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PANDEM

Pandemic Risk and Emergency Management

D2.3 Review of diagnostic technologies

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1 Introduction

Definitive and accurate diagnosis of infectious agents is crucial in a high-impact epidemic or pandemic for effective clinical management and for selecting other appropriate disease control activities such as contact tracing [1].

This report presents a review of diagnostic technologies currently available for the detection and identification of pathogens with pandemic potential.

The first part of this report describes existing diagnostic technologies while the second part presents the outcome of a survey undertaken using a questionnaire. The survey was conducted as part of this deliverable and targeted laboratory managers in Europe. It aims to present currently available diagnostic technologies in Europe, identifies current gaps and lists innovative solutions needed to improve diagnostics for pandemic-prone pathogens.

2 Review of diagnostic technologies

The aim of this review is to present advantages and disadvantages of existing pathogen detection methods. It is to note that a NATO group (HFM RTG-230) with experts from nine countries met regularly from May 2012-May 2016 to develop a "Depository of fast and reliable Detection Methods for Zoonotic Agents" [2, 3]. They published a list of 81 pathogens (30 viruses – 27 bacteria – 24 parasites) and reviewed the first line (field conditions) and second line (lab environment) detection methods available for each pathogen [2]. The final report from the NATO HFM RTG-230 should be issued in the second half of 2016 and will contain a list of diagnostic tests available for the large list of pathogens with pandemic potential [3].

Fast and reliable detection methods do not yet exist for every pathogen. Moreover, if point-of-care (first line methods – field conditions) diagnostics are available for some pathogens, laboratory confirmation is required for pandemic-prone pathogens. Indeed, when a diagnostic method is recommended by ECDC (see the "diagnostic" section in the disease "Factsheet for health professionals" [4]), it is always a second line method (lab environment).

This section will review diagnostic technologies both from the patient bedside and the laboratory environment point of view.

Prior to diagnostic testing, specimens must be collected following "Specimens collection guidelines" issued by official organisations such as WHO or CDC [5]. Recommendations for the packing and transport of samples can also be found in these guidelines.

2.1 Point-of-care testing

The aim of point-of-care testing (POCT) is to provide immediate and easy-to-use diagnostic assays that produce reliable information in order to take immediate clinical management decisions.

POCT is mainly used to perform biochemical monitoring of the patient (glucose, blood gas analysis, electrolytes, lipids, *etc.*) but some POCT tests are also available for rapid pathogen detection and more are under development. When available, POCT usually targets a specific agent linked with patient syndrome. Consequently, in the event of a pandemic or high impact epidemic due to an "unknown" pathogen (a new form of a known pathogen or new emerging pathogen), POCT tests will not be initially available.

The basic principle in most systems is the lateral flow immuno-chromatographic (LFI) test targeting a specific microbial antigen in the patient sample (urine, swab, whole blood), using the ELISA (enzyme linked immuno-sorbent assay) principle [6, 7]. More rarely, the target can be an agent-specific antibody as evidence for past infection.

Benefits of POCT [6-8]

- Short turn-around time;
- Positive patient identification;
- Elimination of blood collection tubes and sample transport;
- Reduced blood specimen volume;
- Few and compact material needed;
- No culture of the pathogenic agent;
- Do not require high level training.

Issues and concerns of POCT [1, 6-9]

- Lack of standardization/ quality control and lack of concordance with laboratory testing;
- Technical characteristics (sensitivity, specificity, positive and negative predictive values) are not always well determined or are determined in a lab environment that is not representative of the field environment;
- Increased risk of operator becoming infected.

New assays based on "Lab-on-a-chip" tests extend the range of targets from proteins to pathogen's nucleic acids, sometimes combining both possibilities on the same support, then allowing pathogen detection and identification with a higher specificity and sensitivity [7, 10]. Those devices are "sample-to-result" closed systems. They cover the whole process from sample preparation to the diagnostic result, reducing to a minimum the manipulations

required, and therefore reducing the risk linked to highly pathogenic sample handling. They do not require laboratory expertise and they can therefore be used after basic training.

However, it is still a matter of discussion whether or not POCTs using nucleic acid targets are really POCTs, as those technologies are more complex and increase both cost and time spent. Moreover, most of them are more "chip-in-a-lab", found within a laboratory instead of the patient bedside [7, 10]. However, further development of such processes and diagnostic devices will accelerate the practical applications of POCT diagnostic system and therefore significant financial support is provided from various foundations and programs to speed up the development to mature technology readiness level, including the Bill & Melinda Gates Foundation and the Research Program of the European Union [10]. A good example of recent POCTs is the simple, field-ready sample-processing workflow which was developed for the rapid and low-cost detection of the Zika virus, involving the detection of Zika RNA (after isothermal amplification) on a paper-based sensor [11].

2.2 Laboratory diagnostics

Different techniques can be used for detecting and identifying pathogenic agents. ECDC factsheets for specific diseases recommend the following diagnostic techniques [4]:

- Serologic testing;
- Microscopic examination;
- Culture (bacterial culture or viral isolation on cell culture);
- Nucleic acid detection by (RT-) PCR[1], sometimes complemented by amplicon sequencing;
- Antigen detection;
- Bioassay (i.e. subcutaneous inoculation of adult laboratory mice).

The serologic testing and PCR are the main used techniques and are usually the first to be developed in case of an outbreak of a new, emerging disease [12].

In addition to the techniques above, more recent technologies including nucleic acid isothermal amplification, matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and next generation sequencing (NGS) will also be reviewed.

[1] (RT)-PCR – reverse transcription polymerase chain reaction

2.2.1 Serology testing

Serological tests allow the identification of the specific antibodies (IgG and IgM immunoglobulins – hemagglutinin – neutralizing antibodies) which can be found in patient serum as a response to an infection [13].

The most widely used technique is ELISA[2]. It is able to detect antibody-antigen interaction; it is simple, inexpensive and rapid [14]. In addition to serum, a variety of sample types (excretions, secretions and tissues) can be used for the detection of an immune response to an agent, or for detection of the agent itself [14].

The most specific and sensitive serologic assay available is the serum neutralization test (SNT) [12], however it requires cell-culture and a long incubation time before results are available [15]. The disadvantages of serology as a diagnostic tool include [4, 12-14]:

- It is usually necessary to obtain acute- and convalescent-phase serum samples to look for a rising titre of IgG antibodies due to the lag between the onset of infection and the development of antibodies to the infecting microorganism;
- Immunosuppressed patients may be unable to mount an antibody response;
- Possible cross-reaction with pathogens from the same genus;
- Serology tests may reflect a past infection, and the current infection may have an entirely different cause. Therefore, results to be interpreted according to the vaccination status, clinical presentation from the patient and their previous exposure to pathogenic agents form the same genus;
- The validation of the tests is challenging because it is dependent on access to a well-characterized serum collection.

Because of these disadvantages, serology testing has to be completed or replaced by other diagnostic tests during the acute phase of an epidemic/pandemic. However, serology is very useful in the preparedness/surveillance phase to determine the prevalence of a disease in a population. Also, as antibodies can be detected over a long period, post-infection serological assays can help to address epidemiological questions about transmission patterns, to observe asymptomatic cases, to analyze disease progression and to identify the origin of the disease [12].

2.2.2 Microscopic examination

Microscopy may identify microorganisms [13]. Parasites, bacteria and viruses can be detected and identified on the basis of morphology.

[2] ELISA - enzyme-linked immunosorbent assay

For parasites and bacteria it is sufficient to have a compound binocular microscope (10-40-100 magnification). Visible traits that can be valuable aids to identification are the cell shape and size, Gram-stain reaction, acid-fast reaction and the presence of special structures, including endospores, granules, and capsules [16]. At that level of magnification, viruses are not visible but their presence can be spotted by the identification of viral inclusion bodies. For virus morphological analysis, electron microscopy (EM) with magnification of around 50,000 are needed [17]. EM can pinpoint additional features useful for bacteria identification such as cell wall flagella, pili, and fimbriae [16].

EM can be applied to many sample types and sample preparation is short. It is a very useful diagnostic method, as it offers an "open view" that allows the detection and morphological assessment of both novel agents and agents overlooked by the clinician [18]. However it has also several pitfalls [18]:

- Detection does not mean specific identification;
- The failure to detect and identify an agent does not mean that it is not there;
- If one is looking for something specific, one will eventually find something that may look alike after a while;
- The presence of a single picture cannot validate the interpretation of morphology;
- EM requires sensitive and costly equipment.

Therefore EM requires a high level of training for sample preparation, analysis and result interpretation and will rarely be available in low-income laboratories. Automatic Particle Detection in EM based on image library opens the way to on-site analysis and interpretation of ME, possibly assisted with remote assistance by EM experts.

2.2.3 Culture

The "gold standard" for pathogen detection and identification is still the culture of the pathogenic agent. Pathogen diagnosis by culture requires high training and might require some prior knowledge of the type of pathogens to lead the choice of protocol and to judge the clinical significance of positive cultures.

Identification of bacteria is based on growth characteristics (time, aerobic/anaerobic), colony and microscopic morphology, physiologic and biochemical characteristics [13].

Culture is complex and contingent on the origin of the sample (sterile [e.g. cerebrospinal fluid]/non-sterile [e.g. faeces]) and the growth time before further analysis can vary from hours to weeks. Moreover, only a small fraction of all bacteria can be successfully cultured, while clinically significant organisms may be slow-growing, fastidious, inert, or unviable [19, 20]. Once an isolate has grown, it is necessary to determine its species by phenotypic

biochemistry. However, this can be difficult as phenotypic and biochemical characteristics of strains from the same species may vary and, conversely, distinct species can share identical phenotypical and biochemical features [21]. If antimicrobial susceptibility has also to be assessed, additional days to weeks are required.

Identification of viruses is usually based on characteristic cytopathic effects in different cell cultures [13]. Novel culture methods reduce the time for virus detection to 24 hours. However, they still need complementary methods for precise identification of viruses [22].

2.2.4 Nucleic acid-based diagnostics

Many of the new diagnostics procedures are nucleic acid-based and replace conventional culture methods [23]. Nucleic acid-based diagnostics involves detection and characterization of both bacterial and viral infection using DNA/RNA methods. Several techniques are available but DNA amplification and sequencing are mainly used for diagnostic and only those will be reviewed here. Those assays can rapidly and precisely detect the presence of microorganisms, including those that are fastidious and slow growing, directly from clinical specimens.

2.2.4.1 Nucleic-acid amplification

Pathogen identification and characterization can be achieved by the detection and amplification of specific genetic sequences (identification of specific polymorphism in a conserved gene, detection of virulence factors, genetic antibiotic susceptibility profile determination). Current amplification techniques are Polymerase Chain Reaction (PCR) and methods for isothermal amplification. Those methods can be developed quickly based upon pathogenic genetic sequences.

Nucleic acid amplification methods are more rapid (hours) compared to culture methods (days). They are very specific and sensitive and can allow multiplexing. Yet the number of target which can be included is limited in order to maintain the test sensitivity. Therefore, to detect a broad range of pathogens, a panel of multiplex tests must be developed [24].

In all current nucleic acid-based assays, detection is based on targeting conserved regions of the pathogen genome and mutations can lead to reduced sensitivity or false negative results. Furthermore, only the targeted pathogens included in the assay will be identified, therefore atypical or emerging pathogens will generally evade detection by gene amplification [24].

It is to note that isothermal amplification methods are more rapid and have shown to be less prone to inhibition than PCR methods and, as they do not require thermal cycles as PCR

do, they can be performed with more simple and compact equipment. Therefore, they are most suitable to develop POCT and are currently used in a lot of diagnostic development [25].

2.2.4.2 Sequencing

First sequencing methods were based on the sequencing of conserved regions nucleic acid amplicons. In that technique, broad-based or universal primers complementary to conserved regions are used so that the region can be amplified (by PCR) from any bacteria. This can differentiate isolates between phylum to genus level and often to species level, but usually no further [26]. However, as this method is based on PCR with a targeted gene, it is still possible that some pathogens evade detection.

New sequencing technologies designs and metagenome approaches hold the promise of identifying all potential pathogens in a single assay without a priori knowledge of the target. The new sequencing techniques have already been used diagnose rare, novel, or atypical infectious aetiologies, in whole genome studies and metagenome studies (Reviewed in [24, 27]).

Since the advent of this next generation sequencing (NGS) techniques, there has been an ongoing expansion of sequencing methods and instruments as well as continual improvements in the quality, quantity, and cost of sequences that are produced. A good understanding of sequencing platforms characteristics and data analysis flows is necessary to choose the best sequencing approach [27, 28].

2.2.5 Antigen detection

Antigen is short for antibody generator. It refers to the protein and polysaccharide located in the outer surface of pathogens (capsule, coats, cell wall, and flagella) that triggers the host immune system into producing antibodies specific to that antigen.

As for serology, the most used technique for antigen detection is immunologic detection by ELISA [13, 14]. Antigen-detection tests are often provided in the form of POCT, with all related advantages and limitations.

2.2.6 Matrix assisted laser desorption ionization-time of flight mass spectrometry

In recent years, matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has emerged as a potential tool for pathogen identification and diagnosis [29]. During the MALDI-TOF MS process, the molecular mass of all cellular proteins is measured to determine the unique global protein profile that is characteristic of the pathogen [30].

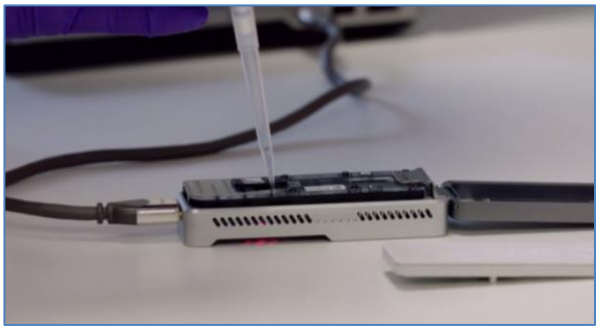
Identification of pathogen by MALDI-TOF MS can be done in minutes at a low cost. However, in some cases, this technology is still not able to discriminate between closely related species that share the same peptide mass fingerprints (PMF) and identification of new isolates is possible only if the spectral database contains PMFs of the type strains of specific genera/species/subspecies/strains [29]. The major constraint of using MALDI-TOF MS in routine microbiological diagnosis is the reproducibility of the PMFs of the same microbial species during different experiments in the same laboratory or during different experiments in different laboratories employing the same/different MALDI-TOF equipment [29].

2.3 POCT – Point of Care Testing - and mobile laboratories

The aim is to have diagnostic laboratories as close as possible of the patient care unit to perform rapid detection and identification of a pathogen, while respecting quality standards [7, 31].

Apart from pathogen culture, which requires a high biosafety level and a long time to achieve the pathogen identification and is therefore precluded from mobile labs, all other diagnostic techniques can be implemented in these laboratories. However, the field environment provides constraints that have to be taken into account: environmental conditions and power supply can be variable, space can be scarce and biosafety level (BSL) is limited (one cannot have a BSL3[3] or BSL4 in field conditions). Those constraints will lead the choice towards safe, rapid, and robust techniques, using small and robust equipment, with adapted protocols (e.g. isothermal nucleic-acid amplification).

One of the current most powerful tools to include in such a facility is “the MinION sequencer” from Oxford-Nanopore. This is a compact and portable NGS device with a small footprint and easy and quick sample preparation; it provides long nucleic acid reads and has a flexible run time for data generation (Figures 1 and 2) [32]. This technology allowed real-time genomic surveillance of the Ebola virus (EBOV) in the field during the EBOV epidemic in West Africa [33, 34].



[3] BSL levels are defined at: https://en.wikipedia.org/wiki/Biosafety_level

Figure 1. MinION device. *Image credit: © 2016 Oxford Nanopore Technologies Ltd [35].*

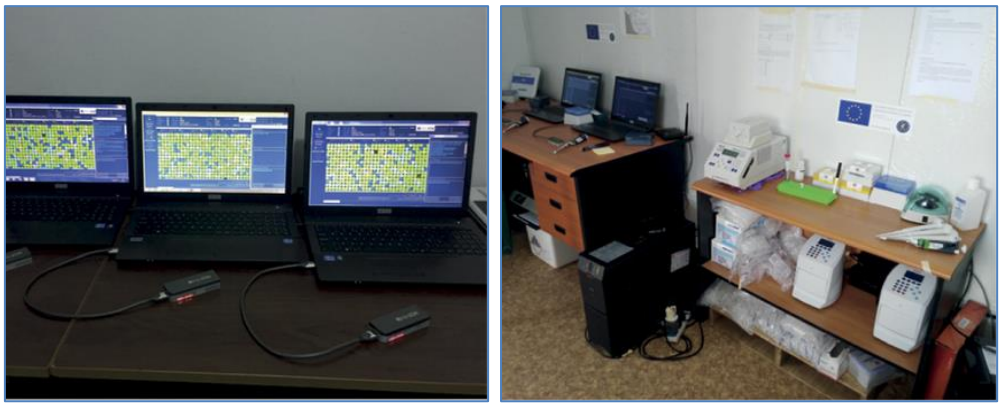


Figure 2. MinION device used on the field for Ebola surveillance [33].

3 Questionnaire on diagnostic technologies

3.1 Introduction

The questionnaire was developed for laboratory managers in Europe, with the second part being specifically directed to mobile laboratory managers. The survey has two aims:

- Collect information on current diagnostic practices:
  How "pandemic-pathogens" are currently monitored, detected and identified?
  How the samples are transported, tracked from sample collection to results delivery?
  How results are interpreted and communicated?
- Identify new solutions and improvement needs in diagnostic practice in the context of a new pandemic:
  Are current technologies matching needs performances and requirements? If not what should be improved?
  What are the current gaps in terms of "pandemic-pathogens" diagnostics?
  Which are the technologies to be improved or developed in order to enhance the "pandemic-pathogens" diagnostic capacity in Europe?

Practitioners usually have very little time for considering external information requests. Therefore, the questionnaire is designed to collect the required information in a format that is easy to read and can be readily completed.

The questionnaire is presented as a "multiple choice" format in a Excel file. It includes answers formats of "Yes or No" (green boxes), lists (blue boxes) and "blanks" (grey boxes) where any appropriate input/comments can be introduced (*The questionnaire template can be found in the Annex*).The questionnaire is divided in three separate sheets. In the first

introductory sheet, the PANDEM project is briefly presented to allow respondents to understand the context and objectives of the questionnaire. In this section all information about the laboratory is collected (type of laboratory, bio-agent diagnosed/studied). The second and third sheets are respectively dedicated to reach-back laboratory managers and to mobile laboratory managers and share a similar structure: a first section contains questions to assess the current practices in the laboratory and a second section to assess the needs and gaps. The questions for the assessment of current situation are structured following the steps of sample processing (divided in pre-analytical, analytical and post-analytical phase) and targeted to gather specific information for situation assessment of EU "pandemic" diagnostic capacities, based on UCL experience (as reach-back diagnostic laboratory and through several fieldable laboratory missions (Table 2, [36-38])). For gaps and needs identification, there were no specific questions and laboratory managers were allowed to list and develop any gap they identify in terms of sample management and diagnostic technologies, and they could define all innovations that are required, following their expertise, to improve pandemic diagnostics.

The list of questions were based on CTMA's laboratory expertise as an academic, clinical and military laboratory, involved in several EU projects targeting best laboratory practices, and by several missions where a tent laboratory was deployed to carry out sample analysis in the field. A snapshot of selected "laboratory practice" related EU projects and CTMA missions with the deployable laboratory is presented in table 1:

Location and date	Exercise	Deployment type	Purpose	Means of verification
Kananga, Occidental Kasai, Republic Democratic of Congo, April 2009		***OPERATION*** (Mil)	Response to outbreak - identification of the monkeypox virus versus varicella in patients with skin rash illness	FL results were validated by the mission stakeholders
Pionki, Poland, April 2014	PIONEX	***DEMO*** FP7-PRACTICE biological (Civ/Mil)	CBRN scenario – a large scale CBRN exercise PIONEX of FP7-PRACTICE project, integration of FL capability of *Bacillus anthracis* detection and identification in the CBRN scenario and integration in the first response system.	External observers of the exercise validated the FL quality performance
N'Zerekore, Guinea, December 2014 – March 2015	EBOLA OUTBREAK	***OPERATION*** B-LiFE / B-FAST	Response to outbreak - Ebola crisis response	FL results were validated by the international, European and local mission stakeholders and on-site operational partners

Munich, Germany, February 2016	CLUELESS SNOWMAN	***EXERCISE*** B-LiFE (Civ/mil)	Training mission – joint international exercise of UCL-CTMA and Bundeswehr Institute of Microbiology in European Space Agency IAP-ARTES 20 B-LiFE project	External observers of the exercise validated the FL quality performance. The OFs and SOPs were compared between two FLs.
Bologna, Italy, April 2016		***DEMO*** FP7-EDEN B-LiFE (Civil)	Validation and use of new technologies on-site - in the new application of "Food Defense" as part of a large-scale CBRN exercise of FP7-EDEN project.	External observers of the exercise validated the FL quality performance. FL certification was performed by Forsvarets Forskningsinstitutt (FFI, Norway)

Table 1. Spectrum of UCL-CTMA Fieldable Laboratory missions

3.2 Participant description

The PANDEM Consortium places high value on the need to protect participants' data and privacy. All participants were informed that the laboratory name and all contacts will be removed and only the country and type of laboratory will be mentioned in the deliverable report.

In total, 47 questionnaires were sent to diagnostic (infectious diseases, virology, bacteriology) laboratories across 24 European countries, including laboratories from the PANDEM consortium institutions and from both infectious bacteria and viruses networks (NIB and NIV). The laboratories linked to these networks are BSL 3 and BSL 4 facilities which are, active in the field of identification of dangerous bacterial and viral human pathogens. One questionnaire was sent to a laboratory in the Democratic Republic of the Congo to obtain information on practices in a developing country with a number of highly infectious disease threats.

In total, 19 laboratories returned a completed questionnaire: 14 laboratories completed the "reach-back[4]" questionnaire, 3 laboratories completed both "reach-back" and "mob lab" questionnaires and two laboratories provided responses to the "mob lab" questionnaire. Reach-back laboratories are where samples are sent from the field by the deployed team.

[4] A **reach-back** laboratory is a **fixed-site** laboratory.

Laboratories description

Figure 3. Participating countries.

Reach-back questionnaire (17): Belgium (3), Democratic Republic of the Congo, Estonia, France, Germany, Ireland, Italy, Lithuania, Norway (2), Poland, Slovenia, Sweden (2), The Netherlands.

Mobile laboratory questionnaire (5): Belgium, France, Germany, Sweden, The Netherlands.

Laboratory types (Figure 4)**:** National defence agency laboratory (1); National institute for public health and the environment (1); National public health institute laboratory (3); National public health institute laboratory and national reference laboratory (6); National public health institute laboratory, national reference laboratory and diagnostic testing (according to contracts) for hospitals, clinics and other private laboratories (1); National reference laboratory (4); Mobile laboratory (2).

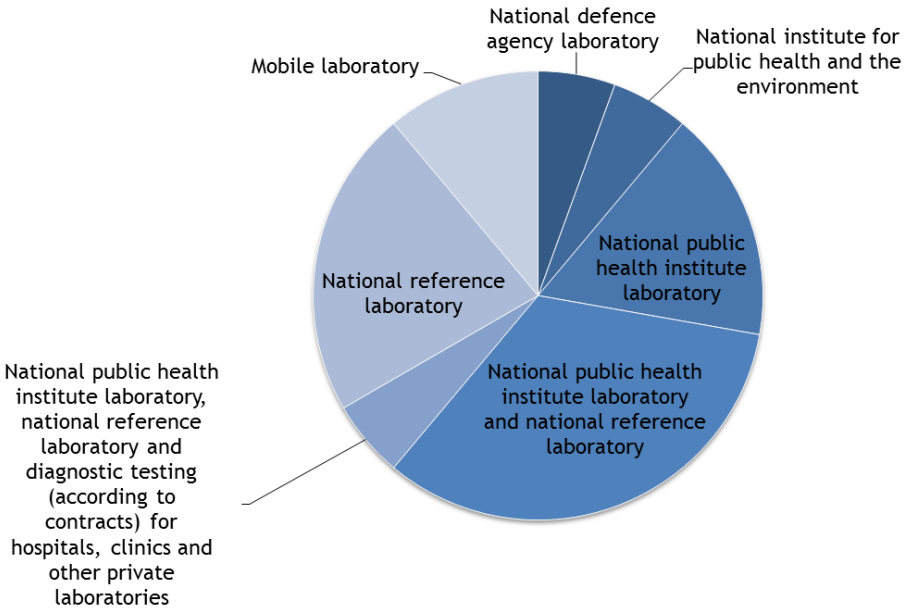


Figure 4. Laboratory type.

Laboratory networks: Antimicrobial resistance interactive database (EARS-Net) (1), Diphtheria Surveillance Network (DIP Net) (1), European Biodefence Laboratories Network (EBLN) (2), European Centre for Disease Prevention and Control (ECDC) (2), European Legionnaires' disease Surveillance Network (ELDSNet) (2), Efficient response to highly dangerous and emerging pathogens at EU level (EMERGE) (5), Epizone European Research Group (1), European Research Infrastructure on Highly Pathogenic agents (ERINHA) (1), European Reference Laboratory Network for Human Influenza (ERLI-Net) [former European Influenza Surveillance Network (EISN)] (5), European Virus Archive global (EVAg) (2), Emerging and Vector-borne Diseases (EVD) [incl, European Network for Diagnostics of "Imported" Viral Diseases (ENIVD)] (5), ECDC Food- and waterborne disease (FWD) Network (2), Global Health Security Action Group (GHSAG) Laboratory Network (1), Global Influenza Surveillance and Response System (GISRS) (2), Global Microbial Identifier (GMI) (1), Global Outbreak Alert and Response Network (GOARN) (1), Global Polio Laboratory Network (GPLN) (2), Invasive Bacterial Diseases Network (IBD-Network) (1), LabNet (1), Measles and Rubella laboratory network (1), National Food Chain Safety Network (1), National Laboratory Network (2), Network of Infectious Bacteria (NIB) (1), Nordic Forum for Biopreparedness Diagnostics (FBD) (1), World Organisation for Animal Health (OIE) (1), *Salmonella-Shigella* network (1), Sexually Transmitted Infection (STI) Network (1), Vaccine-Preventable Diseases (VPD-ECDC) (1), World Health Organisation (WHO) (3), WHO National Influenza Centres (WHO-NIC) (1).

Two of the laboratories who responded did not report being part of any networks. Two laboratories reported being part of networks but did not specify which.

In total, 30 networks were mentioned by respondents. On average, three different networks were cited per lab. The most connected laboratory reported 10 networks. It is notable that only one lab reported a connection with Animal Health Networks.

The most cited networks are:

- European Reference Laboratory Network for Human Influenza (ERLI-Net), an ECDC network, which carries out virological surveillance of human influenza and ensures that data are shared through the European Influenza Surveillance Network (EISN) reporting mechanisms in a timely manner [39].
- EMERGE, an EU funded Joint Action (CHAFEA n° 677 066) that comprises a European network with about 40 diagnostic laboratories focused on risk group 3 bacteria and risk groups 3 and 4 viruses [40]. This was excepted as targeted laboratories were institutions

reported to be part of the infectious bacteria and viruses network (NIB and NIV) that are included in EMERGE.

- The Emerging and Vector-borne Diseases (EVD), an ECDC Programme that contributes to the EU-wide preparedness and response capabilities. The programme supports networks, gathering expertise from institutes, universities, research projects and public health institutions across the EU: the Network for diagnostics of "imported" viral diseases (ENIVD) and the European network for sharing data on the geographic distribution of arthropod vectors, transmitting human and animal disease agents (VectorNet) [41].

This shows that a number of laboratory networks are in place in Europe but none of them is global, targeting a wide range of highly pathogenic agents and emerging diseases AND linking a wide network of laboratories. This constitutes a gap in pandemic management. Indeed, wide networks are useful to establish standards for diagnostic processes and exchange reference biological material. During pandemics, such networks would induce rapid sharing of information and of biological samples, accelerating the development and validation of new diagnostic assays.

Laboratory activities: Diagnostic (5), Diagnostic and new diagnostic tests development (3), Diagnostic and research on pathogens (1), Diagnostic, new diagnostic tests development and research on pathogens (8), Infectious Disease Research, Diagnosis and Screening (1), Research on pathogens (2) are shown in Figure 5.

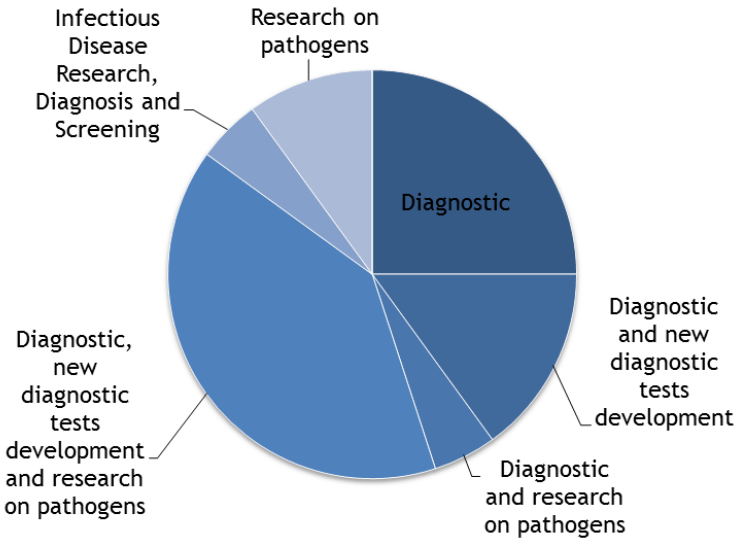


Figure 5. Laboratory activities.

The potential to discover yet unknown pathogens and to develop new tests to improve diagnostic capacities is well present in Europe.

Biosafety level (reach-back)**:** BSL2 (1), BSL2 (BSL3 facilities are being built) (1), BSL2 (access to BSL3 lab according to need) (2), BSL2 and BSL3 (1), BSL3 (9), BSL4 (2), not specified (1) are shown in Figure 6.

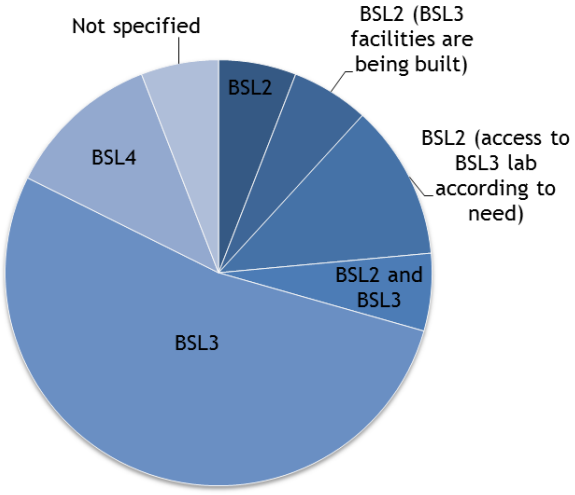


Figure 6. Reach-back laboratory Biosaftey Level (BSL).

The laboratory from Democratic Republic of the Congo is a biosafety level 2 laboratory. All European laboratories [will] have, or have access, to high biosafety level facilities (BSL3 or 4) required to contain highly pathogenic agents.

Certification or accreditation (reach-back)**:** no accreditation/certification (3), accreditation/certification but not specified which (1), ISO 9001 certification (2), ISO 14001 certification (1), ISO 17025 accreditation (7), ISO 15189 accreditation (5), animal facility accreditation (1), not specified (1) are shown in Figure 7.

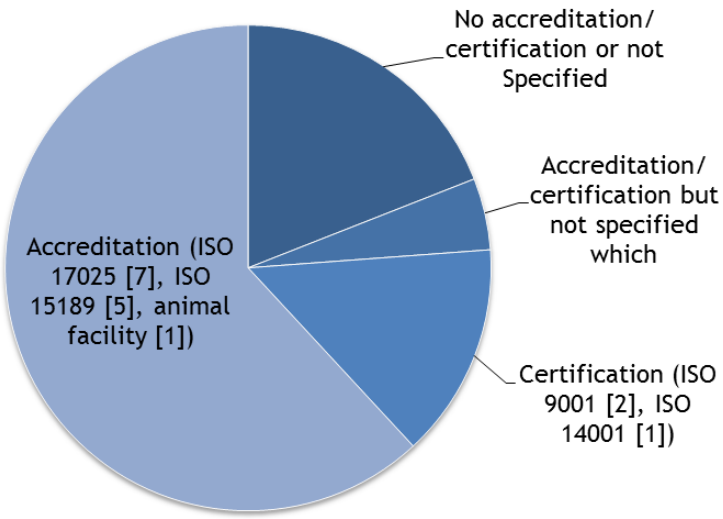


Figure 7. Reach-back laboratory – Accreditation/certification status.

Three labs reported having no accreditation or certification and one reported only certification. One laboratory did not answer the question.

In Europe, commercial *in vitro* diagnostic (IVD) tests and devices must have a certificate of conformity ('CE' mark) [42]. In contrast, home-made tests are not legally regulated and the test validation and guaranteed quality is only ensured by the accreditation processes of the lab (ISO 17025 [generic for testing and calibration laboratories] ISO 15189 [specific for Medical laboratories], or ISO 13485 [for commercial IVD product development and distribution]). As most reported diagnostic tests are home-made, that not all laboratories are accredited is a gap as this means there is no guarantee about validation and robustness of home-made tests in those laboratories.

3.3 List of pathogens

As the bio-agent to cause the next pandemic is unknown, we deliberately did not give any precise list of "pandemic pathogens" (the list of known "pandemic pathogens", was provided in comment as an indication). Knowing which kind of pathogens is considered by the interviewee as a threat for which they are prepared for is part of the assessment.

PATHOGENS	REPORTED CAPACITY
Arenaviruses	Diagnosed (2) - Diagnosed and studied (2): Lassa, Junin, Machupo viruses (1), Lymphocytic Choriomeningitis Virus (LCMV) (1)
Bacillus anthracis	Diagnosed (3) - Diagnosed and studied (2)
Borrelia recurentis	Diagnosed (1)
Brucella sp.	Diagnosed (3) - Diagnosed and studied (1)
Burkholderia sp.	Diagnosed (1) - Diagnosed and studied: *B. mallei* and *pseudomallei* (1)
Clostridium tetani	Diagnosed (1)
Coronaviruses	Diagnosed (7): MERS (2), MERS and SARS (2), Not specified (3) - Diagnosed and studied (2): MERS (1), Not specified (1)
Corynebacterium diphtheriae	Diagnosed and studied (2)
Coxiella burnetii	Diagnosed and studied (1)
Filoviruses	Diagnosed (5): Ebola (1), Not specified (4) - Diagnosed and studied (2): Ebola (1), Ebola, Margburg (1)
Flaviviruses	Diagnosed (6): Dengue (1), Zika, Dengue, Yellow fever virus (1), Not specified (4) - Diagnosed and studied (3): Yellow fever virus (1), Zika, Dengue, Yellow fever virus, etc (1), Not specified (1)
Francisella tularensis	Diagnosed (2) - Diagnosed and studied (2)
Hantaviruses	Diagnosed and studied (1)
Hendra viruses	Diagnosed and studied: Nipah, Hendra (1)
Influenza viruses	Diagnosed (4) - Diagnosed and studied (7): type A and B (1), , highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) (1), Not specified (5) - Studied (1)
Listeria sp.	Diagnosed and studied (1)
Lyssaviruses	Diagnosed (Rabies) (1)
Morbiliviruses	Diagnosed (2) : Measles (1), Not specified (1) - Diagnosed and studied (2)

Mycobacterium sp.	Diagnosed (2) - Diagnosed and studied (2)
Nairoviruses	Diagnosed and studied: Crimean-Congo hemorrhagic fever (CCHF) (2)
Neisseria meningitides	Diagnosed (1) - Diagnosed and studied (1)
Noroviruses	Diagnosed (1)
Orthopox viruses	Diagnosed (3) - Diagnosed and studied (1)
Phleboviruses	Diagnosed and studied (1)
Plasmodium sp.	Diagnosed (3)
Rickettsia sp.	Diagnosed (3) - Diagnosed and studied (1)
Salmonella sp.	Diagnosed (3) - Diagnosed and studied (2)
Shigella sp.	Diagnosed and studied (1)
Togaviridae viruses	Diagnosed (3) : Chikungunya (1), Not specified (2) - Diagnosed and studied (3): Chikungunya (2), Venezuelan equine encephalitis (VEE) (1)
Vibrio cholera	Diagnosed (4)
Yersinia pestis	Diagnosed (4) - Diagnosed and studied (1)
Unknown pathogens	Diagnosed (1)

Table 2. List of pathogens reported in questionnaires.
Known "pandemic pathogens", are underlined in the table

For those who asked it (one laboratory), we gave the following definition: We consider as "pandemic pathogen" any pathogen that can cause a "pandemic", defined in the PAMDEM project as: "An infectious disease that is capable of spreading through human populations, across large regions in multiple continents, or even worldwide during a relatively short time-frame" [43].

All pathogens that are known to have been agents in previous pandemics were referred to, see Table 2. On average, laboratories reported diagnostic capacities for 7 agents (specific species of families). The lab with the biggest diagnostic capacity listed 17 pathogens.

3.4 Assessment of current diagnostic technologies

The questionnaire aimed to evaluate the laboratories in a comprehensive way (tests rather "home-made" or commercial, level of PPE, global approach....).

Some interviewees (4) commented that the questionnaire was not easy to complete. In one case, answers would vary given each specific pathogen as different pathogens are treated in different reference labs in their institution and there are differences from lab to lab in terms of turn-around-time and “track and trace” system. They also stated that different questions did not give sufficient space to express the diversity of their expertise (bacteriology, virology, serology, and parasitology) and the different types of analyses.

We suggested, if easier, that they answer in the context of the threats that are considered in PANDEM scenarios [43] (1.Pandemic influenza - 2.SARS/MERS-CoV - 3.Smallpox - 4.Venezuelan Equine Encephalitis). Three labs filled in the questionnaire in the most comprehensive way they could. One lab returned 5 questionnaires: one for each threat from our scenarios and one for unknown pathogens. We collated the 5 responses to consider them as one institution in our questionnaires analysis.

3.4.1 Mobile laboratory deployment

The question of where the interviewees see the use of a rapidly deployable laboratory capacity in the management of a future pandemic was asked. It is a very hard question that only three out of five labs answered. Mobile labs are necessary in all countries/areas without active laboratory structures for diagnosis and without an effective public health system. Rapidly deployable laboratories would therefore be useful during missions in developing countries and at remote areas inside and outside the EU. In the EU, rapid deployment of mobile laboratories in ports or airports could be useful during a pandemic to perform rapid diagnostic tests on travellers having specific indicative symptoms. Mobile laboratories can also be deployed for rapid assessment in CBRN crises [44].

A number of activation mechanisms for mobile lab deployment exist [44]. The activation mechanisms triggering mobile lab deployment for the questionnaire interviewees was mainly a national decision based on direct contacts with requesting country (4 labs), but deployment can also be triggered through the European Commission Humanitarian Aid and Civil Protection department (DG ECHO) and Emergency Response Coordination Centre (ERCC) (1) and through the WHO Global Outbreak Alert and Response Network (GOARN) (1). The FP7-MIRACLE (MobIle Laboratory Capacity for the Rapid Assessment of CBRN Threats Located within and outside the EU) project recommended “to harmonise the mechanism of activation and to enable a common activation and reciprocal support of national and international (if any) capacities” [44].

3.4.2 Pre-Analytical Phase

Aspects of sample transport and sample reception, as well as safety and security aspects, are considered in the first part of this questionnaire.

3.4.2.1 Sample transportation

(a) Reach-back laboratory

Sample transportation: People from the requesting institution (3), Logistic carrier (1), Mail and logistic carrier (4), People from the requesting institution and by mail (3), People

from the requesting institution and by logistic carrier (2), Mail and logistic carrier and people from the requesting institution (4).

"**Track and trace" system during transportation:** No "track and trace" system (11), Sticker (4), Transportation only by authorised carrier (1), not specified (1).

(b) Mobile laboratory

Test requestor:

Local: Field hospital deployed by WHO/NGO's (1), Both local and WHO/NGO hospitals (2), Crisis expert team. Environmental Assessment Unit, local or national government (1), Not specified (1).

Distant: National authorities of the host country (3), Both national and international authorities (3).

Sample transportation: Provided directly by WHO, NGO's staff and courier (1), Received by courier or direct delivery (1), Provided directly by WHO, NGO's staff and local physicians (1), Samples are delivered either by the police or by the gas tights suit team (1), Depending on mission requirements (1).

"**Track and trace" system during transportation:** No "track and trace" system (2), Sticker (2), Transport form (1).

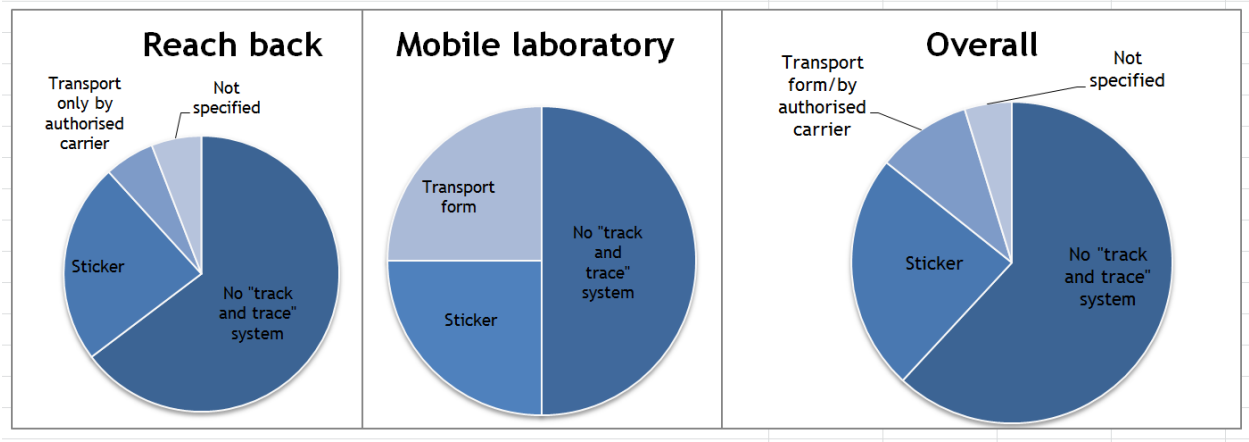


Figure 8. Tracking during sample transportation.

Usually, samples arrive to the laboratory by more than one transportation mean, including a reach-back or mobile structure. No lab reported any robust track and trace system (Figure 8 - tracing only by sticker or by specific carrier forms). This represents a major gap as the transportation of highly infections material holds biosafety and biosecurity risks. It would be therefore very important to know who has been exposed to the sample during transport (biosafety risk) and to know exactly where the samples are collected (geolocation) at all time (biosecurity risk).

3.4.2.2 Samples

(a) Reach-back laboratory

Biological samples:

Any type: Animal (2), Human (5), Human and animal (4), Not analysed (6).

Blood: Animal (2), Human (8), Human and animal (6), Not analysed (1).

Other biological fluids (sample type variable, relevant for the specific pathogen diagnostic. Cited: urine, faeces, serum, cerebrospinal fluid, vitreous fluid, bronchoalveolar/nasal/throat washings, sputum/saliva, tracheal fluids, animal gastric content, sperm and milk)**:** Animal (2), Human (9), Human and animal (5), Not analysed (1).

Tissue sample / biopsy (sample type variable, relevant for the specific pathogen diagnostic. Cited: heart valve biopsy, lymph node biopsy, internal organs [brain, spleen, liver, lung, kidney], autopsy samples): Animal (3), Human (8), Human and animal (5), Not analysed (1).

Swab: Animal (1), Human (8), Human and animal (7), Not analysed (1).

Other samples: Not analysed (13), Yes (4) - specified as: Insects, ticks etc. (2), Animal samples in special circumstances only, as relevant for testing objective (1), Isolated strains from human and animal (2), Retail food specimens (1).

Environmental samples:

Environmental liquid sample: Not analysed (9), in special circumstances only, as relevant for testing objective (1), Yes (7) - specified as: water (raw, sewage and drinking water).

Environmental Sample: Not analysed (8), in special circumstances only, as relevant for testing objective (2), Yes (7) - specified as: vegetation, soil, sand, powder letters, swabs, litter and dust, isolated strains and food.

(b) Mobile laboratory

Sampling: No (1), Environmental sampling (3), Yes (1).

Biological samples:

Any type: Human and animal (3), Not analysed (2).

Blood: Human and animal (4), Not analysed (1).

Other biological fluids (sample type variable, relevant for the specific pathogen diagnostic. Cited: urine, serum, cerebrospinal fluid, saliva, sperm and milk)**:** Human and animal (4), Not analysed (1).

Tissue sample / biopsy (not specified): Human and animal (3), Not analysed (2).

Swab: Human and animal (4), Not analysed (1).

Environmental samples:

Environmental liquid sample: Yes (5) - specified as: (raw) water.

Environmental Sample: Yes (5) - specified as: vegetation, soil, sand, powder letters, swabs, food and air samples.

The diagnostic capacity is available for a wide panel of biological samples types.

For environmental samples, the analytical capacity is more reduced in reach-back but is well present in mobile labs (including the sampling capacity).

3.4.2.3 Request form

(a) Reach-back laboratory

Data recording upon reception: Recording on paper forms/logbook (2), Recording on paper forms/logbook and picture of the request form and of the sample (1), Request form recording on paper and electronic form (6), Electronic data recording from the request form (7), not specified (1).

Request form contains enough information to allow

- **contact tracing:** Yes (17).
- **retrospective epidemiological analysis:** Yes (17), No (2).

(b) Mobile laboratory

Data recording upon reception: Recording on paper forms/logbook (3), Request form recording on paper and electronic form (1), Request form recording on paper and electronic form, and picture of the sample (1).

Specific request form: Yes (5).

Request form contains enough information to allow

- **contact tracing:** Yes (5);
- **retrospective epidemiological analysis:** Yes (5);
- **critical analysis of lab results:** Yes (5).

The majority of reach-back labs (13) electronically record the data from the request form. In mobile laboratories, three out of five labs record the data on paper from upon reception. The fact that not all data are recorded electronically upon reception, with the use of a robust laboratory information management system (LIMS), can be a problem for data preservation.

Data informed on the request form (if it is completed properly) is sufficient to allow contact tracing and, in most cases, retrospective epidemiological analysis. In mobile laboratories, data from the request form also allows critical analysis of the test results.

3.4.2.4 Biosafety issues

(a) Reach-back laboratory

Biosafety guidelines: No guidelines (1), Not specified (2), In-house (2), National guidelines (3), WHO Laboratory Biosafety Manual (4), Biosafety in Microbiological and Biomedical Laboratories (1), National guidelines and Directive 2000/54/EC (1), National guidelines and WHO Laboratory Biosafety Manual (1), National guidelines, Directive 2000/54/EC, WHO Laboratory Biosafety Manual and Laboratory Biorisk Management (CEN CWA 15793) (1), National guidelines, WHO Laboratory Biosafety Manual, WHO Biorisk Management - Laboratory biosecurity Guidance, Laboratory Biorisk Management (CEN CWA 15793 and guidelines for implementation of CEN CWA 16393), UNI CEI EN ISO/IEC 17025, OIE Terrestrial Manual 2015 and FAO "Minimum Biorisk Management Standards for Laboratories Working with Foot and Mouth Disease Virus" (40th General Session of the EuFMD – 22-24 April 2013) (1).

Personal protective equipment (PPE): Depends on pathogen (3), Gloves and labcoat (2), Mask, gloves and labcoat (1), Safety goggles, mask, gloves and labcoat (2), Full PPE (6), Full PPE with active respiratory masks (3).

Cause for sample rejections: No sample rejection (1), No request form (2), Broken sample (4), Incorrect sampling, broken sample (1), Incorrect sampling, Improper packaging (1), Improper/damaged packaging, broken sample, no request form with the sample, missing mandatory information on the request form (8), All pre-cited reasons and if possible explosives and/or high level radioactive content (1).

Most reach-back laboratories (14) follow strict biosafety guidelines. The use of PPE is decided appropriately taking into account the nature of the threat, the lab biosafety level and following biosafety guideline. From the three laboratories that did not inform a biosafety guideline, all reported the use of full PPE (with active respiratory masks for two of them).

(b) Mobile laboratory

Dedicated reception area outside the lab for dangerous samples: Yes (2), No (3).

Biosafety guidelines: Not specified (1), In-house (2), BSL3 guidelines (1), WHO Laboratory Biosafety Manual (1).

Laboratory deployment reviewed by a "Health and Safety Adviser": No (3), All methodologies performed inside the BSL3 lab have been assessed by a biosafety officer (1), Yes - corrective actions on waste management, electrical mapping of equipment, use of PPE (1).

Personal protective equipment (PPE) at the sample reception: Gloves and labcoat (3), Safety goggles, mask, gloves and labcoat (2).

Other PPE, in specific circumstances for sample reception: No (2), Full PPE if biosafety risk (1), Full PPE with active respiratory masks if biosafety risk (1), PPE is scalable and situation tailored - from labcoat and gloves to full PPE with active respiratory masks (1).

Decontamination at sample reception: Bucket with bleach (2), Fumigation and surface disinfection – hydrogen peroxide (1), After each batch of samples - Vircon, nucidex, alcohol (1), With validated products (not specified) (1).

Waste containment at the sample reception: Special biological waste garbage to get all packaging wastes and refused samples after bleach decontamination (1), Plastic containers containing decontamination solutions (1), Use of Bleach - 2,6% of active chlorine (1), The sample reception area is the BSL3 lab (1), No (1).

Cause for sample rejections: No sample rejection (1), Improper/damaged packaging, broken sample, no request form with the sample, missing mandatory information on the request form (3), All pre-cited reasons and if possible explosives and/or high level radioactive content (1).

Personnel trained for lab work in field/outbreak conditions: Yes (4), No (1).

There are no specific biosafety guidelines for mobile laboratories and not all mobile laboratories capacities have been assessed by a biosafety officer. However, laboratory workers are trained to work in such field/outbreak conditions (expect for one lab, but this lab is described as a BSL3 lab environment and not a field environment).

All sample packages are decontaminated upon receptions and the level of PPE can be adapted following biosafety risk assessment.

Therefore, in mobile laboratories, the training (correct donning and doffing of PPE, decontamination procedures, how to make risk assessment) is essential to ensure the biosafety.

3.4.2.5 Biosecurity issues

(a) Reach-back laboratory

Biosecurity measures: Not specified (2), No specific biosecurity measures (1), Biosafety aspect [not biosecurity] (4), Chain of custody (1), Physical locks, according to risk assessment (1), Restricted access (4, one with video monitored area), Restricted access to laboratories and storing areas (2), Restricted access to laboratories and

inventory storing areas, with controlled storing of biological materials (1), Biosecurity measures classified (1).

(b) Mobile laboratory

Biosecurity measures during transport: No specific biosecurity measures (1), Biosafety aspect [not biosecurity] (1), Contact with the carrier and the requestor (1), Transport is performed by police officers (1), Biosecurity measures but not specified (1).

Biosecurity measures in the mobile lab: Biosafety aspect [not biosecurity] (2), Restricted access (1), Sample placed in a locked and secured area (1), Biosecurity measures but not specified (1).

Security of the mobile lab installations: Security team (1), Specific procedures (2), Placed in a locked and secured area (1), Yes but measures not specified (1).

Security of the mobile lab staff: Security team (1), Specific procedures (1), Specific procedures and training (1), Security clearance (1), Yes but measures not specified (1).

Three reach-back labs did not report any biosecurity measures. Five reach-back and two mobile labs reported measures that are not biosecurity but biosafety measure (one reach-back lab also included a biosecurity measure).

This is a big gap - no clear distinction between biosafety and biosecurity and about half of the laboratories do not have any biosecurity measures in place. Training about biosafety/biosecurity and implementation of biosecurity measures are needed.

As a reminder, those terms are well defined in official WHO guidelines:
"**Laboratory biosafety** describes the containment principles, technologies and practices that are implemented to prevent the unintentional exposure to pathogens and toxins, or their accidental release" [45] (in short, keep *bad bugs* away from *people*).
"**Laboratory biosecurity** describes the protection, control and accountability for valuable biological materials within laboratories, in order to prevent their unauthorized access, loss, theft, misuse, diversion or intentional release" [46] (in short, keep *bad people* away from *bugs*).

3.4.2.6 Safety issues (mobile lab)

Safe electric system: Electrical map for equipment repartition given consumption, back-up generator and solar panels for independent electrical alimentation of the cold chain (1), Diesel generator, back-up electricity (1), Inverter, Generator (1), UPS (2h) and external power supply (48h) (1), Yes - measures not specified (1).

Specific measures are taken to provide electrical power. However, only one lab has an uninterruptible power source (UPS) to protect the equipment in case of unexpected power disruption.

Prevention of lab incidents: Specific procedures/training (3), Minimum two staff inside the mobile lab (2), Yes - measures not specified (1).

Other training: No (2), First aid, self-protection, hazard related to electrical problem, fire in the lab, stress, security training in the field … (2), Yes - not specified which (1).

Specific training of mobile laboratory workers is needed to prevent accidents/incidents and to react in the appropriate way in case of incident.

Evacuation/MEDEVAC plans: No (2), Not by default (depend on activation mechanism - is discussed during mission preparation) (1), In coordination with WHO (1), Yes - not specified (1).

3.4.3 Analytical Phase

This part of the questionnaire addresses the sample tracking in the lab and the analytical processes (including biosafety issues for mobile laboratories).

3.4.3.1 Sample tracking and tracing

(a) Reach-back laboratory

"**Track and trace" system during transportation:** Sticker (6), Sticker - Barcode is under development (2), Barcode (9).

Commercial LIMS[5]: No (7), No but to be implemented shortly (1), Internally/specifically developed system (2), Commercial LIMS (7): Star LIMS, Databiotec, CliniSys WinPath, Labware, AS/400 or not specified.

All laboratories trace their samples. However, there seems to be no harmonization in the sample record and tracing (all different solutions tracing the samples and for the LIMS).

(b) Mobile laboratory

"**Track and trace" system during transportation:** Sticker (3), Sticker - Barcode is under development (2), Barcode (1), Regular numbering (1).

Like for reach-back lab, all mobile labs trace their samples but there is no harmonization in the sample record and tracing.

[5] LAB Information Management System

3.4.3.2 Biosafety issues (mobile lab)

Sample inactivated in the glovebox: No (1), Ethanol – Glovebox OWR (2), Inactivation with commercial solution: EUROBIOCONCEPT (1), Commercial solution but not specified (1).

Control of the quality/completeness of the inactivation: Validated procedures (1), Validated procedures and buddy system (2), Validated procedures, double checking, training …(1), Indicators (chemical and biological) (1).

Samples processing after inactivation: On the bench (2), In a depressurised glovebox (3).

PPE used in the mobile lab: Gloves and labcoat (5).

Decontamination inside the glovebox: Sodium hypochlorite 5000 ppm (2), Hydrogen peroxide (1), Hydrogen peroxide and Ethanol (1), A combination (no product specified) (1).

Decontamination in the mobile lab: Sodium hypochlorite 2500 ppm (1), Hydrogen peroxide (2), Sodium hypochlorite 2500 ppm, DDSH and RNAse away (1), A combination (no product specified) (1).

Measures for sample containment/inactivation and for the laboratory and glovebox decontamination are in place in all laboratories. However, all reported protocols are different. As decontamination and sample inactivation are critical points to ensure biosafety, harmonised and validated protocols would be needed. The strict respect of the containment/inactivation/decontamination procedures, and therefore the appropriate training of lab personnel concerning those procedures, is mandatory.

3.4.3.3 Analytical tests

(a) Reach-back laboratory

Microscopy: No microscopy (7), Light/optic microscopy (4), Light/optic microscopy and immunofluorescence (IF) microscopy (2), Light/optic microscopy and sample preparation for electron microscopy (1), Light/optic microscopy and electron microscopy (1), Electron microscopy (2).

DNA- and/or- RNA based identification test:

(Reverse Transcriptase (RT)-) Polymerase Chain Reaction (PCR): No (4), Home-made (13).

Real-time PCR (qPCR): No (3), Home-made (13), Home-made and CDC real-time RT-PCR kit (1).

Recombinase Polymerase Amplification (RPA): No (16), Home-made (1).

Loop-Mediated isothermal AMPlification (LAMP): No (16), Home-made (1).

DNA arrays: No (11), Home-made (4), Commercial (2): FilmArray, Luminex.

Sequencing: No (4), Sanger sequencing (2), Next generation sequencing (NGS) (5), Sanger and pyrosequencing (1), Sanger and NGS (3), Sanger, pyrosequencing and NGS (1), Other but not specified (1).

From the nine labs that reported NGS technology, six lab have one NGS platform, two lab have two NGS platforms and one lab has three different platforms. For labs having more than one platform, one is of the third generation.

NGS technologies cited: Ion Torrent (3), Illumina (6); 3rd generation: PacBio (1), MinION (2).

Other: No (17).

Immuno-analyses

ELISA: No (6), Home-made (1), Commercial or home-made depending on the agent (1), Commercial: Panbio Euroimmun (1).

Lateral flow device: No (13), Home-made (2), Commercial or home-made depending on the agent (2).

Multiplex lateral flow device: No (16), Commercial or home-made depending on the agent (2).

Other: No (14), Slide agglutination (1), Haemagglutination inhibition assay (HI) and Serum Neutralization Test (SNT) (2).

Other

MALDI-TOF: No (13), Yes (3), MALDI BioTyper - Bruker Daltonics (1).

Thin layer chromatography: No (16), Yes (1).

Culture: No (2), Yes (15).

Other: No (13), Yes (4):

- antiviral resistance testing, phenotypic resistance testing on virus isolates (fluorescence-based neuraminidase inhibition assay), genotypic resistance testing on amplified viral RNA in specimens (PyroMark and Sanger sequence analysis);
- fatty acid analysis, conventional tests;
- immunofluorescence antibody test (IFAT);
- Influenza: Antiviral resistance testing (Sequencing, MUNANA).

Biochemical monitoring of patients for laboratory guided clinical care: No (15), Yes (2).

The most represented technologies for pathogen identification are the pathogen culture and identification by PCR and sequencing methods. Diagnostic methods used are mainly home-made methods, with quality validated by the accreditation of the laboratories (or at least the presence of quality controls in the process). This means that the laboratories have the

expertise to develop and validate new diagnostic tests in case of unknown pathogen emergence.

Ten out of 17 labs reported to have an electron microscope and/or next NGS platform and/or MALDI-TOF equipment. This means that only those labs have open detection techniques that hold the potential to detect and identify new/unknown/emerging pathogens.

(b) Mobile laboratory

Preparation of sample for pathological analysis (e.g. microscopy): No (5).

Microscopy: No microscopy (4), Light/optic microscopy (1).

DNA- and/or- RNA based identification test:

(RT)-PCR: No (3), Home-made (2).

qPCR: Home-made (3), Home-made and AnDiaTec and/or Altona kits (2).

RPA: No (4), Home-made (1).

DNA arrays: No (4), FilmArray (1).

Sequencing: No (3), MinION (2).

Other: No (5).

Immuno-analyses

ELISA: No (3), Home-made (1), Commercial (not specified) (1).

Lateral flow device: No (1), Commercial (Standard Diagnostic Bioline or not specified) (2), Commercial or home-made depending on the agent (1).

Multiplex lateral flow device: No (3), Yes (not specified) (2).

Other: No (5).

Other (MALDI-TOF, thin layer chromatography, culture or other): No (5).

Biochemical monitoring of patients for laboratory guided clinical care: No (4), iSTAT and Piccolo Express performed on non-inactivated blood in a depressurized glovebox (1).

In mobile laboratories, there is no pathogen culture reported. The diagnostic is made mainly by qPCR tests. As for reach back laboratories, the majority of the tests are home-made with process validated through quality controls.

Only two laboratories have a NGS device (MinION) allowing detection of new/unknown/emerging pathogens and the follow-up, on the field, of the pathogen genome evolution during the pandemic.

3.4.3.4 Quality control

(a) Reach-back laboratory

Quality controls: Negative and positive controls (3), All required [Multiple and in function of the test] (3), External quality assessments (EQAs) (3), Negative and positive controls and EQAs (1), EQAs and internal quality control system (ICQs) (5), Negative and positive controls, EQAs and monitoring of target (sequence) evolution (2)

As most of the labs have an accreditation, this reflects the high control of quality in those labs. In terms of the labs having no accreditation, two reported participation in external quality exercises and two reported the use of negative and positive controls. The lab that did not answer the question concerning the accreditation/certification reported to have all required controls (but do not list them).

All those factors ensure a certain level of diagnostic quality provided by the laboratories. However, as each lab has their own tests and controls, it would be very difficult to compare the results. Sharing standard reference material between all the labs to validate the home-made tests and to be used as controls would be useful to allow comparisons of result and a step towards harmonisation.

(b) Mobile laboratory

Quality controls: Extraction control and qPCR controls (+/-) (3), Internal controls (3), Controls (1).

There is no certification/accreditation available for mobile laboratories. The confidence of the diagnostic service provided by the mobile laboratories is surveyed internally through test controls. As for reach-back laboratories, the use of the same reference material as controls would improve the confidence in the test and enable a comparison of the results.

3.4.4 Post-Analytical Phase

This third part is related to the data interpretation and result transmission. Specific questions concerning decontamination issues (in reach-back facilities and for mobile laboratories repatriation) are also addressed.

3.4.4.1 Data interpretation

(a) Reach-back laboratory:

Data interpretation: Visual interpretation (3), Interpretation with specific software provided with the analytical instrument (2), Visually or with dedicated (home-

made/software from analytical instrument/commercial/open-source) software (11), Not specified (1).

(b) Mobile laboratory:

Data interpretation in the mobile laboratory: Interpretation with specific software provided with the analytical instrument (2), Visually or with dedicated (home-made/software from analytical instrument/commercial/open-source) software (3).

Data interpretation by distant experts: No (2), Encrypted information sent to reach-back labs (1), Distant experts needed for treatment and analysis of NGS data (1), Distant experts sometimes for PCR results interpretation (1).

As data interpretation is related to testing methods (which are mostly home-made) and to laboratory expertise, there is not one standard method or program for interpretation.

Experts for data interpretation are not always available in mobile laboratories and, sometimes, data needs to be sent to distant experts for result analysis. Therefore, the set-up of secure and fast communication tools within the field laboratory is an asset.

3.4.4.2 Turn-around time (TAT)

(a) Reach-back laboratory:

TAT for (q)PCR: 4 h (2), 4-6 h (1), 6 h (2), 6-8 h (1), 8-24 h for urgent samples (1), For priority testing with suspicion of agent with pandemic potential: same-day or day after receipt (1), 1 working day (1), 24 h (1), 48 h (6-8 h possible for few pathogens) (1), For surveillance - around 48 h, for acute diagnosis - around 4-5 h (1), 48-72 h (1), Urgent testing 6 hours. Routine testing 3-5 days (1), Varied in function of the tests and the studied pathogen (2), No qPCR (1).

TAT for biochemical monitoring of patients: No (15), 1 h (1), 2 h (2).

(b) Mobile laboratory:

TAT for (q)PCR: 2 h (1), 3-4 h (2), around 6 h (1), Depending on the sample! Blood sample and analysis for one specific pathogen: 4-5h (1).

TAT for biochemical monitoring of patients: No (15), 1 h (1), 2 h (2).

Regarding the time for diagnosis, the RDC lab does not perform (q)PCR diagnostics and identification of the pathogen by culture will require at least 48 h. For the molecular diagnostic methods in reach-back laboratories, taking into account the acute diagnosis/urgent testing needed in case of pandemics, the results will be available within a day. In mobile laboratories, results will be available within half a day.

Only three laboratories (two reach-back, including the RDC laboratory, and one mobile laboratory) perform the biochemical monitoring of patients. This procedure is fast (TAT: 1-2 h in reach-back, 20 min in mobile labs) and allows a laboratory-guided and improved clinical care of patients to mitigate adverse effects of diseases/treatments. This is particularly needed in the field during clinical trials of new drug for diseases treatment. Indeed, clinical trials require (ethically compulsory) the monitoring of adverse effects of the experimental drug on the patient, as it was performed during the evaluation of an experimental treatment with Favipiravir for Ebola virus disease [47].

3.4.4.3 Reporting

(a) Reach-back laboratory:

Test report: Tests results (6), Analysis report (1), Depends of the request, mainly the final results (+/-) (2), Type of sample analysed and result for identification (1), Raw data and final report (1), Results (final or sometimes both primary suspected and final) (1), Qualitative results (+/-), information on molecular typing and molecular and epidemiological analyses, titres of positive sera for serological tests (1), Results of the tests, procedures/methods of testing, sample receiving time, time of finishing the tests (1), Diagnostic test result and clinical comment (1), Diagnostic test result, when relevant also with interpretation and advice on follow up including further specimen collection (1), Varies with the requester, from simple result to full detail on analysis (1)

Mode of data transfer: All media possible for data transfer (1), Dependent on the sensitivity of the result (1), By direct contact (paper report) (1), By phone - Also by e-mail in some precise cases (1), By fax, e-mail and mail. By phone preliminary results if urgent (1), By mail and, if requested, by direct contact by phone (1), By mail and through a shared database with the requester (1), By mail, fax or e-mail (2), By mail, by e-mail or by shared database (1), Mail, fax, database of national health authorities (1), By mail, through a shared database with the requester and through dedicated reporting/warning systems (Not specified which) (3), By phone then by mail to requester, through dedicated reporting/warning systems to network (EISN/GISRS) (1), Mail, e-mail, phone, fax, dedicated information system (1), Secure electronic link and by mail (1).

Data communication: Only to the analysis requester (4), To the requester and national health authorities (8), To the requester, national health authorities and ECDC (1), To the requester, national health authorities and WHO (1), To the requester, national health authorities, ECDC and WHO (3).

(b) Mobile laboratory

Data produced:

Pictures: From the request form (if contamination suspected (1), From the sample (3), From the sample, sample packaging and result (1).

Electronic data: Yes (5).

Paper reports: No (1), Yes (4).

Protection of data: No (2), Yes (not specified) (2), Back-up systems (1).

Test report: Tests results (2), Raw data (1), Not specified (2)

Mode of data transfer: By direct contact (paper report) (1), By e-mail and by phone (1), By mail and through a shared database with the requester (1), Through dedicated reporting/warning systems (for security reasons) (1), Mission dependent (1).

Data communication: Only to the analysis requester (2), To the requester, national health authorities and WHO (1), For epidemics: to the requester, local outreach team, national health authorities and WHO (1), Mission dependent (1).

System of communication used: On-site internet connection (1), Proprietary satellite communication (SatCom) or Emergency.lu SatCom and mobile phone with SIM cards (2), Local mobile phones, satellite phones, local infrastructure (1), Not specified (1).

There is no standard for the communication of results (data produced, type of reported data, communication channels). This represents a big gap. In case of a pandemic, there is a need to integrate data from different labs in order to have a global view of the situation for appropriate pandemic management. This is very difficult if the level of information in each report and the report formats are different and if communication channels and reporting systems (having each different reporting characteristic) are disparately used.

In mobile laboratories, having stable and secure communication tools is essential (for communication with experts, for results transmission, for contact with volunteers). The use of satellite communication tools, as reported by two mobile laboratories, offers a broadband, real-time data transfer capacity and represent a strong asset for mobile laboratories, as explored in the ESA IAP-ARTES 20 « Biological Light Fieldable laboratory for Emergencies » (B-LiFE) project [48].

3.4.4.4 Decontamination

(a) Reach-back laboratory

Method: Washing with liquid decontamination solution (7), Washing and fumigation (10).

Agent: Not specified (5), one decontamination solution [cited: Virkon, Ethanol 70 %, Formaldehyde] (3), A combination of more than one agent - different procedures in

different workstation [cited: Hydrogen peroxide (liquid or vapour), Formalin, Ethanol 70 %, Chlorine, DK-DOX chlorin dioxid, Commercial (2% Des Insurance 2000 that contains quaternary ammonium compounds, glutaraldehyde, alcohol, glyoxal); 70% ethanol, 2% glutaraldehyde), UV, 4% bleach, DNA Away, Terralin liquid, Lysoform d, Wofasteril, formaldehyde, Virkon, cetremide, product with iode] (10) .

Assessment of decontamination: No check of the decontamination (4), By contact plates and cultures (6), By swabbing and qPCR (6), By swabbing and qPCR and by contact plates and cultures (1).

(a) Mobile laboratory

Decontamination of glovebox for repatriation: Glovebox not repatriated (2), Fumigation with hydrogen peroxide (2), Washing with sodium hypochlorite 5000 ppm solution (1).

Decontamination of equipment for repatriation: Fumigation with hydrogen peroxide (1), Washing with sodium hypochlorite (2), Fumigation with hydrogen peroxide and washing with ethanol (2), Washing and fumigation (no product specified) (1).

Assessment of decontamination: Not specified (1), No check of the decontamination (2), By swabbing and cultures back in reach-back facility (1), With chemical/biological indicators and by swabbing and qPCR (1).

All laboratories have decontamination procedures but they all vary in terms of decontamination methods and agents. As the labs are dealing with highly pathogenic agents, decontamination is very important to ensure a safe working environment and to avoid a source of contamination. Moreover, four reach-back and two mobile laboratories do not check for the quality of the decontamination, which is a biosafety issue.

3.5 Gaps identification and innovation needs

In this section of the questionnaire, the interviewees were requested to comment on the problems/gaps they identify in the diagnostic process. They were also encouraged to specify what improvements or solutions they would like to see developed in the future in order to address the problems identified.

3.5.1 Gaps identification

Gaps identified concerning the entire diagnostic process (from the transport of samples to the results communication) identified by the interviewees:

Sample transportation

- Lack of secure, prioritised and affordable transportation of samples from suspected cases/outbreak site to the specialized diagnostic lab.

- Problems in transport conditions and sample preservation before the samples reach the laboratory: "*We often receive biological samples from Africa that have been stored for many days in bad conditions and are not in a good state when they arrive in our lab. This could impact the analysis and could be a real problem. But often, the contact with the requester arrives too late to explain the right conditions for storage and transportation*".
- From a recent EC-funded workshop about vector-borne diseases and vectors of transmission[6][49]), several speakers from the Pasteur Institutes of Cambodia and Laos confirmed that bringing precious samples from vectors collected on-site is a major bottleneck. These often precious samples, which are very hard to get, can hardly be shipped because of the potential threat of BSL3/BSL4 contaminating agents in some mosquito species, and cannot be brought quickly enough to reach-back or reference laboratories to prevent the deterioration of the samples, hampering efficient research.

Laboratory capacity

- Some laboratories lack innovative equipment (eg. MALDI-TOF, NGS, etc.).
- Not all laboratories have BSL-3 facilities.
- Capability to study only limited pandemic pathogens.
- No robust and quality controlled decontamination protocols.
- Specific for mobile lab: serology in the field (no inactivation protocol); there is a need for more sensitive and specific hand held test kits and true deep sequencing for the field as an explorative tool.
- One major problem is the availability and accessibility of quality control material when a novel pathogen is identified. There is currently no appropriate repository for biobanking and poor networking and exchange of samples among laboratories.
- RDC laboratory: theses laboratories are lagging behind in terms of quality control, management and improvement. Diagnostic currently capability limited to pathogen culture. Urgent need for to be connected with other laboratories worldwide through communication (mail, telephone...).

Laboratory information management and result communication

- Registration of samples in not always made in a consistent and coherent way with enough detail for subsequent analyses or retrospective epidemiological analysis.

[6] Vectoland: Consequences of change in land use and climate on vector mosquitoes and community structure, 7-9th June 2016, Louvain-La-Neuve, UCL.

- Sample tracking from place of collection to the laboratory steps (registration/laboratory analyses) is not efficient. There is no real chain of custody ensuring traceability of samples and data.
- Gaps in methods and tools for handling information security, information sharing and personal integrity.
- Problems related to the request forms: "*Often, we do not receive the request form with all clinical data with the biological samples, or the form is not fully completed. Sometimes it can be improve by discussion with requester and a right fulfilled form is sent again. But sometimes, request form never arrives to our lab, and in this case it is impossible for us to give the results to prescriber (due to NF S 15189 restrictions).*"
- Paper-based system in regular mail for specimen forms and testing results is cumbersome and slow, and diminishes the value of speeding up lab work.
- No harmonised system for result transmission (defined output format, single reporting system).

<u>Biosafety and biosecurity</u>

- Lack of biosecurity measures.
- No clear distinction between biosafety and biosecurity in people's mind.
- Kack of field trained European lab workers for mobile labs and lack of training for local staff in the field on the concept of biosafety.
- Lack of finance mechanisms to develop and maintain (mobile) laboratories capacity.

3.5.2 Innovations needed

Innovations needed and inferred gaps as specified by the interviewees are listed below:

Sample transport

- Development of a secured, traceable, priority and affordable transport of lab samples.

Laboratory capacity

- Increase the diagnostic capacity among European laboratories:
    - Implementation of high-tech and up-to-date equipment [especially "open detection" equipment: electron microscopy, next generation sequencing and matrix assisted laser desorption ionization-time of flight (MALDI-TOF mass spectrometry].
    - Building more facilities with high biosafety levels: aside those which already exists, it would be efficient to have a "European Reference lab" where European researchers can work and meet for defined period of time (the time of a common scientific project for instance) and build a true European task force while harmonizing the methods, procedure and building a true network by working together rather than by phone or Skype or mails.
- Improving speed of generic diagnostic methods (EM, NGS, MALDI-TOF) and the sensitivity and specificity of those open detection techniques.
- Development of more rapid molecular based test (genetic testing by isothermal amplification, protein detection on later flow) and their implementation in POCT format in order to use them in any environment (patient bedside, reach-back and laboratories). The automatic results transmission from POCT to local databases by Bluetooth or Wi-Fi would be an asset.
- Develop standards for home-made diagnostic tests validation or enforce accreditation for all diagnostic laboratories.
- Develop rapid, easy and standardises methods/protocol for decontamination, and validate them through inter-laboratorial testing.
- Improve laboratories biosecurity measures to prevent the stealing of highly pathogenic material.

The European Commission finances the development of new infectious disease diagnostics (projects like Chips4Life, PARCIVAL, ROUTINE, RiD-RTI, RAPP-ID, EbolaMoDRAD, FILODIAG). However these newly developed tests have to be validated/certified and made available (published, commercialised) before they can be integrated for routine clinical use into laboratories.

The improvement of laboratory capacities must be implemented in collaboration with the EMERGE (Efficient response to highly dangerous and emerging pathogens at EU level) and ERINHA (European Research Infrastructure on Highly Pathogenic Agents) projects, that have as objectives to "provide a common, coordinated and effective response to infectious disease outbreaks at EU level and abroad" and "to enhance basic and finalised research activities and diagnostic activities", respectively.

Specifically for mobile lab:

- Generalise the use of small size true deep sequencing equipment (for instance nanopore 3D generation sequencing or alike portable NGS technologies) in the field for rapid unknown threat identification and for epidemiological surveillance (evolution of the pathogen: genetic drift, acquisition of resistance mechanism) during the high impact epidemic or pandemic.
- Increase the implementation of patient biochemical monitoring capacity (useful for laboratory-guided clinical care and needed for following up the patient status (e.g. response to therapy, potential occurrence of side effects) during new drug clinical trials).
- Develop and validate novel protocols for sample inactivation (especially for serological and antigen analyses, for which it is missing).
- Create new mobile laboratory capacities in Europe that can be scaled up and work jointly.

This capacity building has already been launched by the European Mobile Laboratory Project (EMLab), which main goal is the "Establishment of Mobile Laboratories for Pathogens up to Risk Group 4 in Combination with CBRN Capacity Building in Sub-Saharan Africa", and should be extended by integrating new technologies in the mobile laboratory and extending the number of partners and field trained laboratory workers.

<u>Laboratory information management and result communication</u>

- Develop a robust laboratory information management system (LIMS) that could be used in both reach-back and mobile laboratories.
- Apply the latest technological developments in computational power, data transmission and storage in the management of laboratory data.
- Establish procedures to respect a chain of custody ensuring traceability of samples and data from sampling to results communication and sample storage/destruction. This could be an adaptation of the forensic chain of custody developed in the "Generic Integrated Forensic Toolbox (GIFT)" project [50], taking into account ethics and patient data confidentiality.

- Set up electronic platforms for sharing the request form and test results, which could be completed online by the requester and by the lab, in order to avoid mistakes linked to the re-transcription of data. This system could be a part of the LIMS or should be linked to it to allow direct and easy information transfer.
- Widen the European implementation of One Health/(my care net) systems to link the lab to patient meta-data (complete patient medical file).
- Develop the use of additional data (meta-data), for example photo, video and positioning data (GIS).
- Develop robust systems for sample tracking inside the laboratory, from sample reception to sample storage/destruction.

Specifically for mobile lab:

- Develop methods and tools for integrated work between mobile laboratories and more advanced reach-back facilities. The following items needs to be taken into account: mobile data sharing, cloud computation, real-time data processing, innovated task sharing between different locations and facilities.
- Consider utilization of state of the art IT solutions to ensure an integrated system approach in sampling and mobile laboratory work.
- Develop use of space assets (satellite telecommunications, GNSS (Global Navigation satellite System) data for geo-location and Earth Observation data for site selection and monitoring).

The added value of laboratory management IT tools and space assets to laboratory activities has been proved in the ESA IAP-ARTES 20 « Biological Light Fieldable laboratory for Emergencies » (B-LiFE). Complementarity and interfaces between diagnostic capabilities and IT/communications/geolocation/… assets should be built based on this proof of concept and convince technological providers to help make that work.

Training

- Train people on biosafety and biosecurity issues.
- Increase the number of field-trained lab workers for European mobile laboratories. In their training, besides the specific training on laboratory work in field/outbreak conditions, include training on biosafety (including correct donning and doffing of PPE and risk assessment), first aid, self-protection and management stress and hazards (fire, electrical problem).
- Continue to develop the training (diagnostic technologies and biosafety issues) of local lab workers in developing countries, like the training provided by the Training Programs in Epidemiology and Public Health Interventions Network (TEPHINET), more particularly in Europe: the European Programme for Intervention Epidemiology Training (EPIET) and

the European Programme for Public Health Microbiology Training (EUPHEM), and the Centre for Health Sciences Training, Research and Development (CHESTRAD).

Laboratory network

- Develop a worldwide laboratory network
    - To share protocols/guidelines/standards in order to reach harmonisation concerning: request forms, diagnostic processes, development and validation of new tests, sample inactivation, laboratory and equipment decontamination, result transmission (format and communication channels), biosecurity and biosafety.
    - To organise external quality assessments.
    - Building a legal framework for the exchange and sharing of reference material (for test development, validation and as quality controls), especially exchange of new emerging pathogenic strains.
    - Organise international quality assessments. This would accelerate the development and validation of new diagnostic assays, in accordance with international accreditation requirements.

This network can benefit from the Joint Action EMERGE, (a European network with about 40 diagnostic laboratories that aims to provide a coordinated and efficient response to infectious disease outbreaks of highly dangerous and emerging pathogens at EU level and abroad), and the Joint Action QUANDHIP (Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens), which links and consolidate the objectives of existing European networks: The “European Network for Highly Pathogenic Bacteria“ (ENHPB) and the “European Network of P4 Laboratories (ENP4Lab)”.

4 Conclusion

Important research and innovation needs have been identified to improve diagnostic capacities for pandemic-prone pathogens and build capacity for pandemic management.

Possible improvements have been listed in five main categories: sample transport, laboratory capacity (infrastructure, equipment and diagnostic tests), information management (sample tracing, LIMS and result communication), training and laboratory networks.

These findings will be integrated in the integrated gap analysis and solution specification in PANDEM’s WP5. The outcomes will be reviewed at PANDEM’s next expert workshop to be held in September in Brussels, which aims to identify and analyse priority tasks for technological solutions in the process of developing demonstrator concepts.

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ANNEX - Questionnaire: Diagnostic Technologies

H2020-DSR-4-2014

Questionnaire: diagnostic technologies

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Coordinator: Máire Connolly, National University of Ireland, Galway (NUIG), Ireland

1. Presentation of the PANDEM project

Pandemic Risk and Emergency Management (PANDEM) is a Horizon 2020 crisis management project funded by the European Commission under the Secure Societies Work Programme. The consortium includes the London School of Hygiene & Tropical Medicine, the Public Health Agency of Sweden, Swedish Defence Research Agency, Université catholique de Louvain, the WHO Regional Office for Europe, IGS Strategic Communications and is co-ordinated by National University of Ireland Galway.

The aim of the PANDEM project is to identify innovative concepts to strengthen capacity-building for pandemic risk and emergency management in the EU. The overall objective is to reduce morbidity, mortality, environmental and economic damage from future pandemics by identifying improvement needs for technologies, procedures and systems. Specific attention is being given to enhancing capacity for collaboration on cross-border risk assessment, response and recovery at local, national and EU level. As pandemics are by definition global events, the project is also looking at the needs for strengthening pandemic management beyond Europe and how the EU can support capacity at international level.

The specific objectives of this questionnaire are to identify new solutions and improvement needs in the diagnostic practice in the context of a new pandemic:
- What is available in terms of « pandemic-pathogens diagnosis» (e.g., Environment: Capacities (Mob Lab >< Ref Lab) - Diagnostic Testing: collection, transport, tracking, results (signal processing / interpretation)
- Are current technologies matching needs performances and requirements ? If not what should be improved?

2. Aim of this questionnaire

This questionnaire is addressed to European laboratory managers and has a second part specifically directed to Mobile laboratory manager. It has two aims:

• What is available for « pandemic-pathogens »? Environment: Capacities (Mob Lab >< Ref Lab)

Collect information on current diagnostic practices: how “pandemic-pathogens” are currently monitored, detected and identified, how the samples are transported, tracked from sample collection to results delivery and how results are interpreted and communicated.

• Identify new solutions and improvement needs in the diagnostic practice in the context of a new pandemic: Are current technologies matching needs performances and requirements? If not what should be improved? Identify current gaps in terms of “pandemic-pathogens” diagnostic and identify the technologies to be improved or developed in order to enhance the “pandemic-pathogens” diagnostic capacity in Europe.

The PANDEM project would be grateful if you could complete the following questionnaire, not later than the **22th of May 2016**, and send it to Anne-Sophie.Piette@uclouvain.be.

This questionnaire is presented as a “multiple choice questionnaire” in the form of "Yes or No" (to fill in **green boxes**) or of lists (all **blue boxes**) and also contains boxes (**grey boxes**) you can fill with any input/comments you feel appropriate.
The first part of this questionnaire contains specific questions to allow the quick assessment of current situation. In the second part, focused in identifying gaps and innovation solutions, you provide us with any input/comments you feel appropriate.
The results of this questionnaire will be used as an input to make a review on diagnostic technologies in PANDEM project.
The PANDEM Consortium places high value on the need to protect the project events participants’ data and privacy. Therefore it commits to the following Privacy Policy:

- Personal data (names, email addresses, phone numbers) gathered by PANDEM through its events or website are stored in a separate database.
- PANDEM personal data are not disseminated, and can be accessed only by the staff of the Consortium Partners on a need-to-know basis. They are not passed on to third parties without prior consent of the events attendees or interviewees.

In that regard, Laboratory name and all contacts well be removed and only the country and type of laboratory will be included with the completed questionnaire that will be included in the project deliverable, which dissemination level is Confidential (for members of the consortium, including the Commission Services).

3. Questionnaire: diagnostic technologies

3.1. Contact information and laboratory description

Name of the Laboratory			
Institution			
Laboratory contact			
	Street-n°		
	Zip		
	City		
	Country		
	Phone		
	Fax		
Laboratory manager			
	Name		
	Phone		
	E-mail		
Laboratory type			If other, specify
Is your laboratory part of laboratory network(s) (Yes or No)		If yes, specify which	
Laboratory activities			
Biosafety level			
Does your lab have a certification or accreditation? (Yes or No)		If yes, specify which	

Which “pandemic-pathogens” are diagnosed/studied in the laboratory?

	Diagnosed	**Studied**

Bio-Agent (please specify)	(Yes or No)

Do you also have a Mobile laboratory? (Yes or No)

If **No,** please go to the **"Reach Back Questionnaire"**

If **Yes,** please go to the **"Mob Lab Questionnaire"**

Reach-Back Questionnaire

3.2. Current Practice

3.2.1. Pre-analytical phase related to agents with "pandemic potential"

(a) SAMPLE TRANSPORTATION

How do samples reach the laboratory? If other, specify

(b) TYPE OF SAMPLES

What types of samples do you accept for analysis in your laboratory?

Biological samples	**Human** (Yes or No)	**Animal** (Yes or No)	
Any type			
Blood			
Other biological fluids			if yes, specify
Tissue sample/Biopsy			if yes, specify
Swab			
Other			if yes, specify

	(Yes or No)	
Environmental liquid sample		if yes, specify
Environmental Sample		if yes, specify

(c) REQUEST FORM

Does information mentioned on the Request Form allow easy contact tracing and follow up? (Yes or No)

	Does information on the Request Form allow retrospective epidemiological analysis?		(Yes or No)
(d) SAMPLE TRACKING and TRACING	Is there any "track and trace" system starting from the place of sample collection to the reception area?		(Yes or No)
	During transport, which "Track & trace system" is used?		
	How are the samples recorded upon reception?		If electronic form :
(e) BIOSAFETY ISSUES	What official biosafety guidelines do you follow?		(specify)
	What type of PPE do you use?		
	Can some samples be REJECTED?		(Yes or No)
	If yes, what is/are the reason(s) for rejection?		If other, specify
(f) BIOSECURITY ISSUES	Are there specific measures taken to ensure security of the samples?		If yes, specify

3.2. 2. Analytical phase related to agents with "pandemic potential"

(a) SAMPLE TRACKING and TRACING	How is each sample tracked INSIDE the Lab during the analytical procedure?		If other, specify
	Do you use a commercial Laboratory Information Management System?		If yes, specify
(b) ANALYTICAL TESTS *What kind of diagnostic tests do you carry out?*	**(b1) MICROSCOPY** (Yes or No)		If yes, what is the methodology?
	(b2) DNA- and/or- RNA based IDENTIFICATION TEST	(Yes or No)	
	(RT-)PCR		If commercial, specify
	(RT-)qPCR		If commercial, specify
	Isothermal amplification : RPA		If commercial, specify

Isothermal amplification : LAMP			If commercial, specify
Isothermal amplification : TMA			If commercial, specify
DNA arrays (Luminex, FilmArray,…)			If commercial, specify
Other		If other, specify	

(b3) SEQUENCING ANALYSIS

	(Yes or No)	
Do you perform sequencing?		
Using which technology?		specify

(b4) Immuno-analyses

	(Yes or No)	
ELISA		If commercial, specify
Lateral flow device		If commercial, specify
Multiplex lateral flow device		If commercial, specify
Other		If commercial, specify

(b5) Other

	(Yes or No)	
FilmArray multiplex PCR system		
MALDI-TOF		If commercial, specify
Thin layer chromatography		If commercial, specify
Culture		
Other		If other, specify

Point-of-Care testing

(b6) BIOCHEMICAL TESTING for laboratory-guided clinical care

Do you perform "regular biochemical monitoring" of patients?	(Yes or No)	
If yes, what is the methodology?		If other, specify

(c) QUALITY CONTROL

What type of quality controls do you have to assess your analytical procedures?

3.2. Post-analytical phase related to agents with "pandemic potential" 3.

Results interpretation	How data are they interpreted in the lab?	
Turn-around time	What is the average turn-around time (from sample reception to results communication) for qPCR?	
	What is the average turn-around time (from sample reception to results communication) for biochemical testing?	
Data transfer	What kind of data do you transfer to the test requester?	
	What is the mode of data / results transfer outside the lab?	If needed, specify
	To whom do you transfer the data?	
Biosafety: decontamination	Do you assess the quality of check for decontamination procedure in the lab?	
	Which decontamination method do you use?	
	Which decontaminating agent do you use?	In all cases, specify (product/concentration)

3.3. Gaps & innovations

In the next section, and based on the list of questions above (i.e., from sampling to results communication), please comment on the problems/gaps you identify in this process. To address those problems, specify what improvements or solutions you would like to see developed in the next years

Majors gaps ?

Innovations needed :

Mob Lab Questionnaire

3.1. Contact information and laboratory description

Where do you see the use of a rapid deployable laboratory capacity in the management of a future pandemic? (free comment)

What is the activation mechanism for your MobLab deployment?

If requested, specify

3.2 Current Practice

3.2.1. Pre-analytical phase related to agents with "pandemic potential"

(a) TEST REQUEST

Who are the tests requesters?

Local		If other, specify
Distant		If other, specify

(b) SAMPLES

Sampling

Do you perform sampling? (Yes or No)

if yes, following which protocol?

Type of samples

What types of samples do you accept for analysis in the MobLab ?

Biological samples	**Human** (Yes or No)	**Animal** (Yes or No)	
Any type			
Blood			
Other biological fluids			if yes, specify

Tissue sample/Biopsy			if yes, specify
Swab			
Other			if yes, specify
	(Yes or No)		
Environmental liquid sample			if yes, specify
Environmental Sample			if yes, specify

(c) REQUEST FORM	Is there a specific Request Form?		***If yes, could you send us a pdf copy?***
	Is the own Laboratory Request Form used?		***If yes, could you send us a pdf copy?***
	Does information mentioned on the Request Form allow easy contact tracing and follow up?		
	Does information on the Request Form allow retrospective epidemiological analysis?		
	Does information on the Request Form allow critical analysis of lab results?		
(d) SAMPLE RECEPTION AREA	Is there a special dedicated reception area outside the lab for "dangerous samples"?		
(e) SAMPLE TRACKING and TRACING	How do samples reach the lab (how are they transported)?		If other, specify
	Is there any "track and trace" system starting from the place of sample collection to the reception area?		
	During transport, which "Track & trace system" is used?		If other, specify
	How are the samples recorded upon reception?		If electronic form :
			If your electronic database management is commercial, specify
(f) BIOSAFETY ISSUES	What official biosafety guidelines do you follow?		(specify)
	Has this work organisation inside the MobLab been reviewed by a "Health and Safety Adviser"?		(Yes or No)

	If yes, are corrective actions undertaken?		if yes, addressing which specific points?	
PPE	What type of PPE are normally used at the sample reception?			
	Do you use other kind of PPE, in specific circumstances?		(Yes or No)	
	What type of PPE ?			
	Specify the circumstances of use:			
Decontamination	Do you perform a decontamination protocol for each sample without exception?		if yes, specify which	
	Are special waste containment(s) in use at the sample reception area?		if yes, specify which	
	Which decontaminating agent do you use?			
	In all cases, specify which product/concentration			
Sample rejection	Can some samples be REJECTED?		(Yes or No)	
	If yes, what is/are the reason(s) for rejection?		If other, specify	
(g) BIOSECURITY ISSUES	Are there specific measures taken to ensure security?	(Yes or No)		
	of the staff?		If yes, specify	
	of the Mob Lab installation?		If yes, specify	
	of the samples in the MobLab?		If yes, specify	
	of the samples during transportation?		If yes, specify	
		(Yes or No)		
(h) SAFETY ISSUES	Do you have specific measures to ensure a safe electric system?		If yes, specify	
	Do you have specific measures to prevent lab incidents?		If yes, specify	
	Do you have specific evacuation / MEDEVAC plans?		If yes, specify	
	Does your staff have specific training to perform their lab work in field/outbreak conditions?			

	Does your staff have specific training other than their lab work training? (first aid, self-protection, hazard related to electrical problem, fire in the lab…)		If yes, specify which training	

3.2.2. Analytical phase related to agents with "pandemic potential"

(a) SAMPLE TRACKING and TRACING	How is each sample tracked INSIDE the Mob Lab during the analytical procedure?		If other, specify	
(b) BIOSAFETY ISSUES	Is each sample inactivated in the glovebox before further processing?		If yes, what is the methodology?	
Related to Sample PROCESSING and INACTIVATION			Specify glovebox brand	
	Is there a quality control procedure for assessing the quality / completeness of inactivation?		If Yes, specify which	
Related to WHERE SAMPLES ARE HANDLED	Where are samples processed after decontamination?			
PPE	What type of PPE are use in the MobLab ?			

		Which decontaminating agent do you use?	*In all cases, specify which product/concentration*
Decontamination	Inside the glovebox in the MobLab		
	In the MobLab		

(c) ANALYTICAL TESTS	Do you prepare sample for pathological analysis (e.g. microscopy)?		if yes, which kind of preparation ?	
	(c1) MICROSCOPY (Yes or No)			
	Do you Perform microscopy on-site		If yes, what is the methodology?	
What kind of diagnostic tests	**(c2) DNA- and/or- RNA based IDENTIFICATION TEST**	(Yes or No)		

do you carry out?

(RT-)PCR			If commercial, specify
(RT-)qPCR			If commercial, specify
Isothermal amplification : RPA			If commercial, specify
Isothermal amplification : LAMP			If commercial, specify
Isothermal amplification : TMA			If commercial, specify
DNA arrays (Luminex, FilmArray,…)			If commercial, specify
Other		If other, specify	

(c3) SEQUENCING ANALYSIS

	(Yes or No)		
Do you perform sequencing inside the Mob Lab?		(Yes or No)	
Using which technology?		If MinION, specify SQK-MAP version	
		If other, specify	

(c4) Immuno-analyses

	(Yes or No)		
ELISA			If commercial, specify
Lateral flow device			If commercial, specify
Multiplex lateral flow device			If commercial, specify
Other			If commercial, specify

(c5) Other

	(Yes or No)		
FilmArray multiplex PCR system			
MALDI-TOF			If commercial, specify
Thin layer chromatography			If commercial, specify
Culture			
Other			If other, specify

Point-of-Care testing

(C6) BIOCHEMICAL TESTING for laboratory-guided clinical care

Do you perform "regular biochemical monitoring" of patients?		(Yes or No)	
If yes, what is the methodology?			If other, specify

	Do you take specific biosafety measures to work with this type of non-inactivated samples?		If yes, specify
(d) QUALITY CONTROL	What type of quality controls do you have to assess your analytical procedures?		

3.2 .3. Post-analytical phase related to agents with "pandemic potential"

Results acquisition	What kind of data do you produce?	(Yes or No)		
	Pictures		Type of pictures :	Picture from the sample
	Electronic data			
	Paper reports			
	If other, specify			
Results interpretation	How data are they interpreted in the MobLab?			
	Is there a need for "results interpretation" by distant experts due to the data or results complexity?	(Yes or No)	If yes, specify for which method	
Turn-around time	What is the average turn-around time (from sample reception to results communication) for qPCR?			
	What is the average turn-around time (from sample reception to results communication) for biochemical testing?			
Data transfer	Are data protected?	(Yes or No)	If yes, specify how	
	What kind of data do you transfer?			
	To whom do you transfer the data?			
	What is the mode of data / results transfer outside the lab?		If needed, specify	
	Is internet connexion on-site readily available for data transfer?	(Yes or No)		
	If not, what is the system of communication used?		If other, specify	

Biosafety: decontamination	Is / are glovebox(es) repatriated after the mission?		(Yes or No)
	If yes, how is it decontaminated	*specify*	
	Which decontamination method do you use?		
	Which decontaminating agent do you use?		In all cases, specify (product/concentration)
	Is the Mob Lab equipment decontaminated at the end of mission?		(Yes or No)
	If yes, how is it decontaminated	*specify*	
	Which decontamination method do you use?		
	Which decontaminating agent do you use?		In all cases, specify (product/concentration)
	Do you assess the quality of check for decontamination procedure in the lab?		

3.3 Gaps & innovations

In the next section, and based on the list of questions above (i.e., from sampling to results communication), please comment on the problems/gaps you identify in this process. To address those problems, specify what improvements or solutions you would like to see developed in the next years

Majors gaps ?

Innovations needed :

Lists contents (all blue boxes)

Laboratory type		National reference laboratory
		Public health institute laboratory
		Hospital laboratory
		Research/academic laboratory
		Private laboratory (SME, Industry,…)
		Mobile laboratory
		Other
Laboratory activities		Diagnostic
		New diagnostic tests development
		Diagnostic and new diagnostic tests development
		Research on pathogens
		Diagnostic and research on pathogens
		New diagnostic tests development and research on pathogens
		Diagnostic, new diagnostic tests development and research on pathogens
Biosafety level		BSL1
		BSL2
		BSL3
		BSL4
Who are the tests requesters?		Local practitioners
		Local hospital
	Local	Field hospital deployed by WHO/NGO's
		Both local and WHO/NGO hospitals
		Epidemiologists from WHO/CDC
		Other
	Distant	International organisms: DG ECHO
		International organisms: WHO/CDC
		National authorities of the host country
		Both national and international authorities
		Other
How are samples transported to the lab?		By mail
		By a logistic carrier (as DHL)
		Directly by people from the requesting institution
		By mail and logistic carrier (as DHL)
		Directly and by mail
		Other
		Provided directly by WHO staff
		Provided directly by Red Cross staff
		Provided directly by staff of an NGO (other than RC) treatment centre
		Provided directly by local physicians
		Provided directly by unknown sources
		Provided directly by WHO and NGO's staff
		Provided directly by WHO, NGO's staff and local physicians
		Received by courier
		Other
What could cause sample rejection?		Improper packaging
		Damaged packaging
		Broken sample
		Improper/damaged packaging and broken sample

	No request form with the sample
	Missing mandatory information on the request form
	All above
	Other
How are the samples recorded inside the laboratory?	Recording on paper forms/logbook
	Recording in electronic form
	Recording both on paper and electronic form
If electronic form	Data recording from the request form
	Data recording from the request form and picture of the sample
	Data recording and picture of the request form, and picture of the sample
How are the samples tracked inside the laboratory?	Sticker
	Barcode
	RFID tag
	Other
What PPE do you wear to treat samples suspected to contain “pandemic-pathogens”	Full PPE with active respiratory masks
	Full PPE
	Safety goggles, mask, gloves and labcoat
	Mask, gloves and labcoat
	Gloves and labcoat
	Only gloves
Where are the samples opened	On the bench
	In a glovebox
	In a depressurised glovebox
	In a validated depressurised glovebox
	In a BSL2 hood
	In a glovebox located in a BSL3 lab
	In a glovebox located in a BSL4 lab
Sample inactivation	Bleach
	Ethanol
	Home-made solution
	Commercial solution
	Both home-made and commercial solutions
NGS	Illumina (specify platform)
	Ion Torrent (specify platform)
	Roche (specify platform)
	PacBio (specify platform)
	More than one platform (specify)
	Oxford Nanopore - MinION (specify SQK-MAP version)
	Pyrosequencing (specify platform)
	Other (specify)
What kind of data do you produce?	Picture from the sample packaging
	Picture from the sample
	Picture from test result (if it is not automatically recorded through the equipment software)
	Picture from the sample packaging
	Picture from all three cited above
How do you analyse the data?	Visual interpretation
	Interpretation with home-made software
	Interpretation with specific software provided with the analytical instrument
	Interpretation with other commercial software
	Interpretation with free or open-source software
	A combination of all those solutions

How do you transfer the results to the analysis requester?	By mail
	By e-mail
	By fax
	By phone
	Through a shared database with the requester
	By mail and through a shared database with the requester
	Through dedicated reporting/warning systems (specify)
	By mail, through a shared database with the requester and through dedicated reporting/warning systems (specify)
	Other (specify)
Transmit the results	Only to the analysis requester
	Results are reported to the requester and national health authorities
	Results are reported to the requester and ECDC
	Results are reported to the requester and WHO
	Results are reported to the requester, national health authorities and ECDC
	Results are reported to the requester, national health authorities and WHO
	Results are reported to the requester, national health authorities, ECDC and WHO
	Whole scientific community
	Other
Through which channels?	Laboratory network(s) your lab belongs to
	By e-mail
	By mail
	Through dedicated reporting/warning systems (specify)
	Through scientific papers
	Other (specify)
Decontamination solution	Washing with liquid decontamination solution
	Fumigation
	Washing and fumigation
Decontaminating agent	Alcohol
	Chlorine
	Quaternary ammonium compounds
	Chlorhexidine
	Iodine
	Formaldehyde
	Glutaraldehyde
	Phenolic compounds
	A combination
	Successive use of more than one decontaminating agent
	Commercial solution
	Other
How do you check for decontamination?	No decontamination
	We do not check quality of decontamination
	By swabbing and qPCR
	By contact plates and cultures back in the reack-back facility
Biochemical patient testing?	Piccolo Express® (e.g., HITADO GmbH, Moehnesee, Germany)
	i-STAT (e.g., Abbott Laboratories, Wavre, Belgium)
	Other Point-of-Care device
Microscopy	on-site optic microscopy
	on-site electron microscopy
	on-site optic microscopy and sample preparation for electron microscopy when back home
	Sample preparation for electron microscopy when back home

Communication system	Proprietary SatCom SatCom as a global service for field hospital or medical facility Satellite phone Mobile phone with local SIM card Other
Activation mechanism	1. European Commission Humanitarian Aid and Civil Protection department (DG ECHO) 2. European Commission - Emergency Response Coordination Centre (ERCC) 3. Global Outbreak Alert and Response Network (GOARN). 4. National decision based on direct contacts with requesting country More than one (specify the numbers) Other (specify)