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INMI/Emergency NGO Italian laboratory established in Sierra Leone during Ebola virus disease outbreak in West Africa

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Abstract

The National Institute of Infectious Diseases "L. Spallanzani" led an Italian project for the establishment of a laboratory for the diagnosis of Ebola virus disease (EVD) in the premises of the Ebola Treatment Center (ETC) managed by the Italian Non-Governmental Organization (NGO) Emergency in Goderich, at the periphery of Freetown, Sierra Leone. The activities of the laboratory started on December 12th, 2014 and lasted until June 26th, 2015. Since the opening, the laboratory tested more than 3000 specimens from Emergency NGO ETC and from other referring facilities operating in Western Urban and Rural area of Freetown. This article describes the experience of setting up, implementing and running a field laboratory during the 2013-2016 EVD outbreaks in Sierra Leone. This experience may be of interest for other mobile laboratories in the revision and optimization of their set up methods and procedures.

Abbreviations: EVD: Ebola virus disease, ETC: Ebola Treatment Center, NGO: Non-Governmental Organization, INMI: Italian National Institute of Infectious Diseases "Lazzaro Spallanzani" IRCCS, HIDs: Highly transmissible Infectious Diseases, ECDC: European Center for Disease Prevention and Control, WHO: World Health Organization, GHSAG: Global Health Security Action Group, EMLab: European Mobile Laboratory project, DevCo: EuropeAid Cooperation Office, DGCS: Directorate General for the Development Cooperation, IPC: Infection Prevention Control, SLMoH: Ministry of Health of Sierra Leone, DFID UK-GOV: British Government's Department for International Development, PPE: Personal Protective Equipment, BSCIII: class III Biological Safety Cabinet, SOPs: Standard Operating Procedures, EBOV: Ebola virus, EDTA: ethylenediaminetetraacetic acid, LIS: Laboratory Information System, IC: Internal Control, Ct: Cycle threshold, BSL4: BioSafety Level 4.

The Italian project: INMI and Emergency NGO

The recent Ebola virus disease (EVD) epidemic in West Africa highlighted the importance of a rapid response in the field. Starting from March 2014, when the EVD epidemic was confirmed and recognized, several field laboratories have been progressively set up in the affected countries, contributing to the control of the spread of the infection [1,2].

Since 1994, the Italian National Institute of Infectious Diseases (INMI) "Lazzaro Spallanzani" is recognized as the National Referral Center for the management and diagnosis of highly transmissible infectious diseases (HIDs), such as viral hemorrhagic fevers [3]. INMI is involved in several preparedness and research projects on HIDs, in collaboration with the European Commission, European Center for Disease Prevention and Control (ECDC), World Health Organization (WHO) and other International and Public Health Institutions, and it is a member of the Global Health Security Action Group (GHSAG) since 2001, and of the Global Outbreak Alert and Response Network (GOARN) since 2003. Although INMI was already strongly engaged in previous activities for the implementation of the response directly in African countries (i.e. health cooperation programs in the United Republic of Tanzania since 2005), it had to stretch its capacities to provide assistance in outbreak scenarios of such magnitude. The participation to the European Mobile Laboratory project (EMLab), funded by EuropeAid Cooperation Office EuropeAid Cooperation Office (DevCo) and launched before the EVD outbreak, represented for INMI a first full engagement in projects aimed at promptly responding to epidemic-prone diseases and at facilitating a rapid and effective containment, directly in the field [4,5].

The first confirmed case in Sierra Leone was reported at the end of March 2015 in Kenema district. By July 2015 the disease had spread

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rapidly in the area of the capital city of Sierra Leone, Freetown and the EVD cases dramatically increased [6,7].

INMI was requested by the Directorate General for the Development Cooperation (DGCS) - Ministry of Foreign Affairs to offer its expertise to the Italian project in Sierra Leone for the establishment of a laboratory for the diagnosis of EVD in terms of laboratory and clinical/infection control experts. The project was based on the collaboration with Emergency Onlus, an Italian Non-Governmental Organization (NGO) founded in 1994 to provide medical and surgical care to the victims of war, landmines and poverty [8,9]. Since 2001, Emergency has been active in Sierra Leone with a Surgical and Pediatric Center, which has become the main reference center for trauma surgery in Sierra Leone and neighboring countries. The Center - located in Goderich, on the outskirts of Freetown – has been strongly involved in the EVD fight since the very beginning of the epidemic through the implementation of EVD-oriented Infection Prevention Control (IPC) measures for containing the spread of the disease and preventing infection.

During the peak of the epidemic, the Ministry of Health of Sierra Leone (SLMoH) asked Emergency to build and run a field structure of 22 beds for Isolation of suspected EVD cases in Lakka. The center - open on September 18th, 2014 - was immediately used as a treating facility: the Lakka Holding and Treatment Center, the first unit in the Western Area.

The new laboratory was located in Goderich, in the premises of the new Ebola Treatment Center (ETC) managed by Emergency (Figure 1). The British Government's Department for International Development (DFID UK-GOV), together with Emergency's private donations, financed the construction and most of the running costs of the ETC (including the EVD diagnostic laboratory as masonry building), the only center for EVD in West Africa equipped with an Intensive Care Unit to guarantee an isolated, air-conditioned and clean environment, which could allow sterile maneuvers and invasive procedures.

DGCS funded the deployment of INMI laboratory experts and supported Emergency for the running costs of the laboratory, including reagents and disposables. The project was rapidly developed: the planning and organization of the project started in October 2014, the construction of the laboratory was completed and handed over on December 4th, 2014. The equipment and the setting up of the laboratory occurred together with the progressive implementation of the ETC by Emergency's personnel. The INMI laboratory and Emergency ETC became both operational on December 12th, 2014 and the laboratory activities lasted until June 26th, 2015, also giving an effective support to the surveillance program headed by the SLMoH.

Figure 1. a) Map of Sierra Leone. The Italian EVD diagnostic laboratory run by INMI was located in Goderich, at the periphery of Freetown (red circle).b-c) The ETC managed by Emergency NGO where the Italian laboratory was located. DFID UK-GOV financed the construction of the ETC and of the associated EVD diagnostic laboratory.

The new laboratory in the new ebola treatment center: laboratory concept

The ETC was built by the Royal Engineers as proxy for DFID UK-GOV in cooperation with Emergency's technical and logistic division in the premises of the Olympic Committee field inside the "Milton Margai College" and was provided with 88 beds, including 20 beds in Sub-Intensive Care Unit and 24 beds in Intensive Care Unit. All the admitted patients had a positive result to the Ebola diagnostic test.

The ETC was divided in three different areas based on the risk level evaluated on the proximity to the Ebola-infected patients hospitalized in the ETC: "white-zone", the more external area, considered as a "safe" zone; "green-zone", controlled area, accessible only to authorized personnel; and "red-zone", where the Ebola-infected patients were treated and the authorized and trained personnel could only enter by wearing full personal protective equipment (PPE) [10].

The Italian EVD diagnostic laboratory was placed in the green zone, with an independent entrance outside the center. The laboratory was connected to the red zone through only one window, which can be opened only from inside the laboratory and it was functional for the reception of the samples directly from the ETC (Figure 2).

The laboratory was provided with an air conditioning system, easily cleanable walls, running water, sodium hypochlorite solutions (0.5% and 0.05%) and electric power with UPS system for sensitive electronic equipments (i.e. PCR instruments and biological containment cabinets). The main equipment of the laboratory included one fridge +4°C, two -20°C freezers (one dedicated for the laboratory reagents and residual extracted RNAs, the other one for the storage of residual biological samples), two high-speed refrigerated microcentrifuges, one benchtop centrifuge for the plasma separation, two Real Time RT-PCR instruments and two class III Biological Safety Cabinets (BSCIII), also denominated glove-boxes (Iteco Engineering s.r.l., Italy). The glove-boxes were used for the safe handling of samples; one of the two glove-boxes was exclusively used for the daily diagnostic activities, the second was used mainly as backup [11].

The space was organized according to the workflow of the laboratory activities, as shown in Figure 3. Specifically, the laboratory was provided with separated and dedicated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities. The workflow proceeded in unidirectional manner

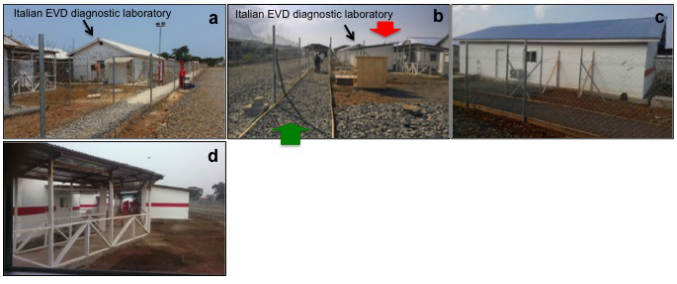

Figure 2. a-b-c) The building of the Italian EVD diagnostic laboratory was placed in the green zone of the ETC and was provided with an independent entrance from outside the center. d) View from the window of the laboratory sodium hypochlorite to the "red zone" where the EVD patients were admitted.

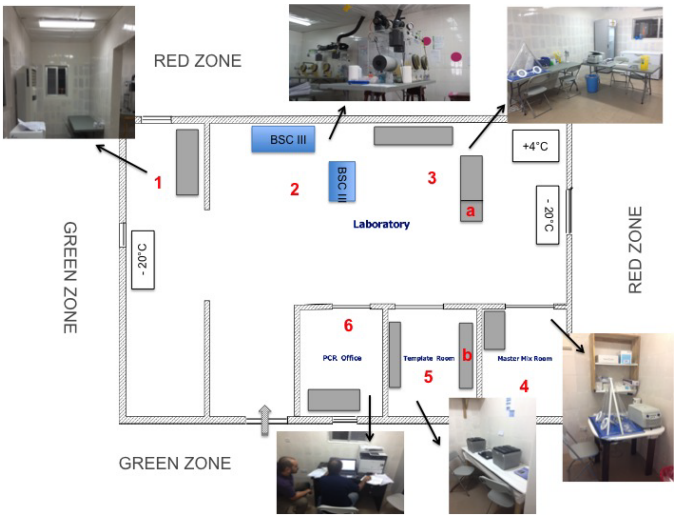

Figure 3. Plan of the Italian EVD diagnostic laboratory according with the workflow of the laboratory activities: (1) acceptance of the samples arriving from Emergency ETC and from other referring centers and hospitals in Freetown; (2) inactivation of the samples performed inside the glove-boxes; (3) viral RNA extraction; (4) PCR master mix preparation; (5) PCR run and (6) office where the data were analyzed and the results were inserted in the SLMoH report and in Laboratory Information System (LIS). Concerning the last 3 spaces, specific rooms were dedicated in order to better separate the activities. The extracted RNA was added to the PCR master mix in the same place of the extraction procedure but on a different bench (a), as for the EBOV positive control, which was added in the room of the PCR instruments, but on a dedicated and cleanable bench using dedicated pipettes and tools (b).

and each analytical phase foresaw the usage of dedicated set of tools and gowns in order to avoid contaminations.

In addition to the Italian EVD diagnostic laboratory, the Emergency' ETC was supported by a general hematology and biochemistry laboratory placed inside the red-zone and run by Emergency staff in full PPE.

The INMI staff deployed for the Italian EVD diagnostic laboratory was organized in teams generally composed of three scientists/laboratory-technicians. Each mission lasted normally five-week, with a one-week overlap between teams. The work schedule envisaged more than ten working hours/day, seven days a week, in order to guarantee an effective and continuous support to the ETC clinicians and to the epidemiologists involved in surveillance activities.

Together with the diagnostic activities, a laboratory biobank consisting of residual samples was established and training sessions for the local lab-technicians were carried out upon request from the SLMoH.

Diagnostics guidelines established by WHO were adopted, and specific Standard Operating Procedures (SOPs) were established for laboratory and biosafety procedures [11].

The EVD diagnosis on live patients was mainly performed on plasma and oral swab samples. The latter samples were generally collected to test the deceased patients or in situations where blood collection was not possible e.g. in children. Indeed, swab collection from live patients was not recommended due to lower sensitivity of RT-PCR.

Urine samples collected from recovering patients after a second negative Ebola virus (EBOV) test result on plasma were also tested. Indeed, the discharge criteria adopted by Emergency's clinicians consisted of two negative results obtained at a distance of at least 24 hours both on plasma and on urine samples [12].

Moreover, other specimens, including axillary swabs, finger pricks, wound swabs, sperm, rectal swabs, dialysis filtrate, collected under special circumstances (e.g. if the patient needed surgery) were also tested [13].

Laboratory methods and procedures

Pre-analytical steps

The Italian laboratory received samples from Emergency ETC and from other referring facilities operating in Western Urban and Rural area of Freetown (i.e. Connaught Hospital, Lumley Hospital, Military Hospital), according to the directive of Sierra Leone public health authorities. The clinical specimens from patients hospitalized in Emergency ETC were hand-delivered directly from the red-zone by the healthcare workers soon after collection. The samples arriving from external centers were delivered by the SLMoH staff to a dedicated reception area. In both cases, the samples were packed in a double container and submerged in a bucket with freshly prepared 0.5% sodium hypochlorite for 10 minutes to allow external decontamination (Figure 4a and 4b) [14].

Plasma was obtained by centrifugation of purple-cap tubes containing ethylenediaminetetraacetic acid (EDTA) at 3500 rpm for 10 minutes, using falcon tubes as sealed centrifuge buckets.

The specimens were accompanied by the appropriate case investigation forms and each sheet of paperwork was decontaminated before examination (Figure 4c). A specific laboratory form, containing the main data of the patient (i.e. patient's name and age, hospital or other provenience, identification code, symptoms and date of symptoms onset) and of the specimen (i.e. type of the specimen, date of collection, tests requested), was developed and used by the laboratory staff as check-list during the analytical procedures to ensure the correct workflow.

Clinical samples arriving at the laboratory were recorded in a Laboratory Information System (LIS), which was developed and adapted on the basis of existing software used at INMI. A unique laboratory identification number was assigned to each sample. This number was indicated on the laboratory forms as well as on the labels,

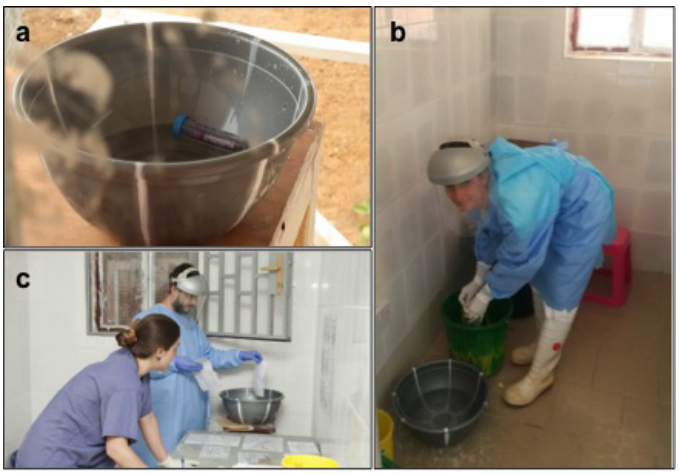

Figure 4. a-b) Samples delivered to the EVD Italian laboratory were submerged in a bucket with freshly prepared 0.5% sodium hypochlorite and left for 10 minutes for decontamination of external surface of the tubes. c) Case investigation forms accompanying the specimens were decontaminated in freshly prepared 0.5% sodium hypochlorite before examination.

which identified the tubes used for the analytical procedures and for the samples' aliquots. Indeed, the amount of samples left over from the diagnostic test was aliquoted (at least two aliquots per sample) inside the glove-box in cryovials and stored in zip-locked bags at -20°C (inside a sealable plastic box). The aliquots were recorded in the laboratory biobank database using a sequential numeration and each aliquot was linked to the clinical and personal data of the patient whenever available.

Analytical steps

EVD diagnosis was performed through direct detection of EBOV RNA, based on Real time RT-PCR method and following the workflow as showed in Figure 3 [15].

The viral RNA was manually extracted using the QIAmp Viral RNA kit (Qiagen, Germany). The inactivation of the biological specimens, the first and most critical part of the viral RNA extraction procedures, was performed inside the glove-box (Figure 5a) [16]. Specifically, for viral inactivation a fixed volume (140 µL) of each sample was transferred to a tube containing 560 µL of the lysis buffer AVL (guanidine thiocyanate-based), that was mixed by repeated up and down flipping. Following 10 minutes of incubation in AVL, the inactivated suspension were transferred to a new tube containing 560 µL of absolute ethanol. All necessary reagents (AVL, ethanol, water) were prepared before starting the activities, as ready-for-use aliquots for each sample outside the cabinet. The AVL was supplemented with an internal control (IC), provided by the diagnostic test kit's producer, to control the good sample preparation and to rule out possible RT-PCR inhibition. For each batch of processed samples, a negative control, prepared using sterile water instead of clinical specimen, was included to check that no contaminations occurred during the analytical procedures.

Dry swabs were swirled in 300 µL of nuclease-free water (Qiagen, Germany) to dissolve biological material, and the necessary amount of suspension was used for RNA extraction (Figure 5b).

The inactivated samples could be taken out from the glove-box through the dedicated pass-box, only after ethanol addition and decontamination of the tube surfaces for 10 minutes in freshly prepared 0.5% sodium hypochlorite (see paragraph 3.4).

Subsequent analytical steps were performed outside of the glove-box. RNA extraction was carried out following the QIAmp Viral RNA kit instructions (Figure 5c). The protocol was modified to accommodate an additional centrifugation step of the extraction columns (10 minutes at approximately 17000 x g, i.e. ~ 13000 rpm) after the last washing step; finally, a double elution step (40 µl each) was performed to increase the yield of viral RNA extracted from the QIAamp Mini column.

RealStar Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics, Germany: sensitivity = 1280 cp/ml), specifically targeting the L gene, was used as the routine reference assay [15,17,18]. Despite the suboptimal sensitivity of the kit, but in reason of the good field performance observed in our previous experiences in Europe and in Africa, we decided to use the Altona kit throughout our activity in the Goderich Laboratory [19]. If needed, RealStar Filovirus Type RT-PCR Kit 1.0 (Altona Diagnostics, Germany) was also available at the laboratory as confirmatory test.

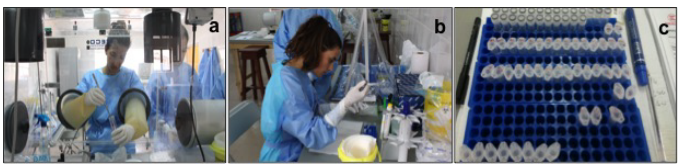

Figure 5. a-b) The analytical steps included chemical inactivation of patients' specimens performed inside the glove-box and the subsequent RNA extraction procedure occurred on the dedicated bench outside the glove-box only after accurate decontamination of the eppendorf tubes surfaces using freshly prepared 0.5% sodium hypochlorite. c) Ready for use aliquots of AVL and Ethanol prepared for each sample outside of the glove-box and before starting the inactivation procedures.

In addition, a fast "ZEBOV - Ebolavirus Zaire Strain" Real Time RT-PCR test developed by Clonit (Clonit s.r.l., Italy) in the framework of EbolaMoDRAD project and for which we are currently finalizing the validation phase, was used in parallel with the reference test to confirm a negative result in some tricky cases. For each batch of analyzed samples, a synthetic viral RNA, provided by the kit producer, was added to a no-sample master mix tube, to serve as a positive reaction control.

Real time RT-PCR was performed using the Smart Cycler system (Cepheid, USA), where each reaction sites is independently programmable and different cycling protocols can be simultaneously performed and started at different times, allowing to perform multiple and different runs at once.

In addition, it was possible to obtain the viral genome quantification (viral load as cp/ml), based on a standard reference curve kindly provided by the kit producer.

When required, Malaria rapid test (SD Bioline Diagnostic, Republic of Korea) as well as HIV-1/2 Combo rapid test (Alere Inc, USA) were performed on whole blood samples from suspected patients. Since both tests had to be carried out on non-inactivated specimen, they were performed using high containment measures inside the glove-box before starting EVD diagnosis [10].

Post-analytical steps

Results from the EBOV RT-PCR assay were evaluated comprehensively with attention paid to both targets, i.e. EBOV target and IC target. The cycle threshold (Ct) cut-off of the EBOV target for this method was set at 45; samples with Ct between 45 and 35 were considered as weak positive, so the test had to be repeated.

Specimens were considered negative when two criteria were met: (1) EBOV L gene was undetected; (2) the IC was detected in an acceptable range of Ct values (approximately 25-28).

In case of failed IC signal (i.e. undetected signal or detected at a Ct value with >3 Ct difference with the Ct of the IC in the negative control), the test results were considered indeterminate, possibly due to the presence of RT-PCR inhibitors in the specimen. If any of the control conditions (i.e. IC within acceptable Ct values and an acceptable signal in the positive control), the analysis of the sample was repeated, possibly on a new sample or, if not available, by diluting the original sample.

According to WHO guidelines, a negative result was considered pending if the specimen was collected <72 hours after onset of clinical illness [11]. In these pending cases, a subsequent follow-up specimen, collected at least 48 hours after the first specimen, was requested for a definitive diagnosis of EVD status. Indeed, individuals suspected to have EBOV infection could be discharged if two negative EBOV RT-PCR tests were obtained on blood samples, collected at least 48 hours apart.

The results were released as indeterminate when the diagnostic

test remained invalid after two separate RNA extractions and RT-PCR test runs. The overwhelming majority of the indeterminate results were obtained on oral swab from dead bodies, whose poor quality was probably due either to improper collection techniques or to excessive transit time and inappropriate shipment conditions to the laboratory.

The results were recorded and gathered in the laboratory software and a specific laboratory result sheet was printed for each sample. A hard copy was delivered to the medical staff or to the responsible authorities. The data obtained were expressed both as Ct values and viral quantification (cp/ml). Telephonic communications were used to promptly report the diagnostic results to others referring centers. The laboratory results were transcribed in a form provided by SLMoH to be sent as an excel file without further "security protections" by e-mail according to the instructions of the local authorities.

Others biosafety procedures used and adaptations developed during deployment

The samples arrived in the laboratory in double packaging, consisting of a primary container (i.e. EDTA collection tube, urine container, swabs arriving both dry or in virus transport medium) within a secondary container. Falcon tubes and zip-locked bags were used as a secondary container since this had to be sealable and washable.

The secondary container was opened only once inside the glove-box. It was important to ensure a higher level of safety for the laboratory personnel in case of accident or damage of the primary container as well as to protect the samples' integrity at the moment of decontamination in sodium hypochlorite (Figure 6) [14].

The glove-boxes were used for the handling and inactivation of infectious specimens as they represent the highest level of BSC, can be used with a minimal PPE and in the presence of air conditioning system [11,20]. They consisted of a solid sealed structure in Plexiglas operating with negative air pressure. They were equipped with arm length gloves and HEPA filters on the incoming as well as exhausted airflow. Exhausted air was not recycled in the laboratory room, but after filtration through double HEPA filters, it was flowed outside the laboratory by a specific canalization running from the cabinet to the laboratory roof. The working pressure in the glove-boxes was set at - 10 mmH_2O and the cabinets were connected to an UPS system. The cabinets were provided both with a pass-box with two interlocked doors to safely introduce or extract materials, and with a further exit window where a double plastic bag was applied for waste disposal.

The PPE worn during the samples inactivation phase included surgical scrubs and boots, disposable level 3 surgical gowns and gloves. The specimens were opened inside the cabinet and handled wearing 3 pairs of gloves: personal latex gloves, the cabinet's arm length gloves, and on top of that, gynecologic latex gloves. This third pair of gloves ensured a higher level of safety for the worker as well as a better cleaning during the procedures (Figure 7a). In addition, for higher comfort and greater dexterity, the third pair of gloves could be selected taking into account the different hand size of the operator working in the glove-box.

During the inactivation phase, a second person ("buddy") supported the operator working inside the glove-box, thus ensuring the correct processing of the samples through check-lists (Figure 7b and 7c).

Daily and periodic maintenance procedures were performed on the BSCIII by the teams working in the laboratory.

The sleeves of the glove-box were inspected for leakage or damages every day before starting the activities. Once every two weeks, or whenever necessary, gloves were replaced using a specific protocol which allowed to remove the old gloves and insert the new pair at the same time (Figure 8a). The integrity of the system and its capacity to keep the negative pressure were checked daily through a vacuum test: glove-box was kept under negative pressure at night and, in the morning, it was checked whether the pressure had remained stable during the night (Figure 8b).

HEPA filters were replaced according to manufacturer's instructions after 400 hours of use recorded by an electronic device.

A complete decontamination of the BSCIII was obtained with fumigation using formalin gas (10 mg/mm^3 formalin for at least 12

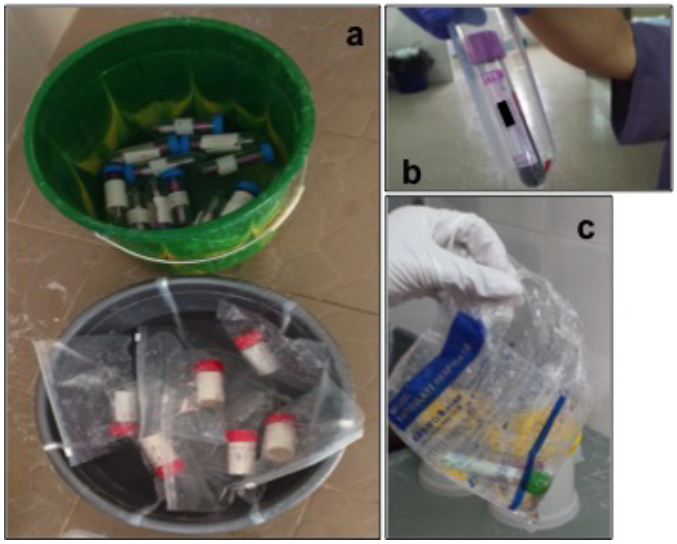

Figure 6. a) Specimens were submerged in 0.5% sodium hypochlorite for decontamination purposes.Falcon tubes and zip-locked bags were used as secondary containers. b) Example of the importance of the secondary container to ensure a higher level of safety for the laboratory personnel in case of accident or damage of the primary container outside the glove-box.c) Case of specimen not properly packed following the decontamination procedures in 0.5% sodium hypochlorite.

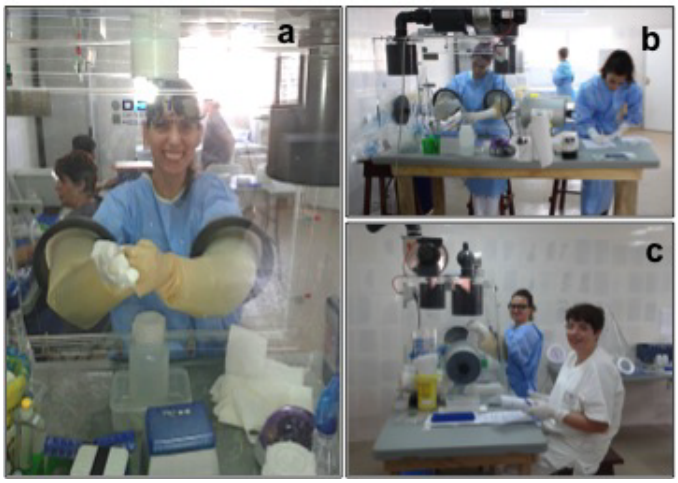

Figure 7. a) The specimens were opened inside the glove-box and handled wearing 3 pairs of gloves: gloves personal latex gloves, the cabinet's arm length gloves, and on top of that, gynecologic latex gloves. b-c) A second person, "buddy", supported the operator working inside the glove-box, thus ensuring the correct processing of the samples through the check-lists.

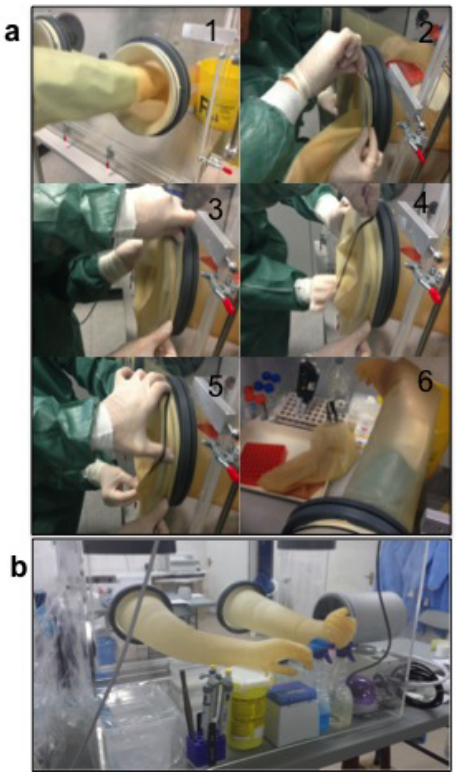

Figure 8. a) The cabinet's arm length gloves (sleeves) were replaced using a specific protocol which allowed to insert the new pair of gloves (1-5) and at the same time, remove the old pair, which are released inside the glove-box to be directly and safely discarded (6). b) The vacuum test was daily performed to check the integrity of the system and its capacity to ensure the negative pressure.

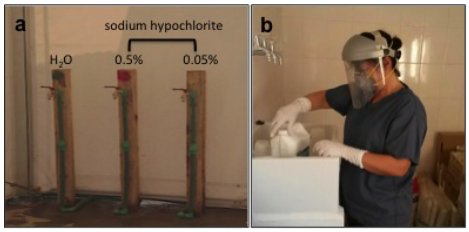

Figure 9. a) Running water and sodium hypochlorite solutions were provided by Emergency NGO logistic division. 0.05% sodium hypochlorite was used for washing and disinfecting hands and other things. 0.5% sodium hypochlorite was used to decontaminate potentially infected materials, tools and surfaces. b) 5% sodium hypochlorite was freshly prepared from the powdered solution and used to decontaminate discarded specimen tubes and tips used for the inactivation procedures occurred inside the glove-box.

hours). It was conducted every 2 months or whenever necessary, and it was required when changing the external filter. A specific procedure allowed to change the internal filters without fumigation of the BSCIII. All the examinations and tests conducted on the glove-box system were promptly and systematically recorded by the laboratory staff.

Freshly prepared 0.5% sodium hypochlorite was daily provided by Emergency logistic division. The surfaces of the tubes containing sample + AVL + ethanol and all the tools or materials which had to be removed from the glove-box (e.g. tubes racks, empty bottles for the water or sodium hypochlorite solution), were disinfected using 0.5% sodium hypochlorite solution for 10 minutes in the cabinet pass-through [20] (Figure 9a).

Discarded specimen tubes and tips used for the inactivation procedures were soaked in 5% sodium hypochlorite before disposal in a waste bag placed inside a solid bucket (Figure 9b). The waste bag was sprayed with 0.5% sodium hypochlorite and then disposed in double biohazard autoclave bags. Generally, the waste bags were removed from the glove-box the next morning or at least after three hours to ensure the chemical inactivation of the materials. The triple-bagged trash was transferred out of the laboratory to the red zone for incineration.

At the end of the working day, a substantial cleaning of the glove-box took place, including surfaces, tools and arm length gloves, using 0.5% sodium hypochlorite. After 10 minutes, they were washed using water to halt the corrosive effect of the sodium hypochlorite.

The laboratory was provided with two dedicated guards, with daily and night shifts, who guaranteed a good level of security for the laboratory personnel and for the residual biological samples stored inside the laboratory in a dedicated - 20°C freezer. In addition, when unattended by the laboratory team, the laboratory was well locked and the freezer containing human samples had a safe padlock.

Strict procedures were adopted for health surveillance of laboratory staff during the mission, including mandatory reporting of any symptoms occurred and anti-malaria prophylaxis in order to avoid infections, which could clinically mimic EVD.

After returning from the mission, according to the Italian Government plan, the laboratory staff underwent a short health checkup upon exiting the airplane at Fiumicino "Leonardo da Vinci" Airport (Rome), as well as the monitoring the body temperature during the 21 days after the return. In case of any symptoms (fever or any other health problems), the INMI staff was instructed to call the INMI coordinator, available 24/7. He would evaluate and predispose testing and isolation procedures, in consultation with the ID clinical team of the Institute. None of the Italian laboratory personnel deployed has been put in isolation.

Challenges and opportunities

Previously, INMI did not have extensive experience in the implementation of field laboratories for the rapid response to outbreaks of such magnitude and, initially, could not count on a large number of staff with previous experience in field laboratories or in risk group 4 pathogens diagnostic.

As mentioned above, the involvement in the EMLab project represented an initial and unique opportunity in this respect [5]. Moreover, INMI has made continuous intense efforts to expand and improve its capacity and expertise. Young and well-motivated INMI scientists have been fully involved in the project. Extensive training sessions and mock deployments of at least a week were organized at INMI's laboratories in Rome before departure in order to provide a solid knowledge about all aspects of the diagnostic processes and the technology used and to prepare the staff to possible technical and environmental issues that could occur during deployment. Specifically, the training mainly focused on bio-safety measures, technical and methodology SOPs to be adopted in the field laboratory, and pre-analytical processes and storage of the samples. In addition, the same equipment, instruments and items present in the field laboratory were employed during the training.

An intense team building activity was pursued by means of information sharing and debriefing sessions: all team participants were kept regularly informed by email (using a dedicated mailing list) and invited to periodical meetings where staff returning from the field normally reported on their experience.

Another remarkable challenge was the lack of INMI logistic structure in the field or any knowledge of the Country. The support by an NGO as Emergency, with a consolidated local presence, a strong logistic capacity and a wide network of contacts with the national and local institutions, was crucial to overcome logistic and cultural issues arising in the field, including laboratory tools and reagents procurement. DGCS also provided valuable support in institutional relationship and in governmental agreements.

The Italian project represented an important opportunity for INMI on many fronts.

First of all, the cooperation with the Emergency NGO clinicians working in the ETC allowed for the development of significant expertise and knowledge about EVD diagnosis and treatment, which was also useful and proved extremely valuable for the management of the two EVD patients successfully treated at INMI.

Secondly, it led to a greater preparedness to outbreak response, including the mentoring and the coaching of young scientists who could provide the basis for future activities and projects.

The project provided an excellent opportunity for the establishment of new international networks and more extensive and intense cooperation with other important research institutions, international organization like WHO, NGOs present in the field and with African public health authorities.

Finally, this experience represented a true example of international cooperation. It was the result of a joint effort by public institutions devoted to the development cooperation such as DFID and DGCS, research and healthcare institutes such as INMI, and NGOs such as Emergency.

Conclusions and recommendations for future deployment

As of March 30th, 2016, there have been 10666 total cases of EVD in Sierra Leone during the 2013-2016 West African epidemics. Of these, 3955 (37%) cases were fatal. Sierra Leone was first declared Ebola-free on November 7th, 2015. Two new cases were later reported in January 2016 and, after 90-days of enhanced surveillance, Sierra Leone was again declared Ebola-free on March 7th, 2016 [6,7].

The role of the Italian laboratory in Goderich was to provide EVD diagnostic for three purposes: (i) testing blood drawn from patients in holding centers to guide their admission to an ETC, (ii) testing patients in the ETC to help with decisions regarding their clinical management and safe discharge, and (iii) testing oral swab samples collected from dead bodies to facilitate contact tracing, the implementation of safe burial protocols and survaillance.

Since the opening of the Emergency ETC, around 120 patients were admitted to the hospital. The ETC closed on June 2nd, 2015 after the last two patients were discharged, whereas the Italian EVD diagnostic laboratory continued implementing the surveillance program by testing swabs collected from deceased people until June 26th, 2015.

The Italian EVD diagnostic laboratory received and processed more than 3000 specimens. It operated non-stop from December 12th, 2014 through to June 26th, 2015 (including Christmas, New Year's and Easter holidays), and 16 INMI scientists, including physicians, virologists, microbiologists and lab-technicians, were deployed in the field laboratory in 8 team shifts.

During that period, the average number of samples tested per day was 25 and the vast majority of the samples (71%) were processed on the day they were received in the laboratory, with the results released the same evening. Samples received too late for a complete processing were tested the next morning. During the drop phases of the epidemic, the laboratory tested an average of 35 oral swab samples per day. Of the processed samples, 80% were negative, 15% positive and 5% indeterminate. The most frequent types of specimen received were blood samples (53% of the total number) and swabs (41% of the samples) (Figure 10). Notably, 68% of the positive blood samples had a very high viral load, i.e. over 10^6 cp/ml. In addition, when the Emergency ETC was operational, longitudinal collection and testing of clinical specimens was requested by clinicians to monitor the viremia for EVD positive patients.

One of the main takeaways from this tragic outbreak is that the efficient interruption of the Ebola virus transmission chains critically depends on reliable and rapid laboratory diagnosis of patients suspected of EVD, and that laboratory results are needed to confirm suspected cases and to execute subsequent isolation measures [21-23].

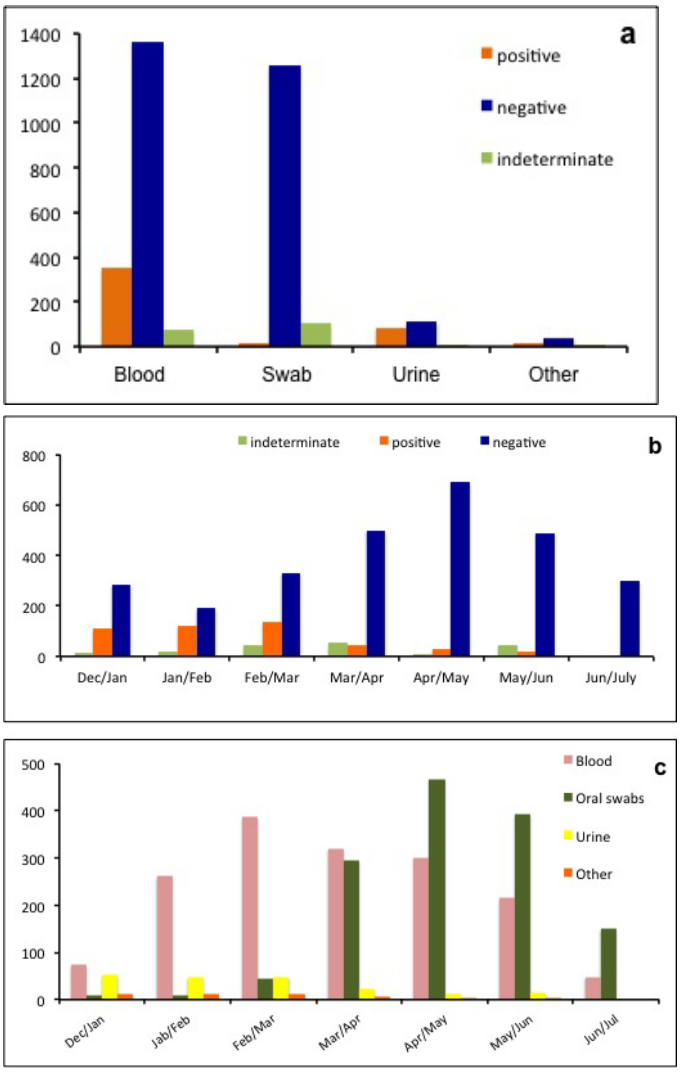

Figure 10. Cumulative number of samples tested for EBOV RNA by Italian EVD diagnostic laboratory (a) by type of specimens and EBOV RT-PCR results and (b-c) related to the time of testing.

Although all the laboratories deployed in Sierra Leone have been an important resource for the clinicians and for the success of the fight against Ebola virus, further improvements of the laboratory services should be sought [5,11,15].

New rapid and safe diagnostic tests are needed, including point-of-care tests that could detect EBOV in blood from a finger prick and, as such, would be invaluable for testing in community settings. Performing a wider differential diagnosis would give a complete pathology picture and could improve the caring of patients suspected of a viral hemorrhagic disease [15,24].

In addition, serology and other immunological assays that could be performed in the field are also needed. Indeed, few studies have been performed in the field and the assays are still restricted to BSL4 facilities. In this respect, IgM detection would be useful to support contact tracing efforts, whilst IgG detection could be used to assess antibodies titers in the blood of EVD survivors and evaluate the immunity status before its transfusion as an emergency experimental treatment or after immunization [15,25,26].

Given the importance of monitoring possible routes of transmission, validated methods to assess samples previously considered unusual and difficult to standardize (e.g. spermatic fluid and breast milk) could also be relevant.

Finally, Real-time field sequencing would be helpful for the epidemiologists and clinicians to support contact tracing and help with the identification of transmission chains [15,27,28].

In conclusion, the Italian laboratory managed by Emergency NGO and INMI is still supporting EVD diagnosis in Sierra Leone in the framework of the surveillance program led by national authorities. Upon request and agreement with SLMoH, the laboratory was moved in July 2015 to the Princess Christian Maternity Hospital (PCMH) in Freetown and is currently run by local staff under the supervision of international scientists.

In addition, further studies and researches have been carried out by INMI scientists exploiting the samples and the related data collected during the project, which resulted in a number of scientific publications [29,30]. The samples and the data are still available and used for research purposes in the framework of European and other international projects in which INMI and Emergency are closely involved. INMI has implemented further research activities, including sequencing methods to monitor the development of mutations in EBOV genomes during the epidemic and serological studies in order to investigate the presence of other pathogens relevant for differential diagnosis and for the outcome of patients in the presence of co-infection and to better understand the immunological response to EBOV infection.

Moving forward addressing many of the unanswered questions about the Ebola virus biology and gaining a deeper knowledge of the disease and the mechanisms of infection and pathogenesis, investigations and scientific research should continue to be conducted even after the end of the epidemic. Moreover, the international community, including all actors involved in the outbreak, should remain engaged and continue supporting national authorities in the development of a stronger healthcare system able to respond effectively in case of epidemics of such magnitude in the future.

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