
Weidmann M1, Bailey D2, de Lamballerie X3, Di Caro A4, Doyon S5, Faye O6, Fleuvaud L7, Fomsgaard A8, Hensley L9, Kioivogu L10, Konneh K11, Koopmans MP12, Magassouba N13, Mirazimi A14, Adomeh DI15, Jansen van Vuren P15, Stroecker K15, Pawska JT15, Picard C17, Sheeley H1, Smit PW16 and Sall AA16

1Institute of Aquaculture, University of Stirling, Stirling, Scotland, UK
2Public Health England, Porton Down, Wiltshire, Salisbury, UK
3Emergence des pathologies Virales,UMR-D 190,Aix-Marseille Université et Institut de Recherche pour le Développement, Marseille, France
4European Mobile Laboratory Consortium and National Institute for Infectious Diseases Lazzaro Spallanzani IRCCS, Rome, Italy
5Médecins Sans Frontières (MSF) Operational Centre Barcelona-Athens (OCBA), Barcelona, Spain
6Arbovirus unit, Pasteur Institute, Dakar, Senegal
7Virus Research & Development Laboratory Statens Serum Institut, Copenhagen, Denmark Infectious Disease Research Unit, University of Southern Denmark, Odense, Denmark
8US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA
9Institut National de Santé Publique, Guinea
10Ministry of Health and Sanitation, Freetown, Sierra Leone
11Public Health Virology, Laboratory preparedness & Response, Department of Viroscience, Erasmus MC, The Netherlands
12Université Gamal Abdel Nasser de Conakry, Laboratoire des Fièvre Hémorragiques en Guinée, Guinea
13Institute for Laboratory Medicine, Dept for Clinical Microbiology, Karolinska Institute, and Karolinska Hospital University, Stockholm, Sweden
14Lassa Fever Diagnostic and Research Laboratory,Institute of Lassa Fever Research and Control, Irrua Specialist Teaching Hospital, Irru, Nigeria
15National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South-Africa
16European Mobile Laboratory Consortium German Center for Infection Research, Hamburg Munich-Marburg-Riems Bundeswehr Institute of Microbiology, Munich, Germany
17Pasteur Institute/Unit of Biology of Emerging Viral Infections, International Center for Infectiology Research, INSERM, CNRS, Lyon 1 University, Ecole Normale Supérieure de Lyon, France

Abstract
During the Ebola Disease outbreak in 2014-2015 in West-Africa about 24 organizations operated laboratories at 40 sites in Guinea, Sierra Leone and Liberia. Representatives of ten organisations which had deployed laboratories to 16 sites across the three countries in West-Africa convened for a two day symposium in Dakar (4-5.02.16) to exchange their experiences. This article summarizes points made during the discussion of the laboratory deployment experiences during the epidemic touching organisational and procedural issues.

Introduction
The unprecedented outbreak of Ebola Virus Disease in West-Africa led to an international response in which all types of existing or budding mobile laboratory concepts were deployed into the field. Apart from some, of their individual experiences described in the preceding papers some experiences were made across the board and here we try to summarize the issues encountered and the lessons learned from them. We hope that this can help other mobile laboratory concepts to revise and optimize their setup and procedures. It is also quite clear that umbrella organisations such as WHO GOARN should take note of these experiences to consider solutions in preparation of potential future mass deployments of mobile laboratories. There already is one publication by the government of the United Kingdom on lessons learned from the outbreak [1]. Here the emphasis is on experiences made by staff working in the mobile laboratories.

The following topics are discussed: infrastructure issues, preanalytical supply chain, analytical and clinical aspects of using molecular assays, laboratory procedures. Finally we summarize the
areas, which need attention after the outbreak has subsided, in a paragraph describing the post-epidemic needs.

Infrastructure

Electricity

Most of the laboratories faced unstable electricity supply with erratic power surges and power cuts. The generators brought by some teams to provide their independent energy supply faced a shortage of supply of the right type of fuel and had to organize their own supply chain. In one case a generator brought by the South African team failed and was luckily replaced by the local government.

Electricity supply can be a major infrastructure issue in poor countries. A lack of electricity supply shuts down laboratory operation and essential cooling of laboratory spaces.

Alternative electricity sources used were cars tapped via power converters. Pickups are very often at their limit if they are tapped and their own systems (e.g. air conditioning) cannot be used at the same time. Uninterruptable power supply units (UPS) turned out to be difficult to charge since local electricity supply was observed not stable/ powerful enough to charge them. Solar panels were used for the suitcase laboratory used by the Institut Pasteur de Dakar team in Guinea.

For future major operations some thought should be put into how to make sure generators run on the same fuel and how the international effort can oversee supply security. One possibility would be to tie in the international oil industry to help establish the supply chain. The fact that electrical engineers were in short supply to maintain electricity connections and repair damage it is seen as another point international umbrella support needs to fix for future outbreak deployments.

Internet

The internet is an essential laboratory infrastructure feature these days to disseminate results but also to maintain modern laboratory devices (e.g. robotic platform systems) some of which are updated and even initiated through the internet. In the case of using the MinIon sequencer it turned out that even data analysis (consensus calling) relied on an internet connection. Instability of internet connections or rigged Wi-Fi connections were experienced by most mobile laboratory teams and it was discussed if this could be solved by fieldable satellite connections e.g. VSAT systems which are of high quality but expensive.

Connections e.g. VSAT systems which are of high quality but expensive. One lesson from all of these experiences is that a generally accepted international case ID form is necessary. Additionally there is the essential requirement for a single patient identifier to be able to track patient movements between health care and treatment centres. All of this should be considered by the WHO GOARN preparing for the next outbreak scenario.

For future major operations some thought should be put into how to make sure generators run on the same fuel and how the international effort can oversee supply security. One possibility would be to tie in the international oil industry to help establish the supply chain. The fact that electrical engineers were in short supply to maintain electricity connections and repair damage it is seen as another point international umbrella support needs to fix for future outbreak deployments.

Pre-analytical supply chain

The pre-analytical supply chain was of great concern to most laboratories. The individual groups of people involved (sampling, packing, transport and delivery) were mostly not aware of what the receiving laboratory team or indeed the various parts of the supply chain actually needed and how they would proceed with the samples on receipt. Samples were brought by all means of transport by people passing them on into the laboratory area.

The most difficult aspect of the supply chain was that uniform case ID forms were not available. Almost every laboratory team tried to solve the situation by using either case ID forms from the system the worked with in their parent institutions, translated version of these, or available forms of an internationally accepted organisation (CDC case ID form). Simply handing out copied forms resulted multiple identical barcodes from the original templates being assigned to several acute cases since staff at the ETC simply repeatedly copied them. Forms had to be handed out with stick on barcodes. The Dutch team for example used the CDC case ID form onto which they stuck their own barcodes.

Case ID forms were all too often delivered inside the “hot” sample pouch. Some were illegible; many were invariably soaked, bloody, or shredded due to being soaked inside the pouch. For that reason most laboratories created an entry portal or barrier to which the samples and their case ID forms had to be delivered and then safely processed before passing them on into the laboratory area.

The Italian team copied the case ID forms on receipt by hand onto new forms, the German team copied them onto decontaminatable paper forms (pretex® 50.150), another team used a Smartphone case ID form, which was sent into the laboratory via Wi-Fi connection.

Also future WHO emergency training packages for local support in the pre-analytical supply chain must convey an understanding of the supply chain to each chain link involved and to what happens to the sample at each chain link.

In a considerable number of cases transportation over long distances took very long and may have had an effect on sample processing and analytical results. Therefore the location of the mobile laboratory next to the ETC proved to be the best solution. It boosted communication and information exchange in some cases especially by joint meetings between the ETC and laboratory staff. It reduced sample delivery time but several concepts of transfer of the sample from hot to green zone were used. The Italian laboratory for example was connected to the hot zone through a window. The samples were left just outside of the windows after external decontamination by spraying with chlorine.

Data management

Laboratory information management systems (LIMS) were not available on site. Improvised systems included the exchange of results in csv files per emails via mobile phone or per SMS. These systems per se breached confidentiality. They were however still used due to the need to convey results. Particularly SMS messages were found to be error prone with the confusion of telephone numbers being a regularly occurring issue. The simple csv format was compatible to all sorts of computer operating systems. However the general opinion was that a mobile phone based app should be developed to allow secure transmission of results from sampling team to the laboratory and from the laboratory to HCW at the ETC. Developed concepts should however also allow easy data sharing with local government authorities while preserving confidentiality for the individual patients.

Analytical and clinical aspects of using molecular assays

Analytical sensitivity

A major discussion point was the analytical sensitivity of the molecular detection assays used. Most laboratories treated real time
PCR (rtPCR) results with a CT > 35 as equivocal and repeated the test, or the extraction and the test, or tried an alternative test. It was generally agreed that in these cases a follow up test after 2 days was necessary. If a rise in virus titre (CT < 35) then matched an evolving clinical picture this measure often helped to identify acute EVD cases. For the opposite outcome (CT > 35, or no CT) the clinical picture also had to match and indicate an end of EVD. However there was no standard approach in releasing formerly rtPCR positive, then negative patients. The detection of rising IgM was seen as a confirmation of beginning convalescence.

In Guinea the following approach was under discussion at the time of the workshop: If a EVD patient shows no more symptoms for a couple of days a blood sample should be tested by rtPCR. If it is negative, blood of the patient should be retested after 48 hrs. If negative again, it would be deemed safe to release the patient.

The South-African team investigated the correlation between CT values and the presence of replicating EBOV. They found that it was possible to isolate EBOV from samples with a CT ≤ 30, but not from samples with a CT > 33. It appeared that there was a grey zone with sporadic isolations from samples with a CT 30-32. Comparing three real time PCR systems the range of RNA copies per millilitre that corresponded consistently to successful virus isolation was from 9.12 x10^9 to 1.33 x10^10 copies/ml serum. Within the range of 1.31 x10^9 to 2.42 x10^9 copies/ml EBOV could not be isolated [2].

Internal positive controls

A variety of internal positive controls (IPC) were used in molecular assays. Molecular IPCs (RNA transcripts from plasmids) were used as inhibition and efficiency controls and in general greater variation of IPC detection was observed in manual extractions than in robotic extraction. The use of negative pick up controls in extractions and test runs was also a common feature used. In general human housekeeping genes were not regarded as reliable as their expression level may vary greatly. During the outbreak however genetic detection of human material in swab samples e.g. by detection of the RNASE P gene was found to be helpful. In contrast to the original protocol disseminated by the CDC, which allows for the detection of the RNASE P DNA, many operators would prefer mRNA detection as this also acts as RNA extraction control. It is quite clear that defined external quality assessment (EQA) panels are needed for the continued development of assays and that quality control of tests should include CT drifts of IPCs over time to avoid loss of IPC efficiency.

A major feature faced by some of the bigger teams was the turnaround of staff and the need for SOPs that could be handed over from one team to the next. Even if the SOP had evolved the documentation of that evolution is necessary to allow teams to take off from the quality level the assay has arrived at without the need to reiterate previous experiences made with the assays during the outbreak.

Procedural aspects

A discussion on turnaround time for real time PCR assays from receipt of sample to result pointed out that manual, semi-manual extraction and molecular amplification pipelines which were used in the various mobile laboratories were just as fast as the GeneExpert system all roughly needing on average 3hrs [2]. The GeneExpert system is simpler when it comes to the number of pipetting steps but overall does not offer much of an advantage over the existing pipelines used.

Although the GeneExpert system is now being advocated as an easily adaptable system because it is already in widespread use through existing HIV and Tuberculosis programmes there is doubt whether this system would be flexible enough as it is for example currently not available for Bundibugyo virus, and although announced it is not clear if and when an open platform of this device allowing free development of assays will be available.

It was also absolutely obvious that the term bed side test is completely misleading as staff at the ETC were so overwhelmed with their task of caring for the patients that there was simply no time nor scope to deal with bedside testing of samples. Bedside sampling was part of the routine and therefore the combination of mobile laboratories next to ETCs proved most efficient.

Clinical sensitivity

The experiences made in the EDV outbreak resulted in the following clear assessment of clinical parameters for molecular assays: Tests used in the EDV outbreak had a high positive predictive value (PPV) due to the sample population tested (Roughly 10% of those tested (case definition triage) were EBOV positive) and in most cases a sensitivity < 1. This meant that in the outbreak situation all positive tests were truly positive but that some positives may have been missed. Doctors were aware of the situation but comforted by the fact that only truly positive patients were admitted to the isolation wards. Positive cases missed in a first test were likely to be picked up in a follow up test if symptoms evolved.

Currently there are suggestions to increase the requirements for point of care robotic platforms to a high PPV and a sensitivity of 1. The consequences of this requirement apparently have not been properly thought through. A sensitivity of 1 raises the spectre of false positive patients being sent to the Ebola isolation ward where they are most likely to pick up the disease with all the consequences that would entail.

This new requirement, if strictly adhered to, transforms a community-based risk by missing some positives into an individual risk by allowing to submit not infected individuals to the isolation ward. In essence the current ETC structure would have to be overhauled to safeguard those false positive non infected patients, which means an infrastructure for individual care. This is regarded as a complicating factor for the infrastructure of ETCs. It would make them more difficult to build, organize and staff.

On the other side of the spectrum it was suggested to include some facilities for the patients awaiting dismissal subject to progressive negative testing as discussed above (e.g. football pitch).

In general it was strongly advised to link the characteristics of the individual molecular assay used to clinical data. Some mobile laboratories held weekly meetings with the clinical staff to discuss cases. This was seen as a very good measure to raise reciprocal appreciation of the expertise available. Upcoming training programmes for mobile laboratories and medical teams should include mutual training units for these very different teams and their different skill sets.

Laboratory procedures

Inactivation

The following approaches for inactivation during RNA extraction were used: AVL-Buffer (lysis buffer containing guanidin thiocynate, Qiagen, Hilden, Germany) + ethanol [3,4], AVL-Buffer + 60°C/15min [5,6], AVL-Buffer + 1% Triton-X-100 [7], SpeedXtract Suspension A (Qiagen, Hilden, Germany) + 95°C/10min [8]. All were shown to inactivate EBOV in patient samples.

...A possibility to prepare inactivation tubes containing a commercially available inactivation buffer which can be easily prepared and used during sampling, was presented during the meeting and has been published in the meantime [9].

Most teams used the following inactivation protocols for serological samples: thermal inactivation at 60°C/15min/30 min [5] or chemical inactivation with 0.1 % Triton-X-100 for 15-20 min [7,10] or chemical inactivation with 4% paraformaldehyde [4].

A major concern was expressed for samples for clinical chemistry. First of all many different types of tubes are used for different analysis systems. To many it was unclear how these samples could be inactivated. Out of caution clinical chemistry devices were therefore used in glovebox systems. However the Piccolo Xpress Chemistry Analyser and i-Stat systems used suffered from the corrosive nature of the decontaminants used (bleach) in the hot zone, and required ambient temperature (air conditioning obligatory). Some of the devices were not suitable for use under negative pressure. A new inactivation protocol for samples for clinical chemistry testing has described the use of 0.1% Triton-X-100/60min [11].

Gloveboxes

A variety of gloveboxes was used by the various teams. There are no internationally accepted standards for larger fixed (stationary in lab tent) or smaller mobile (flexible pop-up plastic or hardplastic) gloveboxes. Most of them used negative pressure but one mobile hardplastic box did not. Specifications should be developed and agree on the negative pressure value to be obtained, the types of filters to be used and on filter exchange intervals. It was suggested to use blower testing to assess negative pressure and integrity of gloves. This however needs some consideration as the complexities of the maintenance requirements of a BSL 3/4 laboratory should not be transferred directly to field laboratories, and guidance on glovebox maintenance as published by Health and Safety Executive in the United Kingdom can only be a starting point for this discussion [12]. A safe but pragmatic approach is needed. Many operators found that using a laboratory glove on top of the fixed glove improved dexterity.

Decontamination

In comparing decontamination protocols it emerged that mostly 0.5% bleach was used for daily cleaning (0.1% used by some in inside the laboratory) and external sample container decontamination, 0.05-0.1% bleach for glove or hand washing, 1% bleach inside the glovebox and 5% for glovebox decontamination.

The French team in Macenta, used peracetic acid to sterilize the class III safety cabinet once a day (after prior inactivation of waste with 0.5% bleach). After external decontamination with 0.5% bleach, the Piccolo and i-STAT instruments and the centrifuge were placed into hermetic bags to avoid entering in contact with the peracetic acid during the sterilization procedure.

The Institute Pasteur de Dakar used Incidin 1%. This disinfectant is accredited for use in French speaking countries of West-Africa and is not corrosive. It should be regarded as an alternative to the use of bleach as respiratory irritation was reported from quite a few of the international staff members that worked in the mobile laboratories [13]. It could also ameliorate the corrosion problems which some laboratory gear suffered.

Post-epidemic needs

An extensive collection of EVD patient samples was made during the outbreak at several sites. The UK and Italy have arranged for contracts with the government of Sierra Leone to allow for regulated transfer to and use of the samples in European laboratories. These exemplary contracts should be evaluated to draft template contracts for future outbreaks. Contracts were not used in all cases and some sample collections were transferred without formal agreements. Everybody present however agreed that formal agreements should be the rule to avoid individual research teams or consortia deciding on samples and to make them available to the scientific community.

Research needs to be addressed were discussed and this shortlist of topics should be put on the research agenda by funding agencies

1. Correlation of molecular testing results with clinical symptoms and progression of the disease
2. Determination of the suitability of detection assays for the various sample types (Blood, urine, swabs)
3. Determination of residual infectivity in low titre samples after resolution of the disease
4. Research on mother-child transmission and transmission to HCW during emergency cesarian section.
5. There is an urgent need to fund research into differential diagnostics for viral haemorrhagic fevers
6. Concepts to improve training of local staff assisting in sampling and transportation of samples
7. Preparation for future outbreak emergencies with special attention to supply logistics in regard to custom clearances, cold chain supply , fuel supply, mobile internet supply.

Acknowledgement

This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement Nº115843. This Joint Undertaking receives support from the European Union’s Horizon 2020 research and innovation programme and EFPIA.

References


