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# Supplementary appendix 2

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# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about the work presented in the main publication by Daniel Mukadi-Bamuleka, François Edidi-Atani, Maria E. Morales-Betoulle, Anaïs Legand, Antoine Nkuba-Ndaye, Junior Bulabula-Penge, Placide Mbala-Kingebeni, et al., titled "**Fatal meningoencephalitis associated with EBOV persistence in two survivors in the Democratic Republic of the Congo: a case report study**"

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# 1 Supplemental Materials and Methods

## 1.1 General information on vaccination strategies and EVD patient and survivor care during the 2018–2020 outbreak in DRC

During the 2018–2020 Ebola virus disease (EVD) outbreak in DRC, vaccination against EVD with the rVSV-ZEBOV-GP vaccine was implemented following recommendations of the Strategic Advisory Group of Experts (SAGE) on immunization in 2017 and 2019.[1,2] In general, a ring or geographic vaccination strategy was used for individuals considered contacts, or contacts of contacts. Vaccination status of the two cases reported here was recorded based on information reported by the patients upon admission to the Ebola Treatment Unit (ETU), or on information available in the various clinical and epidemiological databases. Once admitted to the ETU, EVD patients were managed per WHO recommendations on optimized supportive care for EVD.[3] Some patients were enrolled in a randomized controlled trial (PALM study) and received therapeutics such as the REGN-EB3 that was provided to the two cases described here.[4] Since 2018, the DRC Ministry of Health and the National Institute for Biomedical Research (INRB), with support from WHO and partners, implemented an EVD survivor care program that offered monthly consultations to all EVD survivors, including clinical and psychological care and RT-PCR testing of semen, vaginal fluids, and breast milk. Antenatal care was provided to women like Patient 1, who became pregnant after EVD recovery. A referral system to a higher level of care was organized for complications that could not be managed at the clinic. In case of any emergencies, referrals and hospital admissions were supported by the program. The program was made available to all EVD survivors upon discharge from the ETU, following WHO guidelines.[5]

## 1.2 Next-generation sequencing and genomic analyses

We sequenced blood and cerebrospinal fluid (CSF) samples collected during the relapse episode in 2020, from both patients (Case 1 20FHV036, 20FHV038; Case 2 20FHV037, 20FHV039), along with a sample taken during the initial EVD episode of Case 1 (MAN5036) for comparison. We had previously sequenced a sample from the initial EVD episode in Case 2 (MAN14228) in early 2020 while providing genomic epidemiology support during the 10th EVD outbreak. Samples were extracted using the Qiagen Viral RNA Mini kit (Qiagen, Valencia, CA, USA). MAN5036 and MAN14228 were sequenced using a targeted enrichment technique on the Illumina platform (Illumina Inc., San Diego, CA, USA) as previously described in the Supplement of Mbala-Kingebeni et al.[6] Samples collected during the relapse episode, were sequenced using an amplicon-based approach on the Illumina platform as previously described in the Supplement of Mbala-Kingebeni *et al.*[7] Sequences are available in GenBank under accession numbers: MT778640, and OQ348595-OQ348598.

A Bayesian phylodynamic analysis was performed to construct a time-resolved phylogeny using samples MAN14228, MAN5036, 20FHV036, and 20FHV037, along with 677 other North Kivu outbreak genomes. First, multiple sequence alignment was performed with MAFFT, maximum likelihood tree inferred using IQ-TREE, and tree visualized with TempEst v1.5.3 to assess clock-likeness and absence of outliers (excluding known and suspected persistent infection-related cases).[8,9] Time-calibrated phylogenies were estimated with the MCMC method implemented in BEAST v1.10.4.[10] We used an HKY+G nucleotide substitution model, with a local clock model and a non-informative continuous time Markov chain reference prior on the molecular clock rate with a separate prior for sets of cases known or suspected to be related to persistent infections, and a Skygrid coalescent prior. The analysis was run for 80 million chains, sampling every 8,000. Tracer v1.7.1 was used to assess run convergence (i.e., effective sample size > 200).[11] The

sequence fasta and BEAST XML files are available at https://github.com/cathrnbp/paper-2023-ebolacsf.

## 1.3 Serological testing for anti-EBOV antibodies using CDC's in-house ELISA

### 1.3.1 Specimen inactivation

Blood and CSF specimens were collected from both Case 1 and Case 2 at multiple different time points. Prior to serology testing, specimens were inactivated in a Class III biosafety cabinet (Cleatech HEPA Filtered Glove box, Cleatech LLC, https://www.cleatech.com). For inactivation, 42 µL of blood were diluted in 1 mL of Master Plate buffer containing 5% skim milk (Fischer Sci, Pittsburg, PA, USA) in PBS (Sigma-Aldrich, St. Louis, MO, USA), 0·5% Triton X100 (Sigma-Aldrich, St. Louis, MO, USA), and 0·5% Tween-20 (Sigma-Aldrich, St. Louis, MO, USA). For CSF samples, 50 µL of specimen were diluted in 200 µL of Master Plate diluent. Tubes were then placed in a heat block (Fischer Sci, Pittsburg, PA, USA) at 56°C for 15 min, mixed, and incubated 30 min at 56°C. The samples were then tested in a biosafety level 2 (BSL-2) laboratory using the CDC IgM and IgG ELISA described below.

### 1.3.2 CDC IgM and IgG ELISA

The methods for in-house IgM and IgG ELISAs were previously described.[12,13] Viral antigens were prepared with EBOV-Mayinga strain (spp. *Orthoebolavirus zairense*) in Vero-E6 cells (CDC, Atlanta, GA, USA) in a CDC BSL-4 laboratory. Infected cells were processed by detergent basic buffer extraction for lysate antigen preparations (IgG) and by cell suspension in 0·1 M Tris (pH 8·5), followed by a freeze and thaw cycle to produce a cell slurry (IgM); cell lysate and cell slurry were then inactivated by gamma-irradiation as described.[13,14] Mock control antigen (uninfected Vero-E6 cells) lysate (for IgG) and cell slurry (for IgM) were prepared in a

similar manner. Inactivated specimens were tested in a BSL-2 laboratory by 4-fold serial dilutions from 1:100 (blood) or 1:20 (CSF) to 1:64000. To detect human IgG, anti-human IgG horseradish peroxidase conjugate (Accurate Chemical, Westbury, NY, USA) and $H_2O_2$-ABTS substrate system (KPL, Sera Care, Milford, MA, USA) were used. For IgG, an adjusted OD (difference between values obtained with positive samples and negative controls) of > 0·2 was required for each dilution to be considered positive and for a titer to be assigned accordingly. Specimens were considered positive if the titer was ≥1:400 (for blood) or ≥1:80 (for CSF) and the sum of the adjusted OD was ≥0·95. For IgM ELISA, anti-human IgM antibody (KPL, Sera Care, Milford, MA, USA) was used to capture IgM antibodies from the serially diluted samples, followed by EBOV or mock antigens. Captured antigens were detected using a polyclonal anti-EBOV rabbit antibody produced at CDC, followed by an anti-rabbit horseradish peroxidase conjugate (BioRad, Hercules, California, USA) and the $H_2O_2$-ABTS (Sera Care, Milford, MA, USA) substrate system. For IgM, adjusted OD > 0·1 was required for each dilution to be considered positive and for a titer to be assigned accordingly. Specimens were considered positive if the titer was ≥1:400 (for blood) or ≥1:80 (for CSF) and the sum of the adjusted OD was ≥0·45. Known positive and negative controls were ran in parallel to samples for each ELISA test to ensure adequate performance of the assays.

### 1.3.3 Luminex serology

Plasma and CSF samples from the two cases were transferred to the INRB biobank and stored at -80°C until laboratory analyses were performed. The Luminex assay was performed using four commercially available recombinant viral proteins from EBOV (spp. *Orthoebolavirus zairense)* representing EBOV glycoprotein (Kissidougou strain 2014 glycoproteins [GP-EBOV-k] and Mayinga strain 1976 [GP-EBOV-m]; Sino Biological, Beijing, China), EBOV nucleoprotein (Kissidougou strain 2014 nucleoproteins [NP-EBOV]; Sino Biological]) and EBOV VP-40

(Kissidougou strain 2014 40-kDa viral protein [VP-EBOV; Sino Biological] to detect anti-Ebola IgG on the x.MAP Technology (Luminex, Austin, TX, USA). Briefly, plasma samples were diluted 1:1000 in dilution buffer, composed of deionized distilled water, 1% bovine serum albumin, 5% fetal bovine serum, 1% hypertonic phosphate buffered saline, and 0.2% Tween-20. After mixing the samples with beads previously coupled with a specific antigen, the plates were incubated overnight at 4°C. CSF samples were diluted 1:500 in dilution buffer and similarly treated. Prior to adding the secondary antibody and a fluorescent protein (streptavidin phycoerythrin) successively, the plates were washed five times. The plates were then read using Magpix. Results were expressed in median fluorescence intensity (MFI), which assumes at least 100 beads read per analyte. For each antigen, reported sensitivity and specificity were significantly above 90%.[10,11] We used the following thresholds to determine seropositivity: 400 MFI for GP (Mayinga strain), 600 MFI for NP, and 650 MFI for VP40. These thresholds were previously established in various studies that assessed survivors, their contacts, and a large panel of EBOV-negative individuals.[15,16] Based on the results of the serological tests, patients were considered seropositive when their sample were reactive to at least two of the three EBOV antigens.

# 2 Supplemental information on timelines from exposure to relapse and laboratory testing results of specimens collected during 1st and 2nd EVD episodes for each case

## 2.1 Table S1. Time intervals between initial exposure to EBOV, vaccination, admission to ETU, REGN-EB3 treatment, discharge, and relapse for each case.

| **Time between:** | **Case 1** | **Case 2** |
|---|---|---|
| EBOV exposure[1] → rVSV-ZEBOV-GP vaccination[2] | 5 days | 1–9 days |
| EBOV exposure → EVD symptom onset | 8 days | 5–14 days |
| EBOV exposure → admission to ETU | 14 days | 7–16 days |
| EBOV exposure → treatment with REGN-EB3 | 15 days | 7–16 days |
| rVSV-ZEBOV-GP vaccination → EVD symptom onset | 3 days | 4 days |
| rVSV-ZEBOV-GP vaccination → treatment with REGN-EB3 | 10 days | 6 days |
| EVD symptom onset → treatment with REGN-EB3 | 7 days | 2 days |
| rVSV-ZEBOV-GP vaccination → ETU discharge (1st episode) | 25 days | 25 days |
| Treatment with REGN-EB3 → ETU discharge (1st episode) | 15 days | 19 days |
| First discharge → EVD relapse | 342 days | 137 days |

[1]The exact day of exposure could only be estimated based on the information available in the DRC epidemiology or ETU databases, or case investigation records. For Case 1, exposure was assumed to have occurred at the communal clinic she visited in June, on the same day when two confirmed fatal EVD cases were reportedly present in that health care facility. For Case 2, the period of exposure was estimated based on his records of caring for and assisting to the funeral of a family member who was a confirmed EVD case.

[2]The rVSV-ZEBOV vaccination record information date for each case was obtained from the DRC epidemiology databases or ETU or case investigation records.

## 2.2 Table S2. RT-qPCR, sequencing, and serology results for Case 1 during initial EVD episode and relapse.

Date of onset of 1st episode was in June, 2019, and 2nd episode was in June, 2020. Discharge after 1st episode was in July, 2019, and date of death after 2nd episode was June, 2020. Patient received REGN-EB3* in July, 2019, one day before collection of first sample tested by serological assays. At relapse, blood samples were negative for anti-EBOV IgM and positive for anti-EBOV IgG; CSF samples were negative for IgM and positive for IgG. nd: not done; CSF: cerebrospinal fluid; Kiss: Kissidougou EBOV variant; May: Mayinga EBOV variant.

| Sample type | Lab ID | Days since first EVD onset | Days since second EVD onset | GeneXpert RT-qPCR (Ct) | | Genome coverage (%) | CDC Serology Assay | | | | Luminex Serology Assay IgG Titers and Result | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | GP | NP | | anti-Ebola virus IgM Titer | anti-Ebola virus IgM Result | anti-Ebola virus IgG Titer | anti-Ebola virus IgG Result | anti-NP | anti-$GP_{Kiss}$ | anti-$GP_{May}$ | anti-VP40 | Result |
| **First episode of EVD (symtom onset, June 2019)** | | | | | | | | | | | | | | | |
| Blood | MAN 5036 | 7 | | 30.6 | 27.4 | 99.9 | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Plasma | MAN 5112 | 8 | | 33.1 | 29.2 | nd | 1600 | Positive | 6400 | Positive | 254 | 10308 | 5025 | 59 | Negative |
| Plasma | MAN 5153 | 9 | | 34.7 | 26.6 | nd | 1600 | Positive | 6400 | Positive | 1089 | 8383 | 3536 | 150 | Positive |
| Plasma | MAN 5203 | 10 | | 35.4 | 31.0 | nd | 6400 | Positive | 6400 | Positive | 5967 | 12117 | 6784 | 802 | Positive |
| Plasma | MAN 5250 | 11 | | 36.7 | 31.6 | nd | 1600 | Positive | 6400 | Positive | 7584 | 10432 | 5758 | 1779 | Positive |
| Plasma | MAN 5302 | 12 | | 36.2 | 31.7 | nd | 1600 | Positive | 6400 | Positive | 13816 | 14323 | 9448 | 7386 | Positive |
| Plasma | MAN 5356 | 13 | | 36.3 | 31.4 | nd | 1600 | Positive | 6400 | Positive | 13816 | 14255 | 8932 | 5192 | Positive |
| Plasma | MAN 5395 | 14 | | 37.4 | 33.4 | nd | 1600 | Positive | 6400 | Positive | 11294 | 11786 | 6840 | 2636 | Positive |
| Plasma | MAN 5445 | 15 | | 38.8 | 34.0 | nd | 1600 | Positive | 6400 | Positive | 9354 | 12305 | 7209 | 4390 | Positive |
| Plasma | MAN 5495 | 16 | | 42.3 | 36.5 | nd | 6400 | Positive | 6400 | Positive | 7318 | 7977 | 4303 | 5032 | Positive |
| Plasma | MAN 5528 | 17 | | 39.6 | 36.9 | nd | 6400 | Positive | 6400 | Positive | 12162 | 11287 | 6647 | 5141 | Positive |
| Plasma | MAN 5576 | 18 | | 0.0 | 38.6 | nd | 6400 | Positive | 6400 | Positive | 574 | 5374 | 2156 | 546 | Positive |
| Plasma | MAN 5658 | 19 | | 0.0 | 42.1 | nd | 6400 | Positive | 6400 | Positive | 12160 | 9324 | 4975 | 10870 | Positive |
| Plasma | MAN 5695 | 20 | | 0.0 | 0.0 | nd | 1600 | Positive | 6400 | Positive | 15272 | 14111 | 9443 | 12645 | Positive |
| Plasma | MAN 5778 | 21 | | 0.0 | 0.0 | nd | 1600 | Positive | 6400 | Positive | 12882 | 12791 | 7939 | 7147 | Positive |
| Blood | MAN 5820 | 22 | | 0.0 | 0.0 | nd | 1600 | Positive | 6400 | Positive | 5633 | 6951 | 5019 | 9721 | Positive |
| Blood | MAN 5953 | 24 | | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| **Second episode of EVD (symptom onset, Jun 2020)** | | | | | | | | | | | | | | | |
| Blood | none | 365 | 1 | 35.1 | 33.6 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 366 | 2 | 36.4 | 31.8 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | 20FHV038 | 367 | 3 | 34.1 | 32 | 27.5 | 50 | Negative | 6400 | Positive | 841 | 2432 | 1389 | 603 | Positive |
| CSF | 20FHV036 | 367 | 3 | 20.1 | 16.7 | 99.5 | 10 | Negative | 1280 | Positive | 1170 | 51 | 72 | 101 | Negative |
| Blood | none | 368 | 4 | 36.6 | 33 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

*Note: the antibodies contained in REGN-EB3 cross react with the CDC's IgG ELISA and, if present, would give a positive signal (data not shown); nd = test not done

## 2.3 Table S3. RT-qPCR, sequencing, and serology results for Case 2 during initial EVD episode and relapse.

Date of onset of 1st episode was January, 2020, and 2nd episode was June, 2020. Date of discharge after 1st episode was February 2020, and date of death after 2nd episode was July, 2020. Patient received REGN-EB3* in January, after the first sample collection. At relapse, blood samples were positive for IgM and negative for IgG; CSF was positive for IgM and IgG. nd: not done; CSF: cerebrospinal fluid; Kiss: Kissidougou EBOV variant; May: Mayinga EBOV variant.

| Sample type | Lab ID | Days since first EVD onset | Days since second EVD onset | GeneXpert RT-PCR (Ct) | | Genome coverage (%) | CDC Serology Assay | | | | Luminex Serology Assay IgG Titers and Result | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | GP | NP | | anti-Ebola virus IgM Titer | anti-Ebola virus IgM Result | anti-Ebola virus IgG Titer | anti-Ebola virus IgG Result | anti-NP | anti-$GP_{Kiss}$ | anti-$GP_{May}$ | anti-VP40 | Result |
| **First episode of EVD (symtom onset, January 2020)** | | | | | | | | | | | | | | | |
| Plasma | MAN 14228 | 2 | | 29.7 | 25.0 | 99.7 | 400 | Positive | 100 | Negative | 10 | 995 | 1520 | 403 | Negative |
| Plasma | MAN 14255 | 3 | | 31.2 | 27.3 | nd | 1600 | Positive | 6400 | Positive | 6 | 8580 | 3690 | 9 | Negative |
| Plasma | MAN 14298 | 4 | | 30.5 | 26.6 | nd | 1600 | Positive | 6400 | Positive | 34 | 10841 | 4933 | 23 | Negative |
| Plasma | MAN 14370 | 5 | | 35.4 | 31.8 | nd | 1600 | Positive | 6400 | Positive | 3 | 1118 | 417 | 2 | Negative |
| Plasma | MAN 14413 | 6 | | 38.0 | 34.8 | nd | 1600 | Positive | 1600 | Positive | 52 | 12763 | 6571 | 40 | Negative |
| Plasma | MAN 14450 | 7 | | 39.4 | 35.4 | nd | 6400 | Positive | 6400 | Positive | 155 | 12645 | 6698 | 78 | Negative |
| Plasma | MAN 14505 | 8 | | 40.1 | 36.1 | nd | 6400 | Positive | 6400 | Positive | 141 | 2479 | 196 | 49 | Negative |
| Plasma | MAN 14550 | 9 | | 39.0 | 36.0 | nd | 6400 | Positive | 6400 | Positive | 227 | 10081 | 4595 | 58 | Negative |
| Plasma | MAN 14599 | 10 | | 40.3 | 37.4 | nd | 6400 | Positive | 6400 | Positive | 313 | 13609 | 7484 | 80 | Negative |
| Blood | none | 21 | | 0.0 | 0.0 | nd | 6400 | Positive | 6400 | Positive | 400 | 11039 | 5320 | 400 | Negative |
| **Second episode of EVD (symptom onset, Jun 2020)** | | | | | | | | | | | | | | | |
| Blood | 20FHV039 | 158 | 1 | 0.0 | 35.0 | 97.1 | 6400 | Positive | 50 | Negative | 97 | 252 | 185 | 302 | Negative |
| Blood | none | 159 | 2 | 0.0 | 39.4 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 160 | 3 | 0.0 | 35.4 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 161 | 4 | 0.0 | 36.7 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 162 | 5 | 0.0 | 37.2 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 164 | 7 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| CSF | 20FHV037 | 167 | 10 | 32.6 | 28.4 | 99.7 | 1280 | Positive | 16000 | Positive | 4339 | 66 | 41 | 3576 | Positive |
| Blood | none | 168 | 11 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 169 | 12 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 171 | 14 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 173 | 16 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 175 | 18 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Blood | none | 177 | 20 | 0.0 | 0.0 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| CSF | none | 178 | 21 | 37.4 | 34.4 | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

*Note: the antibodies contained in REGN-EB3 cross react with the CDC's IgG ELISA and, if present, would give a positive signal (data not shown); nd = test not done.

# 3 Additional clinical laboratory results during relapse for both cases

## 3.1 Additional laboratory results for Case 1 during EVD relapse


Figure S1. Rapid diagnostic test results for Case 1. All results are negative. From left to right: hepatitis B virus surface antigen, hepatitis C virus antibody, HIV antigen and antibody, and syphilis rapid tests.

## 3.2 Additional laboratory results for Case 1 during EVD relapse, June, 2020

Table S4. Biological parameter follow-up of Case 1

| Parameter* | Units | Reference range | Day 1 post EVD relapse | Day 2 post EVD relapse | Day 3 post EVD relapse | Day 4 post EVD relapse0 |
|---|---|---|---|---|---|---|
| **Blood chemistry (Piccolo-Metlac 12)** | | | | | | |
| Glu | mg/dL | 73–113 | 151 | 133 | - | 126 |
| BUN | mg/dL | 7–22 | 7 | 6 | - | 6 |
| CRE | mg/dL | 0·6–1·2 | 0·5 | 0·2 | - | 0·2 |
| Na+ | mmol/L | 138–145 | 147 | 133 | - | 138 |
| K+ | mmol/L | 3·6–5·1 | | 3·1 | - | 2·3 |
| CL- | mmol/L | 98–108 | 98 | 100 | - | 99 |
| Tco2 | mmol/L | 18–33 | 16 | 21 | - | 21 |
| Ca | mg/dL | 8–10·3 | <8 | 8·6 | - | 9·1 |
| PHOS | mg/dL | 2·2–4·1 | 3·4 | 2·3 | - | 3·4 |
| Mg | mg/dL | 1·6–2·3 | 0·7 | 1·7 | - | 1·8 |
| Alb | g/dL | 3·3–5·5 | 2·8 | 2·8 | - | 2·6 |
| LAC | mm/L | 0·53–2·1 | 4·8 | 1·8 | - | 1·3 |
| **Hematology (Sysmex)** | | | | | | |
| WBC | $10^3$/µL | 3–15 | 15·2 | 18·9 | - | 19 |
| RBC | $10^6$/µL | 2·5–5·5 | 3.5 | 3.3 | - | 3 |
| HGB | g/L | 8–17 | 10·1 | 9·3 | - | 8·6 |
| HCT | % | 26–50 | 31 | 28·5 | - | 25·4 |
| MCV | /fL | 86–110 | 87·8 | 85·1 | - | 84·4 |
| MCH | /pg | 26–38 | 28·6 | 27·8 | - | 28·6 |
| MCHC | g/dL | 31–37 | 32·6 | 32·6 | - | 33·9 |
| PLT | $10^3$/µL | 150–400 | 450 | 459 | - | 363 |
| LYM | % | 5–55 | 10·5 | 9·3 | - | 6·5 |
| MXB | % | 1–20 | 4·2 | 4·8 | - | 4 |
| NEUT M | % | 45–95 | 85·3 | 85·9 | - | 89·5 |
| LYM | $10^3$/µL | - | 1·6 | 1·8 | - | 1·2 |
| MXD | $10^3$/µL | - | 0·6 | 0·9 | - | 0·8 |
| NEUT | $10^3$/µL | - | 13 | 16·2 | - | 17 |
| RDW-SD | /fL | 37–54 | 62·2 | 56·2 | - | 54·5 |
| RDW-CV | % | 11–16 | 20·4 | 19 | - | 18 |

* Piccolo, and Sysmex pocH-110

## 3.3 Additional laboratory results for Case 2 during EVD relapse, June-July, 2020

Table S5. Biological parameter follow-up during hospitalization of Case 2.

| | | | June 2020 (Days post second EVD onset) | | | | | | | | | July 2020 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter*** | **Units** | **Reference range** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** | **D7** | **D8** | **D10** | **D12** | **D14** | **D16** | **D17** | **D18** |
| **Blood chemistry (Piccolo-Amylte13/Metlac 12)** | | | | | | | | | | | | | | | | |
| Glu | mg/dL | 73–113 | 138 | 131 | 120 | 101 | 110 | - | 171 | 122 | 109 | 119 | 156 | 204 | - | 212 |
| BUN | mg/dL | 7–22 | 18 | 21 | 25 | 21 | 17 | - | 20 | 19 | 20 | 18 | 13 | 17 | - | - |
| CRE | mg/dL | 0·6–1·2 | 1·3 | 1·9 | 1·5 | 1·5 | 1·3 | - | 0·6 | 1·5 | 1·1 | 1·3 | 0·7 | 0·7 | | 1·1 |
| TBIL | mg/dL | 0·2–1·6 | 0·6 | 0·7 | 0·4 | - | 0·5 | - | - | - | - | - | - | - | - | 0·4 |
| Alb | g/dL | 3·3–5·5 | 2·3 | 3·1 | 2·7 | 2·8 | 2·6 | - | 2·6 | 2·9 | 3 | 2·5 | 2·4 | 2·4 | - | 2·4 |
| ALT | U/L | 10–47 | 8 | 11 | 12 | - | 12 | - | - | - | - | - | - | - | - | 46 |
| AST | U/L | 11–38 | 28 | 28 | 25 | - | 22 | - | - | - | - | - | - | - | - | 34 |
| CK | U/L | 30–380 | 400 | 361 | 222 | - | 76 | - | - | - | - | - | - | - | - | - |
| AMY | U/L | 14–97 | 61 | 68 | 112 | - | 74 | - | - | -- | - | - | - | - | - | 69 |
| Na+ | mmol/L | 138–145 | 170 | 138 | 137 | 132 | 133 | - | 119 | 121 | 119 | 119 | | 114 | - | 117 |
| K+ | mmol/L | 3·6–5·1 | 8·5 | 3·8 | 3·7 | 4·6 | 4· | - | 4·3 | 4·9 | 3·9 | 3·9 | 4·9 | 4·8 | - | 5·8 |
| CL- | mmol/L | 98–108 | - | - | - | 100 | - | - | 88 | 87 | 85 | 88 | 82 | 81 | - | - |
| Tco2 | mmol/L | 18–33 | - | - | - | 30 | - | - | 20 | 20 | 21 | 22 | 20 | 22 | - | - |
| Ca | mg/dL | 8–10·3 | 4 | 9 | 9 | 8·7 | 8·6 | - | 8·4 | 8·6 | 8·3 | 8 | 7·8 | 8·3 | - | 9·5 |
| PHOS | mg/dL | 2·2–4·1 | - | - | - | 4 | - | - | 3·8 | 3·2 | 4·7 | 3·1 | 2·7 | 2·8 | - | - |
| Mg | mg/dL | 1·6–2·3 | - | - | - | 1·7 | - | - | 1·8 | 1·8 | 1·8 | 1·6 | 1·6 | 1·7 | - | - |
| LAC | mm/L | 0·5–2·1 | - | - | - | 1·7 | - | - | 4·2 | 3·9 | 1·5 | 1·8 | 2·8 | 3·6 | - | - |
| CRP | mg/L | ≤7·5 | 22·2 | 34·6 | 19·8 | - | 7·5 | - | - | - | - | - | - | - | - | >200 |
| **Hematology (Sysmex)** | | | | | | | | | | | | | | | | |
| WBC | $10^3$/µL | 3–15 | 9· | 7·9 | 7·6 | 8·1 | 6·2 | - | - | - | 10·7 | 4·3 | 3·8 | 5·2 | - | 9·4 |
| RBC | $10^6$/µL | 2·5–5·5 | 3·2 | 3·7 | 3·7 | 2·7 | 3·7 | - | - | - | 3·8 | 3·2 | 3·4 | 3·7 | - | 4·1 |
| HGB | g/L | 8–17 | 9·3 | 10·8 | 10·7 | 10·7 | 10·7 | - | - | - | 11·2 | 9·4 | 10·4 | 10·5 | - | 11·5 |
| HCT | % | 26–50 | 30 | 31·8 | 31·3 | 31·1 | 30·8 | - | - | - | 30·5 | 26·3 | 27·8 | 29·7 | - | 32·7 |
| MCV | /fL | 86–110 | 93·5 | 85·9 | 84·6 | 84·5 | 83 | - | - | - | 80·3 | 81·7 | 80·6 | 80·3 | - | 80 |
| MCH | /pg | 26–38 | 29 | 29·2 | 28·9 | 29·1 | 28 | - | - | - | 29·5 | 29·2 | 30·1 | 28·4 | - | 28·1 |
| MCHC | g/dL | 31–37 | 31 | 34 | 34·2 | 34·4 | 34 | - | - | - | 36·7 | 37·5 | 37·4 | 35·4 | - | 35·2 |
| PLT | $10^3$/µL | 50–400 | 73 | 161 | 160 | 191 | 172 | - | - | - | - | 265 | - | 188 | - | 235 |
| LYM | % | 5–55 | 10·8 | 22·9 | 20·3 | 17·4 | 23 | - | - | - | 13·4 | 10·8 | 18·2 | 7·6 | - | 7·7 |
| MXB | % | 1—20 | 10.0 | 14·5 | 7·4 | 4·9 | 7·3 | - | - | - | 6·8 | 7·5 | 3·5 | 3·9 | - | 1·8 |
| NEUT M | % | 45–95 | 79·2 | 62·6 | 72·3 | 77·7 | 69·2 | - | - | - | 79·8 | 81·7 | 78·3 | 88·5 | - | 90·5 |
| LYM | $10^3$/µL | - | 1 | 1·8 | 1·5 | 1·4 | 1·5 | - | - | - | 1·4 | 0·5 | 0·7 | 0·4 | - | 0·7 |
| MXD | $10^3$/µL | - | 0·9 | 1·1 | 0·6 | 0·4 | 0·5 | - | - | - | 0·7 | 0·3 | 0·1 | 0·2 | - | 0·2 |
| NEUT | $10^3$/µL | - | 7·1 | 5 | 5·5 | 6·3 | 4·2 | - | - | - | 8·6 | 3·5 | 3 | 4·6 | - | 8·5 |
| RDW-SD | /fL | 37–54 | 53·5 | 43·2 | 43·1 | 42 | 40·2 | - | - | - | 39·1 | 40·4 | 39·4 | 40·4 | - | 41·1 |
| RDW-CV | % | 11–16 | 16·2 | 14·2 | 14·2 | 13·8 | 13·5 | - | - | - | 13·8 | 14 | 14·1 | 14·3 | - | 14·5 |
| MPV | /fL | 9–13 | 13·8 | 11 | 11·3 | 11·3 | 12·9 | - | - | - | - | - | - | 0·2 | - | 9·7 |

* Piccolo, and Sysmex pocH-110

## 4 References for Supplementary Appendix


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