Research article

Development and deployment of a rapid recombinase polymerase amplification Ebola virus detection assay in Guinea in 2015

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In the absence of a vaccine or specific treatments for Ebola virus disease (EVD), early identification of cases is crucial for the control of EVD epidemics. We evaluated a new extraction kit (SpeedXtract (SE), Qiagen) on sera and swabs in combination with an improved diagnostic reverse transcription recombinase polymerase amplification assay for the detection of Ebola virus (EBOV-RT-RPA). The performance of combined extraction and detection was best for swabs. Sensitivity and specificity of the combined SE and EBOV-RT-RPA were tested in a mobile laboratory consisting of a mobile glovebox and a Diagnostics-in-a-Suitcase powered by a battery and solar panel, deployed to Matoto Conakry, Guinea as part of the reinforced surveillance strategy in April 2015 to reach the goal of zero cases. The EBOV-RT-RPA was evaluated in comparison to two real-time PCR assays. Of 928 post-mortem swabs, 120 tested positive, and the combined SE and EBOV-RT-RPA yielded a sensitivity and specificity of 100% in reference to one real-time RT-PCR assay. Another widely used real-time RT-PCR was much less sensitive than expected. Results were provided very fast within 30 to 60 min, and the field deployment of the mobile laboratory helped improve burial management and community engagement.

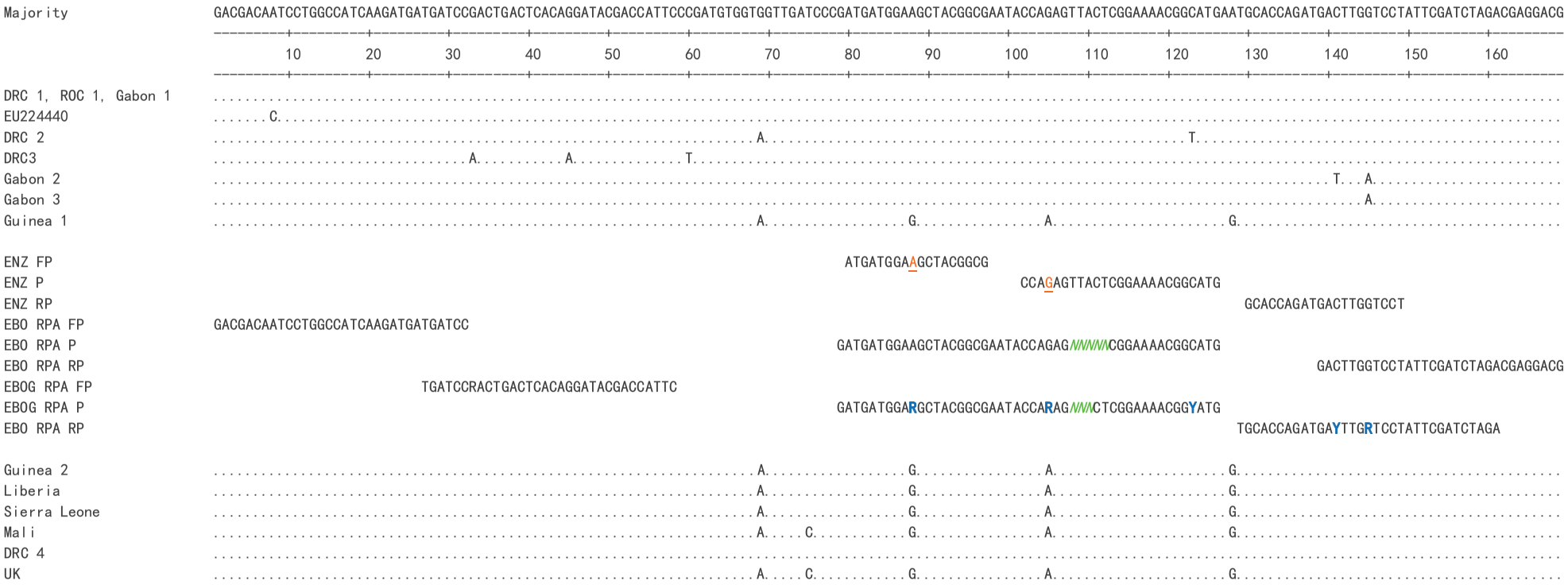
Introduction

As of 11 October 2015, the ongoing Ebola virus disease (EVD) epidemic in West Africa has resulted in more than 28,500 cases and over 11,300 deaths. The early symptoms of EVD (i.e. fever, fatigue, headache, vomiting and diarrhoea) are unspecific and present a challenge for clinical diagnosis [1]. In humans, death occurs generally seven to 10 days after the onset of symptoms. Survivors can be ill for up to 22 days before recovering [2]. Ebola virus (EBOV) infection is mainly diagnosed by various in-house and commercial real-time RT-PCR assays [3], used in up to 38 laboratories implemented at or close to Ebola treatment centres (ETC) in West Africa [4]. Transmission of EVD occurs almost exclusively from human to human by direct contact with body fluids of symptomatic cases. Consequently, the control strategy for EVD epidemics relies on early identification of EBOV-infected patients and corpses for, respectively, isolation and safe burials. It is imperative to trace and follow up contacts and to implement infection control measures.

Therefore, rapid EVD diagnostics impact on outcome of treatment, efficiency of contact tracing and subsequently community engagement, which is central to the successful control of the EVD epidemic. The World Health Organization (WHO) launched a call and consultation for an emergency procedure under its

Figure 1

Alignment of Ebola virus nucleocapsid sequences by country of origin and variant



DRC: Democratic Republic of Congo; ROC: Republic of Congo.

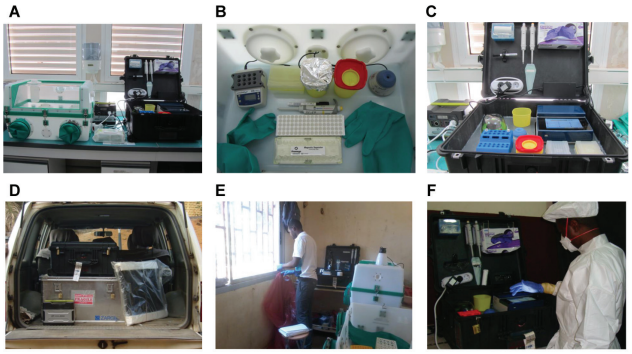
Upper third of the alignment: Sequences from previous and ongoing outbreaks on which the oligonucleotide design was based. GenBank accession numbers: DRC 1 (EBOV Mayinga): NC002549, KC242801, KC242791, AF086833, J04337, AY142960, AF499101, AF272001, KM655246; ROC 1: KM519951; Gabon 1 (EBOV Gabon 94): Y09358, AY058895, EU51640–50, KC242794, KC242798, KC242800; DRC 2: HQ613402–03, KC242784–90; DRC 3 (EBOV Kikwit): KC42796, KC24799, JQ352763, AY354458, AF054908; Gabon 2: EU051639, 2793, KC242795, KC242797; Gabon 3: KC242792; Guinea 1 (EBOV Makona): KJ660346–48.

Centre: Real time-RT-PCR and RT-PRA primers and probes.

Lower third of the alignment: Sequences published since the design. Guinea 2: KP096420–22; Liberia: KM251803, KP178538; Sierra Leone: 99 EBOV genomes [16]; Mali: KP260799–801; DRC 4: KP271018–20; United Kingdom: KP120616, KP184503, KP658432. Degenerated nucleotides are highlighted in bold, mismatches are underlined, NNN represents the gap for the tetrahydrofuran bridge connecting the two sections of the exo probe.

Figure 2

Mobile laboratory for Ebola virus diagnostics



A: Complete mobile laboratory including the solar power pack.

B: The Diagnostics-in-a-Suitcase (DiaS) contains all equipment and reagents to perform up to 100 RT-RPA assays

C: The workspace inside the glovebox contained a heat block mini (VWR International GmbH, Erlangen, Germany), a 96 box each of 100 µl and 1,000 µl sterile filter tips (BRAND, Wertheim, Germany), and two respective automatic micropipettes (Eppendorf AG, Hamburg, Germany), a waste container (Sarstedt, Nuembrecht, Germany), a magnetic separator stand (Promega, Madison, US), a rack for 1.5–2 ml tubes and a marker pen.

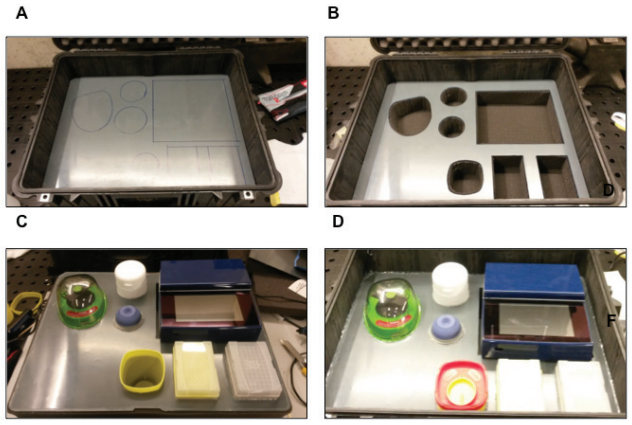
D: Transfer of the mobile laboratory (DiaS, aluminium box containing the glovebox and the PPE, solar panel and power pack).

E: Setup of the glovebox and the DiaS at a hospital in Matoto.

F: Ebola RT-RPA assay in the DiaS.

Figure 3

Assembly of the Diagnostics-in-a-Suitcase



A: A PVC layer was placed on top of the foam filling the bottom of the suitcase.

B: Bespoke insert slots were cut out of the PVC and the foam layer to host the tubescanner, the box for the disinfection wipes, the waste container, the vortex, the minicentrifuge and two boxes of refill pipette tips.

C: Foam, PVC layer and instruments were assembled outside the suitcase. Electricity wires were stowed underneath the foam layer. The equipment was fixed to the PVC layer using hot glue.

D: The setup was placed into the suitcase and the seams were sealed with hot glue.

pre-qualification programme for diagnostic tool assessment [5] to support accelerated development, production and deployment of adapted and rapid Ebola tests. Early in 2015, only three commercial real-time RT-PCR assays (RealStar Filovirus Screen, Altona Diagnostics, Hamburg, Germany; Liferiver Ebola Virus, Shanghai ZJ BioTech Co., Shanghai, China; GeneXpert Ebola virus, Cepheid, Solna, Sweden) and one rapid antigen detection test (ReEBOVTM (Corgenix, Denver, United States (US)) had been approved for emergency use, emphasising the need for such tests. At the time of publication of this article, nine real-time PCR assays for Ebola virus detection have been approved by the WHO.

In this study, we describe the optimisation, evaluation of performance and operational characteristics of a real-time RT-PCR [6] and a rapid RT-recombinase polymerase amplification (RPA) [7] used for diagnosis of suspected Ebola cases, and compare them with the RealStar Filovirus Screen RT-PCR approved for emergency use. In addition, we report the efficient field deployment of the rapid RT-RPA which boosted community engagement for safe and dignified burials.

Methods

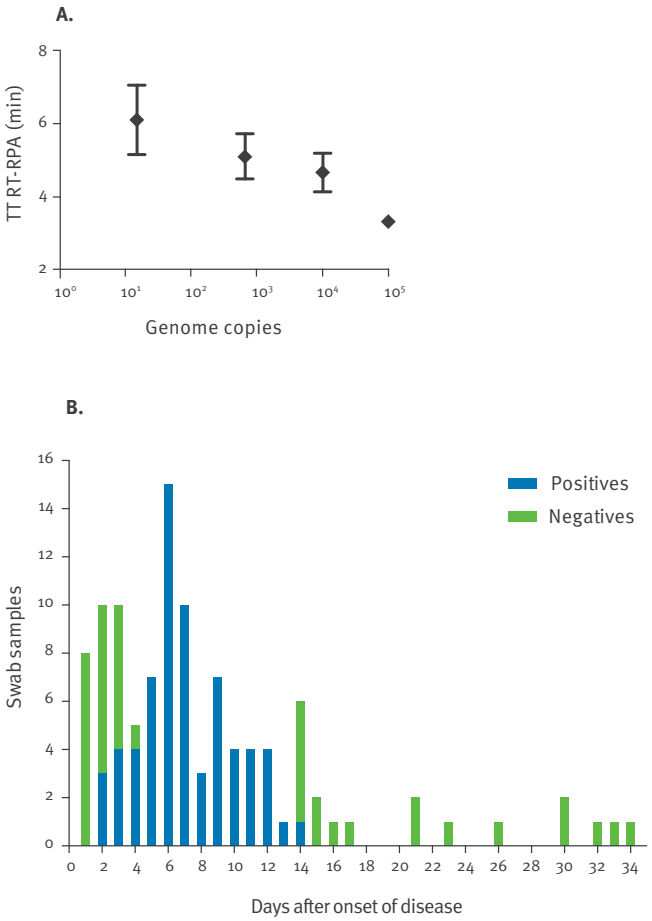
Study design and samples

The study was conducted during the 2014–15 EBOV outbreak in Guinea. On 23 March 2014, the Institute Pasteur de Dakar (IPD), Senegal, upon request of the WHO and the Guinean Ministry of Health deployed a mobile laboratory team to Conakry. An ETC was set up at Donka hospital in Conakry. Serum samples from acute cases and swabs (cheek and tongue) from deceased meeting the WHO definition of a suspected EVD case (see below) were collected in Conakry, Matoto, Télimélé, Coyah and other regions of Guinea between December 2014 and May 2015 and sent to our laboratory for diagnosis. In addition, following an upsurge of EVD cases connected to funeral rites, oral swabs from all deceased were tested at the morgue in Matoto in March and April 2015. During this study, the EBOV RT-RPA was evaluated in parallel to reference methods.

Suspected EVD cases were defined as any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with a suspected, probable or confirmed case of EVD, or any person with sudden onset of high fever and at least three of the following symptoms: headaches, anorexia/loss of appetite, lethargy, aching muscles or joints,

Figure 4

Sensitivity of the Ebola RT-recombinase polymerase amplification test on (A) inactivated Ebola virus and (B) swab samples, Guinea, December 2014–May 2015 (n=138)



RPA: recombinase polymerase amplification; RT: reverse transcription; TT: threshold time.

A: Plasma samples spiked with inactivated Ebola virus (source: ENIVD) extracted and re-quantified by real-time PCR. Extracts (1.5 × 105, 7.0 × 102, 1.0 × 104, 1.0 × 105 Ebola genome molecules /reaction) were tested in triplicate by RT-RPA. The detection limit was 15 Ebola genome molecules/reaction in a maximum of 8 min.

B: Results from 138 swab samples from deceased suspected EVD cases with symptoms among a cohort of 928 samples from deceased with and without symptoms. Positive and negative results are scored over days after onset of disease.

breathing difficulties, and any person with inexplicable bleeding or any sudden inexplicable death.

RNA extraction and inactivation

Two extraction methods were used. In the first method, viral RNA was extracted from 100 µl serum or swab transport medium using the QIAamp Viral Mini Kit (QC; Qiagen, Hilden, Germany). RNA was eluted in 50 µl Tris-EDTA buffer. The second extraction protocol (SpeedXtract Nucleic Acid Kit (SE), Qiagen, Hilden, Germany) was a reverse extraction method extracting protein debris by way of magnetic beads after an initial 10 min heating step at 95°C. It yielded 200 µl supernatant from 20 µl of serum or oral swab transport fluid diluted 1:2 with molecular grade water.

We added 5 µl of either eluate to the W-PCR (EBOV one-step real-time RT-PCR described in [6]) and the optimised RT-RPA, and 10 µl to the A-PCR (The RealStar Filovirus RT-PCR Kit, Altona-Diagnostics, Hamburg, Germany).

To test for inactivation of EBOV by the new SE kit, SE extract dilutions from 10^{-1} to 10^{-5} were added in triplicate onto 2 × 10^5 VeroE6 cells in a 96-well plate and incubated for five days. The supernatant was passaged three times by transfer to a new well, followed by a 3 h incubation, a wash, and another 48 h incubation step. Finally, cells were washed three times and RNA was extracted in 200 µl Trizol and submitted to an EBOV in-house PCR. For each dilution, three more wells to which the supernatants had been added in the same manner were subjected to an immunofluorescence assay after passage 1 [8]. A not extracted patient serum sample was used as positive control and showed virus growth on VeroE6 cells.

Real-time RT-PCR

The W-PCR was performed on the SmartCycler (Cepheid, Sunnyvale, US) using the RNA Master Hybridisation Probes kit (Roche, Manheim, Germany). A dried 10-fold primer and probe mix containing 100 pmol EBOZ FP and EBOZ RP and 50 pmol EBOZ P (TIB Molbiol, Germany) was used. The A-PCR was used on the SmartCycler according to the manufacturer`s instructions. Positive results above cycle threshold (Ct) 35 were regarded as equivocal and repeated for confirmation [9].

RT-RPA assay

The primers and the exo probe of an existing EBOV RT-RPA assay [7] were redesigned to adopt mismatches of the current EBOV outbreak strain (Figure 1, Table 1) following RPA design guidelines [10].

The RT-RPA was performed using a custom-made EBOV-specific exo RT kit with pellets containing optimised enzyme concentrations similar to the commercial TwistAmp RT exo kit [10,11], and additionally containing primers and probe. Briefly, 5 µl of RNA template and 45 µl of customised rehydration buffer containing magnesium acetate were added to each pellet in a strip of eight tubes delivered in vacuum-sealed pouches. In each strip, tubes 1 to 5 were used to test samples, tube 6 was used as negative extraction control and tubes 7 and 8 for a negative and positive RT-RPA reaction control. The reaction tubes were mixed, centrifuged and then placed into the ESEQuant TS2 (QIAGEN Lake Constance GmbH, Stockach, Germany) for real-time monitoring of fluorescence at 42°C for 15 min, with brief mixing and centrifugation of the reaction tubes after 4 min. The resulting curves were analysed by TS2 Studio Version 1.8.2.0 (QIAGEN Lake Constance GmbH, Stockach, Germany). Increase of fluorescence intensity over time above the mean background signal

Table 1

Primers and probe designed for the updated Zaire ebolavirus RT-recombinase polymerase amplification assay

Name	RPA primers and exo probes
EBOG RPA FP	**TGATCCRACTGACTCACAGGATACGACCATT*C**
EBOG RPA RP	TCTAGATCGAATAGGA**Y**CAA**R**TCATCTGGTGC*A
EBOG RPA P	GATGATGGA**R**GCTACGGCGAATACCA**R**AG-**BTF**-CTCGGAAAACGG**Y**ATG-**Ph**

B: thymidine nucleotide-carrying blackhole quencher 1; F: thymidine nucleotide-carrying fluorescein; FP: forward primer; P: probe; Ph: 3' phosphate to block elongation; RP: reverse primer; T: tetrahydrofuran spacer.

* phosphothioate backbone.

Table 2

Pathogen nucleic acids used for evaluation of the Ebola virus RT-recombinase polymerase amplification assay

Pathogen	Strain/source	Ebola RT-RPA TT (min)	Real-time RT-PCR CT value	Real-time RT-PCR assay
Ebola virus	Zaire strain/BNI	4.7	21.0	ENIVD Ebola standard control and [6]
Ebola virus	GIN/2014/Gueckedou-C05/BNI	5	25.4	
Sudan virus	Sudan Virus Maridi	Negative	22.26	[6]
Bundibugyo virus	Bundibugyo virus	Negative	28.7	In-house assay
Marburg virus	Musoke/BNI	Negative	24.5	[6]
Crimean Congo haemorrhagic fever virus	Kosova Hoti/BNI, Afg09–2990/BNI	Negative	20.3 22.4	RealStar CCHFV RT-PCR Kit ((Altona Diagnostics)
Lassa virus	Josiah/BNI, Lib 1580/121/ BNI	Negative	25.9, 34.7	[16]
Yellow fever virus	Asibi AY640589.1 17D RKI	Negative	20.6, 20.0	[17]
Rift valley fever virus	Strain ZH548	Negative	26.2	[18]
Dengue virus 1–4	VR344 (Thai 1958 strain), VR345 (TH-36 strain), VR216 (H87 strain), VR217 (H241 strain)	Negative	24.2 21.3 23.1 22.7	In-house assay
Zika virus	MR766	Negative	20.86	[19]
Chikungunya virus	A26 Strain	Negative	25.13	In-house assay
Plasmodium falciparum	ND	Negative	15.0	In-house qualitative assay

BNI: Bernhard Nocht Institute; ND: not determined; GIN: Guinea; RKI: Robert Koch Institute; RPA: recombinase polymerase amplification; RT: reverse transcription; TT: threshold time.

Ebola RT-RPA assay identified only *Zaire ebolavirus* but not the nucleic acids of other pathogens.

was analysed by threshold validation (mV/min). Slope validation was used to verify that the increase of fluorescence occurred at a sufficiently high rate, and was verified by first derivative analysis.

The mobile laboratory

The mobile laboratory consisted of a glovebox (Bodo Koennecke, Berlin, Germany), a Diagnostics-in-a-Suitcase (DiaS), and a solar panel and power pack set (Yeti 400 set, GOALZERO, South Bluffdale, US). The disassembled glovebox was kept in a metal box (80 × 60 × 41 cm) with other necessary materials (disinfectant solution, extraction kits, filter tips, racks, vortex, heat block, autoclavable plastic bags and personal protective equipment (PPE). The total weight was 28 kg for the box and 16 kg for the DiaS. Sample inactivation and RNA extraction using the SE kit were done in the glovebox (Figure 2A,B). This allowed handling of hazard group 4 samples. The RT-RPA assay was performed in the DiaS (Figure 2A,C) containing the ESEQuant TS2 device with integrated touchscreen to operate the device and display the results (Qiagen, Lake Constance GmbH, Stockach, Germany). The DiaS was assembled using a trolley case (63 × 50 × 30.2 cm, Peli, Düsseldorf, Germany). The bottom layer of the DiaS contains foam to adsorb shocks during transportation which is covered by a PVC top layer fixed around inserted devices to provide water and chemical resistance (Figure 3, [12]).

Statistical methods

Data were analysed using R (version 3.1.1) [13]. Performance parameters of the test (sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive

Table 3

Evaluation of Ebola virus diagnostic assays, serum and swab samples, Guinea, December 2014–May 2015 (n=1,069)

Extraction method [a]	Sample type	Reference test	Analysed test	Ct range	Analysis values[b]	PPV	NPV	Sensitivity	Specificity	n	Analysed method	Reference method	
												Positive	Negative
QC	Serum	A-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	0.82 [0.72–0.9] 3.2×10^{-9}	0.97 [0.88–1.00] 1.19×10^{-14}	0.97 [0.90–1.00] 4.21×10^{-18}	0.79 [0.60–0.88] 1.04×10^{-6}	141	Positive	68	15
											Negative	2	56
		A-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	0.46 [0.27–0.67] 0.8450	0.97 [0.88–1.00] 1.19×10^{-14}	0.86 [0.57–0.98] 0.0129	0.8 [0.69–0.89] 4.30×10^{-7}	84	Positive	12	14
											Negative	2	56
		W-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	1 [0.93–1.00] 0.178	0.86 [0.75–0.94] 1.57×10^{-8}	0.91 [0.83–0.96] 7.58×10^{-17}	1 [0.93–1.00] 1.78×10^{-15}	141	Positive	83	0
											Negative	8	50
		W-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	1 [0.87–1.00] 2.98×10^{-8}	0.86 [0.75–0.94] 1.57×10^{-8}	0.76 [0.59–0.89] 0.0029	1 [0.93–1.00] 1.78×10^{-15}	84	Positive	26	0
											Negative	8	50
		A-PCR	W-PCR	0–40	Estimate: 95% CI: p value:	0.77 [0.67–0.85] 2.51×10^{-7}	1 [0.93–1.00] 1.78×10^{-15}	1 [0.95–1.00] 1.69×10^{-21}	0.7 [0.58–0.81] 0.0007	141	Positive	70	21
											Negative	0	50
		A-PCR	W-PCR	>30	Estimate: 95% CI: p value:	0.41 [0.25–0.59] 0.392	1 [0.93–1.00] 1.78×10^{-15}	1 [0.77–1.00] 0.0001	0.71 [0.59–0.82] 0.0004	84	Positive	14	20
											Negative	0	50
		W-PCR	A-PCR	0–40	Estimate: 95% CI: p value:	1 [0.95–1.00] 1.69×10^{-21}	0.7 [0.58–0.81] 7.67×10^{-4}	0.77 [0.67–0.85] 2.51×10^{-7}	1 [0.93–1.00] 1.78×10^{-15}	141	Positive	70	0
											Negative	21	50
		W-PCR	A-PCR	>30	Estimate: 95% CI: p value:	1 [0.77–1.00] 0.0001	0.71 [0.59–0.82] 0.0004	0.41 [0.25–0.59] 0.3920	1 [0.93–1.00] 1.78×10^{-15}	84	Positive	14	0
											Negative	20	50
SE	Swab	A-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	0.81 [0.71–0.89] 1.39×10^{-8}	1 [1.00–1.00] 1.20×10^{-240}	1 [0.95–1.00] 1.36×10^{-20}	0.98 [0.97–0.99] 2.86×10^{-212}	881[c]	Positive	67	16
											Negative	0	798
		A-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	0.5 [0.31–0.69] 1	1 [1.00–1.00] 1.20×10^{-240}	1 [0.78–1.00] 6.10×10^{-5}	0.98 [0.97–0.99] 1.12×10^{-213}	828[c]	Positive	15	15
											Negative	0	798
		W-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	1 [0.96–1.00] 4.14×10^{-25}	1 [0.99–1.00] 3.69×10^{-127}	1 [0.96–1.00] 4.14×10^{-25}	1 [0.99–1.00] 3.69×10^{-127}	928	Positive	120	0
											Negative	0	808
		W-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	1 [0.88–1.00] 3.73×10^{-9}	1 [0.99–1.00] 3.69×10^{-127}	1 [0.88–1.00] 3.73×10^{-9}	1 [0.99–1.00] 3.69×10^{-127}	863[c]	Positive	55	0
											Negative	0	808

CI: confidence interval; CT: cycle threshold; NPV: negative predictive value; QC: QIAamp Viral Mini Kit; RPA: recombinase polymerase amplification; RT: reverse transcription; PPV: positive predictive value; SE: SpeedXtract nucleic acid extraction kit.

[a] Extraction method for RT-RPA. In all cases the reference test was tested with extracts from QC.

[b] Estimated proportions are given in decimals.

[c] This comparison was tested on a smaller subset.

values (NPV) were estimated for each of the assays using real-time RT-PCR assays as reference test. The 95% confidence interval (CI) of performance parameters was calculated based on the exact binomial test. P values are derived from the exact binomial test. The calculated Se and Sp were considered statistically significant for p values < 0.05. We used Fisher's exact test to compare RT-RPA performance parameters in comparison with W-PCR and A-PCR as the reference method at different Ct ranges.

Results

Inactivation

The inactivation of EBOV by the SE extraction procedure was confirmed in VeroE6 cells inoculated with SE extracts which were all negative in IFA. PCR results at passage 4 ranged from Ct 32 to undetectable. Since the IFA was negative, the PCR results were assumed to be due to remnant input RNA but not to actively replicating virus.

Analytical sensitivity and specificity of the RT-RPA assay

W-PCR and RT-RPA detected RNA standards over a range of 5 to 5×10^5 genome copies (GC)/reaction and 50 to 5×10^5 genome copies/reaction, respectively. RT-RPA assays could detect as little as 5 GC/reaction of a molecular RNA standard (data not shown) and 15 GC/reaction in EBOV-spiked human plasma samples (Figure 4A). No cross-detection of important differential diagnostic pathogens or any other filoviruses was observed for the Ebola RT-RPA assay (Table 2).

Performance of RT-PCR and RT-RPA assay using sera

Using a total of 141 sera extracted with QC, RT-RPA and W-PCR performances were assessed using the WHO-approved A-PCR as reference. Against the

Table 4

Significance of the performance analysis results for Ebola virus diagnostic assays, serum samples, Guinea, December 2014–May 2015 (n=141)

	Ct range	RPA/W-PCR	RPA/A-PCR	Fisher's exact test p value
Se	All	0.91	0.97	0.19
Sp	All	1	0.79	3.45×10^{-4}
PPV	All	1	0.82	3.05×10^{-5}
NPV	All	0.86	0.97	0.09
Se	0–20	1	1	1.00
Sp	0–20	1	1	1.00
PPV	0–20	1	1	1.00
NPV	0–20	1	1	1.00
Se	0–30	1	1	1.00
Sp	0–30	1	0.98	1.00
PPV	0–30	1	0.98	1.00
NPV	0–30	1	1	1.00
Se	>30	0.76	0.86	0.70
Sp	>30	1	0.80	3.27×10^{-4}
PPV	>30	1	0.46	1.09×10^{-5}
NPV	>30	0.86	0.97	0.09

NPV: negative predictive value; RPA: recombinase polymerase amplification; RT: reverse transcription; PPV: positive predictive value; Se: Sensitivity; Sp: Specificity.

A-PCR, the RT-RPA yielded a lower PPV (82% vs 100%, $p = 3.05 \times 10^{-5}$), a higher corresponding Se (97% vs 91%, p = 0.19), a higher NPV (97% vs 86%, p = 0.09) and a lower Sp (79% vs 100%, $p = 3.45 \times 10^{-4}$) than against the W-PCR (Table 3 rows 1 and 3, Table 4). The tendency of the results was even more pronounced in the subset of 84 samples with low viraemia (Ct values > 30, Table 3 rows 2 and 4). The difference between the PCR assays was analysed and revealed a reduced Se (77%) for the A-PCR compared with the W-PCR (Table 3 rows 7–8).

Samples determined as positive by the W-PCR but negative by the RT-RPA were also negative in the A-PCR, which missed some additional samples. There was no case of a negative RT-RPA result being positive in the A-PCR (Table 5).

Performance of RT-PCR and RT-RPA assay using swabs

In a preliminary test of RT-RPA efficiency on SE extracts from 47 swabs from deceased patients, all 47 samples scored positive in the W-PCR and the RT-RPA. Therefore, combined SE extraction and RT-RPA were deployed in the mobile laboratory and altogether 928 post-mortem swab samples (including the 47 preliminary ones) were tested. All 928 samples were also extracted by QC and tested by W-PCR and A-PCR. Overall, 120 samples scored positive both in W-PCR and RT-RPA, and only 67 of a subset of 83 samples scored positive in A-PCR. In reference to QC extraction and W-PCR, SE extraction

Table 5

Concordance of results from Ebola virus diagnostic assays, serum and swab samples, Guinea, December 2014–May 2015 (n=928)

Sera	W-PCR	RT-RPA	A-PCR
Positive	91	83	70
Negative	50	58	71
Total	141	141	141
Swabs	**W-PCR**	**RT-RPA**	**A-PCR**
Positive	83	83	67
Negative	798	798	814
Total	881	881	881

Forty-seven additional swab samples were only tested by W-PCR and RT-RPA. In n = 928 samples, these two assays were absolutely concordant.

and RT-RPA yielded a Se and Sp of 100% each (PPV: 100%; NPV: 100%). Since the results of W-PCR and RT-RPA were concordant, the significance of the results was not calculated (Table 5).

The prevalence of positives as tested by W-PCR and RT-RPA in the 928 swabs was 12.9%. Of the 928 post-mortem samples tested, 790 were from suspected cases for whom no signs of disease were recorded and 138 from suspected cases for whom information on symptoms and onset of disease ranging from 1 to 35 days before death were available. Of the 120 positive cases, 53 belonged to the group without recorded symptoms and 67 belonged to the group with symptoms. Positive results were most frequent around day 6 after disease onset and no positive results were obtained later than 14 days after onset of disease (Figure 4B).

Deployment of the mobile laboratory to the local hospital in Guinea

The mobile laboratory was easy to transport to the point of need (Figure 2D-F). The setup of the mobile laboratory including the assembly of the glovebox and donning the PPE took ca 30 min. The SE step was performed in the glovebox for up to 10 samples in 30 min, while the RT-RPA needed 20 min including pipetting steps and mixing. We were able to power the mobile laboratory (peak energy need: 173 W) with the solar battery for up to 16 hours. Before moving to another spot, the glovebox and DiaS were disinfected with 2% bleach or 0.5% incidine. Altogether, setup, operation and disassembly of the unit was easy to perform in a timely manner.

Four Guinean biologists were equipped with and trained in the use of the mobile laboratory at the IPD in January 2015 in a five-day course. After a pilot phase in Guinea, the mobile laboratories were deployed in the Matoto district of Conakry to support testing of swabs from dead suspected cases, which was introduced to

improve community engagement in the EBOV response as well as community surveillance.

Discussion

In this study, we evaluated the analytical and clinical performance of an updated EBOV RT-RPA compared with reference real-time RT-PCR assays. The isothermal RT-RPA assay, which allows real-time detection of amplification from RNA samples using primers and a fluorescent restriction probe within 3 to 15 min [10]. We improved this assay by adaptating the primers to the new sequences of the EBOV strain circulating in West Africa and incorporating them into dried RT-RPA pellets.

In sera extracted by QC, the RT-RPA scored a Se of 91% and Sp of 100% in reference to the W-PCR (Se: 97% and Sp: 79% in reference to the A-PCR), which means it would miss out some weak positives while identifying all true negatives correctly. Results from SE extracted sera were similar (data not shown). Taking swabs is less invasive than taking serum, which makes it more acceptable to populations, but is also safer and easier for sampling and testing. Since SE extraction does not require the use of a centrifuge, we tried to combine the RT-RPA with SE extraction of swabs to simplify our mobile laboratory procedure.

During the analysis of the results, we noted that the widely used A-PCR was less sensitive than the W-PCR. This lower Se was also described by other teams in Guinea and Sierra Leone [14,15]. A rapid detection test (ReEBOAg, Corgenix, Denver, US) was recently scored against the A-PCR with a Se of 91.8% and Sp of 84.6% and approved by the WHO for emergency use. Another recently described rapid detection test also scored a Se of 100% and a Sp of 96.6% against the A-PCR and was rated as a rule-out screening test by the authors because it would include all positives but would miss out on excluding all true negatives, therefore requiring a confirmatory test [15]. Our data confirm their interpretation that the performance of these tests was underestimated when using the A-PCR as reference test.

Our data show that the combination of SE and RT-RPA is superior to the above rule-out tests as all true positive and negative post-mortem oral swabs are detected. Our previous work has shown that magnetic bead extraction is preferable to centrifuge-based extraction under field conditions as it obviates the need for a high-speed centrifuge (unpublished data). We therefore tested the novel magnetic bead-based SE extraction with its 15 min protocol. The materials for both SE extraction and RT-RPA are stable at ambient temperature (30–35 °C) for up to three months and this cold chain-independent combination proved to be well suited for field diagnostics. It scored very satisfactory results in swab extracts (Table 3, rows 13–14), indicating that the RT-RPA does not need a confirmatory test and can be used on site to correctly include positives and exclude negatives.

The prevalence of EBOV in the 928 swabs tested was 12.9%. The day of death after onset of disease peaked at day 6 (range: 2–14 days) in the group of 67 swab-positive deceased for whom disease symptoms were recorded. For the ongoing EBOV outbreak in West Africa, the mean day of symptom onset is 11 days after infection and sera should ideally be collected during the acute phase of illness, within the first 10 days of the disease [2]. We show here that the same is true for swabs, which could simplify diagnostics tremendously. In 53 positive cases, symptoms were not recorded, which was mainly due to a lack of information in the records of the Safe and Dignified Burial teams that did the sampling.

When new EVD foci erupted in previously not affected western parts of Conakry in April 2015, the mobile laboratory was deployed to Matoto to support teams in charge of safe and dignified burials. Since it had been decided that all deceased should be tested, these teams collected swab samples from deceased of five neighbourhoods of Conakry (Matoto, Ratoma, Dixinn, Matam and Kaloum) and up to 50 samples had to be tested per day. The emergency response results were provided every 30 to 60 min to the field investigators and physicians. The rapidity and mobility of the RT-RPA method in the DiaS, in comparison with the average 3 to 4 h turnover with regular real-time RT-PCR, was appreciated by burial teams, health authorities, response teams and communities, as it allowed rapid clearance for normal burials deceased persons who were confirmed negative. The results also encourage the use of swabs from patients at ETCs. In that context, it would still be necessary to determine if swab samples can replace sera samples.

The deployment demonstrated that the mobile laboratory using glovebox, DiaS, SE and RT-RPA is a very good solution for decentralised biosafe diagnosis of EBOV, resulting in direct impact on community engagement for disease control. Moreover, this small mobile laboratory run by local teams is a sustainable contribution to future outbreak control.

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Conflict of interest

Oliver Nentwich and Olaf Piepenburg are employees of TwistDx Ltd, a wholly owned subsidiary of Alere Inc. The RPA technology is subject to background IP protection and is owned by Alere.

Authors' contributions

AAS, MW, AAEW, PP, OuF designed the study. AAS, MW, AAEW, PP wrote the manuscript. OuF, OsF, BS, AM, DK, AAS, NM collected the data. ON, OP developed and provided primer-in pellets, GF, SK, NF, MKK, AAD, LK, MN organize and support the field deployment. HK, AM performed the inactivation study. All authors contributed to analyse the data and reviewed the manuscript.

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