochemistry capacity was added if required. The standard setup had >2 GeneXpert instruments, along with biochemistry and hematology capacity for patient care and survivor follow-up (viral load in the body fluids, chemistry, and hematology). In the advanced setup, either differential diagnosis (*Sudan* or *Bundibugyo ebolavirus*, in addition to Marburg, dengue, chikungunya, yellow fever, West Nile, Crimean-Congo hemorrhagic fever, or Rift Valley fever viruses) or genomic sequencing capacity was added to the standard setup. Laboratory tests related to research activities, such as administration of EBOV therapeutics (8,9), immunogenicity of Ervebo vaccine (Ebola Zaire vaccine, https://www.fda.gov/vac-cines-blood-biologics/ervebo), and studies of EBOV reservoirs in bats were conducted in standard and advanced setups. After internal discussions, a proposal was presented to the response coordination team to collect feedback, if any. If agreement was reached, the laboratory was deployed according to INRB check-lists for equipment, reagents, and accessories (Appendix, https://wwwnc.cdc.gov/EID/article/29/1/22-1025-App1.pdf). Materials were transported by air, land, or water with >1 INRB staff member assisted by local laboratory personnel. On site, the team set up the laboratory by adapting the workflow to the existing rooms or by rehabilitating or building additional space, if needed. In general, field laboratories were housed in structures located as close as possible to the Ebola treatment centers that they serviced. The average time between decision making and laboratory deployment was 4.7 (range 1–10) days. After deployment, the laboratory was functional within 24 hours. Thirteen field laboratories were deployed in the 3 affected provinces: 6 basic, 4 standard, and 3 advanced laboratories (Table 8).

The laboratories of Beni, Mangina, and Goma were deployed from Kinshasa; the others were deployed from Beni (the main field laboratory); 1 laboratory (Tchowe, South-Kivu) was deployed from Goma field laboratory (Table 9). Throughout the outbreak, there were multiple epicenters, which changed in intensity and locations over time. Deploying a flexible model of laboratories at the epicenters of the outbreak enabled well-structured and coordinated actions involving all pillars of the response. Results were quickly provided on site to enable timely public health interventions and mitigate the risk for security incidents as transportation of samples was reduced. Decentralized setups were maintained to support routine EVD surveillance in remote areas and detect eventual flare-ups. The presence of skillful local laboratory workers strongly decreased the number of national and foreign experts to be deployed and fostered community engagement. Field laboratories helped integrate response activities into the health system.

Seven laboratories were then decommissioned. All instruments were decontaminated according to INRB standard operating procedures, disassembled, and packed in specific boxes or suitcases, shipped to Beni, and later shipped to Goma or Kinshasa. The lab-oratories in Beni, Mangina, Butembo, Bunia, Bukavu, and Mambasa are still functional.

Table 8 Type of setup, activities, and sites in field laboratories for EVD outbreak, eastern Democratic Republic of the Congo, 2018–2020*

Type of laboratory setup	Activities performed	Sites/provinces
Basic	EVD diagnosis: GeneXpert (+)†; Chemistry: Piccolo (+/–)‡	Tchomia/Ituri, Bunia/Ituri, Tchowe/South-Kivu Bukavu/South-Kivu, Biakato/Ituri, Kasindi/North-Kivu
Standard§	EVD diagnosis: GeneXpert (+); Chemistry: Piccolo (+); Hematology: pocH-100i (+)¶; Ebola survivors clinic	Mangina/North-Kivu, Goma/North-Kivu, Komanda/Ituri, Mambasa/Ituri
Advanced§	EVD diagnosis: GeneXpert (+); Chemistry: Piccolo (+); Hematology: pocH-100i; Ebola survivors clinic; EVD differential diagnosis (Smart cycler or RPA) (+) or genomic sequencing (+)	Beni/North-Kivu, Butembo/North-Kivu, Katwa/North-Kivu

*EVD, Ebola virus disease; RPA, recombinase polymerase amplification.
†GeneXpert, (Cepheid, https://www.cepheid.com).
‡Abaxis (https://www.abaxis.com).
§Sysmex (https://www.sysmex.com).
¶Research support: Ebola therapeutics through a Monitored Emergency Use of Unregistered and Investigational Interventions (MEURI) protocol or the Pamoja Tulinde Maisha/Together save lives (PALM) randomized controlled trial; the immunogenicity of rVSV-ZEBOV-GP vaccine; and the study of Ebola virus reservoir in bats.

Table 9 Sites from where field laboratories were deployed overtime, distance covered to deploy the lab and transport means used

Origin of deployment	Laboratory	Distance traveled, km	Means of transport
Kinshasa	Beni	1,669	Airplane
Kinshasa	Mangina	1,692	Airplane/car
Kinshasa	Goma	1,580	Airplane
Beni	Butembo	54	Car
Beni	Tchomia	256	Helicopter
Beni	Bunia	147	Helicopter
Beni	Katwa	60	Car
Beni	Komanda	127	Helicopter
Goma	Tchowe	421	Helicopter
Beni	Bukavu	339	Airplane
Beni	Mambasa	137	Helicopter
Beni	Biakato	70	Helicopter
Beni	Kasindi	75	Car

Decision-making for deployment of field laboratories

Key factors influencing whether and where to deploy field laboratory

1) Increase of confirmed-cases in a defined area not previously reporting cases
2) Lack of pre-existing laboratory capacity in the affected area
3) Possibility of EVD outbreak to reach big cities, from where it can easily spread (presence of roads, airports, harbors…)
4) Population density in the affected area (as this can accelerate the EVD spread)
5) Possible population movements (especially if roads were in good conditions)
6) Possibility of crossing internal and international borders
7) Risk of spread to an unsecured zone (land under rebels' control)
8) Site accessibility of samples shipment and laboratory's supplies refill

Additional points addressed before proceeding to deployment

1) Proximity to EVD treatment units
2) Easy access for surveillance and safe and dignified burials teams
3) Presence of public health facilities in the area to host the laboratory
4) Availability of rooms constructed in durable materials (well ventilated with water/electrical supply, ready to be reconfigured into the desired standards)
5) Presence of an incinerator for biohazardous waste
6) Security measures including an integral fence and guards/units

III. Field Laboratory Composition, Biosafety and Biosecurity, Cleaning and Decontamination

To mitigate the risk for exposure, protect the safety of the laboratory workers, and prevent environmental contamination, EBOV-suspected samples must be handled in a Biosafety Level 3 (BSL-3) laboratory or BSL-3–like conditions (standard personal protective equipment [PPE], negative pressurized glove box, restricted access to the laboratory, laboratory staff trained and vaccinated) (10,11). Hence, deployment of BSL-3–like structures is needed at outbreak locations. An INRB field laboratory contained 3 mandatory areas: hot zone, cold zone, and extra space. Inside the hot zone, we considered the red zone as the space for sample reception, unpacking and testing (Piccolo [Abaxis, https://www.abaxis.com], pocH-100i [Sysmex, https://www.sysmex.com], and iStat [Abbott, https://www.abbott.com]), and PPE doffing; the orange areas were set up for GeneXpert bench (Cepheid, https://www.cepheid.com) and cold chain. The cold zone included multiple-step donning areas. The extra space was used for supply storage and the administrative office (Figure 13). The basic setup had a hot zone and an extra space in which donning PPE, supplies storage, and administrative offices were located (Figure 14). Samples for diagnosis were inactivated and aliquoted within the glove box. Piccolo discs and iStat cartridges were prepared in the glove box and tested with respective instruments at the bench. Because the red and orange zones were contiguous in most settings, the staff working in these areas dressed in full PPE (Tyvek coverall, goggles or face shield, mask [N100 or FFP3], long-cuff nitrile gloves, and laboratory shoes with shoe covers). In cold zones, staff were dressed with light PPE (surgical gown, head covering, and mask [N95 or FFP2]; short-cuff nitrile gloves; and laboratory shoes). Access to the laboratory was restricted to INRB-trained and Ervebo-vaccinated persons (vaccinated >10 days before working).

At the beginning of the day, trained hygienists cleaned the floors with 0.5% bleach and emptied the dustbins while dressed in full PPE (hot zone) or light PPE (cold zone). At the end of the day, laboratory operators disinfected the inner part of the glove boxes and the benches with bleach 0.5% or other solutions (e.g., Rely+On [Virkon, https://relyondisin-fection.com], CDiffend [2XL

Corporation, https:// www.2xlpro.com]), according to INRB standard procedures. In addition to daily cleaning/decontamination, every 2 weeks in each laboratory, workers decontaminated the glove boxes (inside/outside), benches, and all other surfaces and maintained instruments. Cleaning/decontamination activities were recorded in laboratory registers, maintenance sheets, and decontamination sheets.

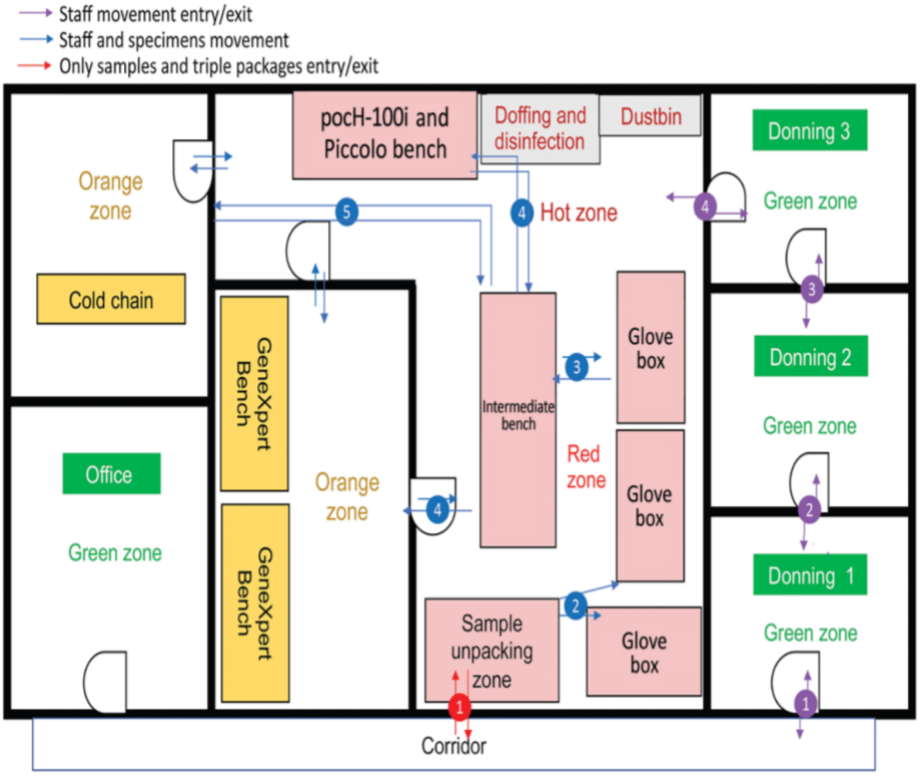

Figure 13. Advanced field laboratory setup used for Ebola virus disease outbreak during chronic insecurity, eastern Democratic Republic of the Congo, 2018–2020. GeneXpert, (Cepheid, https://www.cepheid. com); pocH-100i (Sysmex (https://www.sysmex.com); Piccolo (Abaxis (https://www. abaxis.com).

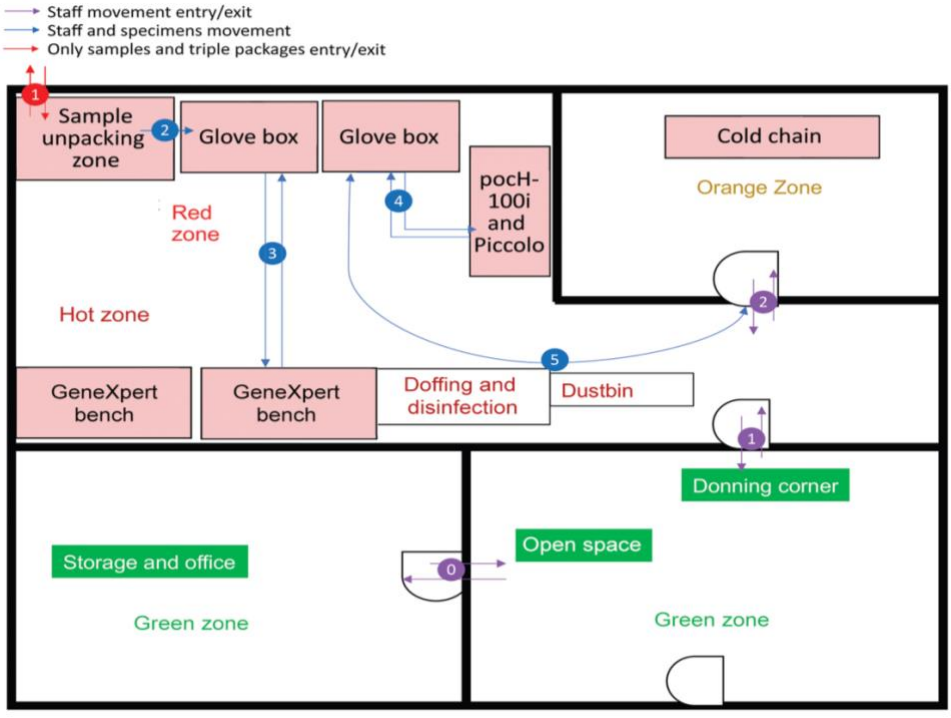

Figure 14. Basic field laboratory setup used for Ebola virus disease outbreak during chronic insecurity, eastern Democratic Republic of the Congo, 2018–2020. GeneXpert, Cepheid, https://www.cepheid. com; pocH-100i, Sysmex, https:// www.sysmex.com; Piccolo, Abaxis, https://www.abaxis.com.

IV. Field Laboratory equipment

Point-of-care devices

Laboratory capacities evolved over the course of multiple EVD outbreaks and followed the level of care provided to persons with confirmed cases. In the past, most EVD laboratories had only reverse transcription PCR testing. Toward the end of the 2013–2016 outbreak in West Africa, cost- effective, rapid, and sensitive tools to detect EBOV at point-of-care and to guide provision of treatments had been developed but were not widely available (12). During the ninth EVD outbreak in DRC (Equateur, 2018), the GeneXpert technology was introduced in a structured way to diagnose EVD in 3 remote laboratories. The tool enables a quick diagnosis (turn-around time <4 hours) and differentiates, by means of its double target (nucleoprotein [NP] and glycoprotein [GP]), between recently Ervebo-vaccinated persons (only GP gene detected) and persons with acute EVD cases (NP and GP detected). From the 10th EVD

outbreak on, a standard Ebola field laboratory included in addition to GeneXpert, pocH-100i (hematology), Piccolo (biochemistry), i-STAT (biochemistry), and a glove box (virus inactivation and sample processing).

A total of 47 GeneXpert IV-modules, 17 Piccolo, 10 iStat, 8 pocH-100i, and 19 glove boxes were deployed. Three types of glove boxes were used: 8 Cleatech HEPA Filtered (Cleatech LLC, https://www.cleatech.com), 8 Könnecke Ultra (Bodo Könnecke, https://www.koen-necke-berlin.de), and 3 Germ Free (Germfree Laboratories https://www.germfree.com) (Table 10, https:// wwwnc.cdc.gov/EID/article/29/2/22-1025-T3.htm).

Maintenance and quality control

Instrument maintenance was performed every 2 weeks, according to INRB standard procedures. Quality control was done monthly on instruments per site by using the Piccolo control kit level 1, 2, 3; the iStat TriControls level 1, 2, 3; the Sysmex Eightcheck3WP-N/L/H; and a GeneXpert positive blood and semen specimen (prepared at the US National Institutes of Health, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, USA). Xpert calibration cartridges were run on all instruments every 6 months. All records were stored in specific binders in the laboratory and uploaded on INRB dropbox and Huddle cloud (https:// us.huddle.com; no longer available).

Cold chain set-up

The standard cold chain included refrigerators (+2°C to +8°C) and freezers (–20°C). The main cold chain located in the Beni laboratory could store up to 40,000 specimens at a time (from different laboratories). It comprised refrigerators and 3 types of freezers (–20°C, –40°C, and –80°C) (Table 10). Power was supplied by three 14-kva generators running alternately, 24 hours/7 days a week. In other laboratories, standard cold chain was continuously connected to five 10–kva generators. Site-to- site trans-port of specimens was achieved by using biological triple-packaging boxes (UN 4H2/GLASS 6.2/14 or BioPack-2 [Airs Sea Containers LTD, https://www. airseadg.com]) filled with frozen icepacks. Given the limited storage capacity in the main cold chain, samples were shipped to the biorepository in Kinshasa. In each laboratory, samples were stored at –20°C and reagents at +2°C to +8°C. Traceable Otio (https:// www.otio.com) trackers were used to monitor the temperature inside refrigerators, freezers, and lab-oratory spaces twice daily. Approximately 120,000 samples were stored in the field and then shipped safely to the Kinshasa biorepository for long-term preservation (3).

Power management

1) Electricity was continuously supplied by 10- 50 Kilo volt Ampere (KVA) generators.
2) Equipment was connected to Uninterruptable Power Supply (UPS) and stabilizersunits to prevent any electricity variation.
3) Backup generators were alternately run to ensure uninterrupted power supply tothe laboratory.
4) Management of the generators required an extensive logistics, continuous fuel, oiland spare-parts provision for almost two years.

V. Logistics for Implementing and Managing Field Laboratories

During the 10th EVD outbreak, field laboratories faced tremendous challenges. The 7 main challenges were: 1) activities interruption after attacks on response teams; 2) movement of contacts and suspect-ed and confirmed case-patients resulting in further spread of the disease; 3) slow resumption of activities after security incidents; 4) evacuation of response staff out of outbreak areas during insecurity events; 5) delayed implementation of activities; 6) disruption to refilling laboratory supplies and fuel; and 7) delayed sample transportation to the laboratory (especially for sequencing unit) (13).

Logistics and financial challenges

1) Limited storage capacity due to important number of samples processed per day (13 laboratories) and also samples stuck in laboratories during insecurity periods.
2) Difficulty to organize more shipments from the field due to high cost of chartered flights
3) Related to equipment maintenance, damage and loss
4) Related to energy requirements
5) Limited financial resources due to steadily increase in human resources, laboratory's needs (reagents and supplies shortage) and logistics to support

Equipment and supplies

Most equipment and supplies were purchased abroad and shipped to Kinshasa. Thereafter, they were airlifted to Beni laboratory, from where the distribution was organized to other sites. Parcels were transported by road, air, and boat by using the World Health Organization and the World Food Program logistics. Finale Inventory software (https://app.finaleinventory.com) was used for the stock management of items at the main warehouses in Beni and Kinshasa. It alerted the laboratory and logistics staff via email regarding the status of items (in critical stock or nearly to be expired). The logistics team could therefore anticipate shortages, prepare timely orders, and prioritize the use of items close to expiration date.

Table 10 Distribution of equipment per laboratory

	Beni	Goma	Butembo	Mangina	Katwa	Kasindi	Tchomia	Bunia	Komanda	Biakato	Mambasa	Bukavu	Tchowe	TOTAL
Gene Xpert	10	4	9	5	4	2	1	2	3	2	3	1	1	47
Piccolo Abaxis	3	1	3	3	2	0	1	0	1	1	1	0	1	17
iSTAT Abbot	3	1	2	2	0	0	0	0	0	0	1	0	1	10
Sysmex pocH-100i	2	1	1	1	1	0	0	0	1	0	1	0	0	8
Glove box	4	2	2	1	2	1	1	1	1	1	1	1	1	19
Fridge (+2 à +8°C)	5	1	1	2	1	2	1	1	1	1	1	1	1	19
Freezer (-20°C)	5	1	1	0	1	1	1	1	1	1	1	0	0	14
Freezer (-40 et - 80°C)	9	0	0	0	0	0	0	0	1	0	0	0	0	10
Laptop	6	1	2	3	2	1	0	1	1	1	1	2	0	21
Printer	5	1	2	1	2	1	0	1	2	1	1	1	1	19
Smart cycler	1	0	0	0	0	0	0	0	0	0	0	0	0	1
RPA	1	0	1	0	0	0	0	0	0	0	0	0	0	2
TOTAL	54	13	24	18	15	8	5	7	12	8	11	6	6	186

VI. Laboratory activities

The most prominent activities conducted in the field laboratories were EVD diagnosis and training. Laboratories contributed to providing investigational therapeutics to treat patients with confirmed cases, and they were equipped with clinical laboratory capacities (8,9). Five laboratories supported survivor activities, 1 conducted genomic sequencing, and 2 performed differential diagnoses (13,14). Throughout the outbreak, 9.3 tons of equipment were deployed across sites (3), 230,936 Ebola Xpert cartridges

were used and subsequently shipped to the Goma laboratory for proper disposal by incineration, as per World Health Organization recommendations. Laboratory performance was evaluated by 2 indicators: proportion of new suspect samples tested within 48 hours and proportion of results delivered within 24 hours. Despite challenging conditions, field laboratories tested 100% of samples with turnaround times of <48 hours.

Waste management

1) Non-biohazardous waste was collected in plastic bags which were wrapped, then sealed with adhesive tape until they were transported to the incinerator.
2) Laboratory waste was treated as biohazardous and separated at each step of samples processing.
3) Items soiled by biological materials (tubes, tissues, pipet-tips etc) were processed in the glovebox and disinfected with 10% bleach or others (Rely+On Virkon®, CDiffend®).
4) After samples' manipulation, soiled materials were wrapped in small biohazard bags, sealed with adhesive tape, sprayed with disinfectant to minimize the microbialload, taken out of the glovebox and dropped into larger biohazard bags which, werealso sealed with adhesive tape.
5) Trained hygienists used plastic garbage to transport all parcels and load them intothe incinerator.
6) Used Xpert® Ebola cartridges were stored across sites and then shipped to the central repository in Beni for temporary storage.
7) Once the Addfield® incinerator (could reach > 1000°C) was purchased and installedin July 2019, all used Xpert® Ebola cartridges were regularly shipped to Goma for appropriated disposal.

Genomic sequencing

1) At the beginning of the outbreak, the first samples of confirmed cases were regularly shipped to INRB-Kinshasa for full genomic sequencing.
2) From September 2019, we implemented a field genomic sequencing laboratory in Katwa (Butembo city) to support the surveillance activities in the monitoring of an epidemic which tended to get out of control (spread in the time and the space) with new confirmed cases emerging from unidentified sources.
3) The near real-time data generated throughout the outbreak helped in the detectionand management of unknown transmission chains [3,15].
4) One aliquot of samples from new confirmed cases was transferred from different sites to the sequencing laboratory in Katwa.
5) The remaining aliquots were rapidly shipped with Ebola negative samples to the central cold chain in Beni.
6) The maximum time taken to transport positive aliquots from their laboratory of confirmation to the sequencing laboratory was 48-72 hours, following the availabilityof transport means (helicopter/plane flights and vehicles).
7) The field sequencing laboratory analyzed 219 samples in total, 60 of them did not have any epidemiological links although 39 of them (65%) gave full genome coverage.

Sample management, sample testing and results communication

Samples were collected by the surveillance team; care team; safe and dignified burials team; or survivors' team. The preferred sample for EBOV diagnosis was whole blood or plasma, although other specimens were oral secretions from cadavers, blood swab samples (patients with circulatory collapse, infants), semen and vaginal secretions from survivors, fetal annexes in delivering survivors, and breast milk in breastfeeding survivors. In care units, samples were collected 1–4 hours after admission during the day (delay mainly resulted from time to stabilize the patient, high number of patients to be sampled, and preparation of sampling material and notification forms). Patients received at night underwent sampling the next morning, after the laboratory was opened. Samples were transported in triple-packaging boxes to the laboratory within 10–20 minutes. At the laboratory, samples were received on a dedicated table, unpacked while personnel dressed in PPE disinfected different layers of the packing, and then transferred through a window into the hot zone for processing.

Chemistry and hematology samples were given priority because those results could help clinicians quickly adjust the care provided (e.g., correction of hypoglycemia, electrolyte imbalance, anemia, shock). Chemistry and hematology samples were processed within 30–40 minutes (from reception to testing). The Piccolo results were read within 12 minutes, whereas pocH-100i outputs were available in 2 minutes. For diagnosis, new suspected samples were processed first to confirm or invalidate their EVD status, and then the follow-up samples were processed. Only the amount of sample required for testing in the GeneXpert lysis buffer (guanidinium thiocyanate) was inactivated. Samples for hematology and chemistry were not inactivated. Cryotubes were labeled (initials of the site, number assigned in an ascending way, type of sample, and type of blood tube), samples were aliquoted in cryotubes, and then aliquots were transferred into 9x9 labeled grids (same type of samples per cryobox) and stored in the cold chain.

Handling Data and Communicating Results

Samples tubes were identified per team collecting the specimen. A notification form accompanying the sample to the laboratory contained the patient identification and sociodemographic, epidemiologic, and clinical information. Additional information (laboratory identification, type of sample, analyses done, type of blood tube, number of aliquots, grid labels, date of testing, EBOV results, and cycle threshold values) were added in the laboratory to make a line list in Microsoft Excel 2016 (https:// www.microsoft.com). All issues related to spelling or transcription errors were deconflicted by using sociodemographic, clinical, epidemiologic information, and laboratory results. The communication of laboratory results was organized by psychosocial teams in close coordination with families and other teams (3). The laboratory head shared results daily with the response coordination via sending the Microsoft Excel line list by email. All laboratory results were centralized in a database (15). Most samples (212,655 [89.1%]) were tested with GeneXpert, 19,227 (8%) by clinical chemistry, and 6,766 (2.8%) by hematology. In total, 207,065 (86.8%) blood, 27,313 (11.4%) oral swabs, and 1,544 (0.6%) semen samples were tested (Table 11, https://wwwnc.cdc.gov/EID/article/29/2/22-1025-T4.htm). We were successful in testing this unprecedented number of samples be-cause of the extended duration of the outbreak (22 months), the number of laboratories deployed, the diversity of activities performed (diagnostic, patient biochemistry and hematology, viral load monitoring, survivor follow-up, and full-genome sequencing), the management of all laboratories by 1 institution (centralized information, data, and samples), and the community-based surveillance (which improved sample flow).

Sample shipments

Most samples collected were shipped to the INRB biorepository in Kinshasa along with corresponding databases by email. Shipments of large batches of samples were done with chartered cargo flights. Site-to-site sample transportation was performed by fleets and vehicles supported by the World Food Program, MONUSCO (https://monusco.unmissions.org), and the World Health Organization. A total of 127,993 samples were shipped from the Beni

laboratory to Kinshasa through 7 large shipments. Decision making for samples shipment was guided by the limitation of the storage capacity on the ground and the need to evacuate the Ebola specimens to a safe area.

VII. Management of human resources

Activities were conducted by medical-biologist doctors, biologists, and laboratory technicians assisted by supporting staff (administrative, logistics, hygienists, security guards, drivers) (Table 12, https:// wwwnc.cdc.gov/EID/article/29/2/22-1025-T5.htm; Figure 15). Local staff members were recruited mostly in the main health facilities of affected provinces or cities; national staff came from INRB (Kinshasa). All laboratory personnel were trained with regard to biosafety and biosecurity; PPE donning and doffing; good clinical and laboratory practices; sample collection, packaging, and transportation; sample manipulations within a glove box; instrument manipulation. and troubleshooting; and safety and emergency management. Refresher trainings were scheduled each trimester, whenever a new staff member started at the laboratory or when an error occurred throughout the testing process. The laboratory was headed by a medical-biologist doctor or biologist assisted by a bench supervisor. The laboratory head was responsible for sample processing, results validation and delivery, quality control and final laboratory records, participation in daily coordination meeting, engaging with other working groups, and drafting the reports and communication. The laboratory head reported daily to the coordinator of all field laboratories who, in turn, presented the global situation at the general coordination meeting. Staff were granted a break after 3 months of work or in any emergency situation. The bench supervisor was responsible for organizing the bench workload and schedules, sample processing and storage, functioning and maintenance of laboratory instruments, cleaning and decontamination procedures, records tracking, and training of new staff. The administrative staff were in charge of preparing paperwork, data entry, data management, results typing, printing, and distribution. Over the course of the outbreak, INRB created a unique database template used by all laboratories, which were shared monthly with the coordinator of field laboratories. We

employed 134 personnel, including 9 medical-biologist doctors, 21 biologists, and 62 laboratory technicians (Table 12). INRB fostered capacity building to quickly hand over Ebola response tools and competences to local staff to empower the health system and decentralize the diagnostics. The local capacity setup at the peripheral level allowed sustained and successful management of 3 successive EVD flare-ups (Butembo in 2021, Beni in 2021 and 2022).

Work force challenges

1) Need of building capacity for local staff in lab techniques such as working in the glovebox, unidirectional flow work, PPE use, decontamination process
2) Biosafety and biosecurity practices (almost new for most of staff)

Table 11 Type of analyses per site

Lab	Analyses performed				Ebola RT-PCR Confirmation		Types of Samples					
	RT-PCR	Biochem.	Hematol	Total	Test +	Samples received	Blood	Oral Swab	Vaginal secretions	Sperm	Others*	Total
	N (%)	N (%)	N (%)	N (%)	n (%)	N	N (%)	N (%)	N (%)	N (%)	N (%)	N
Beni	62706(82.3)	10612(14)	2850(3.7)	76168(31.9)	924(1.5)	61690	68004(89.2)	7035(9.2)	524(0.6)	432(0.6)	173(0.2)	76168
Butembo	69027(90)	5349(7)	2357(3)	76733(32.1)	1129(1.7)	67142	64788(84.4)	9721(12.6)	1189(1.5)	723(1)	312(0.4)	76733
Mangina	21656(93)	980(4.2)	665(2.8)	23301(9.7)	728(3.5)	21077	19511(83.7)	3199(13.7)	253(1)	327(1.4)	11(0.04)	23301
Katwa	34244(94.8)	1140(3.1)	715(2)	36099(15.1)	542(1.6)	34244	32266(89.3)	3825(10.5)	2(0.0)	2(0.0)	4(0.0)	36099
Kasindi	5032(100)	0(0)	0(0)	5032(2.1)	0(0)	5032	4556(90.5)	466(9.2)	0(0.0)	0(0.0)	10(0.2)	5032
Tchomia	125(91.2)	12(8.7)	0(0)	137(0.05)	2(1.7)	115	125(91.2)	10(7.2)	0(0.0)	0(0.0)	2(1.4)	137
Komanda	7522(92.5)	541(6.6)	65(0.7)	8128(3.4)	106(1.4)	7522	5882(72.3)	2077(25.5)	0(0.0)	0(0.0)	169(2)	8128
Biakato	1733(98.5)	26(1.4)	0(0)	1759(0.7)	20(1.2)	1733	1446(82.2)	313(17.7)	0(0.0)	0(0.0)	0(0.0)	1759
Mambasa	10176(93.8)	552(5.1)	114(1)	10842(4.5)	36(0.4)	9687	10062(92.8)	643(6)	76(0.7)	60(0.5)	1(0.0)	10842
Tchowe	434(96.6)	15(3.3)	0(0)	449(0.1)	5(1.2)	434	425(94.6)	24(5.3)	0(0.0)	0(0.0)	0(0.0)	449
Total	212655(89.1)	19227(8)	6766(2.8)	238648 (100)	3492(1.7)	208676 (100)	207065 (86.8)	27313 (11.4)	2044 (0.9)	1544 (0.6)	682 (0.3)	238648

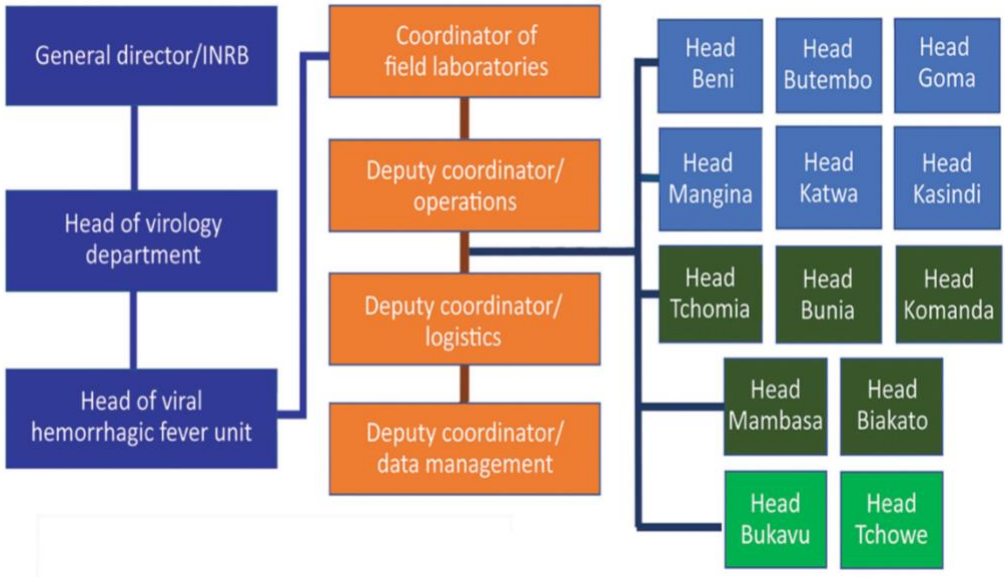

Figure 15. Organizational chart for field laboratories used for Ebola virus disease outbreak during chronic insecurity, eastern Democratic Republic of the Congo, 2018–2020. INRB, Institut National de Recherche Biomédicale.

Table 12 Field laboratory staff deployed or recruited during the outbreak

	Beni	Goma	Butembo	Mangina	Katwa	Kasindi	Tchomia	Bunia	Komanda	Biakato	Mambasa	Bukavu	Tchowe	TOT (%)
Lab Tech	7	6	12	5	1	6	1	3	4	3	4	7	3	62(46,2)
Medical biologist	5	3	3	1	3	0	0	1	1	0	1	3	0	21(15,6)
Data Manager	2	1	2	1	1	1	0	1	1	1	1	1	0	13(9,7)
Medical Biologist Doctor	3	0	3	1	1	0	0	0	0	1	0	0	0	9(6,7)
Hygienist	1	1	1	1	1	1	1	1	1	1	1	1	1	13(9,7
Phlebotomist	0	0	0	0	8	0	0	0	0	0	0	0	0	8(5,9)
Logistician	4	0	1	0	0	0	0	0	0	0	0	0	0	5(3,7)
Administrative	2	0	0	0	0	1	0	0	0	0	0	0	0	3(2,2)
TOTAL (%)	24 (17,9)	11 (8,2)	22 (16,4)	9 (6,7)	15 (11,1)	9 (6,7)	2 (1,4)	6 (4,4)	7 (5,2)	6 (4,4)	7 (5,2)	12 (8,9)	4 (2,9)	134

VIII. Security concerns during the 10th EVD outbreak

The overall societal context of the eastern DRC led to express reluctance toward most EVD counter-measures. Of the 13 laboratories, 10 were deployed within unsafe areas. Thus, on several occasions, teams were attacked by rebels, militia, and other hostile groups. Those threats delayed or impeded deployment and supervision of activities, sample handling, and staff movement (Tables 12, 13). To man-age security in the field, transportation of all personnel movement, equipment, and supplies had to be approved by a security commission. Depending on the context, some convoys had to be escorted by security forces, using military devices such as armored vehicles and pickup trucks with machine guns and bulletproof vests for passengers. In unsafe areas, a curfew was set from 6:00 pm to 6:00 am, except for a few teams, which were circulating under security escort until late to maximize timely results. In unsafe areas, laboratories were protected by armed guards to prevent eventual attacks, sabotage, or disruption. Despite the security incidents encountered, field laboratories operated for an average of 21 months, supporting all pillars of the response.

Access and security challenges

1) Bad road conditions in certain areas
2) Harsh geographical conditions to access certain areas
3) Cost of transport
4) Safety and security threats and attacks on personnel (from rebels, militia and population), equipment (loss or damage) and work (have to stop working several hours even)

IX. Conclusions

In countries at high risk for EVD outbreaks, decentralized laboratories should be strategically positioned to timely detect EBOV. Health authorities should supply them with dedicated equipment and well-prepared teams, built on local know-how to organize efficient responses. A tiered and decentralized policy of laboratories during outbreaks provides flexibility for managing materials and supplies, logistics, and human resources in the health system. The quick handover of competences and capacities to local staff led during the 10th EVD outbreak in the DRC led to sustained and successful management of further out-breaks. Nonetheless, efforts should be made to use new diagnostic and research tools in the laboratory to considerably reduce turnaround time to <24 hours, to strongly improve patient management and research activities, to foster real-time genomic sequencing, and to support follow-up of EVD survivors.

Table 13 Armed groups in Ebola operational zones and time taken to deploy, and operate field laboratory, eastern Democratic Republic of the Congo, 2018-2020

		Results achieved		
Site	Security background	Date of installation	Time taken to deploy laboratory	Time laboratory remained functional, mo
Beni	ADFrebels: near Virunga Parc (ADF stronghold); popular pressure groups and citizens movements; Maï Maï rebel groups	2018 Aug 2	1 d	45*
Mangina	Maï Maï rebel groups; ADF rebels	2018 Aug 11	9 d	45*
Butembo	Maï Maï rebel groups; popular pressure groups; citizens movements	2018 Sep 7	6 d	44*
Tchomia	Maï Maï rebel groups; CODECO rebel Group	2019 Sep 28	6 d	3
Katwa	Maï Maï rebel groups; popular pressure groups	2019 Jan 18	10 d	26
Komanda	Maï Maï rebel groups; ADF rebels	2019 Jan 18	7 d	15
Mambassa	Maï Maï rebel groups; ADF rebels	2019 Sep 2	10 d	18
Tchowe	Maï Maï rebel groups; FDLR rebels	2019 Aug 19	2 d	2
Biakato	Maï Maï rebel groups; ADF rebels	2019 Oct 12	10 d	5
Kasindi	Located in the Virunga Park (Ugandan border); Ugandan rebels stronghold	2019 Oct 28	10 d	6

*As of May 2, 2022. ADF, Allied Democratic Forces; CODECO, Coopérative pour le Développement du Congo; FDLR, Democratic Forces for the Liberation of Rwanda.

Table 14 Major security incidents during the 10th Ebola virus disease outbreak, eastern Democratic Republic of the Congo, 2018-2020

Incident	Site (period)	Consequences
Attack on the response team	Beni (Aug 2018)	Response activities stopped, and recovery took longer to reach full operation
Several days off + murder of civilians	Beni (Sept 2018)	Response activities stopped for few days
Election unrest + destruction of the ETU	Beni/Butembo (Dec 2018)	Response teams evacuated
Attacks on laboratory, surveillance, and coordination teams	Oicha-Eringeti (Dec 2018)	Komanda laboratory deployment delayed
Attackers set fire to Katwa and Butembo ETU	Katwa/Butembo (Feb 2019)	Temporary ETU closure
Attack on the response teams	Butembo (Apr 2019)	Response activities stopped, and 1 foreign doctor killed
Attack on the response teams	Biakato/Beni (Nov 2019)	Evacuation of teams to Goma and Kinshasa, 6 deaths

*ETU, Ebola treatment unit.

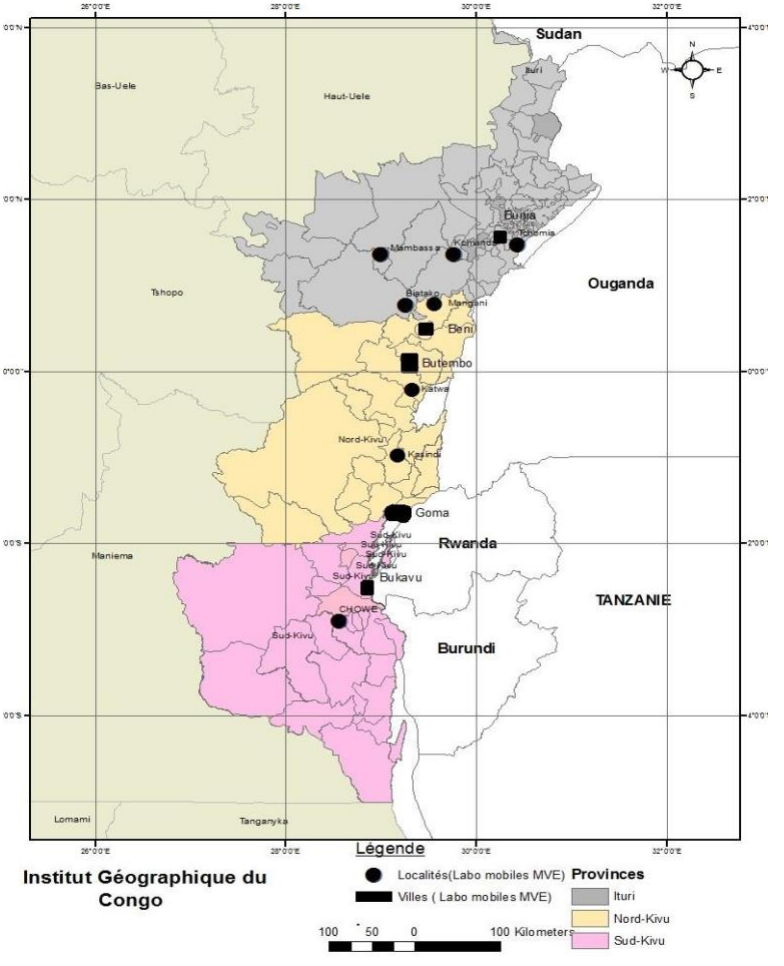

Figure 16: Map of INRB field laboratories during the tenth outbreak

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About the Author

Dr. Mukadi-Bamuleka is a medical doctor and medical virologist at the INRB, Kinshasa, DRC. His expertise includes implementation and coordination of field laboratories during outbreaks (Ebola, COVID-19, monkeypox), serosurveys, and evaluation of diagnostic tools for emerging viruses.

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Chapter 4

Integration of genomic sequencing into the response to the Ebola Virus outbreak in Nord- Kivu, Democratic Republic of the Congo

Integration of genomic sequencing into the response to the Ebola virus outbreakin Nord Kivu, Democratic Republic of the Congo

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Eddy Kinganda-Lusamaki*[1,9], Allison Black*[2,3], **Daniel B. Mukadi***[9], James Hadfield*[3], Placide Mbala-Kingebeni*[1,9], Catherine B Pratt*[4], Amuri Aziza[1], Moussa M Diagne[5], Bailey White[4], Nella Bisento[1], Bibiche Nsunda[1], Marceline Akonga[1], Martin Faye[5], Ousmane Faye[5], Francois Edidi-Atani[1,9], Meris Matondo-Kuamfumu[1,9], Fabrice Mambu-Mbika[1,9], Junior Bulabula[1,9], Nicholas Di Paola[6], Matthias G Pauthner[7], Kristian G Andersen[7], Gustavo Palacios+[6], Eric Delaporte+[8], Amadou Alpha Sall+[5], Martine Peeters+[8], Michael R. Wiley+[4], Steve Ahuka-Mundeke+[1,9], Trevor Bedford+[2,3], Jean-Jacques Muyembe Tamfum+[1,9]

* These authors contributed equally.

+ Co-senior authors.

Affiliations

1. *Institut National de Recherche Biomédicale, Kinshasa, DRC*
2. *Department of Epidemiology, University of Washington, Seattle, WA, USA*
3. *Vaccine and Infectious Disease Division, Fred Hutchinson Cancer ResearchCenter, Seattle, WA, USA*
4. *Department of Environmental, Agricultural, and Occupational Health, Universityof Nebraska Medical Center, Omaha, NE, USA*
5. *Institut Pasteur de Dakar, Dakar, Senegal*
6. *Center for Genome Sciences, United States Army Medical Research Instituteof Infectious Diseases, Frederick, MD, USA*
7. *Department of Immunology and Microbial Science, The Scripps ResearchInstitute, La Jolla, CA, USA*
8. *TransVIHMI, Institut de Recherche pour le Développement, Institut National de la Santé et de la Recherche Médicale, Université de Montpellier, Montpellier, France*
9. *Service de Microbiologie, Cliniques Universitaires de Kinshasa, Kinshasa,Democratic Republic of the Congo*

Abstract

On 1 August 2018, the Democratic Republic of the Congo (DRC) declared its tenth Ebola virus disease (EVD) outbreak. To aid the epidemiologic response, the Institut National de Recherche Biomédicale (INRB) implemented an end-to-end genomic sur- veillance system, including sequencing, bioinformatic analysis and dissemination of genomic epidemiologic results to frontline public health workers. We report 744 new genomes sampled between 27 July 2018 and 27 April 2020 generated by this surveillance effort. Together with previously available sequence data (n = 48 genomes), these data represent almost 24% of all laboratory-confirmed Ebola virus (EBOV) infections in DRC in the period analyzed. We inferred spatiotemporal transmission dynamics from the genomic data as new sequences were generated, and disseminated the results to support epidemiologic response efforts. Here we provide an overview of how this genomic surveillance system functioned, present a full phylodynamic analysis of 792 Ebola genomes from the Nord Kivu outbreak and discusshow the genomic surveillance data informed response efforts and public health decision making.

Introduction

Since the first documented outbreak of EVD in Yambuku, DRC, in 1976, further outbreaks have occurred sporadically in that country. In June 2018, laboratory capacity for performance of whole-genome EBOV sequencing was established in the DRC at the INRB in Kinshasa. The establishment of sequencing capacity enabled genomic surveillance over the entire duration of the Nord Kivu EVD outbreak (1 August 2018 to 25 June 2020). At the time of writing, we had generated 792 full andpartial genome sequences representing ~24% of laboratory-confirmed cases of EVD in the region.

Comparative analysis of pathogen genomes can support traditional epidemiologic surveillance by improving the capacity to detect and define clusters of related infections, thereby facilitating detailed investigations of spatiotemporal disease dynamics. During the 2013–2016 West African EVD outbreak, analysis of viral genomic data was used to differentiate sexual EVD transmission from standard human-to-human transmission (1), and to demonstrate that large, sustained case counts were attributable to many cocirculating transmission chains of varying size (2).Genomic data were also used to detect the emergence of the A82V variant that roseto high frequency during the epidemic, per- haps due to the variant's increased infectivity in humans (3,4).

Despite its utility, genomic surveillance presents challenges for many public health agencies. Assembly and analysis of pathogen genomic data can require both advanced computational infrastructure and analysts trained in disciplines that have not historically been a part of public health, including bioinformatics, computational biology and data science (5). This means that the ability of public health agencies toanalyze and interpret genomic data within an epidemiologic context often lags behind laboratory capacity to perform sequencing (6).

We sought to increase the utility of viral genomic data during the Nord Kivu EVD outbreak by regular generation and analysis of EBOV sequence data, releasing the results as genomic epidemiology situation reports. These reports, written in both English and French, allowed representation of

interactive genomic data visualization alongside written scientific interpretations. Here we provide an overview of this end- to-end genomic surveillance system, describing sequencing intensity over the courseof the Nord Kivu outbreak and patterns of data release. We then describe the broad epidemic dynamics inferred from phylogeographic analysis of all 792 publicly available EBOV genomes. Finally, we discuss how the genomic data supported publichealth decision making and issues that impacted the actionability of the data.

Results

Overview of the genomic surveillance system

Between July 27, 2018 and June 25, 2020, clinical diagnostic specimens were collected from individuals presenting with EVD-like symptoms. A convenience sampleof EBOV-positive specimens were selected for sequencing, which occurred at a mobile laboratory in Katwa or at INRB in Kinshasa. In total, 792 EVD genomes were sequenced: forty-eight of these sequences were previously published (7) and 744 sequences are analyzed here for the first time. Samples were sequenced over the full temporal span of the outbreak (Figure 17). While the complex geographical and political situation in eastern DRC affected sequencing intensity over time (Figure 17),there is minimal geographic bias. The number of sequenced cases from each healthzone (the operational jurisdiction for health service in the DRC) is proportional to thetotal number of confirmed cases reported from that health zone (Figure 17).

To promote open data sharing and to facilitate insights from the international scientificand public health community, genomic data were released publicly on GitHub as they were generated, accompanied by de-identified metadata (https://github.com/inrb- drc/ebola-nord-kivu). As the genomic surveillance system matured over the outbreak,the time between sequencing and data release decreased (Figure 17). Initially, genomic findings were communicated through haplotype maps which were manuallyannotated with epidemiologic information. We shared these visualizations, along witha short description of the findings, with the response team as PDFs. The reports werealso presented

and discussed at emergency operations meetings in Goma, a city closer to the outbreak that served as a major hub for the response.

In September 2019, we transitioned from generating and manually annotating haplotype maps to using an automated pipeline to construct divergence and temporally-resolved phylogenies. We also shifted from sharing the haplotype map towriting interactive situation reports, deployed as Nextstrain Narratives (8). These interactive reports allowed users to access more detailed information about the genomic data on demand, facilitating further self-guided exploration of the data if desired. The reports were available online in both English and French, and were circulated by email as PDFs that could be viewed offline. These situation reports werealso presented to the public health response team at emergency operations centre meetings. While the original reports contain sensitive patient information which preclude public release, we have provided five de-identified reports, initially released in September and October 2019, as examples (https://nextstrain.org/community/narratives/blab/ebola-narrative-ms/).

Adopting an automated analysis pipeline increased the efficiency and scalability of analyses and reduced the average time between sequencing and private sharing of phylogenetic information (Figure 17). After adoption of the automated analysis pipeline, we shared data and analyses with the frontline response team on average within 6.6 days after sequencing (standard deviation 7.8 days). Public release of thedata occurred on average 13.4 days later. The transition away from haplotype mapsalso enabled us to include genomes that were less than full length in analyses and toexplicitly incorporate temporal information, thereby improving the utility of these analyses for understanding disease transmission dynamics.

When circumstances were ideal, we performed diagnostic testing, sample transportation, and sample preparation for sequencing in as little as 4 days, with sequencing and data analysis taking an additional 2 to 3 days. This timeline made itpossible to deliver genomic epidemiological inferences to the response team in as few as 7 days after sample collection. However, the time period between sample collection and sequencing was typically longer.

Before September 1st 2019, we sequenced and analyzed 33% (169 of 508) of samples within 30 days of collection. After September 2019 we sequenced and analyzed 48% (128 of 264 samples) within
30 days of specimen collection from the patient. Notably, these proportions are conservative. Over the course of the outbreak, we performed additional retrospectivesequencing of archival isolates, which by definition have longer lag times between sample collection and sequencing.

Broad-scale dynamics of EVD circulation

From phylogeographic analysis of 792 publicly available EBOV genomes collected between July 27, 2018 and April 27, 2020, we inferred broad patterns of spatial transmission over time. Previously, phylogenetic analysis indicated that the Nord Kivu outbreak resulted from a single zoonotic spillover event (7). We inferred that this event likely occurred in July 2018 in the Mabalako health zone (Figure 18), which agrees with case surveillance data (7). Transmission to the nearby health zones of Beni and Mandima occurred early in the outbreak (Figure 18), with multiple introductions of EVD from Mabalako into Beni (Figure 18). One of these introductions,which occurred in August 2018 (95% CI: Aug 15, 2018 – Aug 20, 2018), establisheda lineage, termed the primary outbreak clade (defined by A7312G) that became the primary circulating lineage during this outbreak (Figure 18). We also observed migration of viral lineages back into previously affected health zones. For example, the primary outbreak clade moved from Beni into Kalunguta around the end of August2018 (95%CI: Aug 16, 2018 – Sept 12, 2018), and then was introduced to Katwa multiple times between October 2018 and January 2019. One of the lineagescirculating in Katwa then migrated back into Beni in mid-April 2019 (Figure 18).

A secondary, sustained lineage, termed the secondary outbreak clade, resulted froman introduction from Beni into Katwa sometime between August and October 2018 (Figure 18). This lineage later circulated in Mandima and Rwampara, and migrated back into Katwa. Although smaller than the primary outbreak clade, this secondary lineage persisted throughout much of the outbreak, with some genome sequences sampled as late as September 2019

clustering within this clade.

The frequent movement of viral lineages between health zones in Nord Kivu, with limited periods of local transmission after introduction, is consistent with the dynamics that sustained the West African EVD outbreak (2). In that outbreak, phylogenetic analysis demonstrated that many affected regions experienced frequent independent EBOV introductions, but that the subsequent transmission chains were short-lived, causing on average only 75 EVD cases before dying out or moving to a new region (2). Given similar apparent dynamics (Figure 19, Figure 20), we sought to quantify the frequency of EBOV introductions into health zones and the duration of local circulation after an introduction event.

In total, we detected 188 independent introduction events where the source and recipient health zones could be inferred with at least 80% confidence. Amongst thesehigh confidence events, there were 60 distinct combinations of source health zone (where a viral lineage originated) and sink health zone (where a viral lineage movedto). Of 23 affected health zones, 11 health zones acted only as sinks, meaning that viral lineages were introduced into that health zone, but were never exported from that health zone to a different one. The majority of introduction events into new healthzones were seeded from only 5 source health zones: Beni, Mabalako, Katwa, Kalunguta, and Mandima. Each of these five health zones seeded transmission in a different health zone at least 20 separate times.

In general, viral lineages migrated between health zones that were geographically proximal (Figure 19), although the geography and infrastructure of Eastern DRC means that straight-line distances may be misleading. Once introduced to a health zone, the majority of lineages circulated locally within that health zone for less than 8weeks (Figure 19). In a minority of cases, lineages appeared to circulate locally in a health zone for much longer (Figure 19, Figure 20). It is possible that sexualtransmission events from persistently-infected EVD survivors artificially lengthened some of these periods, as persistently-infected survivors maintain the infecting lineage over long periods of time even though that lineage is not actively

circulating in the community (1). On average, circulating viral lineages seeded 2.97 introduction events into new health zones, although this was highly variable (standard deviation 5.3, Figure 19). The length of time that a lineage circulated in a health zone was weakly, but significantly, correlated with the number of times that lineage seeded introductions into other health zones ($r^2=0.21$, $p<0.001$).

Since these sequences represent a convenience sample of the outbreak, we performed a sensitivity analysis to evaluate the robustness of our phylogeographic inference procedure to the sampling frame. As discussed in Hall et al (9), phylogeographic analysis of sequences sampled uniformly across time and space performs similarly well to sampling demes in proportion to incidence. Thus, we sampled a fraction of the full dataset to create two more equitably subsampled datasets. One dataset included three viruses sampled per health zone per month, theother included five viruses sampled per health zone per month (full and subsampled builds are available at https://nextstrain.org/community/blab/ebola-narrative-ms/). Phylogeographic analysis of these equitably-subsampled datasets recapitulated the dynamics observed in analysis of the full dataset.

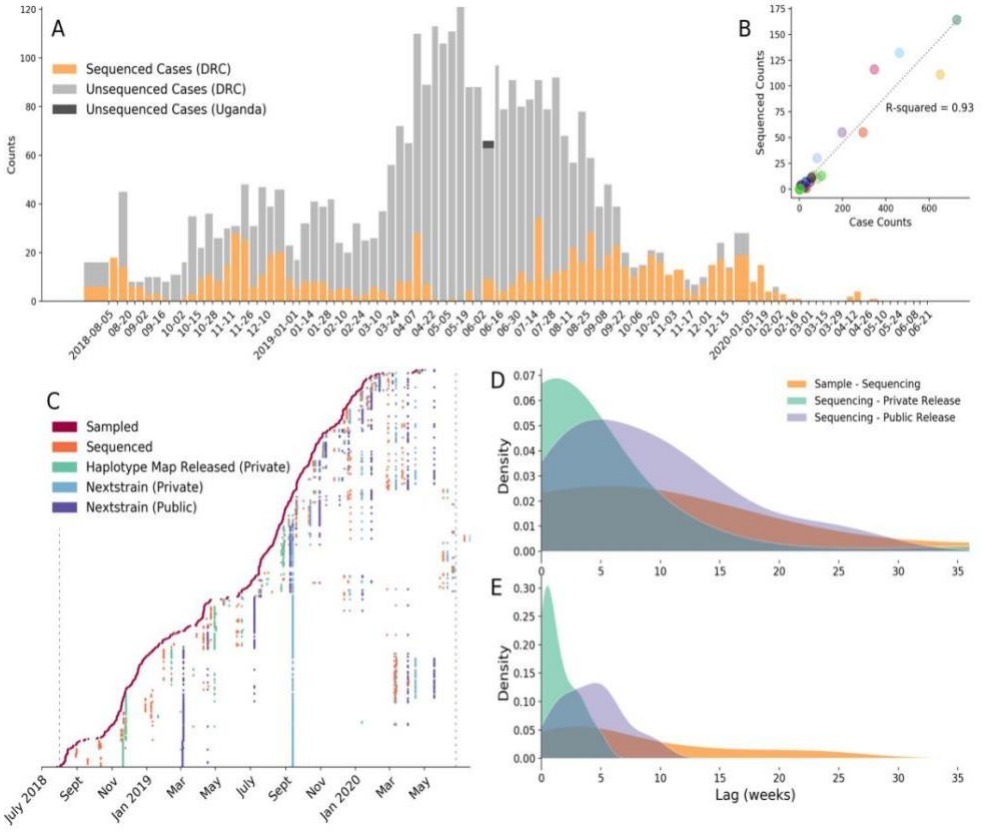

Figure 17 Progress in genomic surveillance over the course of the outbreak

a, Total numbers of sequenced (orange) and unsequenced (gray) laboratory- confirmed cases of EVD as reported in WHO (World Health Organization) situation reports. **b**, Correlation between the numbers of laboratory-confirmed and sequencedcases reported in individual health zones. **c**, Time lags between sample collection and release of phylogenetic analyses. In this figure, each row represents a sample.

The *x*-axis position of a colored dot represents the date when a specific action occurred, and the color represents the action. Thus each row shows the amount of time that passed between different events for a single sequenced sample. Vertical lines represent events that occurred for a large proportion of samples; the dashed black lines represent when WHO declared that the outbreak started and ended. **d**,**e**,Kernel density estimates of lag times between sample collection and sequencing (orange), between sequencing and private release of the data (teal) and between sequencing and public release of the data (purple), before September 2019 (**d**) and after switching to privately released Nextstrain Narrative situation reports in September 2019 (**e**).

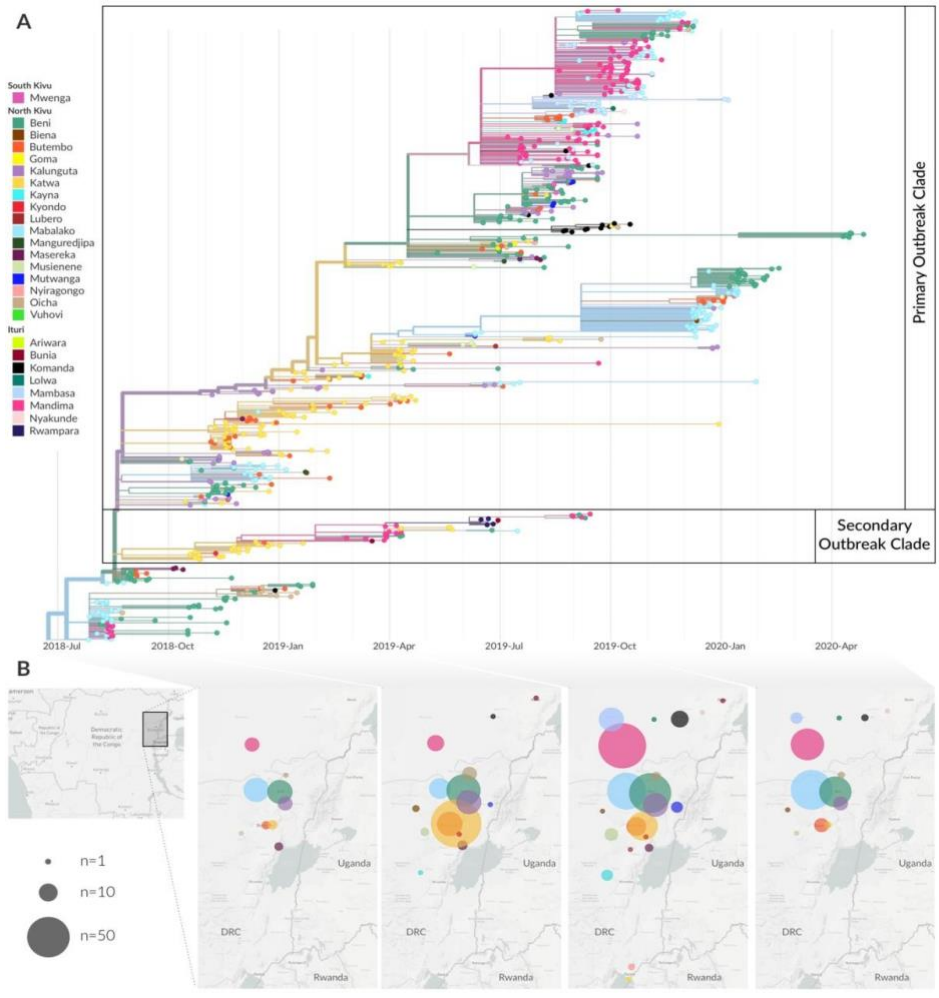

Figure 18 Broad-scale spatiotemporal dynamics of EVD in Nord Kivu.

a,Temporally resolved phylogenetic tree of 792 EBOV genomes, colored according to reporting health zone. The health zone of internal nodes is inferred via a discrete model, and reduced confidence is conveyed by transitioning colors to gray.

b, Geographical spread of samples over four disjoint time intervals spanning the entire outbreak. Figure adapted from Nextstrain visualizations. Note that three health zones—Manguredjipa (two samples), Rwampara (four samples) and Mwenga (four samples)—are located outside of the area on the map shown here.

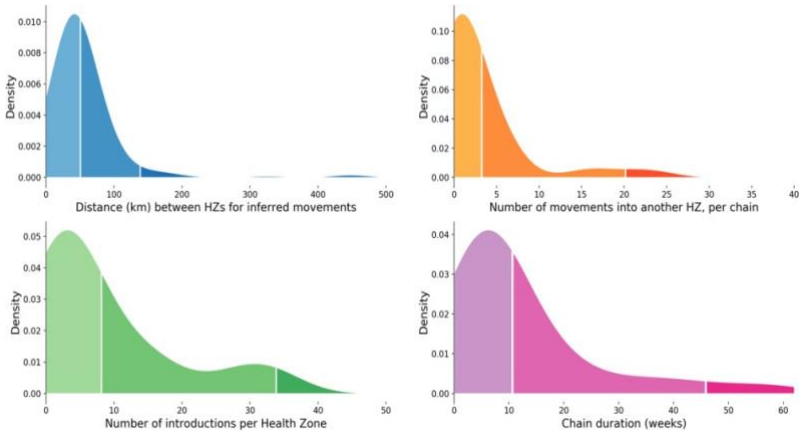

Figure 19 Transmission dynamics within and between health zones

a, Kernel density estimate of the inferred distance between a source and a sink health zone for 188 high-confidence events where a viral lineage moved between two health zones (HZs): 50 and 95% of movement events occurred between health zones <49 and <200 km apart, respectively. **b**, Kernel density estimate of the number of timesa lineage was introduced into a different health zone: 50 and 95% of lineages seededfewer than five and 25 introduction events, respectively. **c**, Kernel density estimateof the number of times EBOV was introduced into each health zone: 50 and 95% of health zones experienced fewer than three and eight introduction events, respectively. **d**, Kernel density estimate of the duration of time a lineage circulated within a single health zone: 50 and 95% of lineages circulated within a single health zone for <10 and 40 weeks, respectively.

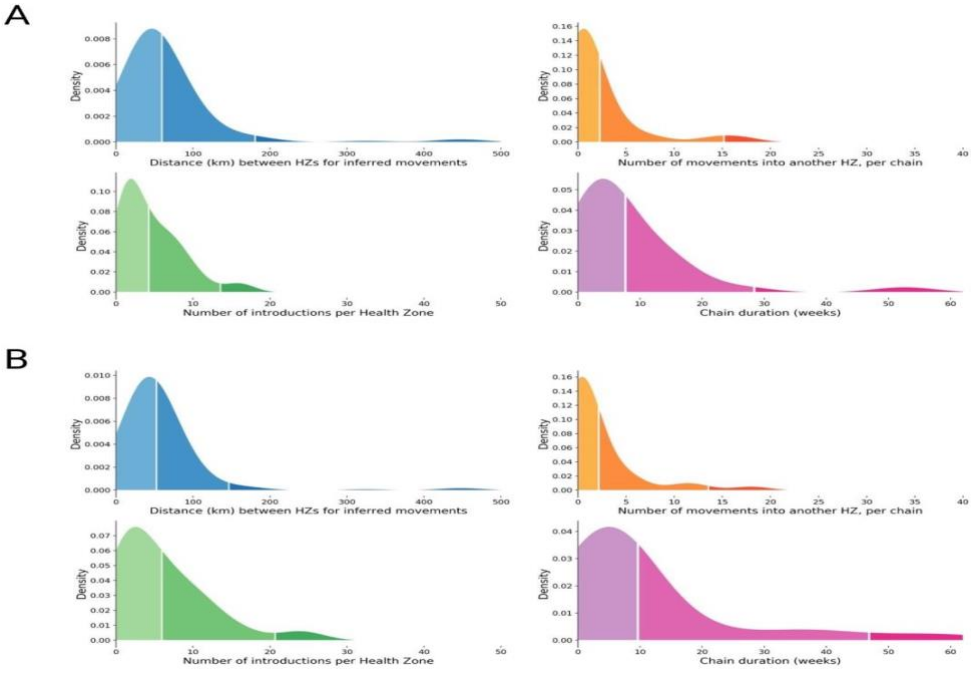

Figure 20 Inferred transmission dynamics are robust to sampling

a, Kernel density estimates for the same metrics presented in Fig. 3. This analysis used a dataset subsampled to include 3 genomes per health zone per month (total *n* = 323 genomes). **b**, Kernel density estimates for the same metrics presented in Fig. 3.This analysis used a dataset subsampled to include 5 genomes per health zone per month (total *n* = 433 genomes). Inferences from the subsampled datasets recapitulate the findings shown in Fig. 3, suggesting that phylogeographic inferences are robust tosampling frame

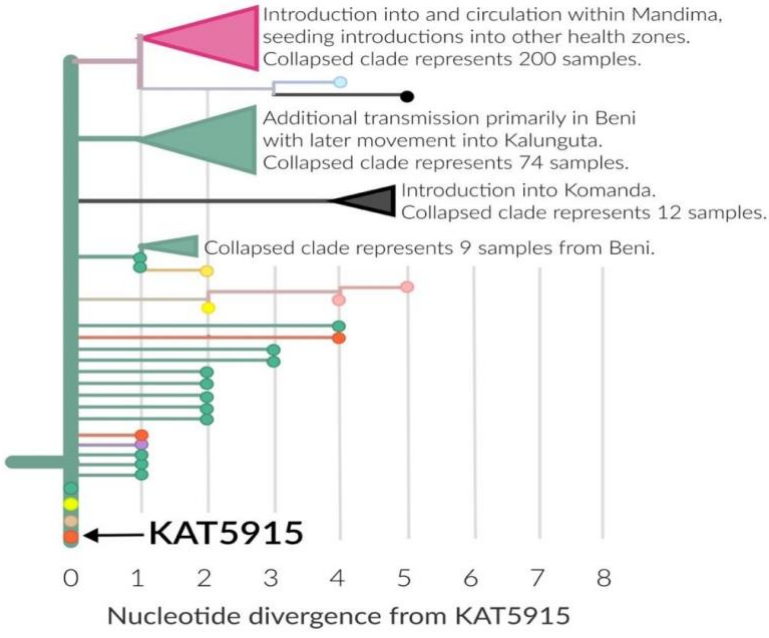

Figure 21 Genomic characterization of transmission after unsafe burial of a pastor
The horizontal axis represents nucleotide substitutions relative to the EBOV genome sequence from the pastor (KAT5915, orange). Three other samples had identical genome sequences to KAT5915. One case was from Oicha (light brown), one casewas from Ariwara (neon yellow), and another was from Beni (green). Additional cases diverged by only one nucleotide were detected in Beni (green), Butembo (orange), and Kalunguta (purple)

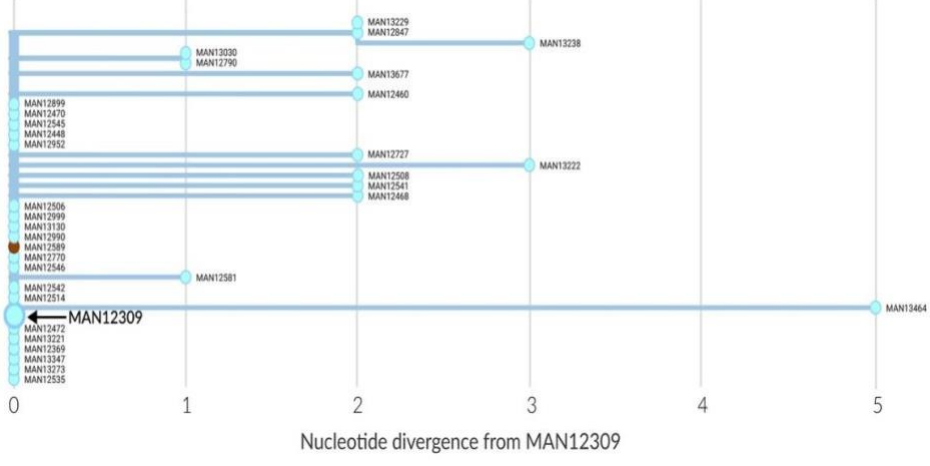

Figure 22 Secondary transmission associated with infection of a motorcycle taxi driver

The horizontal axis represents nucleotide substitutions relative to the EBOV genome sequence from the infected motorcycle taxi driver (MAN12309). Twenty other samples had identical genome sequences, as indicated in the figure by their positionat 0 nucleotides diverged. Distance along the y-axis has no meaning, and only servesto separate samples for visualization. Additional sequenced cases in Mabalako weremore genetically diverged from MAN12309, indicating additional propagated transmission following this event.

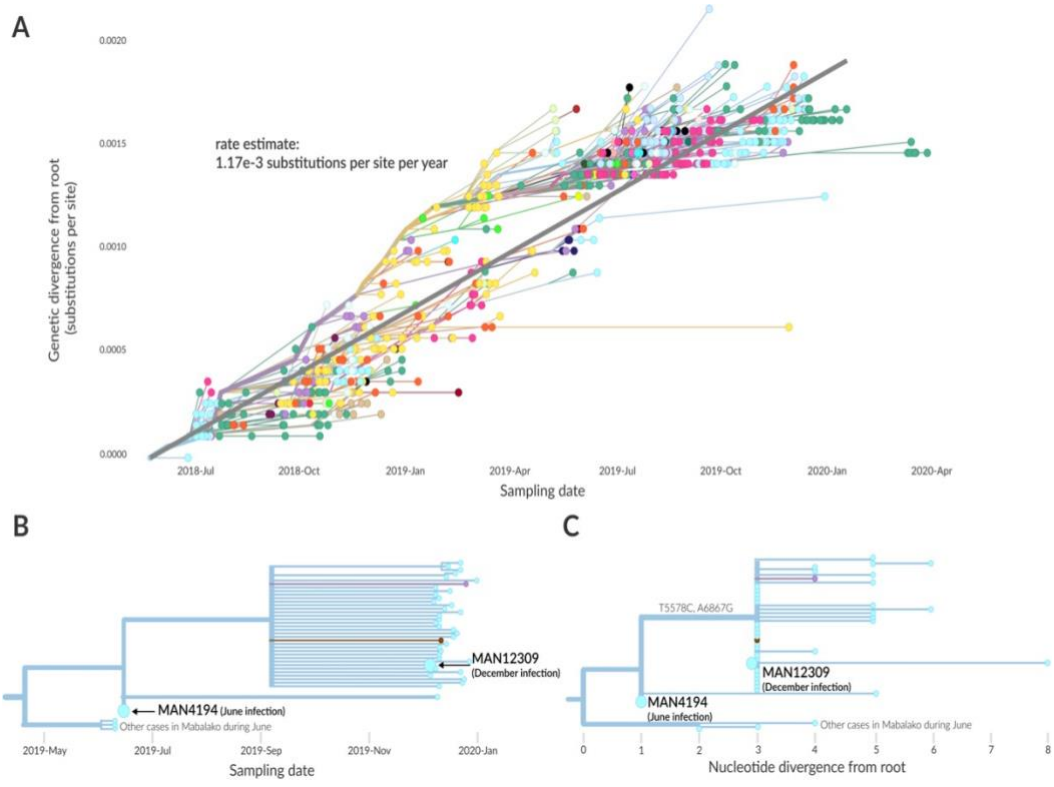

Figure 23 Initial genomic evidence for an infection relapse event

a, Root-to-tip plot showing genetic divergence of all 792 genomes as a function of their sampling date. The regression line indicates the average substitution rate acrossthis outbreak (1.17×10^{-3} substitutions per site per year, as annotated).

b, Temporally resolved phylogenetic tree showing patient's June sample (MAN4194) and December sample (MAN12309). **c**, Phylogenetic tree showing nucleotide divergence from the root of this clade. The June infection (MAN4194) and Decemberinfection (MAN12309) are diverged by only two substitutions, T5587C and A6867

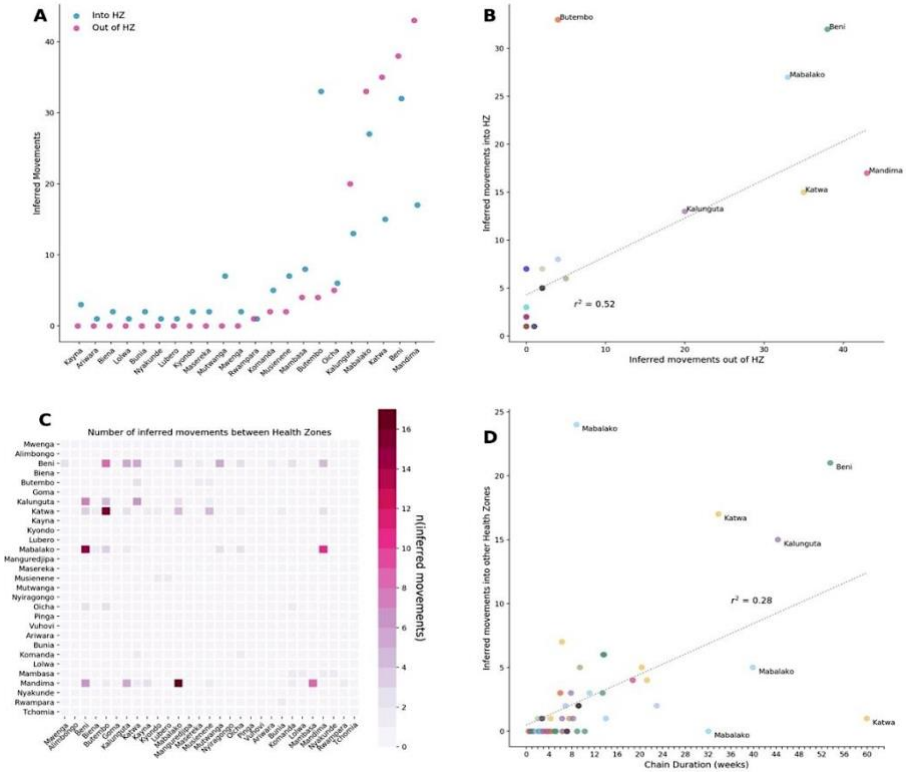

Figure 24 Patterns of transmission between health zones

a,**b**, The number of introductions of EVD into a health zone positively correlates with the number of exportations out of a health zone (r^2 = 0.48, p < 0.001), with most movement events occurring into and out of the same 5 health zones (Mabalako, Kalunguta, Katwa, Beni, and Mandima). State reconstructions that are less than 80% certain are excluded. **c**, Heatmap showing the frequency of lineage migration betweenall pairs of affected health zones. A migration event is counted only if the phylogeographic reconstruction for both the source and the sink health zones is at least 80% certain. **d**, The duration of time that a lineage circulated within a health zoneis weakly correlated with the number of introduction events that a lineage seeded intoother health zones (r^2 = 0.21, p < 0.003)

Case study 1: Using genomic surveillance to guide vaccine allocation by detecting superspreading.

Following development and testing during the West African EVD epidemic, both rVSV-ZEBOV-GP (10) and Ad26-ZEBOV/MVA-BN-FILO (11) vaccines were available for use during the Nord Kivu outbreak. However, given the limited supply, vaccination efforts primarily focused on contacts and contacts-of-contacts of confirmed positive cases, with preemptive vaccination only available to healthcare and frontline public health workers.

We monitored the genomic data for evidence of other settings or occupations that could be associated with high amounts of secondary transmission. Consistent with previous EVD outbreaks, the data suggested that infections in clergy could contributenumerous secondary infections. For example, KAT5915 was a pastor who died of EVD in Beni. His body was transported from Beni to Butembo for burial, and the funeral, which did not follow EVD safe burial protocols (12), was widely attended. Exposure at the funeral led to additional cases in Beni, Butembo, Ariwara, and Oicha (Figure 21). Three of these cases had identical viral genome sequences to KAT5915, while another 5 cases had sequences that differed from KAT5915 by only one nucleotide (Figure 21). In total, 320 sequenced infections descended from this founder event.

The genomic data also suggested that secondary cases could be linked to infected motorcycle taxi drivers. For example, MAN12309 worked as a motorcycle taxi driver,including while symptomatic with EVD in December 2019. Contact tracers sought toidentify exposed clients, and diagnostic specimens from clients who developed EVD were sent for sequencing. Twenty of the driver's contacts had identical EBOV genome sequences to him, indicating that the driver was the likely source of their infection (Figure 22).

In response to these findings, the vaccination policy was expanded to recommend preemptive vaccination for clergy and motorcycle taxi drivers in addition to healthcareand public health workers.

Case study 2: Differentiating between reinfection and relapse of a previous EVDinfection.

In December 2019, a male patient presented at a local health clinic with symptoms of EVD infection. In June 2019 he had been infected with EVD and sought treatment atan Ebola Treatment Unit in Mangina where he recovered 14 days later.

When he tested positive for EVD again in December, his diagnostic specimen was sent for sequencing. Genomic analysis indicated that his December infection was geneticallymore similar to viral lineages that had circulated in Mabalako during June 2019 than it was to viral lineages circulating in Mabalako in December 2019.

This finding prompted sequencing of his original June 2019 diagnostic specimen (Figure 20, annotated on the tree as MAN4194). We detected only two nucleotide differences between the driver's June and December samples (Figure 23), fewer substitutions than one would expect if that viral lineage had circulated in the community for 6 months.

The genomic data thus support a scenario in which the patient relapsed after recovering from his initial EVD infection, rather than having been re-infected with a different EBOV strain circulating in Mabalako in December 2019.

Differentiating between these two scenarios was a key question as the patient had been vaccinated against EVD and had also received experimental monoclonal antibody treatment during his June 2019 infection. Determining whether he had relapsed or had been reinfected was important for regulators seeking to understand which intervention might require further investigation. A full case report of this patient's infections is discussed elsewhere (13).

Discussion

In response to the ongoing Ebola outbreak in Nord Kivu, Democratic Republic of theCongo, we implemented an end-to-end genomic surveillance system. This system included viral whole genome sequencing, bioinformatic analysis, and dissemination of genomic epidemiologic results to frontline public health workers. We used the genomic surveillance data to broadly describe epidemic dynamics. Our findings suggest that the frequent movement of viral lineages between health zones sustainedthe epidemic, with only a small number of lineages circulating locally within a health zone over longer periods of time. While such large-scale descriptive inferences provide important context during outbreaks, frontline public health workers also needspecific, actionable pieces of information in close to real-time. To meet this need, wealso explored fine-scale transmission dynamics of the outbreak, monitoring forsuperspreading events and differentiating between relapse and reinfection events.

We began developing sequencing capability at INRB towards the end of the 2018 Equateur EVD outbreak. Our original intention was to develop the infrastructure andworkforce to conduct genomic surveillance at INRB over time. However, the start of the Nord Kivu outbreak in August 2018 necessitated a faster ramp up than we had originally intended. While the end-to-end system performed well generally, we encountered various challenges that impacted how quickly we could receive and sequence samples and thus how actionable the inferences were.

For example, sequencing capacity was initially only available in Kinshasa, roughly 2,600 km from Nord Kivu. This meant that prior to sequencing, diagnostic specimenshad to be transported from 11 regional diagnostic labs across various health zones to Beni, then from Beni to Goma (~240km), and then finally to Kinshasa (~2400km). Arranging specimen transport was complicated. Initially all commercial airlines flying between Goma and Kinshasa refused to carry EBOV-positive specimens. While specimen transport flights were later arranged by the World Health Organization, transport times contributed to large lags between sample collection and sequence availability. This issue was partially mitigated by adding sequencing

capacity at the Katwa diagnostic laboratory, starting in February 2019.

While the sequencing lab in Katwa improved turnaround times between sample collection and sequencing, various infrastructural, logistical, and funding challenges continued to impact the speed and consistency with which we could generate sequence data. In Katwa, equipment such as gloveboxes for RNA extraction were shared between diagnostic and sequencing teams, with diagnostic teams given priority. This meant that sequencing could only proceed when diagnostic assays werecomplete. The high level of conflict in the region further exacerbated these delays bylimiting the number of people allowed access to the lab and the amount of time they could spend there. At baseline, the Katwa sequencing lab could not have more thantwo scientists working at a time. During periods of heightened violence, such as whenthe Katwa Ebola Treatment Unit located next to the lab was destroyed by arson, access to the building was completely banned. Other times, access to the Katwa labwas only permitted with armed escorts, and only for two hours at a time, which provided insufficient time to complete steps of sequencing protocols between safe stopping points. Beyond the direct experience in Katwa, these security challenges also meant that supporting scientists were unable to travel to the outbreak area, andhad to provide technical support from a distance. These virtual connections were severely hampered during major internet outages, such as the 3-week long shutoff that occurred during the federal election in January 2019.

Finally, while funding was provided to pay for the laboratory staff and space, there was no consistent funding source for purchasing reagents. When reagents could be purchased, they were almost entirely hand-carried into the DRC by visiting international and returning Congolese scientists, as traditional shipping mechanismsusually led to delays in Customs, during which reagents thawed and degraded. Inconsistency in the supply of sequencing reagents contributed to periods where wecould not conduct sequencing despite having access to samples.

Beyond addressing these physical and logistical challenges, we believe that

genomicsurveillance will be more efficient and useful if it is fully integrated with traditional epidemiologic response efforts. We found that insufficient staff, limited time, and the inability to travel easily to the frontline impeded communication between scientists conducting genomic surveillance and epidemiologists coordinating response efforts.This is unfortunate, as drawing inferences from multiple data sources can provide greater confidence in inferred epidemiologic dynamics and pinpoint weaknesses or erroneous findings across data streams. Integrated genomic and epidemiologic responses would also have allowed us to quantitatively evaluate how frequently genomic and surveillance epidemiological inferences aligned. A weakness of our study is that without that integration we were unable to conduct this type of evaluation. Notably, evaluating genomic surveillance systems will be critically important for ensuring that expensive investments yield sufficient benefits, especially in low resource settings. To support integrated surveillance systems, we will need unified databases that provide all public health responders with access to well-linked epidemiologic information, laboratory information, and genomic data for cases. We also believe the system will work best if genomic and traditional epidemiologists collaborate closely in real-time during outbreak response.

An additional consideration when performing genomic surveillance for outbreak response is how sampling could impact phylogeographic inference. Ideally, sampledsequences should represent the full genetic diversity of the circulating pathogen. Thisidealized sampling frame is often not achievable with convenience sampling during outbreaks. Therefore, as genomic surveillance becomes more common, the field would benefit from additional simulation-based work exploring how genomic epidemiologic interpretations may change as a function of sampling. Finally, phylogenetic inferences may change with the addition of more sequence data. This does not necessarily mean that the inferred dynamics are wrong; rather, one can think of the phylogeny as incomplete due to lack of data. Increasing genomic surveillance capacity such that even higher proportions of cases are sequenced will go far in alleviating these limitations. In the meantime, genomic epidemiologists should be careful to accurately convey the meaning of the data, as well as

sources of uncertainty, to surveillance epidemiologists who may be less familiar with interpretingphylogenetic trees.

Our work during the 2018-2020 EVD outbreak in Nord Kivu shows how far genomic surveillance for outbreak response has progressed. At the time, the 2013-2016 WestAfrica EVD epidemic was notable for its high density of sequenced cases, representing ~5% of reported EVD cases (2). The vast majority of those sequences were generated by external scientists who came to West Africa, and very little sequencing capacity was left behind once the outbreak was declared over. Althoughthe Nord Kivu outbreak was smaller, we sequenced close to 24% of confirmed EVD cases, with all sequencing, and now most bioinformatic analysis, occurring within the DRC. The value of building capacity within-country is demonstrated not only by our work here, but also by the sustainability of a system that can be shifted to other surveillance efforts as well. Indeed, using this same genomic surveillance system we are now providing much needed epidemiologic support for understanding SARS- CoV-2 epidemiology in the DRC.

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Methods

Ethics statement

Diagnostic specimens were collected as part of the DRC Ministry of Health public health emergency response; therefore, consent for sample collection was waived. Allpreparation of samples for sequencing, genomic analysis, and data analysis were performed on anonymized samples identifiable only by their laboratory or epidemiological identifier. Institutional review boards at both the United States Army Medical Research Institute of Infectious Diseases and University of Nebraska Medical Center determined that the generation of sequencing data for public health responsedid not constitute research.

Sequence data generation

As described previously (8), clinical diagnostic specimens were collected from individuals presenting with EVD-like symptoms. Specimens were tested for the presence of EBOV RNA using the GeneXpert Ebola Assay (Cepheid, Sunnyvale, CA,USA). We sequenced a subset of all EBOV-positive samples; generally, samples were sequenced if they represented an epidemiologically important case or if the casehad an unusual contact history. Once samples were selected for sequencing, samples were sent to either the field genomics laboratory in Katwa or to INRB in Kinshasa. Samples were handled in a glovebox and RNA was extracted from the diagnostic specimen using the Viral RNA Mini kit (Qiagen, Hilden, Germany). Samples were processed for sequencing using a hybrid capture method as describedpreviously (8) or with an amplicon-based method (14). For hybrid capture sequencing,we used the KAPA RNA HyperPrep library preparation kit (KAPA Biosystems,Wilmington, MA, USA) with a spike-in of 20 ng HeLa RNA (Thermo Fisher, USA) andxGen Dual Index UMI Adapters (Integrated DNA Technologies, IA, USA). The libraries were enriched for EBOV using biotinylated probes (Twist Biosciences, USA)with the TruSeq Exome Enrichment kit (Illumina, San Diego, USA). For amplicon sequencing, the ThermoFisher 1st strand synthesis system was used to reverse transcribe RNA to cDNA. We amplified overlapping EBOV-specific amplicons according to a primer scheme generated from PrimalSeq (14) using Q5 DNA High- Fidelity Mastermix (New England Biolabs, Ipswich,

MA) according to manufacturer'sspecifications (primers are in Supplementary Information Table 1). Amplicons were quantified with the Qubit dsDNA High Sensitivity assay on the Qubit 4.0 instrument (Life Technologies, Carlsbad, CA) and then diluted to <500 ng for input into library preparation. Sequencing libraries were prepared using the Illumina Nextera DNA Flexkit (Illumina, San Diego, CA) with IDT for Illumina Unique Dual indexes. Libraries from both methods were quantified by qPCR with the KAPA Universal Library Quantification kit or by Qubit with the dsDNA High Sensitivity assay, and run on an Illumina iSeq 100 or Miseq System for 2 x 150 cycles.

Bioinformatic and phylogenetic analysis

We used a custom bioinformatic pipeline to generate consensus genomes from the raw FASTQ-formatted sequencing output (8,15). De-identified metadata about the patient, diagnostic lab, and sequence quality were paired with the consensus genome. This additional data included the laboratory identifier of the sample, the epidemiologic identifier for the patient, the patient's symptom onset date, the sample collection date, health zone, province, lab that performed the diagnostic testing, the sequencing date, and the percent genome coverage of the sequence. Phylogenetic analysis of all consensus genomes was performed using Nextstrain (16), with updated builds occurring each time new sequences were released. Alignments were verified manually in Geneious (https://www.geneious.com/).

Our specific phylogenetic analysis pipeline utilises Augur version 6.3.0 (a componentof Nextstrain), which performs a multiple sequence alignment with MAFFT v7.402 (17), computes a maximum likelihood phylogeny using IQ-TREE v1.6.6 (18), and temporally resolves this phylogeny using TreeTime v0.7.2 (19). We infer the health zone at internal nodes in the tree using the discrete trait inference found in TreeTime.Resulting data are visualised using Auspice (a component of Nextstrain) which allowsinteractive exploration of the data.

Generating and deploying situation reports

Upon release and analysis of new sequence data, we examined the

phylogenies to determine where the new sequences clustered and to investigate epidemic dynamicsapparent in the genomic data. These situation reports were written in English and French, and were shared as PDFs that could be viewed offline and as interactive reports available from a password-protected instance of nextstrain.org. Situation reports released to frontline public health workers contained sensitive patient information which necessitated private sharing. However, to illustrate what these situation reports are like, we have provided five narratives originally shared during September and October 2019, with sensitive information redacted. Links to the online interactive versions of these narratives are available at https://nextstrain.org/community/narratives/blab/ebola-narrative-ms/.

Data Availability

All genomic surveillance data, including consensus genomes and de-identifiedmetadata, were released publicly over time at https://github.com/inrb-drc/ebola-nord-kivu. The exact datasets analyzed in this manuscript are available at https://github.com/blab/ebola-narrative-ms. Interactive phylogenies for the fulldataset and the subsampled datasets can also be explored on Nextstrain at https://nextstrain.org/community/blab/ebola-narrative-ms/full-build, https://nextstrain.org/community/blab/ebola-narrative-ms/subsampled/3, and https://nextstrain.org/community/blab/ebola-narrative-ms/subsampled/5.

Code Availability

All of the code for the analyses presented in this paper, including the analysis pipeline and code for generating figures, is available at https://github.com/blab/ebola- narrative-ms/. Nextstrain Augur and Auspice are open-source and all source code can be found at https://github.com/nextstrain/augur and https://github.com/nextstrain/auspice.

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Author contributions

E.K-.L., A.B., J.H., P.M.-K., C.B.P., M.R.W. and T.B. designed the study. E.K.-L., A.B., J.H., P.M.-K. and C.B.P. performed bioinformatic analysis and genomic epidemiologic interpretation of the data over the course of the outbreak and for this paper. D.B.M. and P.M.-K. communicated genomic analyses to frontline workers. C.B.P., B.W., M.R.W., G.P., N.D.P., E.D., M.G.P., K.G.A. and M.P. supported
sequencing throughout the outbreak by both training INRB scientists and providing reagents. A.A., M.M.D., B.W., N.B., B.N. and M.A. performed the sequencing for thisstudy. M.F., O.F., A.A.S., F.E.-A., M.-M.K., F.M.-M. and J.B. interfaced between the INRB and the frontline response. A.B., J.H., P.M.-K. and C.B.P. wrote the manuscript.G.P., E.D., A.A.S., M.P., M.R.W., S.A.-M., T.B. and J.-J.M.T. supervised this work.

Competing interests

The authors declare no competing interests.

Part 3: Evaluation of the performance of Ebola rapid diagnostic tests versus RT-PCR during outbreaks in eastern of the Democratic Republic of the Congo

Chapter 5

Clinical evaluation of QuickNavi™-Ebola in the 2018 outbreak of Ebola Virus Disease in the Democratic Republic of the Congo

Clinical Evaluation of QuickNavi™-Ebola in the 2018 Outbreak of Ebola VirusDisease in the Democratic Republic of the Congo

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Sheila Makiala[1,2], **Daniel Mukadi** [1,2], Anja De Weggheleire [3,4], Shino Muramatsu[5], Daisuke Kato[5], Koichi Inano[5], Fumio Gondaira[5], Masahiro Kajihara[6], Reiko Yoshida[6],Katendi Changula[7,8], Aaron Mweene[7,8], Placide Mbala-Kingebeni[1,2], Jean-Jacques Muyembe-Tamfum[1,2], Justin Masumu[1], Steve Ahuka[1,2] and Ayato Takada[6,7,8,9,*]

1) Institut National de Recherche Biomédicale, Avenue de la Démocratie, Kinshasa/Gombe-P.O.Box 1197, Kinshasa I, Democratic Republic of the Congo
2) Service de Microbiologie, Cliniques Universitaires de Kinshasa, Kinshasa,Democratic Republic of the Congo
3) Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium
4) Outbreak Research team, Institute of Tropical Medicine, Antwerp, Belgium
5) Denka Seiken Co., Ltd., 1359-1, Kagamida, Kigoshi, Gosen, Niigata 959-1695, Japan
6) Division of Global Epidemiology, Research Center for Zoonosis Control, HokkaidoUniversity, Kita-20, Nishi-10, Kita-ku, Sapporo 001-0020, Japan
7) School of Veterinary Medicine, the University of Zambia, Great East Road Campus, Lusaka, Zambia
8) Africa Center of Excellence for Infectious Diseases of Humans and Animals, the University of Zambia, Lusaka, Zambia
9) Global Station for Zoonosis Control, Global Institution for Collaborative Researchand Education, Hokkaido University, Kita-20, Nishi-10, Kita-ku, Sapporo 001- 0020, Japan

*****Correspondence: atakada@czc.hokudai.ac.jp; Tel.: +81-11-706-9502

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Abstract

The recent large outbreaks of Ebola virus disease (EVD) in West Africa and the Democratic Republic of the Congo (DRC) have highlighted the need for rapid diagnostic tests to control this disease. In this study, we clinically evaluated a previously developed immunochromatography-based kit, QuickNavi^{TM}-Ebola. During the 2018 outbreaks in DRC, 928 blood samples from EVD-suspected cases were tested with QuickNavi^{TM}-Ebola and the WHO-approved GeneXpert. The sensitivity and specificity of QuickNavi^{TM}-Ebola, estimated by comparing it to GeneXpert-confirmed cases, were 85% (68/80) and 99.8% (846/848), respectively. These results indicate the practical reliability of QuickNavi^{TM}-Ebola for point-of-care diagnosis of EVD.

Keywords: Ebola virus; EVD; rapid diagnostic test; immunochromatography; QuickNavi; DRC

1. Introduction

Ebola virus (EBOV) is known to cause severe hemorrhagic fever in humans and/or nonhuman primates with human case fatality rates of up to 90% [1]. Five distinct ebolavirus species are known: Zaire ebolavirus (i.e., EBOV), Sudan ebolavirus, Taï Forest ebolavirus, Bundibugyo ebolavirus, and Reston ebolavirus. Ebola virus disease(EVD) poses a significant public health threat as shown by the largest EVD epidemic during the years 2013–2016 in West Africa. The second largest EVD outbreak is currently ongoing in the Democratic Republic of the Congo (DRC) where a cumulativetotal of 2181 confirmed and probable EVD cases and 1459 deaths have been reportedsince the beginning of the outbreak (as of 17 June 2019) [2]. These large-scale outbreaks emphasize the need for sensitive, easy-to-use, and robust rapid diagnostictests (RDT) to enable quick and reliable screening of suspected EVD cases at the point-of-care. By facilitating early detection, RDTs can contribute to controlling the spread of the virus, especially in a context with known resistance to centralized EVD care.

The WHO-approved GeneXpert® (Cepheid) technology, which is currently used in thefield to diagnose EVD, is a major step forward compared to the conventional reverse transcription-PCR (RT-PCR) methods used previously in terms of turnaround time, ease-of-use and performance. However, this method still requires trained technicians and an uninterrupted power supply and is therefore considered a ‘near’ point-of-care test. Immunochromatography (IC)-based RDT assays for EVD could be a complementary first-line screening strategy at decentralized ‘points-of-care’ in the community.

We developed an immunochromatography-based (IC-based) RDT for EVD, QuickNavi™-Ebola, using mouse monoclonal antibodies (mAbs) specific to the ebolavirus nucleoprotein (NP) [3]. This kit can detect ebolaviruses in the species Zaireebolavirus, Bundibugyo ebolavirus, and Taï Forest ebolavirus with equally high sensitivity, but cannot differentiate these viruses. Since 2016, QuickNavi™-Ebola hasbeen continuously provided to the Institut National de Recherche Biomédicale (INRB), DRC, and experimentally used for early

diagnosis of suspected EVD cases. It is particularly noted that QuickNavi^{TM}-Ebola supported the diagnosis of initial EVD casesconfirmed by INRB in May and August in the 2018 outbreaks in the DRC [4] (Table 15).

Currently, there are four RDTs approved by the FDA and/or WHO, ReEBOV
(Corgenix), OraQuick Ebola (OraSure Technologies, Inc., Bethlehem, PA, USA), SD Q Line Ebola (SD Biosensor, Inc., Suwon, Korea), and DPP Ebola Antigen (Chembio,Medford, OR, USA) [5,6]. Of these, only OraQuick Ebola, which is based on the detection of the EBOV matrix protein, was available and used in the 2018 DRC outbreaks [4,7]. During the outbreak, QuickNavi^{TM}-Ebola has also been experimentallyused to assist screening of EVD-suspected patients at the outbreak sites. The RDT was used alongside confirmatory molecular testing, but its results were not used for clinical decision making. We report in this paper on the diagnostic performance of theQuickNavi^{TM}-Ebola based on field data from the North-Kivu/Ituri EVD outbreak.

2. Materials and Methods

2.1. Devices

IC-based QuickNavi^{TM}-Ebola devices were produced by using mouse mAbs ZNP105- 7 and ZNP108-2-5 specific to the ebolavirus NP as described previously [3] (Figure 28). Samples (10–30 µL) were applied onto the sample pad of the device, followed bythe addition of 2 drops (approximately 40 µL) of the sample buffer supplied together with the devices. Results were interpreted 10–15 min later. Stability tests for QuickNavi^{TM} were performed according to a standard procedure in Denka Seiken Co.,Ltd. The test kit devices were stored in a storage room kept at 33 ◦C. Using virus-likeparticles consisting of the viral glycoprotein (GP), viral protein 40 (VP40), and NP, performance data were periodically obtained at the specified time and the sensitivity and specificity in the experiment condition were monitored up to 24 months.

2.2. Realtime PCR

RealStar Filovirus Screen RT-PCR Kit (Altona Diagnostics, Hamburg, Germany), LightMix® Ebola Zaire rRT-PCR Test (Roche Diagnostics, Basel, Switzerland), and GeneXpert® (Cepheid, Sunnyvale, CA, USA) were performed according to the procedures provided by the manufacturer.

2.3. Sample Collection and Detection of the Virus

Blood samples obtained by venous blood draw were collected from suspected EVD patients at Ebola Treatment Centers or health facilities in North-Kivu province (Mangina and Beni) and Ituri province (Tchomia, Bunia, and Komanda) in the DRC andsubjected to rapid screening with QuickNavi^{TM}-Ebola followed by testing with RT-PCR- based detection of the viral RNA genome (i.e., GeneXpert approved by WHO). Both tests were performed in INRB-managed field laboratories linked to the above-mentioned health facilities. Most of the samples were analyzed within 5 h after sample correction. The technicians applied strict biosafety measures and adheredto the manufacturers' instructions. Blood collection and clinical evaluation during the outbreak investigations were approved as standard care by the Ministry of Health of the DRC and oral consent was obtained from all patients before blood sampling.

3. Results and Discussion

During the outbreak caused by an ebolavirus (Zaire ebolavirus) in 2018–2019 [8], 928whole blood samples collected from suspected EVD cases were tested with QuickNavi^{TM}-Ebola and the WHO-approved GeneXpert. Whenever QuickNavi^{TM} testswere available, they were used in a systematic manner on the first blood sample (andrepeat if symptomatic less than 72 h at first blood draw) of each new EVD-suspect case. QuickNavi^{TM} testing was always performed and read before GeneXpert testing.The definition of positive and negative cases is as follows. Negative: GP not detected/NP not detected, GP not detected/CT of NP ≥ 40, or GP detected/NP not detected (considered negative or vaccinated case depending on vaccination history). Positive: GP detected/NP detected (CT < 40) or GP not detected/NP detected (CT < 40).

The sensitivity and specificity of the QuickNavi™-Ebola assay were estimated by comparing its results to the GeneXpert-results and confidence intervals (CI) based onthe F-distribution approximation were calculated (Table 16). Of the 80 GeneXpert-positive samples, 68 samples were positive with QuickNavi™-Ebola, which representsa sensitivity of 85% (95% CI; 75.26–92.00). Most (10 of 12) were GeneXpert-positive,but QuickNavi™-Ebola-negative samples had high Ct-values (30 <) both in NP and glycoprotein (GP) gene targets, and half of these samples were negative for detectionof the GP gene (Table 17). Indeed, the distribution of Ct-values for NP gene detectionindicated that the median, interquartile range, and mean of QuickNavi™-Ebola-positive samples were remarkably lower than those of QuickNavi™-Ebola-negative samples (Table 18). The QuickNavi™-Ebola-negative samples that showed low CT values might result from a prozone effect or aggregation of too many antibody–antigen complexes which might restrict a flow on the membrane. Importantly, most of the GeneXpert-negative samples (846/848) were also negative with QuickNavi™-Ebola, giving a specificity of 99.8% (95% CI; 99.15–99.97).

Table 15 Diagnosis of initial Ebola virus disease (EVD) cases in the two 2018 outbreaks in the Democratic Republic of the Congo (DRC)

Sample ID	Outbreaks (Month)	QuickNavi™-Ebola	Real-Time PCR
18M-1	1st (May)	Negative	Negative†
18M-2	1st (May)	Positive	Positive (23.6/28.7)†
18M-3	1st (May)	Negative	Negative†
18M-4	1st (May)	Negative	Negative†
18M-5	1st (May)	Negative	Negative†
18M-6	1st (May)	Positive	Positive (22.3/22.3)†
18A-1	2nd (August)	Negative	Positive/Negative (39.6/NA)‡§
18A-2	2nd (August)	Negative	Negative‡
18A-3	2nd (August)	Positive	Positive (21.6/25.0)‡
18A-4	2nd (August)	Positive	Positive (28.3/30.7)‡
18A-5	2nd (August)	Negative	Negative‡
18A-6	2nd (August)	Positive	Positive (20.1/23.7)‡

† RealStar Filovirus Screen RT-PCR Kit (Altona Diagnostics) and LightMix® Ebola Zaire rRT-PCR Test (Roche Diagnostics). CT values of positive samples are indicated in parentheses (Altona/Roche). ‡ GeneXpert (Cepheid) to detect NP and GP genes. CT values of positive samples are indicated in parentheses (NP/GP). § Positive for NP, but negative for GP. NA: not applicable.

Table 16 Performance of QuickNaviTM-Ebola at the outbreak sites

	GeneXpert Ebola		
	Positive	Negative	Total
QuickNavi™-Ebola Positive	68	2	70
QuickNavi™-Ebola Negative	12	846	858
Total	80	848	928

Sensitivity: 85% (68/80) (95% CI; 75.26–92.00); Specificity: 99.8% (846/848) (95% CI; 99.15–99.97); Negative predictive value: 98.6% (846/858); Positive predictive value: 97.1% (68/70); Agreement rate: 98.5% (914/928).

Table 17 Details of QuickNavi™-Ebola-negative samples in PCR-confirmed cases

Sample	QuickNavi™-Ebola	GeneXpert Ebola			
		NP	CT (NP)	GP	CT (GP)
1	Negative	Positive	38.7	Negative	NA†
2	Negative	Positive	39.9	Negative	NA
3	Negative	Positive	38.1	Positive	40.2
4	Negative	Positive	38.7	Negative	NA
5	Negative	Positive	26.1	Positive	31.6
6	Negative	Positive	34.2	Positive	42.3
7	Negative	Positive	36.0	Positive	41.1
8	Negative	Positive	33.6	Positive	37.9
9	Negative	Positive	38.0	Negative	NA
10	Negative	Positive	36.8	Negative	NA
11	Negative	Positive	37.7	Negative	NA
12	Negative	Positive	13.9	Positive	19.1

† NA: not applicable.

Table 18 Distribution Ct-values of QuickNavi™-Ebola positive and negative samples

Statistics	QuickNavi™-Ebola Positive (n = 68)	QuickNavi™-Ebola Negative (n = 12)
Minimum	14.20	13.90
25 percentile	17.98	34.05
Median	21.00	37.25
75 percentile	24.88	38.25
Maximum	35.60	39.90
Mean	21.76	34.31
Standard deviation	4.69	7.40

Considering that PCR-based assays generally show higher sensitivity than IC-based RDTs, it was quite reasonable that most samples false-negative with QuickNavi^{TM}- Ebola showed relatively high CT values in real-time PCR-based GeneXpert (Table 17).In experimental conditions, the limit of detection (LOD) of QuickNavi^{TM}-Ebola was 103–104 focus forming units/mL for infectious Ebola virus and 33 ng/mL for the purified recombinant EBOV NP [3]. According to the manufacturer, the LODs of FDA/WHO- approved OraQuick Ebola and ReEBOV were 1.64 × 106 TCID50/mL and 106 plaqueforming units/mL, respectively, and their sensitivities estimated with whole blood clinical samples were 84% (95% CI: 63.9–95.5) (OraQuick Ebola) and 78–96%(ReEBOV), respectively [5,9]. QuickNavi^{TM}-Ebola had 99.8% specificity in this study, whereas the reported specificities of OraQuick Ebola and ReEBOV were 98% and 73– 91%, respectively. Taken together, these data may implicate at least a comparable oreven better performance of QuickNavi^{TM}-Ebola than OraQuick Ebola and ReEBOV, as indicated by its lower LOD, similar sensitivity, and higher specificity, although this comparison should ideally made using the same set of clinical samples (e.g., head-to-head comparison on stored samples). It is also worth noting that our ongoing study suggests that QuickNavi^{TM}-Ebola has a shelf life of at least 24 months at room temperature.

Ebolavirus particles consist of seven structural proteins [1]. Of these, NP, VP 40, and GP are known to abundantly present in the viral particles. In general, viral envelope glycoproteins are antigenically variable and thus thought to be unsuitable for IC tests that require the capacity to widely detect virus variants. The four RDTs approved by the FDA and/or WHO (i.e., ReEBOV, OraQuick Ebola, SD Q Line Ebola, and DPP Ebola Antigen) have been designed to detect VP40 [5,6]. In contrast, we used NP- specific mAbs to produce QuickNavi^{TM}-Ebola. The EBOV particle contains approximately 3200 NP molecules which form large complexes of the nucleocapsid consisting of the helical nucleoprotein–RNA complex [10,11]. The NP antigen appearsto be one of the ideal targets for IC assays for EVD because of the presence of common epitopes among ebolavirus species, its strong antigenicity, and the large oligomeric structure of NP complexes providing multiple antibody binding sites,

whichmay enhance the sensitivity of the test [12]. The use of mAbs is also one of the advantages of QuickNavi^{TM}-Ebola since the antibodies can be stably produced and can avoid the use of live animals to produce polyclonal antisera. IC-based RDTs are currently used for various viral diseases such as influenza, Human adenovirus infection, and norovirus infection with significant reliability [13].

Currently available EVD RDTs cost around 10–20 US dollars/test, while some other commercialized RDTs based on the immunochromatography generally cost around 1–10 US dollars depending on their targeted diseases. Since the clinical manifestations of EVD are usually non-specific and similar to those of other infectious diseases present in EVD endemic areas, IC-based RDTs can be powerful tools for diagnosis even in remote areas in African countries. Since it is not predictable where and when EVD outbreaks will occur in the future, it is important to quickly discriminate between EVD and other viral diseases for early cases of suspected EVD, which may enable usto respond immediately to potential outbreaks, followed by initiating EVD-specific countermeasures once it is confirmed [14]. Given the simplicity of the procedure of QuickNavi^{TM}-Ebola, it would also be of benefit if repeated testing were done for patientswho were QuickNavi^{TM}-Ebola-negative in initial screening since increased plasma viralloads in such patients should ensure the detection in a few days.

The present study demonstrates that QuickNavi^{TM}-Ebola has good sensitivity and specificity in clinical field conditions. The results were similar to or tended to be even better than those obtained for other Ebola RDTs with WHO and/or FDA approval for emergency use. Owing to its significant practical utility, including simplicity, high stability, and the absence of requirements for special equipment and training, QuickNavi^{TM}-Ebola is expected to be a useful tool for point-of-care screening of EVD.

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Chapter 6

Postmortem Surveillance for Ebola Virus using OraQuick® Ebola Rapid diagnostic tests, Eastern Democratic Republic of the Congo, 2019- 2020

Postmortem Surveillance for Ebola Virus using OraQuick® Ebola Rapiddiagnostic tests, Eastern Democratic Republic of the Congo, 2019-2020

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Daniel Mukadi-Bamuleka[1], Yibayiri Osee Sanogo[1], Junior Bulabula-Penge[1], Maria E.Morales-Betoulle, Patrice Fillon, Patrick Woodruff, Mary J. Choi, Amy Whitesell, Alison M. Todres, Anja De Weggheleire, Anaïs Legand, Jean-Jacques Muyembe- Tamfum[2] Pierre Formenty, John D. Klena[2] Joel M. Montgomery[2], Steve Ahuka- Mundeke[2], RDT Working Group[3]

1. These authors contributed equally to this article
2. Senior co-authors
3. Members of the group who contributed data are listed at the end of this article

Author affiliations:

Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo (D. Mukadi-Bamuleka, J. Bulabula-Penge, J. Muyembe-Tamfum, S. Ahuka- Mundekea); Kinshasa Teaching School of Medicine, University of Kinshasa, Kinshasa (D. Mukadi-Bamuleka, J. Bulabula-Penge, J. Muyembe- Tamfum, S. Ahuka-Mundekea); US Centers for Disease Control and Prevention, Atlanta, Georgia, USA (Y.O. Sanogo, Maria E. Morales-Betoulle, M.J. Choi, A. Whitesell, A.M. Todres,
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Abstract

After a pilot study, we tested 443 cadavers using Ora-Quick Ebola rapid diagnostic tests during surveillance after the 10th Ebola outbreak in the Democratic Republic of the Congo. No false negative and 2% false-positive results were reported. Quickly returning results and engaging the community enabled timely public health actions.

1. Introduction

The 10th outbreak of Ebola virus (EBOV) disease (EVD) in North Kivu, Democratic Republic of the Congo (DRC), was the longest (August 1, 2018–July 25, 2020) and largest EVD outbreak in DRC; 2,287 persons died and 1,171 survived. A case of EVDrecrudescence (recorded June 15, 2019) resulted in 91 additional infections in 6 health
zones (1–3).

Challenges in controlling this EVD outbreak included security threats, widespread community mistrust in response activities, and low acceptance of systematic safe anddignified burials (SDBs). The difficulty with SDBs was in part because of long turnaround times (4 h to 72 h) of required quantitative reverse transcription PCR (RT-PCR) results for burial to bereaved families.

During the post epidemic period, enhanced surveillance of EVD is critical for controlling outbreaks because of potential flare-ups from undetected transmission chains or recrudescence in survivors (4–7). The objective of this study was to strengthen laboratory surveillance by quickly returning test results to families for timely public health interventions and to improve community engagement and acceptance of SDBs.After a pilot study conducted during active EVD transmission, we used OraQuick Ebola rapid diagnostic tests (RDTs; OraSure Technologies, Inc., https://www.orasure.com) to screen for EBOV infection in decedents within the community and in healthcare facilities during the post epidemic enhanced surveillance period using real-time field data reporting and molecular confirmation.

2. The Study

OraQuick Ebola is the first RDT licensed by the US Food and Drug Administration for EVD screening using blood or cadaver fluid samples (8). The US Centers for Disease Control and Prevention and the World Health Organization have recommended its usefor testing cadaver fluids of suspected EVD patients (9). Ethics approval and participant consent were not deemed necessary because specimens were collected for outbreak response and data were de-identified before analysis. A consortium of laboratory, epidemiology, communication, and community engagement professionals, led by the DRC

Ministry of Health, formed an RDT technical working group to coordinate field implementation, including SDBs, community engagement, and data collection. A steering committee composed of senior leaders from the Institut National de Recherche Biomédicale (INRB) and international partners advised the RDT Working Group.

The pilot study was conducted during active EBOV transmission (October 31–December 31, 2019) in Mambasa, Mandima, and Beni health zones. Trained healthcare workers conducted RDTs in communities and healthcare facilities. Data were collected manually. Samples were shipped to the INRB lab for confirmation by RT-PCR. SDBs were systematically performed on all cadavers regardless of RDT results. Some community reticence was encountered during the pilot study; violence led to change of location from Mambasa to Beni.

Of 196 cadavers tested by RDTs during the pilot study, 12 (6%) were reactive, of which 4 were negative by RT-PCR (2% false positive) (Table 19). Positive predictive value was 66% and negative predictive value 100% (no false negatives). Among confirmed cases, EBOV gene cycle thresholds ranged from 15.8 to 27.7 for nucleoprotein and 12.3 to 31.4 for glycoprotein. Lessons learned from the pilot study included the need for better community engagement, improved data collection and reporting, and more in-depth healthcare worker training.

Table 19 Summary results of OraQuick RDT* pilot study performed on cadaveroral fluid in Mambasa, Mandima, and Beni health zones during active transmission of Ebola virus, North Kivu, South Kivu, and Ituri provinces, Democratic Republic of the Congo

	PCR Results		
	Confirmed	Not confirmed	Total
Reactive	8	4	12
Nonreactive	0	182	182
Invalid	0	2	2
Total	8	188	196

*DRC, Democratic Republic of the Congo; RDT, rapid diagnostictest. (OraQuick, OraSure Technologies, Inc, https//www.orasure.com

After the pilot study, RDT post epidemic (August 1–October 31, 2020) surveillance wasconducted on cadavers in 19 health areas of the Beni health zone (Figure 25), the lasthealth zone affected during the out- break. RDTs were administered by 32 teams of locally trained healthcare workers, each composed of a laboratorian or nurse, a hygienist, a community engagement specialist, and a supervisor. The laboratorian/nurse collected 1 swab sample with the pad of the OraQuick device for the RDT and stored another swab sample in viral transport medium for quantitative RT-PCR confirmation. The hygienist oversaw biosafety practices and ensured that biologic waste (used RDT kits and personal protective equipment) was properly incinerated. A community engagement specialist communicated with the family, provided psychosocial support, and engaged the community using media and interactions with local leaders. The supervisor assumed responsibility for RDT qualitycontrol. Field teams were provided with the testing algorithm (Figure 26), a field training manual, and communication materials to assist with community engagement. SDB teams were on standby for safe burials as requested by families or if a sample was reactive/invalid.

Data were collected using tablets outfitted with a free, open-source, Kobo–based mobile data collection tool (https://www.humanitarian response.info/fr/ applications/kobotoolbox), developed for this purpose using a set of 75 questions in French. The data collection tool operated offline. RDT data, collection site geolocations, and photographs of RDT results were transmitted daily to the Kobo server when inter- net connection was available. A dashboard displaying key indicatorswas updated automatically twice a day. We used R software (10) to assess the diagnostic accuracy of the RDTs, using quantitative RT-PCR results as the standard.

After receiving permission from decedents' families, the laboratorian/nurse hygienist performed the test following instructions in the manual (S2). Results were read, interpreted, and photographed at 30 minutes, according to the manufacturer's instructions. If the RDT was nonreactive, families could proceed with traditional burial.If the RDT was reactive or invalid, the sample in viral transport medium, packaged in cooler boxes with ice packs, was transported

immediately to an INRB lab for confirmation by GeneXpert Ebola quantitative RT-PCR (Cepheid, https://www. cepheid.com), with result turnaround time under 24 hours. An RDT was considered invalid when, after 1 repeat, no line appeared in the C area of the test, a purple background obscured the results, or a partial line appeared in the C or T area after 30minutes.

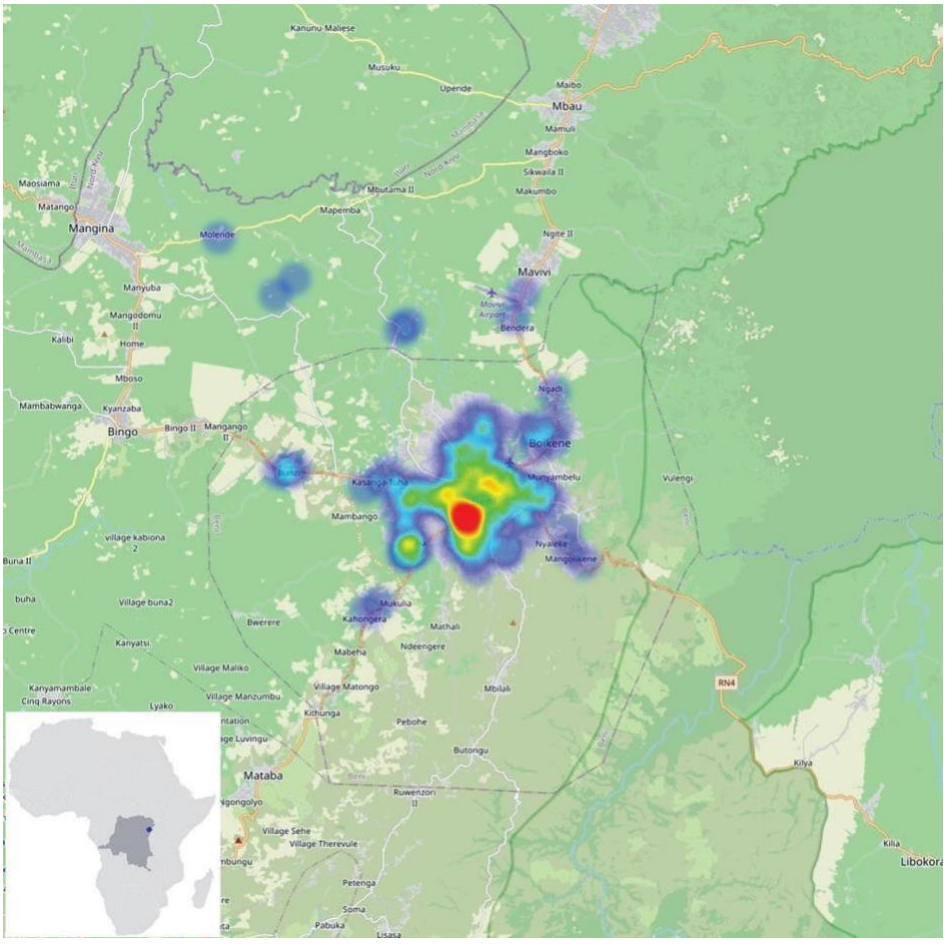

Figure 25 Beni Health zone with sites of Ebola virus disease sample collection for study on postmortem surveillance for Ebola virus using OraQuick (OraSure Technologies, Inc., https://www.orasure.com) Ebola rapid diagnostic tests, eastern Democratic Republic of the Congo, 2019–2020. The numbers and the geolocation rapid diagnostic testing are provided in heatmaps from blue (fewercases)to red (most cases). Most of the cases were from the health care facilitiesin Beni township. Inset shows location of the Beni Health zone in the Democratic Republic of the Congo and in Africa

During post epidemic surveillance, 443 cadavers were tested (3 cadavers were removed by families before RDTs were performed): 235 (53%) were from mortuaries,111 (25%) from the community, and 97 (22%) from hospitals. Swab specimens were

collected from 272 (61%) male and 171 (39%) female cadavers; 27% were children <5 years. Of the 443 samples, 425 (96%) had nonreactive RDTs, 11 (2%) were invalid, and 7 (2%) were reactive. Reactive, invalid and non- reactive samples testedby quantitative RT-PCR (363) were all negative, yielding 6 false-positive and no false-negative results (Table 20). One reactive RDT was not confirmed by quantitative RT-PCR. Although no EVD cases were confirmed among decedents, 32 SDBs were requested by families.

Table 20 Summary results of OraQuick RDTs* performed on cadaver oral fluids in the Beni health zone during the 90-days enhanced surveillance periodfollowing the 10th EVD outbreak in North Kivu, South Kivu, and Ituri provinces,DRC, 2019–2020†

	PCR results			
RDT results	Positive	Negative	Not done	RDT totals
Reactive	0	6	1	7
Non-reactive	0	348	77	425
Invalid	0	9	2	11
PCR Total	0	363	80	443

*RDTs †Fifteen RDTs performed to retest invalid andnonreactive initial RDTs are not included.

3. Conclusions

Trained local healthcare workers successfully used OraQuick Ebola RDTs for enhanced postmortem surveillance after the 10th EVD outbreak in DRC. Molecular testing revealed no false-negative RDT results, suggesting that quick public health actions can be based on RDT results alone. The low cycle thresholds observed in positive samples during the pilot study (Appendix Table, https://wwwnc.cdc.gov/ EID/article/28/2/21-0981-App1.pdf) support using RDTs in cadavers, in which viral loads are expected to be high (11–13). Our study shows that RDTs can detect EVD- related deaths and reduce the risk for community transmission. The utility of this tool in EVD surveillance is supported by recent observations that SDBs were not conducted during early stages of recent EVD resurgences in North Kivu and Guinea (CDC 2021 Ebola Response, unpub. data).

In conclusion, postmortem OraQuick Ebola RDTs effectively complemented outbreak- response efforts, improved community trust, and decreased the number of SDBs. However, the reported 2% false-positive tests required further confirmation and were not immediately actionable. SDBs requested by families despite nonreactive RDT further highlight the need for further community engagement.

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About the Authors

Dr. Sanogo is a microbiologist and epidemiologist in the Emergency Response and Recovery Branch (Global Rapid Response Team), Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention. His research interests encompass laboratory diagnostics of emerging pathogens, including vectorborne diseases, hemorrhagic fever viruses, next-generation sequencing, and strengthening laboratory capacity in resource-limited countries.

Dr. Mukadi is a physician and medical virologist at the Institut National de Recherche Biomédicale in Kinshasa, DRC. His expertise includes setup and coordination of field laboratories in response to Ebola outbreaks and evaluation of rapid diagnostic tests.

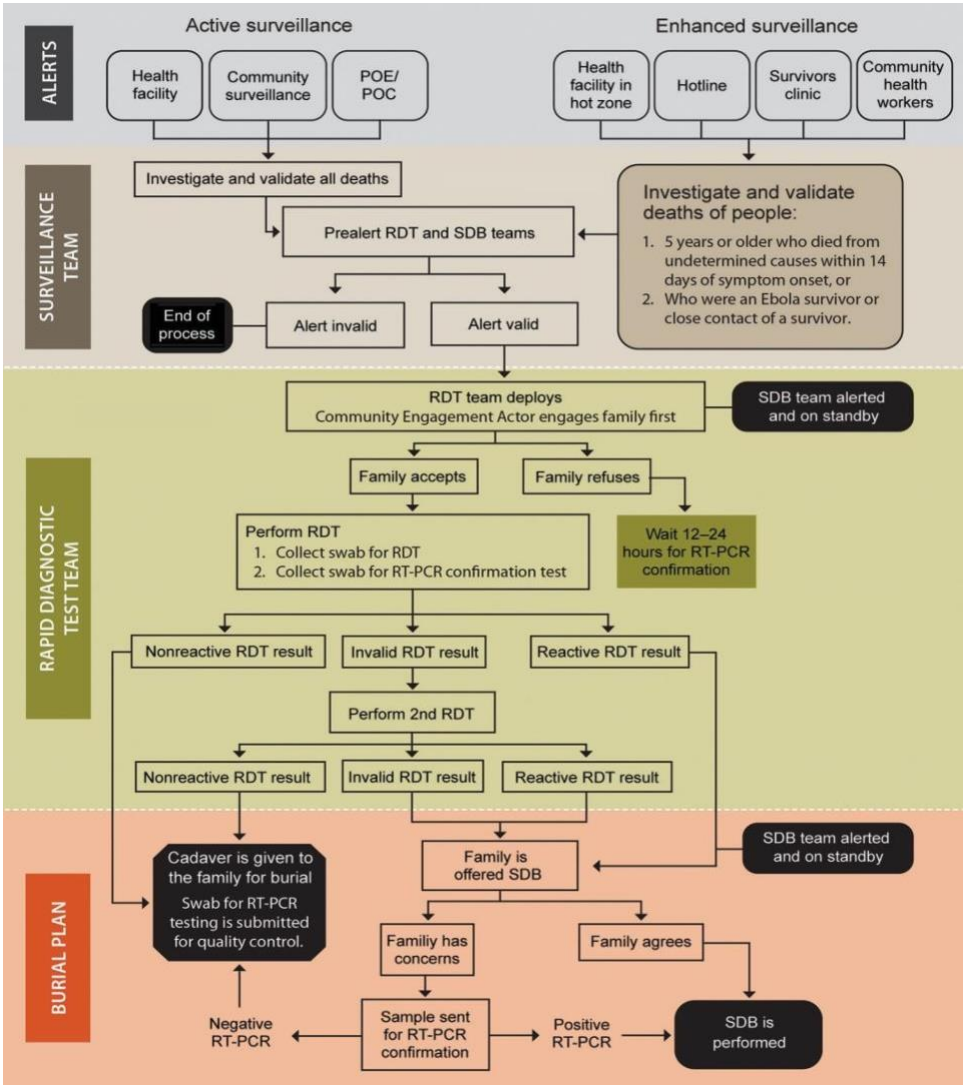

Figure 26 Algorithm of Ebola virus disease RDT implementation in North Kivu in the Beni health zone during active transmission (active surveillance) and postepidemic enhanced surveillance (enhanced surveillance), Democratic Republicof the Congo, 2019–2020.

This information was used to inform burial planning and SDBs when indicated. EVD, Ebola virus disease; RDT, rapid diagnostic test (OraQuick, OraSure Technologies, Inc., https://www.orasure.com); RT-PCR, reverse transcription PCR; SDBs, safe and dignified burials.

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Chapter 7

Field performance of three Ebola rapid diagnostic tests used during the 2018–20 outbreak in the eastern Democratic Republic of the Congo: a retrospective, multicentre observational study

Field performance of three Ebola rapid diagnostic tests used during the 2018–20 outbreak in the eastern Democratic Republic of the Congo: a retrospective,multicentre observational study

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Daniel Mukadi-Bamuleka, Junior Bulabula-Penge, Anja De Weggheleire, Bart K M Jacobs, François Edidi-Atani, Fabrice Mambu-Mbika, Placide Mbala-Kingebeni, Sheila Makiala-Mandanda, Martin Faye, Cheick T Diagne, Moussa M Diagne, Oumar Faye, Masahiro Kajihara, Ousmane Faye, Ayato Takada, Amadou A Sall, Jean-Jacques Muyembe-Tamfum, Johan van Griensven, Kevin K Ariën, Steve Ahuka-Mundeke

Department of Virology, Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of the Congo (D Mukadi-Bamuleka MD, J Bulabula-Penge MD, F Edidi-Atani MD, F Mambu-Mbika MD, P Mbala-Kingebeni PhD, S Makiala-Mandanda PhD, Prof J-J Muyembe-Tamfum PhD, Prof S Ahuka-Mundeke PhD); Service de Microbiologie, Département de Biologie Médicale, Cliniques Universitaires de Kinshasa, Université de Kinshasa, Kinshasa, Democratic Republic of the Congo (D Mukadi-Bamuleka, J Bulabula-Penge, F Edidi-Atani, F Mambu-Mbika, P Mbala- Kingebeni, S Makiala-Mandanda, Prof J-J Muyembe-Tamfum, Prof S Ahuka- Mundeke); Department of Clinical Sciences (A De Weggheleire MD, B K M Jacobs PhD, Prof J van Griensven PhD) and Department of Biomedical Sciences (Prof K K Ariën PhD), Institute of Tropical Medicine, Antwerp, Belgium; Institut Pasteur de Dakar,Dakar, Senegal (M Faye PhD, C T Diagne PhD, M M Diagne PhD, Oumar Faye PhD,Ousmane Faye PhD, A A Sall PhD); International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan (M Kajihara PhD, Prof A Takada PhD); Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium (Prof K K Ariën)

Summary

Background The Democratic Republic of the Congo has confronted 13 outbreaks of Ebola virus disease since 1976. Rapid diagnostic tests (RDTs) detecting viral antigens have been developed to circumvent difficulties encountered with RT-PCRfor diagnosis in remote low-resource settings, but there is still uncertainty about theirperformance characteristics and usability during outbreaks. We aimed to assess thefield performance of three antigen detection RDTs compared with the gold-standardCepheid GeneXpert Ebola assay results.

Methods We conducted a retrospective, multicentre observational study using complete and de-identified databases of five mobile laboratories (managed by the Institut National de Recherche Biomédicale) to assess the performance of three Ebola virus disease RDTs (QuickNavi-Ebola, OraQuick Ebola Rapid Antigen Test, and Coris EBOLA Ag K-SeT rapid test) run on blood samples of patients with suspected Ebola virus disease in direct comparison with the Cepheid GeneXpert Ebola assay reference test during the 2018–20 outbreak in the eastern DemocraticRepublic of the Congo. We estimated the sensitivity and specificity of each test through generalised linear mixed models against the GeneXpert Ebola assay reference test and corrected for cycle threshold value and random site effects.

Findings 719 (7·9%) of 9157 samples had a positive GeneXpert Ebola assay result.The QuickNavi-Ebola RDT had a sensitivity of 87·4% (95% CI 63·6–96·8) around the mean cycle threshold value and a specificity of 99·6% (99·3–99·8). The OraQuick Ebola Rapid Antigen Test had a sensitivity of 57·4% (95% CI 38·8–75·8)and specificity of 98·3% (97·5–99·0), and the Coris EBOLA Ag K-SeT rapid test hada sensitivity of 38·9% (23·0–63·6) against the GeneXpert Ebola assay reference and specificity of 97·4% (85·3–99·6). The QuickNavi-Ebola RDT showed a robust performance with good sensitivity, particularly with increasing viral loads (ie, low cycle threshold values), and specificity.

Interpretation The three RDTs evaluated did not achieve the desired sensitivity andspecificity of the WHO target product profile. Although the RDTs cannot triage and rule out Ebola virus infection among clinical suspects, they can still help to sort people with suspected Ebola virus disease into high-risk and low-risk groups while waiting for GeneXpert Ebola assay reference testing.

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1. Introduction

Outbreaks of Ebola virus disease are public health emergencies. The Democratic Republic of the Congo is the most affected country, with 13 outbreaks of Ebola virus disease reported since 1976, of which the past five occurred in rapid succession over the past 6 years (1). In humans, outbreaks have been caused by different species, including Sudan ebolavirus, Bundibugyo ebolavirus, and Zaire ebolavirus (also known as Ebola virus). Zaire ebolavirus has affected the widest geographical areas and caused the most outbreaks. Fatality is generally high, ranging from 25% to 90% in humans who do not receive treatment. However, the survival rate improves markedly with early supportive and specific treatment (2, 3). Outbreaks of Ebola virus disease typically start with a zoonotic spillover to humans, after which the virus further spreads via human-to-human transmission, amplified by funeral rituals and poor infection control conditions in health facilities (2, 4).

Identification and control of outbreaks of Ebola virus disease, and also individual survival, relies on rapid and reliable diagnosis of patients with Ebola virus disease. As the clinical case definition is insufficiently accurate (5), laboratory confirmation of Ebola virus disease is an absolute requirement (6). Generally, laboratory confirmation is based on detection of the viral RNA with RT-PCR technology. In many African countries, including the Democratic Republic of the Congo, laboratory confirmation is done with the Cepheid GeneXpert Ebola assay, a rapid and fully automated quantitative RT-PCR for Ebola virus that targets the NP and GP genes (7-13). Although the assay combines high sensitivity and specificity, it also has several disadvantages, as it is costly and demanding in terms of laboratory infrastructure, training of staff, and electricity supply. These factors prevent wide-scale use of the assay in remote areas of countries such as the Democratic Republic of the Congo, because the conditions needed for use are often available only at the provincial level and rarely at lower levels of the health system. Rapid diagnostic tests (RDTs) could help to screen living and deceased people with clinically suspected Ebola virus disease either at the point of care or in the community, shorten the time to detection, and allow

quicker decisions for patient referrals or required precautions for burials, especially in context of community resistance (13, 14).

RDTs detecting viral antigens have been developed specifically to circumvent the logistical and organizational difficulties encountered with quantitative RT-PCR testing at the point of care and in low-resource settings. Different structural proteins of the various strains of Ebola virus are targeted in these tests, predominantly NP, VP40, GP, or a combination (14, 15). In 2014, WHO published a target product profile for point-of-care tests for Ebola virus disease, stipulating a desired clinical sensitivity of at least 98% and a specificity of at least 99% (16).

Several RDTs were evaluated towards the end of the 2013–16 outbreak of Ebola virus disease in west Africa. The OraQuick (Orasure Technologies), ReEBOV (Corgenix), SD Q Line Ebola (SD Biosensor), and the DPP Ebola Antigen System (Chembio Diagnostics) received emergency use authorisations from the US Food and Drug Administration (FDA) or WHO (14, 17, 18). However, questions exist regarding diagnostic performance and usability in operationally challenging outbreak contexts, because the decisions to authorize for emergency use were mostly based on small- scale evaluations (ie, few samples with generally high viral load) that were done in ideal laboratory conditions. Moreover, most previous studies included a range of different reference PCR tests and generally not the Cepheid GeneXpert Ebola assay platform, which is the current gold standard for diagnosis of Ebola virus disease. Two of these RDTs that received emergency authorization (ie, ReEBOV and SD Q Line Ebola) are no longer commercially available and none had a sensitivity of 98%. Several other tests have been developed since the end of the west African outbreak of Ebola virus disease (2013–16), aiming for increased sensitivity (14, 19-24).

From August, 2018, to June, 2020, the Democratic Republic of the Congo faced its tenth and, so far, largest outbreak of Ebola virus disease, with 3470 confirmed cases, in the eastern provinces of North Kivu, Ituri, and South Kivu (25).

In response to this outbreak, the Institut National de Recherche Biomédicale

(INRB), which is the National Reference Public Health Laboratory, progressively deployed 13 field laboratories for diagnosis of Ebola virus disease in the three affected provinces. As per WHO recommendation, the Cepheid GeneXpert RT-PCR system was used as the preferred diagnostic platform for Ebola virus disease (26).

During the outbreak, these mobile laboratories tested more than 250 000 samples for diagnostic and clinical follow-up purposes with the Cepheid GeneXpert Ebola assay. Several RDTs for Ebola virus disease were, when available, run in parallel with the Cepheid GeneXpert assay in some of these field laboratories. As data for their diagnostic accuracy were scarce, the RDT results were documented in the laboratory registers and databases but were not used for patient management.

The many clinical samples that were tested across a wide range of cycle threshold values, and simultaneously with the Cepheid GeneXpert Ebola assay and one or several RDTs, provide a unique opportunity to enhance the evidence base on the diagnostic accuracy of RDTs when used in field circumstances during an outbreak. We conducted a retrospective study to assess the field performance of three different antigen detection RDTs performed on blood samples of living people with suspected Ebola virus disease compared with the gold-standard, Cepheid GeneXpert Ebola assay results.

2. Methods

Study design and participants

We conducted a retrospective, multicentre study using deidentified databases from the INRB mobile laboratories, where samples from people with suspected Ebola virus disease were analysed by the Cepheid GeneXpert Ebola assay (Sunnyvale, CA, USA) and at least one of the following Ebola virus disease RDTs: QuickNavi- Ebola (Denka Seiken, Niigata, Japan), OraQuick Ebola Rapid Antigen Test Kit (OraSure Technologies, Bethlehem, PA, USA) and Coris Ebola Ag K-SeT (Coris BioConcept, Gembloux, Belgium). During the outbreak (ie, August, 2018, to June, 2020), RDTs were mainly used on diagnostic samples from people

with suspected Ebola virus disease, and rarely for follow-up testing once confirmed to be positive for Ebola virus disease. This study covers data for blood samples that were analysed in five field laboratories (in Beni, Bunia, Butembo, Katwa, and Mangina) between Aug 2, 2018, and Sept 30, 2019, corresponding to the period during which Ebola RDTs were available.

For our analysis, we used data related to living adults and children with suspected or confirmed Ebola virus disease for whom a venous blood sample was taken as a diagnostic or follow-up sample and who had a documented valid GeneXpert Ebola assay result and at least one RDT result from one of the five field laboratories. We excluded all data related to other sample types, such as samples obtained on swabs or of other body fluids. We also excluded data related to RDT results if a particular RDT had been performed 20 times or less at a site (ie, field laboratory) to avoid bias in the performance analysis due to inexperienced handlers.

The deidentified data used for this study were collected as part of the standard procedure of the response to the outbreak of Ebola virus disease in the eastern Democratic Republic of the Congo and were guided by the recommendations of the national multisectorial response coordination secretariat (Secrétariat Technique du Comité Multisectoriel de lutte contre la Maladie à Virus Ebola). Therefore, this study did not require specific ethical approval in the Democratic Republic of the Congo and did not present any risk for the participants, as databases were deidentified before analysis.

Procedures

As per the routine approach for living people with suspected Ebola virus disease, a blood sample for diagnostic testing was obtained by venous blood draw on arrival to the Ebola treatment unit (ETU), or in the community for the people refusing an immediate transfer to the ETU.

The EDTA (edetic acid)-treated blood samples were thereafter transported in temperature-monitored cool boxes (2–8°C) to the nearest INRB field laboratory, according to the standard operational procedures, as applied in the tenth outbreak of Ebola virus disease in the Democratic Republic of the Congo (27). However, in exceptional cases, samples obtained in the

community could not be transported according to this protocol because of emergency (ie, security) situations or a temporary absence of cold chain. The delay between obtaining the sample and reception at the laboratory depended on the distance and available transportation means. Samples from nearby ETUs arrived in the laboratory generally within 30 min to 2 h after they were obtained, whereas those from the community could take from several hours to 1 day to reach the laboratory, sometimes in suboptimal conditions.

On reception, the parcel was unpacked and sample tubes were decontaminated, identified, registered, and transferred into the glovebox. Routinely, all diagnostic samples were processed and submitted for analysis by the GeneXpert Ebola RT- PCR. Whenever Ebola virus disease RDTs were available, they were run in parallel with the reference GeneXpert Ebola assay. Visual interpretation and documentation of RDT results was done before results of the GeneXpert Ebola assay were available, as the processing time of RDTs was shorter. Only qualified laboratory staff from either INRB or local level were involved in sample manipulations. To be appointed into INRB field laboratories, all personnel had to complete a general training on biosafety, biosecurity, obtaining samples, packaging, transportation, and storage. Additionally, specific training was provided for donning and doffing techniques for personal protective equipment, Cepheid GeneXpert Ebola assay and RDT procedures, bench work in the gloveboxes, and other biosafety standard operating procedures. Bench aids for each test, in French, were available in each field laboratory. Only the result of the GeneXpert Ebola assay was communicated back to the response teams. The GeneXpert Ebola assay result of a diagnostic sample was considered to be positive if NP cycle threshold was less than 40 cycles and considered to be negative if NP was not detected or NP cycle threshold was at least 40 cycles. All invalid results on the GeneXpert Ebola assay were rerun with the same sample. In case of failure, a second sample was obtained, when feasible, by the surveillance or outreach team, the ETU, or the safe and dignified burial teams. Result interpretation for the RDTs was performed at the recommended time to read results according to the

manufacturer's instructions. The RDT result was considered to be positive if visible lines appeared both in the test and control reading window and considered to be negative if a line was visible only in the control window. Samples with invalid results (ie, no visible control line) were not included in our analysis.

Repeat diagnostic testing was done within 48–72 h after the start of symptoms whenever the first GeneXpert Ebola assay result was classified as negative and the patient was less than 72 h symptomatic on initial diagnostic testing. Repeat diagnostic testing followed the same procedures as initial testing, with GeneXpert Ebola assay as the reference test and an Ebola virus disease RDT run in parallel whenever available. Besides diagnostic testing, RDTs were used in some rare occasions on follow-up samples of patients with confirmed Ebola virus disease.

During the study period, three Ebola virus disease RDTs were used on a sufficiently large sample set (ie, there was a clear gap between widely used tests and rarely used tests, and given the prevalence of Ebola virus disease at the time of testing, few covariates, and scarcity of existing estimates of diagnostic accuracy, we considered that more than 500 samples was sufficient).

All three RDTs were antigen-detection lateral flow immunoassays, and characteristics are listed in the appendix.

In addition to diagnostic testing for Ebola virus disease, blood samples of people with suspected Ebola virus disease were also, whenever tests were available, submitted to malaria RDT testing (SD Bioline Malaria Ag P.f/Pan from Standard Diagnostics, Gyeonggi-do, South Korea).

For data capture, each laboratory held a Microsoft Excel 2016 sample line list, which was filled with data from the standard suspect-case notification form and the laboratory test results. For this study, DM-B, JB-P, and ADW retrieved data in June– July, 2020, for the following variables: age, gender, date and location of sample analysis, duration of symptoms, type of sampling (ie, initial diagnostic sample, repeat diagnostic sample, or follow-up sample), and laboratory results (ie, GeneXpert Ebola assay, Ebola RDT, malaria

RDT). Information on vaccination status was not available in the laboratory databases.

The data in the laboratory databases were routinely collected as part of the response to the Democratic Republic of the Congo's tenth outbreak of Ebola virus disease under the supervision and guidance of the Secrétariat Technique du Comité Multisectoriel de lutte contre la Maladie à Virus Ebola.

Samples that had more than one RDT result were included in the results and analyses for each individual test. Additionally, we explored the results of the 807 samples that were tested with at least two RDTs.

Statistical analysis

Data were analysed with Stata/IC 15 and R version 4.0. Basic characteristics of participants and samples were summarized with descriptive analyses. The diagnostic performance of the RDTs was assessed with the GeneXpert Ebola RT- PCR as the reference standard.

Sensitivity was defined as the probability that patients with a positive GeneXpert Ebola assay result had a positive RDT result. Specificity was defined as the probability that patients with a negative GeneXpert Ebola assay result had a negative RDT result. Sensitivity and specificity were estimated through two independent generalized (ie, logistic) linear mixed models by use of the lme4 package in R. We modelled the binary RDT outcome (ie, positive or negative) as a function of test type, random site effect for Beni, Bunia, Butembo, Katwa, or Mangina, and cycle threshold value (ie, proxying for viral load). Inclusion of interactions and random slopes were tested at the 5% significance level. 95% Cis were constructed by use of the profile likelihood when possible and with the asymptotic Wald approximation otherwise. Equality of sensitivity and specificity of different RDTs was tested with Tukey-style pairwise comparisons by use of the multcomp package in R. Positive and negative predictive values were estimated through the reverse model, adjusted for random site effect.

Role of the funding source

There was no funding source for this study.

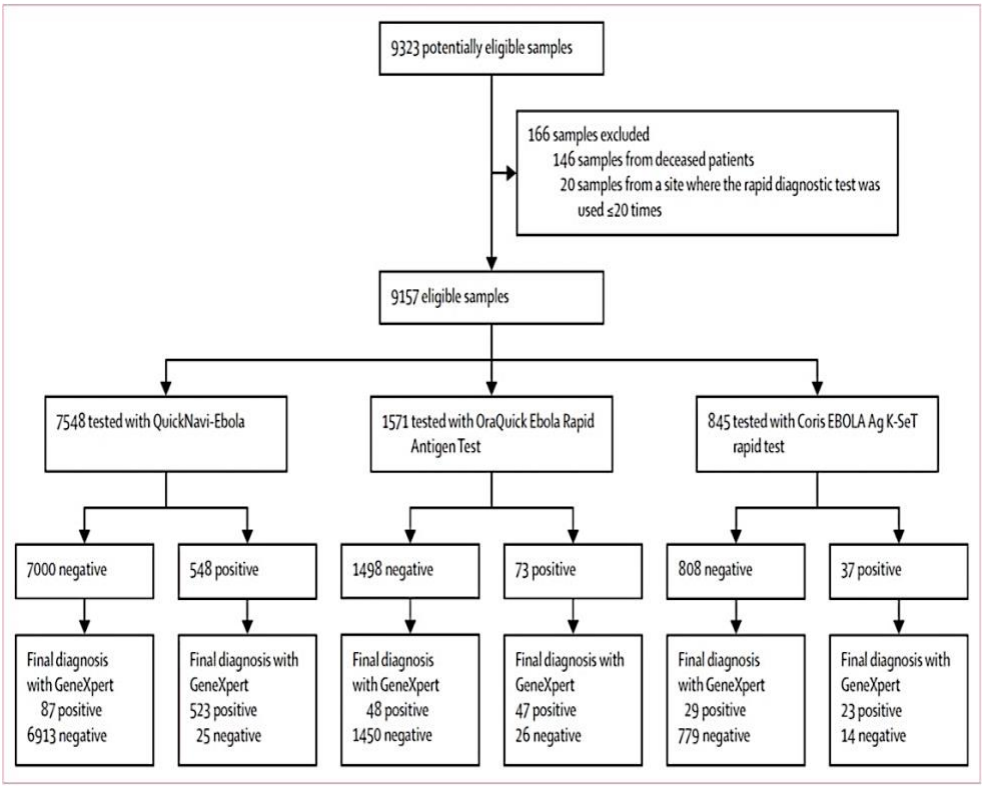

Figure 27 Study profile

3. Results

Data for 9157 venous blood samples with a valid GeneXpert Ebola assay and at least one RDT result were available for analysis (Figure 27). These samples originated from 8805 living patients and were distributed as follows: 8489 patients with one diagnostic sample, 323 patients with two diagnostic samples (ie, initial negative result on GeneXpert Ebola assay and a repeat sample taken 48–72 h later), and a small group of 13 patients with an initial positive GeneXpert Ebola assay and at least one follow-up sample during Ebola virus disease care (table 21). Most data originated from the Beni field laboratory, followed by Mangina, Butembo, and Katwa (table 21).

The gender and age distribution of the patients was similar across sites and in line with the overall suspect case population in the ETUs. Diagnostic samples were obtained at a median of 4 days (IQR 3–7) after symptom onset. The mean NP cycle threshold was 24·1. The QuickNavi-Ebola and Coris EBOLA Ag K-SeT rapid tests were mostly used in the Beni laboratory, whereas the OraQuick Ebola Rapid Antigen Test was mostly used in Mangina and Butembo.

The sensitivity and specificity were adjusted for cycle threshold value and site effect (table 22). Taking the GeneXpert Ebola assay result as reference, the QuickNavi- Ebola RDT misclassified 87 of 610 Ebola virus disease-positive samples as false negative and 25 of 6938 Ebola virus disease-negative samples as false positive. After adjusting for cycle threshold value and site effect, the QuickNavi-Ebola RDT had a sensitivity of 87·4% (95% CI 63·6–96·8) and a specificity of 99·6% (99·3– 99·8). The negative predictive value was 98·8% (95% CI 98·5–99·0) and, after adjusting for site effect, 97·8% (95·3–99·0), whereas the positive predictive value was 95·4% (93·2–97·0) and 97·8% (95·0–99·1) after adjusting for site effect.

The OraQuick Ebola Rapid Antigen Test correctly detected 47 of 95 GeneXpert Ebola assay positive samples for a sensitivity of 57·4% (95% CI 38·8–75·8) after adjusting for cycle threshold value and site effect, and the Coris EBOLA Ag K-SeT rapid test correctly detected 23 of 52 GeneXpert Ebola assay positive samples for a sensitivity of 38·9% (23·0–63·6). The specificity was similar for both tests, with 98·3% (95% CI 97·5–99·0) for the

OraQuick Ebola Rapid Antigen Test and 97·4% (85·3–99·6) for the Coris EBOLA Ag K-SeT rapid test (table 22).

The OraQuick Ebola Rapid Antigen Test has a positive predictive value of 65·9% (95% CI 42·6–83·5) and negative predictive value of 97·1% (95·2–98·2) in our dataset after adjusting for site, whereas the Coris EBOLA Ag K-SeT rapid test has a positive predictive value of 45·4% (15·5–79·0) and negative predictive value of 96·4% (92·4–98·3). The appendix 2 shows the positive predictive value and negative predictive value as a function of the prevalence of Ebola virus disease in the tested population assuming a similar distribution of viral load among true positives, but without adjustment for site effect.

Table 21 Characteristics of samples by rapid diagnostic test

	Samples with at least one rapid diagnostic test result (n=9157)	QuickNavi-Ebola test (n=7548)	OraQuick Ebola Rapid Antigen Test (n=1571)	Coris EBOLA Ag K-SeT rapid test (n=845)	QuickNavi-Ebola and Coris EBOLA Ag K-SeT rapid test (n=378)	QuickNavi-Ebola and OraQuick Ebola Rapid Antigen Test (n=407)
Type of sampling						
Initial	8805 (96·2%)	7332 (97·1%)	1455 (92·6%)	819 (96·9%)	376 (99·5%)	403 (99·0%)
Repeat	323 (3·5%)	200 (2·6%)	104 (6·6%)	25 (3·0%)	2 (0·5%)	4 (1·0%)
Follow-up	29 (0·3%)	16 (0·2%)	12 (0·8%)	1 (0·1%)	0	0
Median duration of symptoms,*† days (IQR)	4 (3-7)	4 (3-7)	5 (3-7)	4 (3-6)	4 (3-6)	4 (3-7)
GeneXpert result						
Negative	8438 (92·1%)	6938 (91·9%)	1476 (94·0%)	793 (93·8%)	366 (96·8%)	387 (95·1%)
Positive	719 (7·9%)	610 (8·1%)	95 (6·0%)	52 (6·2%)	12 (3·2%)	20 (4·9%)
Median *NP* cycle threshold range of positive samples (IQR)	22·6 (19·3-27·8)	22·5 (19·1-27·6)	24·2 (20·9-29·4)	21·9 (18·9-25·7)	20·1 (18·1-23·3)	22·5 (18·6-28·2)
***NP* cycle threshold range categories**						
<15	13/719 (1·8%)	12/610 (2·0%)	1/95 (1·1%)	1/52 (1·9%)	0/12	1/20 (5·0%)
15-20	199/719 (27·7%)	181/610 (29·7%)	13/95 (13·7%)	16/52 (30·8%)	6/12 (50·0%)	5/20 (25·0%)
20-25	231/719 (32·1%)	191/610 (31·3%)	35/95 (36·8%)	19/52 (36·5%)	5/12 (41·7%)	6/20 (30·0%)
25-30	146/719 (20·3%)	120/610 (19·7%)	25/95 (26·3%)	10/52 (19·2%)	0/12	7/20 (35·0%)
30-35	71/719 (9·9%)	56/610 (9·2%)	13/95 (13·7%)	5/52 (9·6%)	1/12 (8·3%)	1/20 (5·0%)
>35	59/719 (8·2%)	50/610 (8·2%)	8/95 (8·4%)	1/52 (1·9%)	0/12	0/20
Malaria rapid diagnostic test result‡						
Negative	4647/5967 (77·9%)	3990/5235 (76·2%)	587/783 (75·0%)	613/739 (82·9%)	273/366 (74·6%)	251/403 (62·3%)
Positive	1320/5967 (22·1%)	1245/5235 (23·8%)	196/783 (25·0%)	126/739 (17·1%)	93/366 (25·4%)	152/403 (37·7%)

Data are n (%), unless otherwise indicated. Some samples had more than one rapid diagnostic test result and have been included more than once in this table. The 22 samples tested with Coris Ebola Ag K-SeT rapid test and OraQuick Ebola Rapid Antigen Test are not included here. *Excluding follow-up samples. †Data were missing for 781 (8·5%) of 9157 samples. ‡Data were missing for 3190 (34·8%) of 9157 samples.

Table 1: Characteristics of samples by rapid diagnostic test

Table 22 Rapid diagnostic test performance compared with GeneXpert Ebola assay as reference standards

	True positive	False positive	True negative	False negative	Sensitivity,* % (95% CI)	Specificity, % (95% CI)	Positivity, %	Positive predictive value, %	Negative predictive value, %
QuickNavi-Ebola	523	25	6913	87	87·4% (63·6–96·8)	99·6% (99·3–99·8)	7·3%	97·8%	97·8%
OraQuick Ebola Rapid Antigen Test	47	26	1450	48	57·4% (38·8–75·8)	98·3% (97·5–99·0)	4·6%	65·9%	97·1%
Coris EBOLA Ag K-SeT rapid test	23	14	779	29	38·9% (23·0–63·6)	97·4% (85·3–99·6)	4·4%	45·4%	96·4%

All diagnostic accuracy measures were adjusted for site effect. *At the mean cycle threshold value in the data, which is 24·1 cycles.

Table 3: **Rapid diagnostic test performance compared with GeneXpert Ebola assay as reference standard**

Table 23 Distribution of patients, samples, and RDTs by field laboratory

	Beni (6231 samples; 6023 patients)	Mangina (1071 samples; 960 patients)	Butembo (853 samples; 843 patients)	Katwa (717 samples; 707 patients	Bunia (285 samples; 272 patients)
Gender*					
Female	3065/5869 (52·2%)	498/960 (51·9%)	440/837 (52·6%)	345/706 (48·9%)	134/270 (49·6%)
Male	2804/5869 (47·8%)	462/960 (48·1%)	397/837 (47·4%)	361/706 (51·1%)	136/270 (50·4%)
Age†					
<1 year	20/5672 (0·4%)	25/956 (2·6%)	40/838 (4·8%)	21/698 (3·0%)	6/263 (2·3%)
1–5 years	655/5672 (11·5%)	99/956 (10·4%)	92/838 (11·0%)	37/698 (5·3%)	32/263 (12·2%)
5–15 years	1265/5672 (22·3%)	205/956 (21·4%)	182/838 (21·7%)	116/698 (16·6%)	56/263 (21·3%)
15–60 years	3500/5672 (61·7%)	589/956 (61·6%)	498/838 (59·4%)	484/698 (69·3%)	157/263 (59·7%)
>60 years	232/5672 (4·1%)	38/956 (4·0%)	26/838 (3·1%)	40/698 (5·7%)	12/263 (4·6%)
Dates of RDT use	August, 2018, to July, 2019	September, 2018, to September, 2019	April–July, 2019	January–July, 2019	November, 2018, to August, 2019
Samples on which RDTs were performed, n‡					
QuickNavi	6050	950	253	243	52
Oraquick	..	510	558	270	233
Coris	534	33	54	224	..
QuickNavi and Coris	353	18	..	7	..
QuickNavi and Oraquick	..	404	1	2	..
Coris and Oraquick	..	..	11	11	..

Data are n (%), unless otherwise indicated. Data for gender and age are the number of patients. RDT=rapid diagnostic test. *Data were missing for 163 (1·9%) of 8805 patients. †Data were missing for 378 (4·3%) of 8805 patients. ‡Includes samples that were tested with more than one test.

Table 2: **Distribution of patients, samples, and RDTs by field laboratory**

The sensitivity of the QuickNavi-Ebola was significantly higher than that of the Coris EBOLA Ag K-SeT rapid test (p<0·0001), but no significant differences were found between the OraQuick Ebola Rapid Antigen Test and either of the other RDTs (QuickNavi-Ebola vs OraQuick Ebola Rapid Antigen Test, p=0·16; OraQuick Ebola Rapid Antigen Test vs Coris EBOLA Ag K-SeT rapid test, p=0·44). The specificity of QuickNavi-Ebola was significantly higher than the specificity of OraQuick Ebola Rapid Antigen Test (p<0·0001) and Coris EBOLA Ag K-SeT rapid test (p=0·023), whereas no significant difference in specificity was found between the OraQuick Ebola Rapid Antigen Test and the Coris EBOLA Ag K-SeT rapid test (p=0·85).

The sensitivity of all three RDTs declined significantly with decreasing Ebola viral load (ie, increasing NP cycle threshold; p<0·0001; appendix 2 p 2). This effect seemed to be consistent for the three RDTs (p=0·90) and was estimated to have an odds ratio of 0·77 (95% CI 0·73–0·80). Therefore, for each RDT, the odds of being positive was estimated to be 23% lower for each difference of one additional cycle in cycle threshold value (ie, half as much viral load in the sample). For samples with high viral load, the sensitivity was still quite low for the Coris EBOLA Ag K-SeT rapid test, exceeding 50% only for cycle threshold values of 22 and lower and 90% for cycle threshold values lower than 15 (Figure 28). For the OraQuick Ebola Rapid Antigen Test, a sensitivity of more than 50% was estimated for cycle threshold values of 25 and lower, whereas 90% was reached for cycle threshold values lower than 17. The QuickNavi- Ebola reached 90% at a cycle threshold value of 23. In samples with low viral load, the Coris EBOLA Ag K-SeT rapid test did not reach 10% sensitivity for cycle threshold values of 30 and higher. The OraQuick Ebola Rapid Antigen Test also missed most cases, with sensitivity dropping below 20% for cycle threshold values higher than 30, whereas the QuickNavi-Ebola still correctly identified more than 50% of cases for cycle threshold values of 31 and higher and exceeded 10% up to a cycle threshold value of 39. Outcomes of the 807 samples tested with two different RDTs were in line with these results (appendix 2).

Considerable variation was observed between the different testing sites with respect to sensitivity (p<0·0001). Additionally, this site effect was not

constant across the different RDTs (p<0·0001), even after adjusting for cycle threshold value. The highest between-site variation was identified for the QuickNavi-Ebola (SD 1·489 on the log-odds scale), followed by the Coris EBOLA Ag K-SeT rapid test (0·535) and the OraQuick Ebola Rapid Antigen Test (0·332). Some between-site variation, similarly not constant across RDTs, was observed for specificity (p=0·045). The largest variation existed for the Coris EBOLA Ag K-SeT rapid test (SD 1·316 on the log-odds scale). The OraQuick Ebola Rapid Antigen Test (0·074) and the QuickNavi-Ebola (0·088) showed negligible between-site variation with respect to their specificity.

Malaria RDT results were available for 5967 diagnostic samples (table 20). After adjusting for site effect, the specificity of the QuickNavi-Ebola RDT was 99·5% (95% CI 99·2–99·7) in malaria RDT negative samples and 99·8% (99·4–100·0) in malaria RDT positive samples. For the OraQuick Ebola Rapid Antigen Test, the specificity was 97·2% (95% CI 95·2–98·5) in malaria-negative samples versus 98·9% (96·7–100·0) in malaria-positive samples, and the specificity for Coris EBOLA Ag K-SeT rapid test was 97·4% (81·7–99·7) for malaria negative samples versus 92·1% (59·8–98·9) for malaria positives. Malaria infection did not significantly affect the specificity to detect Ebola virus disease with the three RDTs.

4. Discussion

Our study retrospectively assessed the diagnostic field performance of three Ebola antigen detection RDTs during the tenth Ebola virus disease outbreak in eastern Democratic Republic of the Congo compared with the Cepheid GeneXpert Ebola assay. Results for 9157 blood samples, including 719 samples that were positive for Ebola virus disease (NP cycle threshold range 12·9–39·9 cycles), were available for analysis. The most robust estimates were obtained for the QuickNavi-Ebola RDT (ie, with NP as the target) as this test was most frequently used (ie, for 7548 samples).

Overall sensitivity was 87·4% (95% CI 63·6–96·8) but varied between nearly 100% in samples with an NP cycle threshold value lower than 20 cycles and less than 50% in samples with an NP cycle threshold value of at least 31 cycles. Specificity was 99·6% (95% CI 99·3–99·8). These findings support the first field results that were reported for the QuickNavi-Ebola RDT by Makiala and colleagues in 2019 for a smaller set of samples, but also in an operational outbreak setting and with the same reference standard (28).

The OraQuick Ebola Rapid Antigen Test had a sensitivity of 57·4% (95% CI 38·8– 75·8), but varied between more than 80% for samples with an NP cycle threshold value of 20 or lower and less than 50% if the NP cycle threshold value was at least 25, and a specificity of 98·3% (97·5–99·0). The Coris EBOLA Ag K-SeT rapid test had a sensitivity of 38·9% (95% CI 23·0–63·6), ranging from more than 70% in samples with an NP cycle threshold value lower than 20 cycles and less than 10% in samples with an NP cycle threshold value of at least 30 cycles, and a specificity of 97·4% (85·3–99·6). Sensitivity sharply declined with decreasing Ebola viral load for all tests at a rate of 23% lower odds of being positive for a halved viral load (cycle threshold value 1 higher). The sensitivity for the OraQuick Ebola Rapid Antigen Test obtained in our study differs substantially from the 84·0% (95% CI 65·3–93·6) sensitivity reported in the FDA and WHO evaluation for emergency use reports (19, 29). These reported performance characteristics were based on a small-scale evaluation with stored samples from the 2013–16 outbreak of Ebola virus disease in west Africa. The lower cycle threshold range of the samples (ie, all lower than cycle threshold 34), the use of an

earlier generation reference standard that was possibly less sensitive (ie, VP40 RT-PCR method) than the Cepheid GeneXpert Ebola assay, and the more controlled laboratory working conditions in this post- outbreak evaluation than in our study could explain the difference. Similar arguments might also clarify the difference between our sensitivity estimate for the Coris EBOLA Ag K-SeT rapid test and the estimates obtained on stored samples by Colavita and colleagues (88·6%, 95% CI 81·1–93·3) (22). The poor performance of the OraQuick Ebola Rapid Antigen Test and the Coris EBOLA Ag K-SeT rapid test on samples with low Ebola viral load was also apparent in all earlier evaluations (22, 29). Notably, specificity did not seem to be affected by malaria co-infection for any of the three RDTs.

The challenging field conditions during the outbreak, and the fact that operators knew that only the GeneXpert Ebola assay result would be returned to the response teams, might have affected test performance and explain heterogeneity between sites. We observed considerable variation in sensitivity between the different sites for all RDTs (resulting in quite imprecise overall sensitivity estimates) and variation in specificity between sites for the Coris EBOLA Ag K-SeT rapid test. Obvious challenges were the high workload and pressure, which might have led to insufficient time for briefing and training on the specifics of each test and to errors in the test procedures and result reporting; completion of bench work in glove boxes, which might have hampered reading of faint test lines; ensuring the quality of the samples in terms of varying storage conditions and duration until arrival in the laboratory, especially when samples were taken in the community (although samples were taken in the community in few patients and only when instant referral to an ETU was refused); and possibly suboptimal field storage conditions of the RDTs. Although some of these challenges can be mitigated during future outbreaks, the working conditions will always be suboptimal in outbreak settings and diagnostic tools should be designed to be as robust and simple as possible to avoid a large gap between performance in controlled laboratory circumstances and the actual setting (ie, during outbreaks in remote areas where technical or qualified staff are scarce and optimal storage conditions and operating procedures

cannot always be guaranteed).

Rather than considering these possible shortcomings as a weakness of the study, we suggest that they reflect the actual conditions in which RDTs are used, to which diagnostic development should be tailored, and urge for careful interpretation of data from controlled laboratory settings and subsequent extrapolation to the real-world setting. The field conditions were similar for all three RDTs, but different robustness (eg, thermostability, proportion of faint lines, susceptibility to slight non-compliance in test procedures, such as number of buffer drops, reading timeframe, etc) cannot be excluded. Notably, different antigens are used in the QuickNavi- Ebola RDT (NP) and the OraQuick Ebola Rapid Antigen Test (VP40) and Coris EBOLA Ag K-SeT rapid test (VP40).

Whether this difference has any role in the observed higher sensitivity of the QuickNavi-Ebola RDT than of the OraQuick Ebola Rapid Antigen Test and Coris EBOLA Ag K-SeT rapid test requires further study.

After adjusting for cycle threshold value and random site effect, we identified that the QuickNavi-Ebola had a significantly higher sensitivity than did the Coris EBOLA Ag K-SeT rapid test. No such difference was shown between the OraQuick Ebola Rapid Antigen Test and either of the other tests due to the small sample size and large variation between sites. Additionally, the specificity of the QuickNavi-Ebola was significantly higher than of both other RDTs. Although in a small sample size, the two sets of samples (n=407 for QuickNavi-Ebola vs OraQuick Ebola Rapid Antigen Test and n=378 for QuickNavi-Ebola vs Coris EBOLA Ag K-SeT; appendix 2) that were tested by two RDTs in addition to the GeneXpert Ebola assay, by the same operator, provide further substantiation for the reliability of the overall study outcomes in favour of the QuickNavi-Ebola RDT.

In summary, none of the RDTs achieved the desired levels of both sensitivity and specificity as set out in the WHO target product profile. The QuickNavi-Ebola RDT performed better than did the OraQuick Ebola Rapid Antigen Test and Coris EBOLA Ag K-SeT rapid test both on positive and negative samples. The samples that were incorrectly identified as negative by the RDTs were mainly samples with low Ebola viral load (ie, from people

presenting early or late in the course of disease). However, we noted that some samples with high viral load were misclassified as negative. Whether high concentrations of the targeted antigen can result in false negative results in any of these tests (ie, as a result of the hook effect) should be further investigated (30).

Ideally, RDTs would be used to triage and rule out Ebola virus disease (requiring high sensitivity but potentially accepting a lower specificity) among people with clinically suspected Ebola virus disease at the peripheral care level. None of the evaluated RDTs fulfilled these criteria in our study, However, the available RDTs could help to sort people with a high-risk of suspected Ebola virus disease from people with a low-risk of suspected Ebola virus disease while waiting for confirmatory GeneXpert Ebola assay reference testing at the triage site. The QuickNavi-Ebola RDT achieved the WHO-desired clinical specificity of at least 99%. In patients with a high clinical suspicion of Ebola virus disease, a positive test could be sufficient to reliably make a diagnosis of Ebola virus disease. In the context of low prevalence of Ebola virus disease in the community (eg, at the end of an outbreak or after community vaccination) the high negative predictive value could support the use of RDTs for triaging, under the assumption that sensitivity is the same in low prevalence settings, requiring confirmatory testing with GeneXpert Ebola assay. Use for triaging could entail the development of algorithms, including clinical and epidemiological information. Finally, our study showed that the use of RDTs during an Ebola virus disease outbreak is feasible in low-resource settings.

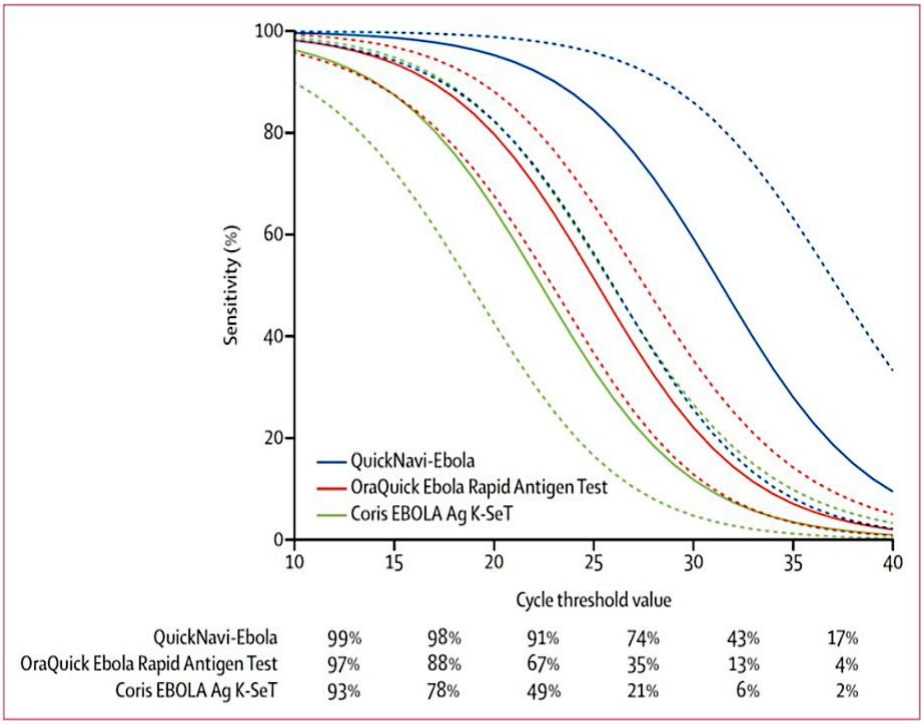

Figure 28 Sensitivity of the rapid diagnostic tests as a function of GeneXpert cyclethreshold value (Dashed lines indicate 95% CIs)

Contributors

DM-B, JB-P, ADW, JvG, KKA, and SA-M conceived the study and methods and wrote the original draft. DM-B, JB-L, FE-A, FM-M, MMD, MF, OumF, and OusF performed investigation and data curation. DM-B, ADW, JB-P, CTD, and BKMJ performed formal data analysis. BKMJ, PM-K, SM-M, CTD, MMD, MF, OumF, OusF, and AAS edited the manuscript. DM-B, ADW, BKMJ, MF, CTD, MK, AT, and J-JMT visualised the study documents. JvG, KKA, and SA-M validated the study documents and the manuscript. DM-B, JB-P, and ADW accessed and verified all of the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

WHO donated GeneXpert cartridges and laboratory supplies used during the tenth outbreak of Ebola virus disease in the Democratic Republic of the Congo. WHO, Africa Centers for Disease Control and Prevention, Institute of Tropical Medicine Antwerp, and Japan International Cooperation Agency donated GeneXpert instruments for response purposes. US National Institute of Allergy and Infectious Diseases/National Institutes of Health provided GeneXpert instruments and laboratory supplies in the context of the PALM randomised controlled trial during the tenth outbreak of Ebola virus disease. Centers for Disease Control and Prevention– Atlanta donated OraQuick Ebola Rapid Antigen tests for the response.

Denka and Hokkaido University, Sapporo, Japan, provided QuickNavi-Ebola rapid tests via the Japan International Cooperation Agency.

Coris Bioconcept supplied Coris K SeT Ag tests via Institut Pasteur de Dakar. DM- B is a Clinical Research During Outbreaks fellow supported by the Belgian Directorate-general for Development Cooperation and Humanitarian Aid. All other authors declare no competing interests.

Data sharing

The data statement is shared separately in the appendix 2 (data sharing agreement will be available 3 months after official paper release).

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Chapter 8

Head-to-head comparison of diagnostic accuracy of four Ebola rapid diagnostic tests versus GeneXpert® in eastern Democratic Republic of the Congo: a prospective observational study

Head-to-head comparison of diagnostic accuracy of four Ebola rapid diagnostictests versus GeneXpert® in eastern Democratic Republic of the Congo outbreaks: aprospective observational study

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Daniel Mukadi-Bamuleka, MD[1,2,3,] Junior Bulabula-Penge, MD[1,3], Bart K.M. Jacobs, PhD[4], Anja De Weggheleire, MD[4], François Edidi-Atani MD[1,2,3], Fabrice Mambu-MbikaMD[1,3], Anaïs Legand, MPH[5], John D. Klena, PhD[6], Peter N. Fonjungo, PhD[6], Placide Mbala-Kingebeni, PhD[1,3], Sheila Makiala-Mandanda, PhD[1,3], Masahiro Kajihara, PhD[7],Ayato Takada, PhD[7], Joel M. Montgomery, PhD[6], Pierre Formenty, MPH[5], Jean- Jacques Muyembe-Tamfum, PhD[1,3], Kevin K. Ariën, PhD[4,8], Johan van Griensven, PhD[4], Steve Ahuka-Mundeke, PhD[1,3] and **Study Group***

Study Group*: Hugo Kavunga-Membo, PhD[1,2], Elie Ishara-Nshombo, DVM[2], Stijn Roge, PhD[4], Noella Mulopo-Mukanya, MSc[2], Espérance Tsiwedi-Tsilabia, MSc[2], Emile Muhindo-Milonde, MSc[2], Marie-Anne Kavira-Muhindo, MSc[2], Maria E. Morales- Betoulle[6], Antoine Nkuba-Ndaye, PhD[1,3]

Affiliations

1. Institut National de Recherche Biomédicale, INRB, Kinshasa, DRC
2. Rodolphe Mérieux INRB-Goma Laboratory, Goma, North-Kivu, DRC
3. Service de Microbiologie, Departement de Biologie Médicale, Cliniques Universitaires de Kinshasa, Université de Kinshasa, DRC
4. Institute of Tropical Medicine, Antwerp, Belgium
5. Health Emergencies program, World Health Organization, Geneva, Switzerland
6. US Centers for Disease Control and Prevention, Atlanta, Georgia, United States ofAmerica
7. International Institute for Zoonosis Control, Hokkaido University, Sapporo, Japan
8. University of Antwerp, Antwerp, Belgium

Corresponding Author: Daniel Mukadi-Bamuleka: drmukadi@gmail.com, +243 812 965 243, Department of Virology, Institut National de Recherche Biomédicale, INRB, 5345,Avenue de la Démocratie (Ex Des Huileries), Kinshasa-Gombe, DR Congo

Summary

Introduction Ebola virus disease (EVD) outbreaks have emerged in Central and West Africa. EVD diagnosis relies principally on RT-PCR testing with GeneXpert®, which has logistical and cost restrictions at the peripheral level of the health system. Rapid diagnostic tests (RDTs) would offer a valuable alternative at the point-of-care to reduce the turn-around time, if they show good performance characteristics. We evaluated the performance of four EVD RDTs against the reference standard GeneXpert® on stored EVD positive and negative blood samples collected between 2018-2021 from outbreaks in eastern Democratic Republic of the Congo (DRC).

Methods We conducted a prospective and observational study in the laboratory on QuickNavi-Ebola™, OraQuick® Ebola Rapid Antigen, Coris® EBOLA Ag K-SeT, and Standard® Q Ebola Zaïre Ag RDTs using left-over archived frozen EDTA whole blood samples. We randomly selected 450 positive and 450 negative samples from the EVD biorepositories in DRC, across a range of GeneXpert® cycle threshold values (Ct-values). RDT results were read by three persons and we considered an RDT result as "positive", when it was flagged as positive by at least two out of the three readers. We estimated the sensitivity and specificity through two independent generalized (logistic) linear mixed models (GLMM).

Findings 476 (53%) of 900 samples had a positive GeneXpert Ebola result when retested. The QuickNavi-Ebola™ showed a sensitivity of 56·8% (95% CI 53·6-60·0) and a specificity of 97·5% (95% CI 96·2-98·4), the OraQuick® Ebola Rapid Antigen test displayed 61·6% (95% CI 57·0-65·9) sensitivity and 98·1% (95% CI 96·2-99·1) specificity, the Coris® EBOLA Ag K-SeT showed 25·0% (95% CI 22·3-27·9) sensitivity and 95·9% (95% CI 94·2-97·1) specificity, and the Standard® Q Ebola Zaïre Ag displayed 21·6% (95% CI 18·1-25·7) sensitivity and 99·1% (95% CI 97·4-99·7) specificity.

Interpretation QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen Test did not achieve the “desired or acceptable level” of sensitivity as set out in the WHO target product profile for EBOV tests, although their respective specificities were close to 99% (97·5% and 98·1%). Nonetheless, both RDTs can be used as frontline tests for triage of suspected-cases while waiting for RT-qPCR confirmatory testing.

Role of funding source Institute of Tropical Medicine-Antwerp/EDCTP PEAU-EBOV-RDC project.

Key-Words: EVD, EBOV, GeneXpert, RDT sensitivity, specificity

Introduction

Ebola virus disease (EVD) outbreaks have emerged in Central and West Africa where they posed major threats to global health security (1, 2, 3). Over the last ten years (2012-2022), twelve EVD outbreaks were reported in the world among which the two largest and deadliest caused 11,310 and 2,287 deaths, in West Africa (2013-2016) and in eastern Democratic Republic of the Congo (DRC) (2018-2020), respectively (2, 4, 5, 6). Early diagnosis is key to control EVD outbreak as it allows triaging and isolation of cases, case finding and follow-up of their contacts, investigation of the cause of death and clinical management of cases, implementation of therapeutics and vaccines clinical trials, post-epidemic surveillance and survivor's follow-up (7-13).

During EVD outbreaks, diagnosis currently relies principally on the GeneXpert®, a semi-automated Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) closed system. This technique offers a lower-risk of contamination, high sensitivity (>99%) and specificity (>95%), ease of the use (minimal technical knowledge and few steps required), and short turn-around time (<2 hours) (14, 15). However, the use of GeneXpert® is costly and requires skilled personnel, infrastructure and equipment, reagents and uninterrupted power supply to be continuously run at the peripheral healthcare of the health system. As most of these requirements are not usually available at the peripheral level, the use of the GeneXpert® is strongly hampered, calling out for reliable rapid diagnostic tests (RDTs) which are suitable for field settings (14-18).

RDTs are easy-to-use and less-resource intensive assays detecting viral antigens in the blood and other bodily fluids without requiring any power supply, cold chain, sophisticated equipment or highly trained-personnel compared to the GeneXpert®. Thus, RDTs can complement the GeneXpert® in the detection of EVD cases at the point-of-care. RDTs offer several advantages as they 1) considerably reduce the turn-around time, 2) allow a quick decision-making on case management and death screening during or after outbreaks, 3) can help patients with reactive RDT to be transferred to the adequate health facility, and 4) can increase the access and acceptability

of EVD testing at the point-of-care (7, 11, 12, 14, 16, 17, 19, 20, 21). However, RDTs need to be highly sensitive and specific to allow early detection of viremic patients during the course of the disease and reduce the consequences of false-positive/negative results during the public health interventions (10, 22, 23).

Most of the previous Ebola virus (EBOV) RDTs evaluated (including outbreaks in the DRC) displayed variable performance characteristics, without reaching the desired (sensitivity >98%, specificity >99%) or acceptable (sensitivity >95%, specificity >99%) level of sensitivity and specificity as set out in the World Health Organization target product profile (TPP) for EBOV tests (11, 17, 18, 24). Therefore, some concerns with RDTs still unresolved such as 1) the consistency in the performance characteristics in suboptimal or controlled settings, 2) the necessity to use RDTs to triage and rule out EVD in symptomatic patients at the peripheral care level, and 3) the development of algorithms to be used at the point-of-care in low resource settings (17).

The Institut National de Recherche Biomédicale (INRB) as the National Reference Laboratory of the DRC has long-term storage for left-over samples from different EVD outbreaks, including those from eastern DRC (2018-2021). As a large and well-characterized EVD number of negative and positive samples were available at INRB biorepositories, a unique opportunity emerged to establish a head-to-head comparison of several RDTs with the same batch of samples. In this study, we estimated the performance characteristics of four EVD RDTs against the GeneXpert® as the reference standard on a set of stored EVD positive and negative whole blood samples from eastern DRC outbreaks.

Materials and Methods

Study Design, population and setting

We conducted a prospective laboratory study to evaluate two lots of QuickNavi-EbolaTM and Coris® EBOLA Ag K-SeT, and one lot of OraQuick® Ebola Rapid Antigen and Standard® Q Ebola Zaïre Ag RDTs. We used archived frozen whole blood samples from North Kivu (Beni, Butembo,

Mangina and Katwa) and Ituri (Komanda, Mambasa, Tchomia, Bunia and Biakato) provinces, during DRC EVD outbreaks (2018-2021). Those samples managed and tested within INRB field laboratories were collected in patients admitted in Ebola Treatment Units for diagnosis. The left-over samples following primary testing were temporarily stored across sites, and later shipped to INRB biorepositories in Kinshasa or Goma for long-term storage.

Sample selection, sample processing and Data collection

The sequences of the study procedures are summarized in Figure 29.

We initially used field laboratory datasets to randomly select 450 positive and 450 negative samples (plus 50 additional per category to foresee any replacement) based on GeneXpert® results at the time of outbreak response. This sample size was chosen to obtain sufficiently precise estimates of the sensitivity and specificity, and be able to find clinically meaningful differences in them with sufficient power.

Thereafter, we sorted out the selected samples from biorepositories in Kinshasa and Goma to check for their corresponding volumes. For EVD positive patients, we included one aliquot from the first diagnostic sample or the first positive sample of the patient's follow-up, in case of insufficient volume of the initial sample. Finally, we included 476 positive and 424 negative samples having a volume > 500 µl and complete information in the 'study dataset' (ID, type of specimen, timing of sampling, Xpert Ebola results, Cycle threshold value, availability of epidemiological data).

The lab testing (GeneXpert® and RDTs) was carried out at the Biosafety level-3 of Rodolphe Mérieux INRB Laboratory in Goma (North Kivu Province, DRC) by experienced lab-technicians. The final set of samples was randomly mixed, relabeled, aliquoted (one copy per RDT brand and per lot, and one for the GeneXpert® retesting), and stored in identified cryoboxes (one aliquot specimen per cryobox). The study personnel completed a training on the protocol, Good Clinical and Laboratory Practices, GeneXpert® and RDTs proficiency prior to the study initiation. For standardization purposes between operators, we used the package insert of each kit to develop practical bench-aids for RDTs and the GeneXpert® assay. All the samples were re-run with

the GeneXpert®, their results recorded into the 'study database' and double-blinded to operators while running the RDTs.

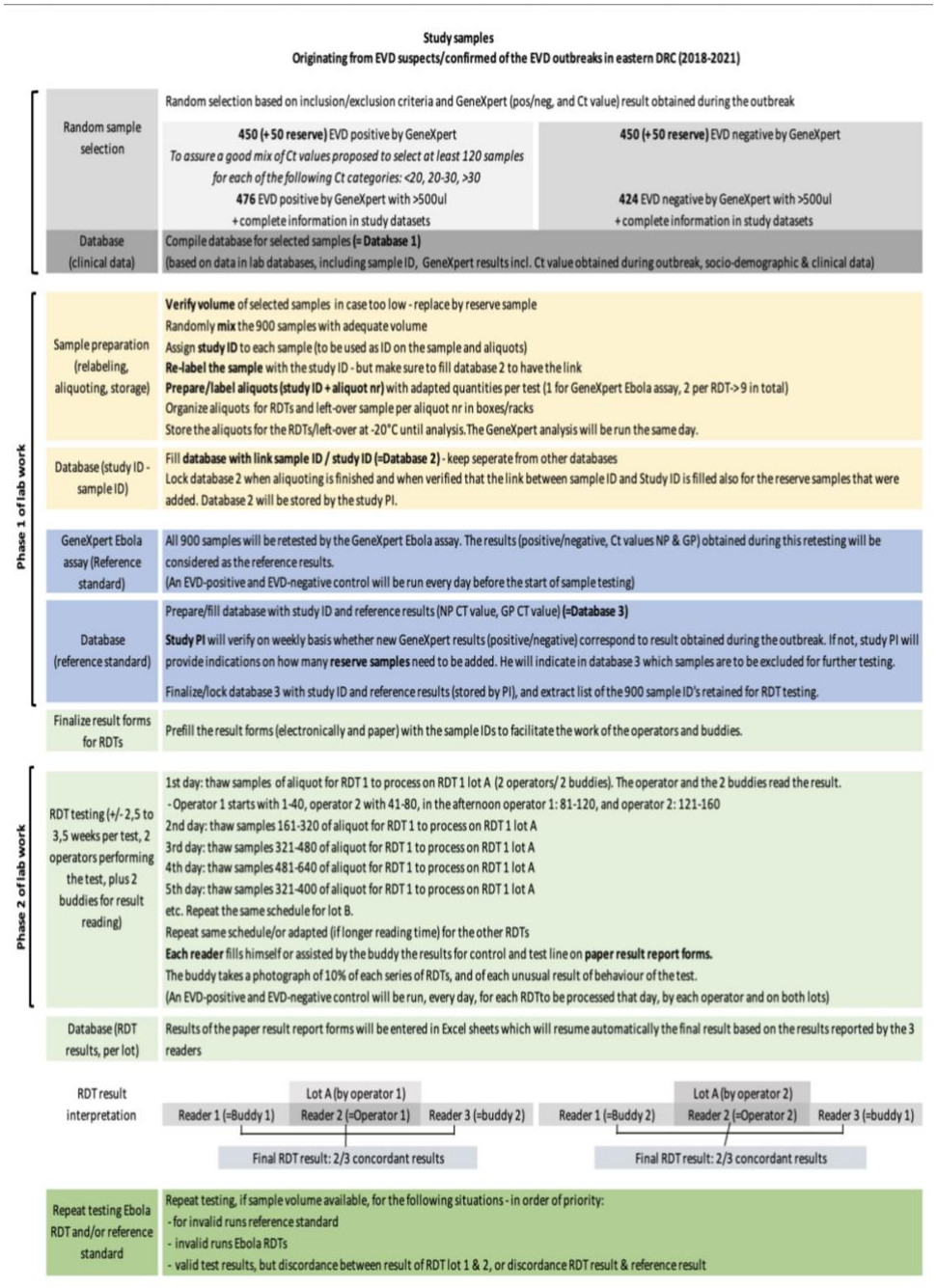

Figure 29 Study procedures & data collection

Based on the cycle threshold values (Ct-values) of Ebola virus targets glycoprotein (GP) and nucleoprotein (NP), sample results were categorized as follows: EVD negative 1) GP not detected/NP not detected, or 2) GP not detected/Ct of NP ≥ 40, or 3) GP detected/NP not detected; EVD positive 1) GP detected/NP detected and Ct < 40, or 2) GP not detected/NP detected and Ct < 40.

Samples with invalid results were re-run from the original tube at the end of all the GeneXpert® testing. Samples were processed per RDT brand and per lot: only one brand was run at a time by two operators, one operator per lot assisted by his/her buddy. As there was a risk that operators got used to the reactivity patterns of the tests while processing aliquots in the same sequence, we randomly allocated the samples boxes to be processed for the same lot while changing also the operators and buddies.

RDTs results were directly read from the device by three persons following this order: the buddy of the operator, the buddy of the other operator and the operator themself. The first two readers independently wrote the results on their individual reporting form then, asked for the operator themself to give their result which was noted on another form by the buddy. We considered an RDT result as “positive”, when it was flagged as positive by at least two out of the three readers. After daily sample processing, the results were entered into an Excel sheet which automatically displayed the final result based on the reports of two out of three readers.

Data analysis

Data were analyzed using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria). Basic characteristics of participants and samples were summarized using descriptive analyses. The diagnostic performance of each of the RDTs was evaluated against the GeneXpert® reference standard for the primary outcome. Sensitivity was defined as the probability that patients with a positive GeneXpert® Ebola assay result had a positive RDT result. Specificity was defined as the probability that patients with a negative GeneXpert® Ebola assay result had a negative RDT result. Sensitivity and specificity were estimated directly using proportions and associated Wilson confidence intervals (CIs), and through two independents

generalized (logistic) linear mixed models (GLMM) using the lme4 package in R (25) when adjusting for different viral loads (measured through the proxy variable Ct-value) and for sources of technical variation, as well as to compare the diagnostic accuracy between the RDTs.

We modelled the binary RDT outcome (positive or negative) as a function of test type and Ct-value (for sensitivity). We considered including sample, lab technician and RDT lot as a random effect. Inclusion of interaction (between RDT and Ct-value) and random slopes (i.e. per RDT rather than jointly) were tested at the 5% significance level when relevant. CIs were constructed using the profile likelihood when possible and with the asymptotic Wald approximation otherwise. Equality of sensitivity and specificity of different RDTs was tested with Tukey-style pairwise comparisons using the multcomp package in R (26).

Ethical Issues

This study received the approval of Kinshasa School of Public Health Ethics committee (Approval Number: ESP/CE/06/2022).

Role of funding source

This study was funded by the Institute of Tropical Medicine-Antwerp, the EDCTP PEAU-EBOV-RDC project under grant agreement RIA2018EF-2087, and through the FA5 DRC Program funded by the Directorate General for Development Cooperation and Humanitarian Aid (DGD) of the Belgian government.

The authors had full access to the study datasets and had the final responsibility for the decision to submit for publication. The funders have not played any role in the study design, data collection, data analyses, interpretation and writing of report.

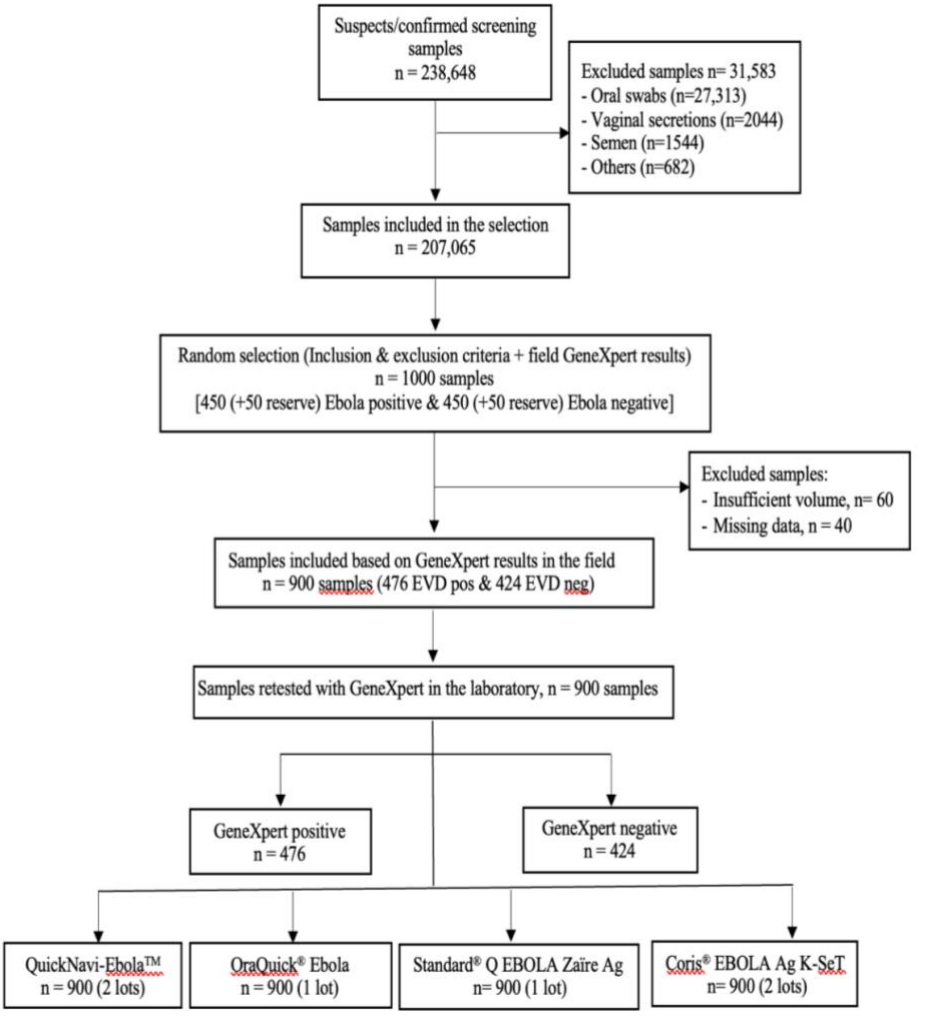

Figure 30. Samples flow diagram

Results

We tested a total 900 whole blood samples in the laboratory (Figure 30). Out of 900 samples re-tested on the GeneXpert®, 476 (53%) were EBOV positive and 424 (47%) EBOV negative. For EBOV positive samples, the median NP Ct-value was 27·7 (IQR 22·5-35·0), with minimum of 15·9 and maximum of 39·9. The distribution of NP Ct-values in EBOV positive samples followed a bimodal pattern, with large number of samples in the range of 19-26 and 34-39 (Figure 31).

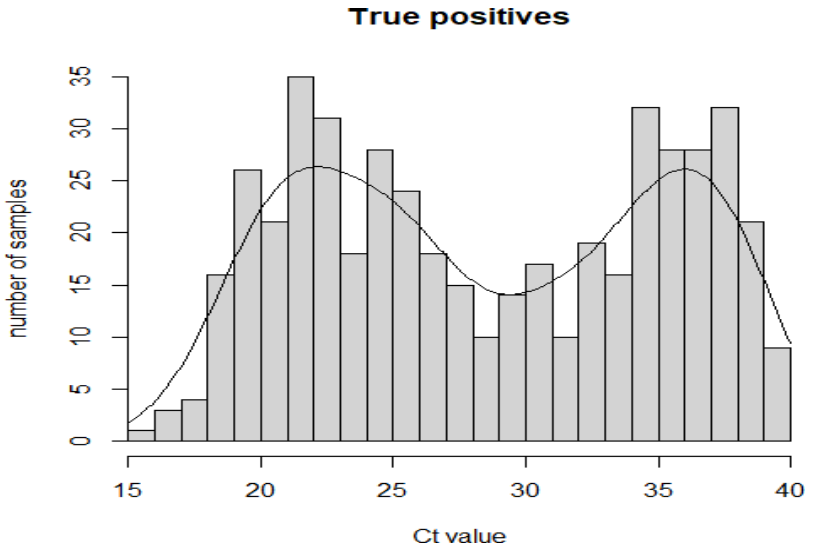

Figure 31. Distribution of NP Ct-values in EBOV positive samples

Table 24. Rapid diagnostic test performance versus GeneXpert Ebola assay as reference standard

RDT		Individual	Positive by 1/3 reader	Positive by 2/3 readers	Positive by 3/3 readers
Coris	Sensitivity	24·9% (23·3-26·5)	27·8% (25·0-30·8)	25·0% (22·3-27·9)	21·8% (19·3-24·6)
	Specificity	95·6% (94·8-96·4)	94·8% (93·0-96·2)	95·9% (94·2-97·1)	96.2% (94·7-97·4)
OraQuick	Sensitivity	61·4% (58·8-63·9)	64·1% (59·6-68·4)	61·6% (57·0-65·9)	58·6% (54·0-63·1)
	Specificity	97·8% (96·8-98·5)	96·0% (93·5-97·6)	98·1% (96·2-99·1)	99·3% (97·8-99·8)
QuickNavi	Sensitivity	56·4% (54·5-58·2)	58·2% (55·0-61·3)	56·8% (53·6-60·0)	54·1% (50·9-57·3)
	Specificity	97·1% (96·3-97·7)	96·1% (94·5-97·3)	97·5% (96·2-98·4)	97·6% (96·3-98·5)
Standard Q line	Sensitivity	21·2% (19·1-23·5)	22·5% (18·9-26·6)	21·6% (18·1-25·7)	19·5% (16·1-23·4)
	Specificity	98·7% (97·9-99·3)	98·1% (96·2-99·1)	99·1% (97·4-99·7)	99·1% (97·4-99·7)

Note: estimate (95% confidence interval)

Table 24 provides sensitivity and specificity while interpreting RDT positive results compared to the GeneXpert® as the reference-standard by 1/3, 2/3, 3/3 readers, aswell as individual reads (all reads were considered as independent observations).

Sensitivities and specificities of the four RDTs evaluated versus the reference standard GeneXpert® are shown in table 24.

When we considered 1/3, 2/3, 3/3 or individual reads (all reads taken as independent observations) flagging a test as positive, the sensitivity and the specificity were similar for each RDT (Table 23). The overall performance of all RDTs observed showed that QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen were the most sensitive tests whereas the Standard® Q Ebola Zaïre Ag was the most specific (98·7%) (Table 24). We also noted a good agreement between the different lab-operators, as for all six lots we found at least 95% agreement between all three readers (all positive or all negative) (Table 25).

Table 25. Agreement between readers on positive samples per Rapid diagnostic test

	Coris lot 1	Coris lot 2	OraQuick	QuickNavi 1	QuickNavi 2	Standard Q Line
0 positive	771 (85·7%)	720 (80·0%)	578 (64·2%)	605 (67·2%)	608 (67·6%)	785 (87·2%)
1 positive	11 (1·2%)	25 (2·8%)	21 (2·3%)	12 (1·3%)	13 (1·4%)	8 (0·9%)
2 positive	14 (1·6%)	19 (2·1%)	19 (2·1%)	6 (0·7%)	21 (2·3%)	10 (1·1%)
3 positive	104 (11·6%)	136 (15·1%)	282 (31·3%)	277 (30·8%)	258 (28·7%)	97 (10·8%)
Agreement	875 (97·2%)	856 (95·1%)	860 (95·6%)	882 (98·0%)	866 (96·3%)	882 (98·0%)

This table shows the percentage of agreement between all 3 readers for each of the 6 lots. Agreementis defined. As all 3 readers independently classifying a sample as negative (0 positive) or positive (3 positive), irrespective of a sample's reference state defined by PCR.

The estimated sensitivity at different observed Ct-values is displayed in Figure 32 (continuous lines). The very steep curves indicate the large impact of Ct-value (output of a model with different slopes). The sensitivity of the four RDTs declined as the Ct-values increased. The QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests showed respectively 98% and 97% sensitivity in samples at 17·5 NP Ct-values compared to the Coris® EBOLA Ag K-SeT (sensitivity: 75%) and Standard® Q Ebola Zaïre Ag (sensitivity: 81%) tests. At an NP Ct-values of 22·5, the sensitivity of the QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen was maintained above 90%, whereas it has significantly dropped to under 50% for Coris® EBOLA Ag K-SeT and Standard® Q Ebola Zaïre Ag. We observed a drop in sensitivity under 20% at NP Ct-values > 35·0 for the QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen, whereas the threshold of < 20% sensitivity was already reached with the NP Ct-values > 27·5 for the Coris® EBOLA Ag K-SeT and Standard® Q Ebola Zaïre Ag tests (Figure 32: continuous lines).

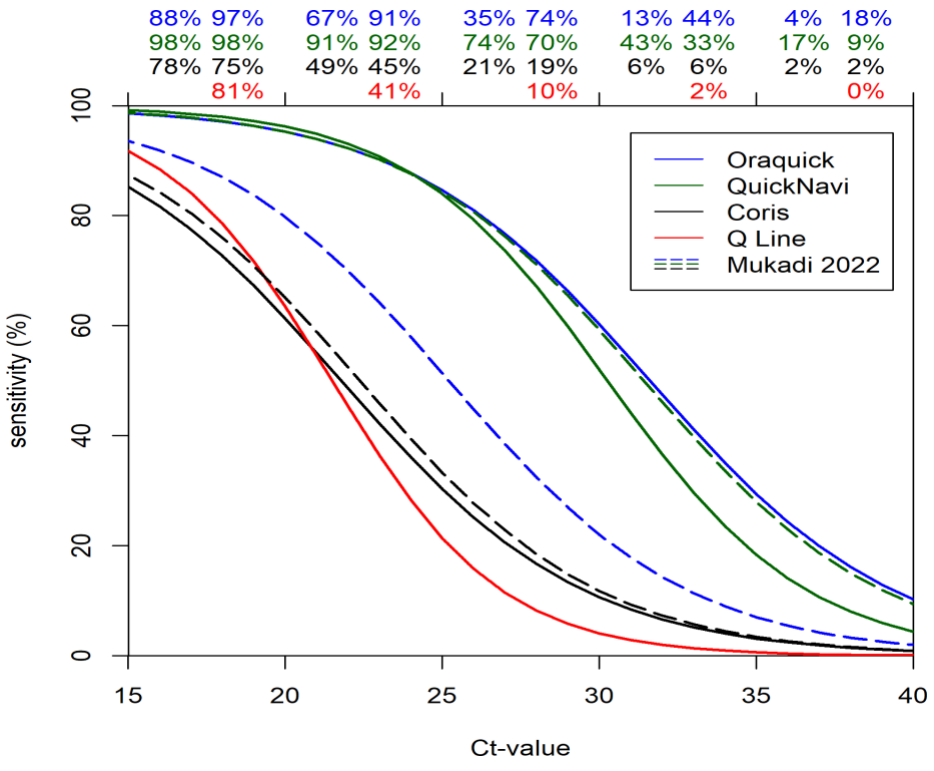

Figure 32 Sensitivity of the rapid diagnostic tests as a function of GeneXpert Ct-value. Continuous lines indicate the sensitivity of RDTs in the current study, whereas the dashed lines indicate the sensitivity of RDTs in the previous study (Mukadi et al). Theestimates of sensitivity on top of the graph are for Mukadi et al (left) (Lancet Infect Dis.2022 Mar 14:S1473-3099(21)00675-7) and the current study (right), respectively. The very steep curves indicate the large impact of Ct-value (output of a model with different slopes). The percentages on the figure do not show the estimated sensitivityin the bins, but instead give the modelled sensitivity in the middle of the bin (i.e. at 17·5, 22·5, 27·5 etc.) which is an estimate for the sensitivity conditional on a sample with an observed concentration that corresponds with a Ct-value of such a number.

Figure 33 shows the RDT positivity for GeneXpert® positive samples per category of Ct-value. The QuickNavi-EbolaTM and OraQuick® Ebola Rapid Antigen detected more positives in samples with low and medium Ct-values (high and medium viral loads), but they detected lower positives in specimens with high Ct-values (low viral load). The Coris® EBOLA Ag K-SeT and Standard® Q Ebola Zaïre Ag had low sensitivity around the median Ct-values. Among 103 true positives samples with the Standard® Q Ebola Zaïre Ag test,

99 detected VP40 protein (89 by three readers); 54 detected GP protein (46 by three readers) including 4 samples that were VP40 negative; and 9 detected NP protein (data not shown).

The lot effect was small and not significant for both the sensitivity (p=0·92) and specificity models (p=0·89). The random sample ID effect was highly significant for both sensitivity and specificity (p<0·0001), even after adjusting for Ct-value.

The final model for sensitivity included the type of RDT, Ct-value, and sample ID as random effect. All differences in sensitivity between the four RDTs were significant except for the Coris® EBOLA Ag K-SeT versus Standard® Q Ebola Zaïre Ag (p=0·16).

The p-value between QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen was 0·03, all others were <0·0001. Despite the significant difference, OraQuick® Ebola Rapid Antigen Test and QuickNavi-Ebola™ performed similarly, and considerably better than Coris® EBOLA Ag K-SeT and Standard® Q Ebola Zaïre Ag. The significant difference between QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen seems to be explained by the OraQuick® Ebola Rapid Antigen test performing slightly better than QuickNavi-EbolaTM in samples with high Ct-values especially (Figure 33). All the tests showed high specificity, except for the Coris® EBOLA Ag K-SeT which performed significantly worse than Standard® Q Ebola Zaïre Ag (p=0·005). We combined overall sensitivities and specificities by Ct-values for different RDTs to get testing strategies. Combining OraQuick® Ebola Rapid Antigen and QuickNavi-Ebola™ tests as a panel would lead to a modest increase of sensitivity compared to a single test, with only a small reduction of specificity. Adding either Coris® EBOLA Ag K-SeT or Standard® Q Ebola Zaïre Ag to the panel was not likely to increase the sensitivity much and would in case of Coris® risk a considerable reduction in specificity. Combining OraQuick® Ebola Rapid Antigen Test and QuickNavi-Ebola™ with an “AND” criterion would further reduce the sensitivity to undesirable levels (Table 26).

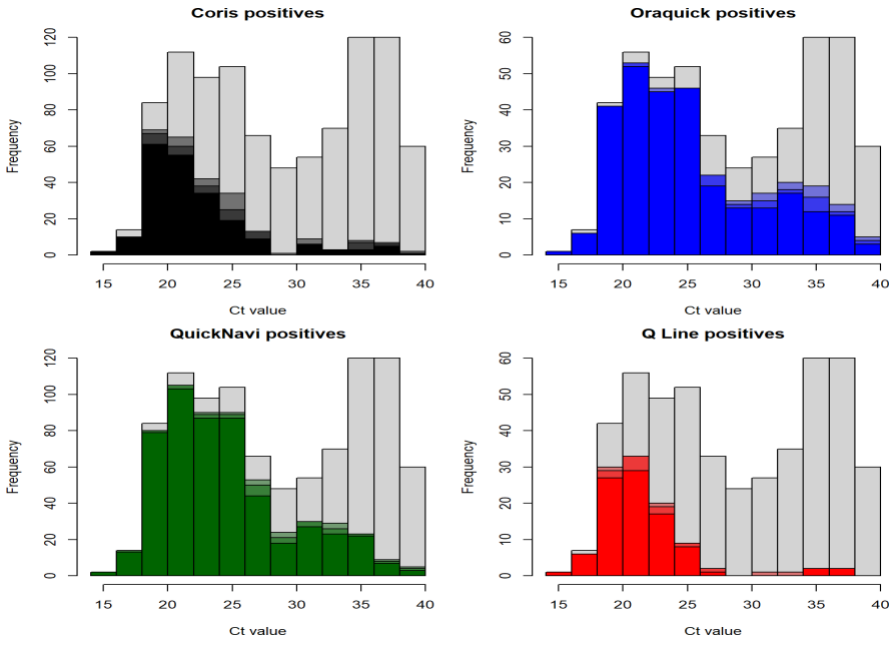

Figure 33 RDT positivity for GeneXpert positive samples by Ct-value

This histogram describes the frequency of RDT positive (coloured) and negative (grey) samples compared by type of RDTs per Ct-value among Xpert positive samples. Thegrey bars indicate the negative results as flagged by three readers. Colours indicate the positivity by RDT and by Ct-value, the darkest colour indicates a test being positivefor all 3 readers, and the lighter colours for 2 and 1 reader positive (Each colour represents RDT brand).

Table 26. Raw sensitivity and specificity of testing strategies of 1 or more RDTs

Positive (+) if	**sensitivity**	**specificity**
Oraquick +	61·6% (57·0-65·9)	98·1% (96·2-99·1)
QuickNavi +	56·8% (53·6-60·0)	97·5% (96·2-98·4)
OraQuick + OR QuickNavi +	68·8% (65·7-71·7)	96·0% (94·4-97·2)
OraQuick + OR QuickNavi + OR Coris +	69·6% (66·6-72·5)	92·6% (90·5-94·2)
OraQuick + OR QuickNavi + ORQ Line +	68·8% (65·7-71·7)	95·3% (93·6-96·6)
OraQuick + AND QuickNavi +	49·6% (46·4-52·8)	99·6% (98·9-99·9)

Discussion

Throughout this study, the QuickNavi-Ebola™ and OraQuick® Ebola RDTs did not reach the “desired or acceptable level” of sensitivity following the WHO TPP for EBOV tests. However, both tests showed specificities close to the “desired or acceptable level” of specificity (>99%) with 97·5% and 98·1%, respectively.

A previous evaluation of plasma samples with Coris® EBOLA Ag K-SeT showed sensitivities of 98·7% (samples with low Ct-values), 62·1% (samples with high Ct-values), and 88·6% (overall sensitivity) (27). However, the sample size was small (n=210), the majority of samples had low Ct-values (<34·0), and the reference standard used was less sensitive (27). During the 2018-2021 EVD outbreaks in DRC, the Coris® EBOLA Ag K-SeT was run on a large number of blood samples (n=819 and n=900), but it displayed poor performances (38·9% and 25·0% sensitivity, 97·4% and 95·9% specificity) compared to GeneXpert® (17). QuickNavi-Ebola™ was reported to have a low limit of detection compared to OraQuick® Ebola Rapid Antigen Test (14). In outbreak settings, the QuickNavi-Ebola™ showed good performances in two studies (14, 17), while less impressive performance was observed in the current study (56·8% sensitivity versus 97·5% specificity). However, as shown previously (17), test performance is strongly dependent on the Ct-values of the tested samples. We therefore compared not only the overall performance, but also taking into account the Ct-value (Figure 33).

Since our study has more samples with higher Ct-values than previously (17), we could expect results for overall sensitivity to be worse. However, when the results are analyzed by Ct-value for QuickNavi-Ebola™ and Coris® EBOLA Ag K-SeT, the differences are generally small and its performance is in line with the previous study (17). In summary, the QuickNavi-Ebola™ test showed good performance characteristics in samples with low Ct-values, but performs poorly in samples with high Ct-values. Conversely, the OraQuick® Ebola Rapid Antigen test overall estimates are in line with the previous study (57·4% sensitivity and 98·3% specificity) (17), which masks that it performed better in this study taking into account the Ct-value, with performance especially better for the middle and lower Ct-values, compared to our

previous study (Figure 33). These findings are also consistent with previous reports (manufacturer: 84% sensitivity, 98% specificity at mainly low Ct-values) (13, 20, 28). The Standard® Q Ebola Zaïre Ag (designed to detect both VP40 and NP) showed a very low sensitivity (21·6%) for a good specificity (99·1%). This finding is in line with a previous study (11), that showed high specificity for low sensitivity compared to other RDTs. Additionally, the Standard® Q Ebola Zaïre Ag showed excess pooling on the sample reception pad, resulting in failure to flow on the membrane (11), as we noticed too while using whole blood samples.

A few technical conditions could be pointed out as potential source for the suboptimal performance of the RDTs evaluated here. First, the storage and transportation conditions could have modified the quality of the biological specimens used. During the outbreak, samples were subjected to multiple shipments and variable cold chain conditions from primary sites of testing to the temporary and long-term storage sites. Technical requirements in the implementation of this study such as freeze-thaw process and aliquoting could have changed the quality of the specimens. In order to compensate possible sample quality loss due to the material storage over time, we kept one aliquot to be re-tested with the GeneXpert®. Decay of antigens in the samples could have partially affected the detection of these antigens in the RDTs. Viral proteins such as VP40 and NP are usually abundantly expressed in infected organisms and thus represent ideal targets for RDTs. We consequently expected our RDTs to detect more positives as those abundant viral proteins were targeted.

Using archived whole blood samples instead of plasma could have slowed the migration over the membrane. However, during EBOV outbreaks the laboratory procedures do not allow specimen centrifugation, in order to mitigate the risk of environmental contamination. Wonderly et al observed a reduced specificity of RDTs using plasma versus EDTA whole blood, but no effect on the sensitivity was found (11). This observation warrants for separate performance evaluation with whole blood and plasma specimens. In our study, we only had EDTA whole blood available for testing. Therefore, care should be taken before extrapolating these results to plasma or fresh

samples tested in the field (11). In samples with low Ct-values, the high concentration of antigens could have formed antibody-antigen complexes sticking to the membrane and reducing flow across the sample pad (prozone effect).

In our previous study (17), we evaluated QuickNavi-Ebola™, OraQuick® Ebola Rapid Antigen, Coris® EBOLA Ag K-SeT, and added Standard® Q Ebola Zaïre Ag test in the current study. The QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests did not achieve the "acceptable level" of performance as stated by the WHO TPP for EBOV tests (11, 17, 18, 24), although their respective specificities had almost reached 99% (97·5% and 98·1%). Nonetheless, we can propose their use as frontline diagnosis in remote areas to triage and isolate suspected-cases. All individuals will be isolated separately in the triage ward while waiting for RT-qPCR results i.e. those with at least one positive RDT in the high-risk area and those with negative results in low-risk area. To use RDTs in the field, we should take into consideration 1) the performance characteristics of the most sensitive tests, 2) the conditions of biosafety, and 3) the ease of use.

In the current study, RDTs performance was ranked as follows (sensitivity and specificity): 1) OraQuick® Ebola Rapid Antigen Test (61·6% and 98·1%) and QuickNavi-Ebola™ (56·8% and 97·5%), 2) Coris® EBOLA Ag K-SeT (25·0% and 95·9%) and Standard® Q Ebola Zaïre Ag (21·6% and 99·1%). In our previous study, RDTs were categorized in the following manner (sensitivity and specificity): 1) QuickNavi-Ebola™ (87·4% and 99·6%), 2) OraQuick® Ebola Rapid Antigen Test (57·4% and 98·3%) and 3) Coris® EBOLA Ag K-SeT (38·9% and 97·4%) (17). At the point-of-care, we propose a screening panel consisting of the QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen Test (high combined sensitivity and specificity) using finger prick or venous blood with an "OR" criterion for inclusion (Table 26). To our knowledge, Ebola RDTs and GeneXpert® evaluated in this study can only be applicable to EBOV species. Therefore, it will be useful to test those diagnostic tools against other Ebola species in further studies. Using this panel of two RDTs at a time will allow the detection of at least one target viral protein (NP or VP40), as the presence of antigens may vary in positive cases

according to the stage of the infection. At this stage, RDT use will not generate cost savings for EVD testing as RT-qPCR will still be needed to discriminate positives from negatives among suspected-cases. From our studies, we learned that both QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests can be easily run and interpreted in field conditions. However, their implementation will require 1) adequate training on their correct use, 2) accurate reporting of their results, 3) correct shipment of clinical specimens to the laboratory for confirmatory RT-qPCR with venous blood collected after RDTs analysis, in order to exclude any false positives and false negatives (5, 14, 17). In the meantime, stringent and adequate measures should be taken to carefully triage and manage isolation of suspect-cases in care units, in order to mitigate the risk of EVD nosocomial transmission. Furthermore, the implementation of these RDTs should be supported by a clear at-risk communication plan, an excellent psychosocial environment and messaging, and detailed clinical and epidemiological data.

Contributors

DM-B, ADW, BKJ, JvG, KKA, SA-M: conceived the study, the methodology and wrote the original draft. DM-B, JB-P, FE-A, FM-M, NMM, EMM, MAK-M, ET-T, EI-N: performed investigation and data curation. DM-B, BKJ, EIN, JB-P, FE-A, FM-M and ADW: accessed and verified all underlying data. BKJ, JvG, DM-B, and ADW: performed formal data analysis. JDK, HK-M, BKJ, PM-K, SM-M, MK, AT, PF, JMM, JJM-T, MEM, PNF, SR, AL, AN-N: edited the manuscript. DM-B, ADW, BKJ, JDK, PF, JMM, MK, AT, PNF, JJM-T, AL, AN-N, SR: visualized the study documents. JMM, KKA, JvG, SA-M: validated the study documents and the manuscript.

All authors read and approved the final version of the manuscript, and ensure it is the case.

Declaration of interests

US CDC provided the Xpert® Ebola cartridges. FIND purchased OraQuick Ebola Rapid Antigen tests and donated to INRB through US CDC partnership. Institute of Tropical Medicine-Antwerp purchased Coris® Ag K-SeT and Standard® Q line Zaïre Ebola Rapid diagnostic tests with the financial support of the EDCTP PEAU- EBOV-RDC project under grant agreement RIA2018EF-2087, and through the FA5 DRC Program funded by the Directorate General for Development Cooperation and Humanitarian Aid (DGD) of the Belgian government.

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Data sharing Statement

The data statement is shared separately in the supplementary documents. The data sharing agreement will be available three months after official paper release.

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Part 4: General Discussion and Conclusions

The main objective of this PhD was 1) to show how the implementation and management of field laboratories and genomic sequencing played a key role in the control of the outbreak; and 2) to improve the quality of EVD diagnosis by the use of new tools such as RDTs and RT-qPCR at the point-of-care and in the community.

The throughput of information generated by field laboratories during the tenth EVD outbreak in eastern DRC, allowed the dissemination of accurate and specific results (RT-qPCR and genomic sequencing) to guide public health decision-making and successful management of the outbreak. The QuickNavi™-Ebola RDT showed high specificity and quite good sensitivity over other RDTs while tested with clinical specimens within outbreak conditions, in comparison to the reference standard (GeneXpert®). Despite the significant difference, OraQuick® Ebola Rapid Antigen Test and QuickNavi-Ebola™ performed similarly, and considerably better than Coris® EBOLA Ag K-SeT and Standard® Q Ebola Zaïre Ag in laboratory conditions, whereas Standard® Q Ebola Zaïre Ag showed to be more specific than others.

4.1. Discussion of the main results

4.1.1. Efficiency of Field Laboratories for Ebola Virus Disease Outbreak during Chronic Insecurity, Eastern Democratic Republic of the Congo, 2018-2020

Deploying field laboratories at the epicenter of an EVD outbreak enabled all the pillars of the response to organize well-structured and coordinated on-site activities. Quick results provided on-site enabled timely public health interventions within a short time window. Throughout the 2018-2020 EVD outbreak, we observed simultaneous multiple epicenters constantly changing their location over time. In these conditions, field laboratories outlined future new pathways to conduct Ebola response while targeting prompt, efficient, accurate and multidisciplinary approaches. New laboratory capacities brought in the field supported substantial activities. The testing was conducted with point-of-care, or near point-of-care instruments to provide EVD diagnosis, biochemistry and hematology analytes in patients under specific therapeutics

plus standard of care. EVD survivors' follow-up has become an important component of the routine activities during and after outbreaks. Survivors' follow-up helps preventing or reducing the risk of reintroduction of the virus from a human reservoir to the community, especially in areas with a large number of survivors such as eastern DRC. Field laboratories deployed were used as one of the response tools to EVD outbreaks. Later on, as the capacity was maintained in place, those laboratories became frontline sentinels for the surveillance of EVD outbreaks in the concerned regions. Those functional laboratories detected three flare-ups of EVD in Butembo and Biena (February 2021), and in Beni (October 2021 and August 2022).

For countries with limited laboratory capacities, they should convert health emergency events (eg. outbreaks, flare-ups) to opportunities for building perpetual capabilities with the partners' support. Those laboratories will include the use of POC and near POC for outbreaks diagnosis, biological follow-up, and genomic sequencing. The DRC used EVD, Covid and Monkeypox outbreaks as an opportunity to setup laboratory networks in the southern (Lualaba province), northern (Equateur province) and eastern (North-Kivu, Ituri, South-Kivu provinces) part of the country. Starting with testing for one disease (Ebola), additional analyses were added to the panel (Covid, blood chemistry, hematology, malaria, plague, Cholera, meningitis etc). From one referral genomic sequencing laboratory in Kinshasa, a second hub was implemented in Goma (North-Kivu) to serve the eastern part of the DRC. In the meantime, genomic sequencing mobile laboratories are operating in Kongo Central, Lualaba and Equateur provinces as of today to support SARS-CoV-2, Poliovirus, Cholera and Plague sequencing. This approach reinforced the surveillance system and fostered joint partnerships to support the ongoing and additional activities in those laboratories. The same strategy is actually being used in Kole (Sankuru province) with a partner to build long-term capacity for research and surveillance of Monkeypox, other zoonotic diseases and prevalent infections in the region.

Empowered local human resources successfully managed the three aforementioned events with only technical advices received from experts at the national level. The profit of a quick hand over to local resources strongly

decreased the number of national and foreign experts to be deployed in the field. This strategy has improved the knowhow of local workers, reinforced the health system asthe zonal approach was implemented, and has not raised any community reluctance or resistance during the three Ebola flare-ups in 2021 and 2022. Additionally, human resources should also be trained accordingly to improve their skills in samples handling, manipulation of new equipment, management of laboratories, and capacity to deploy and support surrounding areas, if necessary.

Field laboratories helped to store for a short-term, samples processed during the outbreak, before their final shipment to INRB biorepositories in Kinshasa and Goma. Those valuable samplesobtained through this model of field laboratories are being used actually in several research projects involving researchers of all the world. Additionally, Ebola field laboratories capacity was strongly used to face Covid-19 pandemic in the eastern DRC, as the facilities and personnel were available and ready for emergency challenges. The experience of managing field laboratories in insecurity and hostility circumstances helped to quickly and efficiently address other emergency situations (Covid-19), and improve the capacity of resilience of personnel and structures.

4.1.2. Integration of genomic sequencing into the response to EVD outbreak in eastern DRC

The genomic surveillance by sequencing provided valuable data to support quick decision-making for public-health interventions such as contact tracing, preemptive vaccination of contacts and frontline workers etc. The large amount of genomic sequencing data provided throughout the outbreak improved the understanding of the dynamics of Ebola virus. The comprehension of the viral lineage's movements coupled to the population dynamics, determined specific types of transmission between healthzones. Genomic sequencing results were increasingly available to be used for unknown alive/death confirmed-cases as they supported the investigation, transmission chain building, and preemptive vaccination. Understanding the mechanisms leading to the emergence of the virus, its spread and maintaining within the community could help the response team to strategize adequate measures for outbreak prevention and control. Genomic sequencing became an essential tool in predicting 1) the pathogen

behavior/spread, (2) identifying at-risk populations and health zones exposed, (3) assessing the efficiency of medical countermeasures by identifying potential adaptive mutations as the outbreak progressed over time. The genomic sequencing has clearly highlighted the need to re-structure the health system in order to include new components the toolbox for outbreak response. The health system should quickly adopt new strategies, appoint additional staff, accept new technologies' opportunity to vehicle health information (bioinformatics), and allocate enough financial, human, material resources to sustain that transition at each level of the system.

Building on the same basis than diagnostic laboratories deployed throughout outbreaks (Ebola, Covid, Monkeypox), we can also convert outbreaks opportunities as starting point for implementation of new sequencing mobile units in the regions affected by outbreaks, including equipment and training of local staff (lab technicians, bioinformaticians and data managers). Those decentralized units can work in outbreak affected areas to enable public-health decision making while using portable technologies such as MinIon® (Oxford, Nanopore, UK) or iSeq100® (Illumina, California, USA). However, in areas with less probability for EVD or other outbreaks occurrence, specimens can be collected and shipped to the intermediate (provincial) or national level of the health system to support sequencing activities. In DRC, a decentralized approach was used for sequencing laboratories during EDV10, EVD11, EVD14 and Covid-19 in Goma; whereas a centralized approach was carried out during EVD10, EVD12, EVD13, EVD15 and Covid-19 in other parts of the DRC.

4.1.3. Evaluation of the performance of rapid diagnostic tests in suspect andconfirmed cases during eastern DRC EVD outbreaks

In this thesis, we estimated the performance of four Ebola RDTs against the GeneXpert® as the reference standard throughout three separated studies. In outbreaksettings, the QuickNavi™-Ebola RDT performed well as it showed high specificity and overall, quite good sensitivity, although it missed some samples, as they were detectedas positive with the GeneXpert® (Makiala et al, Mukadi et al). The OraQuick® Ebola and Coris® Ebola Ag K-SeT showed both a poor sensitivity for higher specificity under outbreak conditions. Thus, the

QuickNavi™-Ebola performance suggested a possibilityof its use at the triage or point-of-care under specific conditions such as in suspected-cases with EVD symptoms, as we assume that the viral load will be high enough to bedetected by RDTs. However, it is unlikely that all patients will come at the same stageof the infection, and the clinical manifestations may differ from one person to another regardless of the viral load. In laboratory conditions, the QuickNavi-Ebola™ showed less impressive performancecompared to our previous studies (Makiala et al, Mukadi et al). The OraQuick® Ebola Rapid Antigen test performance was almost in line with our previous findings (Mukadiet al), which masks that it performed better in this study after taking into account the Ct-value, with performance especially better for the middle and lower Ct-values, compared to our previous study. However, the manufacturer and other results reportedquite higher performances (Broadhurst et al, VanSteelandt et al, US FDA). In our study, the Standard® Q Ebola Zaïre Ag was only tested in laboratory conditions as it showeda very low sensitivity for a good specificity. In a previous study, Standard® Q Ebola Zaïre Ag was categorized as a test with the highest specificity and lower sensitivity compared to the other RDTs studied (Wonderly et al). The test also showed excess pooling on the sample reception pad, resulting in failure to flow on the membrane (Wonderly et al), as we noticed as well during our evaluations.

The disparate performance observed throughout our different studies can be discussed as follows:

1) False negative results could have been caused by a prozone effect. Indeed, in samples with high concentration of antigen, the flow across the sample pad can bestuck by multiple antibody-antigen complexes. It is possible to use some technicalities such as sample dilution, to reduce the prozone effect and improve thesensitivity of the test. However, these conditions can only be met in the laboratory setting, whereas the greatest benefit of using RDTs is at the triage or point-of-care for a quick decision-making. It is possible to dilute the samples in the laboratory onlyfor patients with very presumptive EVD symptoms associated to a negative Ebola RDT at the triage or POC. Those patients can wait a little bit longer to get their results while the sample

is being processed at the nearest laboratory for a maximumof safety. But the response team should determine, how opportune it is to wait for sample dilution while running RDTs, unless the RT-PCR is not available. In this case, sample dilution can be used as an alternative. Nonetheless, it will require venous blood instead of finger prick draw for example, as the sample has to be shipped to the laboratory.

2) The storage and transportation conditions could have modified the quality of the biological specimens used. During the outbreak, samples were subjected to multiple shipments and variable cold chain conditions from primary sites of testing to the temporary and long-term storage sites. Technical requirements in the implementation of the head-to-head study such as freeze-thaw process and aliquoting could have changed the quality of the specimens. In order to compensate possible sample quality loss due to the material storage over time, we kept one aliquot to be re-tested with the GeneXpert® (head-to-head comparison). Viral proteins such as VP40 and NP are usually abundantly expressed in infected organisms. We consequently expected our RDTs to detect more positive cases, as those abundantviral proteins were targeted. However, compared to the previous study (Mukadi et al), the general performance was almost identical for QuickNavi-Ebola™ and Coris® EBOLA Ag K-SeT, somewhat better for OraQuick® Ebola Rapid Antigen, and lowerthan expected for Standard® Q Ebola Zaïre Ag (designed to detect both VP40 and NP). From this angle, it clearly appears that decay of antigens in the samples couldhave partially affected the detection of these antigens in the RDTs.

3) Using archived whole blood samples instead of plasma could have slowed the migration over the membrane. However, during EBOV outbreaks the laboratory procedures do not allow specimen centrifugation, in order to mitigate the risk of environmental contamination. Thus, crude samples can only be shortly decanted or directly aliquoted. With the availability of additional testing (chemistry, hematology, sequencing and differential diagnosis) started from DRC tenth outbreak and onwards, additional volume of specimens was required and that diminished to the amount of plasma to be used in our study. Wonderly et al observed a reduced specificity of RDTs

using plasma versus EDTA whole blood, but no effect on the sensitivity was found. This observation warrants for separate performance evaluation with whole blood and plasma specimens. In our study, we only had EDTAwhole blood available for testing. Therefore, care should be taken before extrapolating these results to plasma or fresh samples tested in the field (Wonderlyet al).

4) From a commercial point of view, the interest to improve, develop, manufacture and stockpile Ebola RDTs is still a big problem. However, the situation of the 2013-2016 and 2018-2020 EVD outbreaks, respectively in West Africa and east of the DRC, has shown the possibility of the outbreak to spread beyond the natural sites of its emergence. From these two emergency situations, it raised the need to synergize all efforts towards the manufacturing of much better Ebola RDTs 1) to detect the virus in prevalent regions (Central and West Africa), 2) to prevent any importation of cases from the susceptible regions, 3) and to improve the surveillance system in at-risk areas.

From arguments highlighted above, the disparate performance of different RDTs clearly depicts the difficulty to outline clear decisions regarding their use in absence of the reference standard test. The QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests did not achieve the desired (sensitivity >98%, specificity >99%) or acceptable (sensitivity >95%, specificity >99%) levels of sensitivity and specificity as stated by the WHO TPP for EBOV tests (Wonderly et al, Mukadi et al, Emperador et al, WHO). But their respective specificities (>99% and 98%) were close to the acceptable level (>99%) of WHO TPP in most our studies. Nonetheless, we can propose QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests to be used as frontline diagnosis in remote areas to triage and isolate suspected-cases. All individuals will be isolated separately in the triage ward while waiting for RT-qPCR results i.e. those with at least one positive RDT in the high-risk area and those with negative results in low-risk area. To use RDTs in the field, we should take into consideration 1) the performance characteristics of the most sensitive tests, 2) the conditions of biosafety, and 3) the ease of use.

Based on the performance of RDTs in the field (Makiala et al/Viruses, Mukadi

et al/ postmortem, Mukadi et al/retrospective) and in the laboratory (Mukadi et al/head-to-head), the screening panel proposed at the point-of-care will consist of the QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen Test (high combined sensitivity and specificity) using finger prick or venous blood with an "OR" criterion for inclusion. Using this panel of two RDTs at a time will allow the detection of at least one target viral protein (NP or VP40), as the presence of antigens may vary in positive cases according to the stage of the infection.
At this stage, RDT use will not generate cost savings for EVD testing as RT-qPCR will still be needed to discriminate positives from negatives among suspected-cases. Additionally, RDTs should be sustainably available for application in the field.

From our studies, we learned that both QuickNavi-Ebola™ and OraQuick® Ebola RapidAntigen tests can be easily run and interpreted in field conditions. However, their implementation will require 1) adequate training on their correct use, 2) accurate reporting of their results, 3) correct shipment of clinical specimens to the laboratory for confirmatory RT-qPCR with venous blood collected after RDTs analysis, in order to exclude any false positives and false negatives (Mukadi-Bamuleka et al/Postmortem, Makiala et al, Mukadi-Bamuleka et al/Field performance). In the meantime, stringent and adequate measures should be taken to carefully triage and manage isolation of suspect-cases in care units, in order to mitigate the risk of EVD nosocomial transmission. Furthermore, the implementation of these RDTs should be supported bya clear at-risk communication plan, an excellent psychosocial environment and messaging, and detailed clinical and epidemiological data.
WHO TPP for EBOV tests aimed for a "desired level" of >98% sensitivity and >99%, and an "acceptable level" of >95% sensitivity for >99% specificity. QuickNavi-Ebola™ showed 85%, 87.4%, and 56.8% sensitivity; 99.8%, 99.6%, and 97.5% specificity on whole blood specimens in three different studies, respectively. OraQuick® Ebola displayed 100% sensitivity versus 97.8% specificity in cadavers, while it showed 57.4% and 61.6% sensitivity versus 98.3% and 98.1% specificity, in whole blood samples in two studies, respectively.

Based on the performance obtained from our studies, QuickNavi-Ebola™ and OraQuick® cannot confirm EVD cases, but they can suggest a high probability of infection among suspect-cases at the POC. A reactive test from at least one RDT should lead at a separated isolation of suspect-cases, while waiting for the RT-qPCR. Screening asymptomatic high-risk contacts may not be useful as the viremia raise is mostly associated with symptoms in infected individuals. For postmortem surveillance, a SDB should be carried out for any cadaver with a reactive or invalid OraQuick® RDT, as the assay showed to be more sensitive on oral fluid. However, to improve the likelihood of detection in alive suspect-cases, the association of both tests can provide a strong added value as a combination of performance using two different EBOV viral targets in screened samples.

It will be useful to provide innovative technologies to support automated reading of RDTs in place of naked eye reads, in order to minimize the risk of errors in the field. For future outbreak laboratories, providing training on the RDTs use, interpretation and reporting will be critical. Additionally, RDTs reading can use new technologies such as smartphone or electronic tablets with special software or application, to capture RDTs images and provide automated interpretation. However, this new approach should comply with biosafety and biosecurity measures, as the risk of contamination can be increased among staff handling those instruments. The ongoing trend is to decrease the number of steps to be carried out by lab-workers while processing highly pathogenic microorganisms in the field, in order to mitigate the risk of exposure to pathogens. Therefore, the devices to use in the field laboratories should be adapted to the environment with a well-defined circuit 1) to get high resolution and interpretable images, 2) to transmit collected images to a centralized system for real-time interpretation and archiving, 3) to ensure power supply of those devices, 4) to ensure maximum biosecurity of the personnel, including proper decontamination process. However, smartphone and tablets might not be easy to handle while operators are donned with full or light PPE (gloves, faceshield or goggles, etc).

4.1.4. Postmortem surveillance for EVD using OraQuick® Ebola RDT in theeastern DRC, 2019-2020

OraQuick® Ebola RDT was used in the postmortem surveillance during outbreak and in the post-EVD period in Mambasa (Ituri province) and Beni (North-Kivu province). The communities had a poor uptake of postmortem surveillance activity, as they were mostly opposed to systematic safe and dignified burials (SDBs) caried out during activeresponse period. OraQuick® Ebola RDT was brought up to sensibly decrease the number of SBDs to be performed in corpses with non-reactive results. On-site quick result delivery aimed to decrease the tension observed around deaths between response teams and communities, in order to associate the community to the decisionof carrying out a SDB or not. As OraQuick® Ebola RDT detected the virus in cadaverswith high viral load (low Ct-values), it was therefore suitable for large-scale use while screening cadavers in which EBOV viral load is mostly expected to be higher. Thus, in decedents with history of EVD suggestive symptoms such as fever, headache, bleeding, vomiting, diarrhea, jaundice etc, the OraQuick can be proposed to rule out any EVD case. However, as the implementation of postmortem activity is very sensitive, it will require leaders' support to foster the community engagement, which is the foundation of a successful response in the African context. Beyond the communityengagement, response teams noticed the need to improve data collection and reporting as well as in-depth training among local healthcare workers. All these resultswere obtained during the enhanced surveillance period. As the aftermath of this new strategy, the community strongly engaged into activities with high uptake, and local staff successfully conducted activities. In these conditions, the support of local leadersand community engagement led families to accept a SDB whereas the OraQuick® testwas non-reactive and could normally call for a traditional burial.

Postmortem surveillance with OraQuick® Ebola RDT can be included and maintained beyond the three months of post-EVD period. This tool can be used for routine testingon cadavers in the health facilities and communities, but it will

require regular provisionof field supplies (RDT, PPE etc), motivation fees for human resources, logistics (vehicles to transport teams, materials, fuel) etc. National leadership should be ready to go beyond outbreaks to perpetuate this activity across sites with high risk of EVD emergence in order to possibly detect unknown circulating EBOV strains and preventnew flare-ups in the concerned areas.

4.2. Limitations of our studies

The limitations and challenges related to different studies were separately discussed in each chapter. In this thesis, limitations encountered were mostly linked tothe retrospective character and the peculiar context in which these studies were conducted in the field.

The study on the QuickNavi™-Ebola RDT was not conducted on the same set of samples. The head-to-head comparison study would be the best option to determine the real performance of the tests. However, during EVD outbreaks, conditions for a head-to-head comparison are rarely met due to the emergency situation. We also thinkthat it would also be of benefit if the testing could be repeated in patients with negativeresults at the initial screening. Indeed, in some patients, the increase of viral loads should ensure the viral detection by RDTs a few days later.

In the pilot stage of the postmortem surveillance with OraQuick® Ebola RDT, violence against response teams and activities had led to relocation of the intervention site (from Mambasa to Beni). Upon the resumption of these activities in Beni health zone, there was no EVD confirmed case detected among cadavers. These aforementioned eventsstrongly impeded the dynamics of the postmortem surveillance in the concerned healthzones. For the post-epidemic period, all corpses tested did not reveal any EVD confirmed-case in Beni (the last health zone affected). Therefore, the performance of OraQuick® Ebola RDT, as shown in this study, might have been biased by the low number of positive cases as the surveillance was conducted at the tail-end of the outbreak (pilot phase) and in the enhanced surveillance period (post-epidemic) whereno confirmed-cases were reported.

The retrospective evaluation of three Ebola antigen RDTs versus the GeneXpert® results showed disparate performances. As the operators knew that only Xpert® Ebolaresults will serve for patient management, that could have altered their judgment in theinterpretation of the RDTs. The high workload and pressure in the field, the operators'variations in pipetting, reading time frame, number of buffer drops added and compliance with procedures might have

introduced variation in test results. Insufficienttime taken on-site for briefing, training and correction of errors could explain the variation between results of sites. The work in the glovebox with goggles or face shieldmight have altered the reading of faint test lines. The quality of samples collected, the delay between sample collection and testing, the samples and RDT storage conditions in the field could have also led to disparate performances. The three RDTs were evaluated on different sets of clinical samples with the same reference standard. However, a head-to-head comparison could address issues related to 1) the role of viral excretion kinetic in the bodily fluids considered, 2) the stage of the infection in thehost where the testing was performed and 3) the limit of the detection of the RDT considered.

In the head-to-head study, a few technical conditions could be pointed out as potentialsource for the suboptimal performance of the RDTs evaluated here: 1) archived specimens used with a possible decay of some viral target proteins, 2) storage conditions (freezing-thawing cycles), 3) only EDTA whole blood samples available, and 4) multiple shipments.

4.3. Conclusions

Decentralized and strategically positioned diagnostic laboratories quickly helped to mitigate the risk of Ebola virus spreading towards cities. Deployment and tiered management of field laboratories enabled all the pillars of the response, to conduct a well-structured response across sites. Field laboratories were supplied with dedicatedequipment, and well-prepared local personnel, drawing the new pathways to conduct prompt, efficient, accurate and multidisciplinary response. Thus, survivors' biological follow-up, hematological and biochemical testing in patients under specific therapeutics have become a classical component of EVD response starting from tenthoutbreak and onwards. The quick hand over of competences and capacities from staffof the national level to local teams led to sustained and successful management of further health emergency situations with less foreign experts (national and international) deployed. The empowerment of local staff was perpetuated by field laboratories, which were continuously run in certain concerned areas where they still be functional as frontline tools for the surveillance of EVD. By this way, they detected three EVD flare-ups [Butembo/Biena (February 2021), and Beni (October 2021 and August 2022)] and supported Covid-19 testing in the eastern DRC.

The sequencing laboratory provided a real-time dissemination of viral genomic data, to guide public health decision-making throughout the outbreak (orientation of epidemiological investigations, contact tracing, preemptive vaccination of contacts and frontline workers, determination of unknown transmission chains and sources of infection). Genomic sequencing can help understanding the dynamic of the virus spread in the community and defining better strategies to develop prevention and control measures as well as medical countermeasures against EVD.

The QuickNavi™-Ebola RDT showed high specificity and quite good sensitivity than OraQuick® Ebola rapid antigen and Coris® Ebola Ag K-SeT in outbreaks conditions. Inlaboratory conditions, QuickNavi-Ebola™ showed less impressive performance compared to the previous studies (Makiala et al, Mukadi et al).

The OraQuick® Ebola Rapid Antigen test performance was almost in line with previous findings (Mukadi et al), although it performed better for the middle and lower Ct-values, taking into account the Ct-value. The Standard® Q Ebola Zaïre Ag was only tested in laboratory conditions where it showed a low sensitivity for a good specificity. None of the four Ebola RDTs evaluated throughout our PhD studies, achieved the desired (sensitivity >98%, specificity >99%) or acceptable (sensitivity >95%, specificity >99%) levels of sensitivity and specificity as stated by the WHO TPP for EBOV tests. However, respective specificities of the QuickNavi™-Ebola (>99%) and OraQuick® Ebola (98%) in most studies were close to the acceptable level of performance (>99%).

Based on the overall performance (from the field and laboratory) observed in our studies, we propose a screening panel at the point-of-care consisting of the QuickNavi™ and Oraquick® Ebola Rapid Antigen tests (high combined sensitivity and specificity) using a finger prick or venous blood with an “OR” criterion for inclusion. This panel will support the triage and isolation of suspect-cases in remote areas, while they are waiting for the RT-qPCR results. Additionally, the results expectancy will be done in separated areas, individuals with at least one positive RDT will be in the high-risk area, whereas those with negative RDTs results will be isolated in low-risk area.

For postmortem surveillance, OraQuick® Ebola RDT effectively complemented the outbreak response efforts, improved the community trust, and decreased the number of systematic safe and dignified burials (SDBs) to be performed in corpses with non- reactive RDT. The strategy used during the implementation of the postmortem surveillance 1) decreased the tension between response teams and communities, 2) improved community engagement, 3) and led families to voluntarily request SDBs despite non-reactive OraQuick® Ebola tests in cadavers.

4.4. Lessons learned and ways forward

After eight successive EVD outbreaks in the last six years, the DRC has learned a lotand should therefore scale-up adapted strategies for the implementation and management of Ebola field laboratories, the diagnosis packages to provide to the laboratories or health facilities, and the organization of the surveillance system in this new configuration.

1) In the provinces at-risk for EVD outbreaks (Equateur, Kasai-central, Nord-Kivu, Ituri), it is advisable to maintain or deploy permanent frontline laboratories which will be considered as surveillance sentinel sites. Those frontline laboratories should be supplied with dedicated equipment to conduct biological follow-up (viral load measurements, biochemistry and hematology among treated patients, vaccinees, and survivors), well-prepared local personnel and additional resources (power source, water supply, transportation means, usable reagents/supplies, motivation fees) in order to quickly scale-up an efficient response against new EVD flare-ups.

   Activities to be conducted in those laboratories will include preemptive surveillance, long-lasting resilience and outbreak research activities. The preemptive surveillance will detect new spillover, relapse or resurgence of the virus among suspected-cases (alive or deaths) within the community. Long-lasting resilience will complement the preemptive surveillance with postmortem screening, and biological follow-up in EVD survivors. Research activities in the field laboratories will target subjects who benefited from medical countermeasures (vaccines & therapeutics) or those who survived from EVD.
2) The genomic sequencing activities and staff should be totally integrated to the response system. Therefore, the viral genomic data will be included into the different pillars of EVD response (laboratory, care management, vaccination, survivors' activities, and surveillance databases), and publicly shared in the SitRepas a part of routine activities during outbreaks.
3) Throughout this thesis, our findings on Ebola RDTs suggest the possibility of using a panel of tests based on the overall (field and laboratory) performances while including clinical manifestations (in alive suspected-

cases or reported symptoms in decedents), stage of the infection in patients (if considering symptoms or date of disease onset), epidemiological information, biosafety conditions and friendlinessin RDTs use. This screening panel will be used at the point-of-care, combining the QuickNavi-Ebola™ and OraQuick® Ebola Rapid Antigen tests with an "OR" criterion for inclusion on finger prick or venous blood specimens 1) to triage and isolate suspected-cases waiting for RT-qPCR, and 2) to separately isolate suspect-cases waiting for their RT-qPCR in two distinct areas (those with at least one positive RDT in the high-risk area, and those with negative results in low-risk area). The OraQuick® Ebola Rapid Antigen test can be used for permanent postmortem surveillance in the community and health facilities, as local personnel were empowered and demonstrated full proficiency during past EVD outbreaks activities in eastern DRC.

This strategy will enable the training of healthcare workers (either at the point-of-care or in the community) on the correct use of RDTsin suspected-cases (alive or death), on the accurate and real-time reporting of results to the surveillance system, and on the shipment of clinical specimens to the reference laboratory for confirmatory testing by RT-PCR.

DRC national authorities and their supporting partners should provide required RDTs to the frontline laboratories or health facilities of health zones at-risk for of EVD outbreaks emergence.

Beyond RDTs provision, national and supranational governments of countries at-risk for EVD emergence should commit to fund and support the development of assays within their borders. This strategy includes 1) building local work force, 2) building of adequate facilities outfitted with adapted equipment and other essentials, 3) creating hubs to import novel technologies, 4) involving local knowhow at each level of the decision-making, manufacturing and distribution of different assays, 5) producing antigens, antibodies, proteins, nucleic acids and other biological materials locally, 6) quick sharing of tools developed with other concerned regions or countries to build a surveillance network, 7) increasing the demand within the network to motivate local manufacturers.

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Scientific Curriculum vitae

Personal information

- Name: Daniel MUKADI-BAMULEKA
- Gender: Male
- Date of birth: 20-January-1984
- Marital status: Married (2 children)
- Country of citizenship: Democratic Republic of CongoAddress: B3J641, Salongo-Lemba, Kinshasa, DRC
- Email: drmukadi@gmail.com, daniel.mukadi@inrb.net
- Phone: +243 812 965 243, +243 998 273 290
- Place of Work : Laboratoire Rodolphe Mérieux INRB-Goma
- Work address : 29, Avenue des Orchidées, Les Volcans, Goma, DRC
- Language : English (Fluent), French (Fluent), others (Lingala, Kiswahili, Kikongo)
- Current Position: Regional Director of the P3/P2/P2 Rodolphe Mérieux INRB-GomaLaboratory

Education & Trainings

- 2008: Medical Doctor, Faculty of Medicine, University of Kinshasa, DRC
- 2012-2014: Specialization part I (Master I), Bacteriology and Mycology, KinshasaTeaching School of Medicine, University of Kinshasa
- 2015-2017: Specialization part II (Master II), Virology and Vaccine preventablediseases, Kinshasa Teaching School of Medicine, University of Kinshasa
- 2014: Clinical Immunology (long course: certificate of completion), Suez CanalUniversity, Ismailia, Egypt
- 2015: A Master level course of vaccinology (certificate of completion), CentreInfectieux Charles Mérieux, Mali, Bamako
- 2017: Vaccinology short course (certificate of completion), International VaccineInstitute (IVI), Seoul, South-Korea
- 2018: Molecular Biology and genomic sequencing (Intensive training), Robert KochInstitute, Berlin, Germany

- 2019: PhD Student, Institute of Tropical Medicine, Antwerp, Belgium
- 2022: Outbreak Investigation and Research short course (certificate of completion),Antwerp, Belgium
- 2022: 11th Advanced course on Diagnostics (ACDx), September 2022, Veyrier du Lac, Annecy, France

Personal Statement

This PhD is the fruit of continuous field work conducted throughout EVD outbreaks in DRC in which, I have implemented and managed several field laboratories. I have experienced surveillance, diagnosis and research activities with tools such as RT- qPCR, rapid diagnostic tests, genomic sequencing and other near point-of-care.

For several years, I have deployed, managed and coordinated field laboratories in thehinterland in Democratic Republic of Congo for surveillance and research purposes.

My skills encompass several fields of the laboratory such as Molecular Biology, Genomic Sequencing, Virology, Serology, Bacteriology, Immunology and Clinical- biology.

During the last six years, I was involved in the last eight EVD outbreaks in my country,the DRC. I have coordinated the laboratory response for the last six EVD outbreaks inDRC:

- Eighth (Bas-Uele province, 2017): Head of post-EVD ecological researchlaboratory
- Ninth (Equateur province, 2018): Head of Ebola field laboratory in Itipo
- Tenth (North-Kivu, Ituri and South-Kivu provinces, 2018-2020): Coordinator of Ebola INRB field laboratories in eastern DRC (13 laboratories managed)
- Eleventh (Equateur province, May-September 2020); I managed five laboratories
- Twelfth (Butembo/North-Kivu, February-May 2021)

- Thirteenth (Beni/North-Kivu, October-December 2021)
- Fourteenth (Mbandaka/Equateur, April-July 2022)
- Fifteenth (Beni/North-Kivu, August-September 2022)

Since the start of the Covid-19 pandemic in DRC (March 2020) until now, I am the Coordinator of INRB Covid-19 laboratories in the eastern DRC. I have implemented and still lead all Covid-19 activities 'Surveillance and Research' activities within INRB laboratories in the eastern of the DRC.

- Provinces of North-Kivu: 3 laboratories (Goma, Beni, Butembo),
- Province of Ituri: 1 Lab (Bunia)
- Province of Haut-uele: 2 Labs (Durba and Isiro)
- South-Kivu (Bukavu)

In 2018, the WHO, the Ministry of health of DRC (MoH), INRB and other partners decided to create a follow-up program for EVD survivors in DRC. Starting from the tenth outbreak up to now, I am leading the Laboratory of EVD Survivors program in DRC. Actually, three mobile clinics are opened in Beni, Butembo and Mangina (North-Kivu province) and three others in Mbandaka, Bikoro and Itipo (Equateur Province).

I was personally involved in several research activities:

- Monkeypox Therapeutics "A randomized, placebo-controlled, double-blinded trial ofthe safety and efficacy of tecovirimat (TPOXX®) for the treatment of adult and pediatric patients with monkeypox virus disease" clinical trials with NIH/NIAID(Clinical Laboratory Specialist Co-Lead)
- PALM Study Ebola Therapeutics "A Randomized Controlled Trial" with NIH/NIAID (Laboratory Coordinator)
- Immunogenicity of Johnson & Johnson Ebola vaccines (Ad26.ZEBOV & MVA-BN- Filo) with LSTHM & Epicentre/MSF (Laboratory Manager);
- International study (InVITE) on Covid-19 Vaccines Immunogenicity, reactogenicity and efficacy in DRC with NIH/NIAID (Laboratory Coordinator);
- Survey on incidence and prevalence of SARS-CoV-2 among healthcare workers inDRC: A longitudinal study with US CDC (Co-Investigator)

- Monitoring of immunity induced by the rVSV-ZEBOV-GP vaccine against Ebola virus in healthcare providers previously and recently vaccinated in DRC“EBOSURV” with ITM-Antwerp/EDCTP (Co-Investigator)
- International study on clinical characterization of Covid-19 in Africa with ALERRT- CCP/ITM-Antwerp/INRB (Co-Investigator)
- The impact of COVID 19 on primary health care service provision and utilization in Tanzania, Sierra Leone and the Democratic Republic of Congo with LSHTM (Lab coordinator)
- Impact of Cholera mass vaccination on the excretion of vibrio in feces among vaccinees in DRC with Epicentre & John Hopkins University (Lab Coordinator)
- Biological follow-up of EVD survivors “Les vainqueurs” with INSERM and IRD Montpellier (Laboratory Coordinator)
- Assessment of immunity against Poliomyelitis, measles and other vaccine preventable diseases in children of 6-59 months in Haut-Lomami, DRC with UCLA-Health and Training Program (Lab Manager)
- Ebola antibodies detection among healthcare workers in DRC (Haut-Lomami,Mongala, Tshuapa) with UCLA-Health and Training Program (Lab Manager)
- Prevalence, sexual practices and genetic diversity of HIV/HBV/HCV among men who have sex with other men in DRC with University of Kinshasa/Laboratoire Mixte International-IRD-Montpellier (Principal Investigator)

I conducted investigation and research in the field of emerging infectious diseases such as (Ebola, Monkeypox, Measles, Poliomyelitis, HIV, HBV, HCV, Arbovirus, Covid-19 etc).

My knowledge of the field, especially of the eastern part of the DRC, my capacity of leadership and management supported my appointment as the Regional Director of the Rodolphe Mérieux INRB-Goma, the first branch of Institut National de Recherche Biomédicale (INRB) which actually serves 5 different provinces of DRC.

Positions, Scientific Appointments, and Honors

- **Positions and Employment**

- June 2020-: Regional Director of the P3/P2/P2 Rodolphe Mérieux INRB-GomaLaboratories, Goma, DRC

  (Member of the GABRIEL Network)
- August 2018-: Field Coordinator of INRB Ebola Field Laboratories in Eastern DRC(Nord-Kivu, Sud-Kivu, Ituri and Haut-Uele Provinces)
- June 2020-: Field Coordinator of Covid-19 INRB Field Laboratories in Eastern DRC(Nord-Kivu, Sud-Kivu, Ituri and Haut-Uele Provinces)
- August 2021-: Lab Coordinator in "International Study on COVID-19 Vaccine to assess immunogenicity, reactogenicity and efficacy (InVITE), funded by NIH/NIAID
- November 2018-: Lab Coordinator of Ebola Survivors Laboratories in DRC and National Head-Deputy of Ebola National Survivors Follow-up program
- June 2020-: Head-Deputy of Ebola Rapid diagnostic Tests consortium for Postmortem surveillance and testing in eastern DRC
- November 2018-December 2020: Lab Coordinator in "A Multicenter, Multi- Outbreak, Randomized, Controlled Safety and Efficacy Study of Investigational Therapeutics for the Treatment of Patients with Ebola Virus Disease" funded by NIH in the DRC (NIH/LEIDOS/TMG Ebola PALM Base Operations Initiative).
- June 2015-July 2017: Lab Manager at UCLA Health Training Program in DRC
- 2014-: Head of Research Unit on key populations,

  Virology Unit, Department of Medical Biology, Kinshasa teaching

  School of Medicine, University of Kinshasa, DRC
- November 2019-: PhD Student at Institute of Tropical Medicine-Antwerp/Universityof Antwerp, Belgium
- February 2021 to now: Member of Pedagogic Committee, Advanced

course onDiagnostics in Africa (ACDx)

- **Honors**

- 2014: Scholarship for “International Course of Clinical Immunology”, Suez CanalUniversity, Ismaïlia, Egypt
- 2015: Scholarship for “Vaccinology in Africa- A Master Level course”, Centre d’Infectiologie Charles Mérieux, Bamako, Mali
- 2017: European Congress of Tropical Medicine and International Health (ECTMIH) 2017, Antwerp, Belgium, Yearly meeting, Travel Award: Oral presentation
- 2017: Scholarship for “Vaccinology Course”, International vaccine Institute, Seoul,South-Korea
- 2019: Scholarship from Belgian Directorate-General Development Cooperation fora PhD in Biomedical Sciences
- 2022: Travel Grant for “11ème Conférence internationale francophone, AfraVIH2022 », Marseille, France : Oral presentation
- 2022 : Scholarship grant for Outbreak and investigation research course, ITM-Antwerp, Belgium
- 2022 : Travel Grant for “11th Advanced course on Diagnostics (ACDx)”, 18-23September 2022, Veyrier du Lac, Annecy, France
- 2022: Best young researcher/Best scientific production Award, University ofKinshasa researchers’ ranking, November 2021-May 2022.

Supervised students

1) Junior BULABULA PENGE, Performance et implémentation du test rapide Oraquick® Ebola chez les décès communautaires pendant la dixième épidémie en République Démocratique du Congo, Medical Biologist specialist Degree, June 2021
2) François EDIDI ATANI, Persistance du virus Ebola dans les humeurs biologiques des survivants de la dixième épidémie de la Maladie à virus Ebola en RD Congo, Medical Biologist specialist Degree, June 2021
3) Fabrice MAMBU MBIKA, Déploiement et gestion des laboratoires mobiles Ebola dans un contexte d’insécurité, Medical Biologist specialist Degree, December 2021

4) Meris MATONDO KUAMFUMU, Apport du séquençage sur terrain dans le retraçage des chaînes de transmission des cas pendant la dixième épidémie de la Maladie à virus Ebola en RD Congo, Medical Biologist specialist Degree,June 2022

Contributions to Science

1. **Coronaviruses Are Abundant and Genetically Diverse in West and Central African Bats, including Viruses Closely Related to Human Coronaviruses**

   Dowbiss Meta Djomsi, Audrey Lacroix, Abdoul Karim Soumah, Eddy Kinganda Lusamaki, Asma Mesdour, Raisa Raulino, Amandine Esteban, Innocent Ndong Bass, Flaubert Auguste Mba Djonzo, Souana Goumou, Simon Pierre Ndimbo-Kimugu, Guy Lempu, Placide Mbala Kingebeni, **Daniel Mukadi-Bamuleka**, Jacques Likofata, Jean-Jacques Muyembe Tamfum, Abdoulaye Toure, Eitel Mpoudi Ngole, Charles Kouanfack, Eric Delaporte, Alpha Kabinet Keita, Steve Ahuka-Mundeke, Ahidjo Ayouba and Martine Peeters, **Viruses 2023, 15, 337.** https://doi.org/10.3390/v15020337

2. **Efficiency of Field Laboratories for Ebola Virus Disease Outbreak during Chronic Insecurity, Eastern Democratic Republic of the Congo, 2018-2020.**

   Mukadi-Bamuleka D, Mambu-Mbika F, De Weggheleire A, Edidi-Atani F, Bulabula-Penge J, Mfumu MMK, Legand A, Nkuba-Ndaye A, N'kasar YTT, Mbala-Kingebeni P, Klena JD, Montgomery JM, Muyembe-Tamfum JJ, Formenty P, van Griensven J, Ariën KK, Ahuka-Mundeke S. **Emerg Infect Dis. 2023 Jan;29(1):1-9. doi: 10.3201/eid2901.221025.** PMID: 36573642.

3. **Development of Ebola virus disease prediction scores: Screening tools for Ebola suspects at the triage-point during an outbreak.**

   Tshomba AO, **Mukadi-Bamuleka DR**, De Weggheleire A, Tshiani OM, Kitenge RO, Kayembe CT, Jacobs BKM, Lynen L, Mbala-Kingebeni P, Muyembe-Tamfum JJ, Ahuka-Mundeke S, Mumba DN, Tshala-Katumbay DD, Mulangu S. **PLoS One. 2022 Dec 16;17(12):e0278678.** doi:10.1371/journal.pone.0278678. PMID: 36525443; PMCID: PMC9757576.

4. **Preventing future pandemics and epidemics through A North-Southcollaboration on genomic surveillance in Africa**
   Vittorio Colizzi, Claudia Alteri, Stefano D'Amelio, Ibrahim Hassan Garba, MartaGiovanetti, Florence Komurian-Pradel, José Lourenço, Stella Mazeri, Adrian Muwonge, Carlo Federico Perno, Gianluca Russo, **Daniel Mukadi-Bamuleka Journal of Public Health in Africa. 2022; 13:2319.** https://doi.org/ 10.4081/jphia.2022.2319
5. **"The evolving SARS-CoV-2 epidemic in Africa: Insights from rapidly expanding genomic surveillance" in Science.**
   Tegally H et al. **Science. 2022 Sep 15:eabq5358.** doi:10.1126/science.abq5358.Epub ahead of print. PMID: 36108049.
6. **Virus kinetics and biochemical derangements among children withEbolavirus disease.**
   Lindsey Kjaldgaard, Kasereka Masumbuko Claude, **Daniel Mukadi-Bamuleka**, Richard Kitenge-Omasumbu, Devika Dixit, Francois Edidi-Atani, Meris Matondo Kuamfumu, Junior Bulabula-Penge, Fabrice Mambu-Mbika, Olivier Tshiani-Mbaya,Janet Diaz, Sabue Mulangu, Anais Legand, Placide Mbala-Kingebeni, Pierre Formenty, Steve Ahuka-Mundeke, Jean-Jacques Muyembe-Tamfum and Michael T.Hawkes
   eClinicalMedicine2022;53:101638,https://doi.org/10.1016/j.eclinm.2022.101638
7. **Ebola virus: DRC field laboratories' rapid response.**
   Mukadi-Bamuleka D, Ahuka-Mundeke S, Ariën KK. **Nature. 2022 Apr;604(7905):246.** doi: 10.1038/d41586-022-01005-6. PMID: 35414664.
8. **Added value of an anti-Ebola serology for the management of clinically suspect Ebola virus disease patients discharged as negative in an epidemiccontext.**
   Nkuba-Ndaye A, **Mukadi-Bamuleka D**, Bulabula-Penge J, Thaurignac G, Edidi- Atani F, Mambu-Mbika F, Danga-Yema B, Matondo-Kuamfumu M, Kinganda- Lusamaki E, Bisento N, Lumembe-Numbi R, Kabamba-Lungenyi G, Kitsa- Mutsumbirwa D, Kambale-Sivihwa N, Boillot F, Delaporte E, Mbala-

Kingebeni P, Ayouba A, Peeters M, Ahuka-Mundeke S.

J Infect Dis. 2022 Feb 18:jiac057. doi: 10.1093/infdis/jiac057. Epub ahead of print.PMID: 35176762.

9. **Field performance of three Ebola rapid diagnostic tests used during the 2018-20 outbreak in the eastern Democratic Republic of the Congo: aretrospective, multicentre observational study.**

   Mukadi-Bamuleka D, Bulabula-Penge J, De Weggheleire A, Jacobs BKM, Edidi- Atani F, Mambu-Mbika F, Mbala-Kingebeni P, Makiala-Mandanda S, Faye M, Diagne CT, Diagne MM, Faye O, Kajihara M, Faye O, Takada A, Sall AA, Muyembe-Tamfum JJ, van Griensven J, Ariën KK, Ahuka-Mundeke S.

   Lancet Infect Dis. 2022 Mar 14:S1473-3099(21)00675-7.

   doi: 10.1016/S1473-3099(21)00675-7. Epub ahead of print. PMID: 35298901.

10. **Postmortem Surveillance for Ebola Virus Using OraQuick Ebola Rapid Diagnostic Tests, Eastern Democratic Republic of the Congo, 2019–2020**

    Daniel Mukadi-Bamuleka, Yibayiri Osee Sanogo, Junior Bulabula-Penge, Maria E. Morales-Betoulle, Patrice Fillon, Patrick Woodruff , Mary J. Choi, Amy Whitesell,

    Alison M. Todres, Anja De Weggheleire, Anaïs Legand, Jean-Jacques Muyembe- Tamfum, Pierre Formenty, John D. Klena, Joel M. Montgomery, Steve Ahuka- Mundeke, RDT Working Group. **Emerging Infectious Diseases • www.cdc.gov/eid Vol. 28, No. 2, February 2022,** DOI: https://doi.org/10.3201/eid2802.210981,

11. **Clinical Evaluation of QuickNaviTM-Ebola in the 2018 Outbreak of Ebola Virus Disease in the Democratic Republic of the Congo**

    Sheila Makiala, **Daniel Mukadi**, Anja De Weggheleire, Shino Muramatsu, Daisuke Kato, Koichi Inano, Fumio Gondaira, Masahiro Kajihara, Reiko Yoshida, Katendi Changula, Aaron Mweene, Placide Mbala-Kingebeni, Jean-Jacques Muyembe- Tamfum, Justin Masumu, Steve Ahuka, Ayato

Takada

Viruses 2019, 11, 589; doi:10.3390/v11070589, www.mdpi.com/journal/viruses, PMID: 31261668

12. **Zoonotic risk factors associated with seroprevalence of Ebola virus GP antibodies in the absence of diagnosed Ebola virus disease in the DemocraticRepublic of Congo**

    Nicole A. Hoff, Reena H. Doshi, Adva Gadoth, Megan Halbrook, Patrick Mukadi, Kamy Musene, Benoit Ilunga-Kebela, D'Andre Spencer, Matthew S. Bramble, David McIlwan, J. Daniel Kelly, **Daniel Mukadi**, Placide Mbala-Kingebeni, Steve Ahuka-Mundeke, Emile Okitolonda-Wemakoy, Jean-Jacques Muyembe-Tamfum, Anne W. Rimoin. **PLoS Negl Trop Dis 15(8): e0009566.** https://doi.org/10.1371/journal.pntd.0009566 PMID: 34383755, PMCID: PMC8384205

13. **Risk factors for Ebola exposure in healthcare workers in Boende, Tshuapa Province, Democratic Republic of the Congo**

    Reena H Doshi, Nicole A Hoff, Anna Bratcher, Patrick Mukadi, Adva Gadoth, BradlyP Nicholson, Russell Williams, **Daniel Mukadi**, Matthias Mossoko, Joseph Wasiswa, Alexis Mwanza, Cyrus Sinai, Vivian H Alfonso, Rupal Shah, Matthew S Bramble, Benoit Ilunga-Kebela, Emile Okitolonda-Wemakoy, Jean-Jacques Muyembe-Tamfum, Anne W Rimoin

    J Infect Dis. 2020 Dec 3;jiaa747. doi: 10.1093/infdis/jiaa747.

    PMID: 33269402

14. **Investigating the Circulation of Ebola Viruses in Bats during the Ebola VirusDisease Outbreaks in the Equateur and North Kivu Provinces of the Democratic Republic of Congo from 2018**

    Audrey Lacroix, Placide Mbala Kingebeni, Simon Pierre Ndimbo Kumugo, Guy Lempu, Christelle Butel, Laetitia Serrano, Nicole Vidal, Guillaume Thaurignac, Amandine Esteban, **Daniel Mukadi-Bamuleka**, Jacques Likofata, Eric Delaporte, Jean-Jacques Muyembe Tamfum, Ahidjo Ayouba, Martine Peeters and Steve Ahuka Mundeke

    Pathogens 2021, 10, 557. https://doi.org/10.3390/pathogens10050557, PMID: 34064424, PMCID: PMC8147758

15. **Role of Wildlife in Emergence of Ebola Virus in Kaigbono (Likati), DemocraticRepublic of the Congo, 2017**

Sophie Gryseels, Placide Mbala-Kingebeni, Innocent Akonda, Roger Angoyo, Ahidjo Ayouba, Pascal Baelo, **Daniel Bamuleka Mukadi**, Elie Bugentho, Trenton Bushmaker, Christelle Butel, Sébastien Calvignac-Spencer, Eric Delaporte, Birgit De Smet, Ariane Düx, François Edidi-Atani, Robert Fischer, Corneille Kahandi, Jimmy Kapetshi, Servet Kimbonza Sumba, Léonce Kouadio, André MalekaniBendeke, Claude Mande, Guy Midingi Sepolo, Joseph Moudindo, Eitel Mpoudi Ngole, Prescott Musaba, Patrick Mutombo, Innocent Ndong Bass, Casimir Nebesse, Steve Ngoy, Simon-Pierre Ndimbo Kumogo, Stephanie N. Seifert, Jacques Tanzito, Dudu Akaibe, Nicaise Amundala, Kevin K. Ariën, Guy-Crispin Gembu, Fabian H. Leendertz, Herwig Leirs, Jean-Claude Mukinzi, Vincent Munster, Jean-Jacques Muyembe-Tamfum, Martine Peeters, Erik Verheyen, SteveAhuka-Mundeke

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 9, September 2020,

DOI:https://doi.org/10.3201/eid2609.191552, PMID: 32818404, PMCID: PMC7454093

16. **Rapid confirmation of the Zaire Ebola Virus in the outbreak of the Equateur province in the Democratic Republic of Congo: implications for public healthinterventions.**

Placide Mbala Kingebeni 1,2,3, Christian-Julian Villabona-Arenas 3, Nicole Vidal 3,Jacques Likofata4, Justus Nsio-Mbeta 5, Sheila Makiala-Mandanda 1,2, **Daniel Mukadi** 1,2, Patrick Mukadi 1,2, Charles Kumakamba1,2, Bathe Djokolo 5, AhidjoAyouba 3, Eric Delaporte 3, Martine Peeters 3, Jean-Jacques Muyembe Tamfum 1,2, Steve Ahuka Mundeke 1,2

Lancet Infect Dis. 2019 Jun;19(6):648-657.doi: 10.1016/S1473-3099(19)30118- 5. Epub 2019 Apr 15. PMID: 29961823 PMCID: PMC6321851

17. **Medical countermeasures during the 2018 Ebola virus disease outbreak in the North Kivu and Ituri Provinces of the Democratic Republic of the Congo:a rapid genomic assessment**

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