LASSA Fever

Brief instructions for handling and transport of samples from suspected cases and exposed contacts, including referral for diagnostic confirmation

EMERGE Network (Work Package 5)

Guidance for local laboratories for selection, preservation and local transport of samples from suspected cases and exposed contacts	
Specimens	Preferred specimen: blood (serum, plasma or whole blood). Other possible specimens: urine, saliva, post-mortem biopsy, others. Although a comprehensive analysis of laboratory data is still to be completed, some preliminary observations can be drawn based on experience in LASV endemic countries. • Blood samples (serum, plasma and whole blood) are the first choice for testing. The results from these specimens show the best correlation with both infectivity and clinical course. • Reverse Transcription-Polymerase Chain Reaction (RT-PCR) can be used in the early stage of disease. No commercial assays are available, even if some are in an advanced phase of development; the high degree of sequence divergence of Lassa genomes is the major problem affecting the development of molecular diagnostic tests. Different PCR protocols are available in the literature, although most are not quantitative: – Olschläger S et al. Improved detection of Lassa virus by reverse transcription–PCR targeting the 5' region of S RNA. J Clin Microbiol. 2010;48:2009–13 . 10.1128/JCM.02351-0; – Drosten C, Gottig S, Schilling S, Asper M, Panning M, Schmitz H, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. Journal of clinical microbiology. 2002; 40(7):2323–30. – Bowen MD, et al. Genetic diversity among Lassa virus strains. J Virol. 2000;74:6992–7004 . 10.1128/JVI.74.15.6992-7004.2000; – Vieth S, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. Trans R Soc Trop Med Hyg. 2007;101:1253–64; – Leski TA, et al. Sequence variability and geographic distribution of Lassa virus, Sierra Leone. Emerg Infect Dis. 2015 Apr;21(4):609-18. – Demby AH, et al. Early diagnosis of Lassa fever by reverse transcription-PCR. Journal of

	clinical microbiology. 1994; 32(12):2898–903. – Lunkenheimer K, et al. Detection of Lassa virus RNA in specimens from patients with Lassa fever by using the polymerase chain reaction. Journal of clinical microbiology. 1990; 28 (12):2689–92. – Trappier SG, et al. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. The American journal of tropical medicine and hygiene. 1993; 49(2):214–21. – Coulibaly-N'Golo D, et al. Novel arenavirus sequences in Hylomyscus sp. and Mus (Nannomys) setulosus from Cote d'Ivoire: implications for evolution of arenaviruses in Africa. PloS one. 2011; 6(6):e20893. Lassa fever can be diagnosed using enzyme-linked immunosorbent serologic assays (ELISA) or IFA, which detect IgM and IgG antibodies and/or Lassa antigen. The virus can be cultured in 7 to 14 days, after which virus can be detected by RT-PCR and/or IFA, c.p.e. is not always detected. Viral culture must be performed only in a high containment BSL4 laboratory. Immunohistochemistry, performed on formalin-fixed tissue specimens, can be used to make a post-mortem diagnosis. http://www.cdc.gov/vhf/lassa/diagnosis/index.html • For differential diagnosis of suspected patients other viral haemorrhagic fever (VHFs) should be considered (according to epidemiological data) as well as malaria, enteric fever, tuberculosis and any specific pathogens with similar symptoms that are currently associated with outbreaks in the geographical region where the infection originated. **For all the reasons previously reported is really important that LASV diagnostic tests must be performed in specialized laboratories (at least BSL3) with consolidated experience in VHF inactivation and diagnosis.**
Bed-to-local laboratory transport	• Plastic air-tight and leak-proof containers are obligatory. The external surface of the container should be disinfected with 3% sodium hypochlorite. If the specimen container is a tube, it must be tightly capped. All samples should be labelled with a unique patient ID. Specimens should be accompanied with a documentation sheet (packaged separately from the sample) including the patient's unique ID, date/time/place of sampling, type of specimen, test requirements, clinical data including travel history and exposure to a suspected or confirmed case. For the transport of the primary container from the patient bed to the lab a hard-sided/impact resistant outer secondary container is recommended.
Preservation	Mainly depends on the type of testing for which the sample is intended. Please contact your referral laboratory for specific guidance. As a general guide: • Unfixed/fresh solid samples (e.g. swabs) should never be allowed to dry. These samples should be placed either in viral transport medium (preferable) or 0.9% NaCl solution. • Samples for histopathology and/or electron microscopy (e.g. glutaraldehyde fixed) do not need additional preservation measures and can be transported at room temperature • Specimens expected to arrive at referral laboratory within 2 hours should be stored at room temperature • Specimen that can be sent to a referral laboratory within 24 hours should be transported either on dry ice or at +4°C • Sample which cannot be transported within 24h should be stored at -70°C
Handling	Detection of Arenaviruses can be safely performed in a proficient BSL-3 reference laboratory. For viral culture of LASV a BSL-4 facility is required. In any case we strongly suggest that the diagnostic confirmation should be performed in laboratories with specific and consolidated expertise in

	differential diagnosis for VHFs. A BSL-4 is required for animal infections as well. We recommend contacting a referral laboratory before undertaking any procedure. In all cases an approach based on a "risk stratification" is advisable, as described in "Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence", available at: https://www.gov.uk/government/publications/viral-haemorrhagic-fever-algorithm-and-guidance-on-management-of-patients. Criteria for risk stratification analysis can differ among the European member states (EU MS) and the appropriate local guidance should be consulted. Referral **inactivation procedures** are described in the following documents: – Public Health Agency of Canada http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/lassa-eng.php. – Canadian contingency plan for viral haemorrhagic fevers and other related diseases, available at: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/97vol23/23s1/index.html – and Notice to Readers Update: Management of Patients with Suspected Viral Haemorrhagic Fever -- United States, available at: http://www.cdc.gov/mmwr/preview/mmwrhtml/00038033.htm
Transport	Samples for primary diagnostic procedures must be sent to national referral VHF laboratories according to national rules. It is necessary that the transfer of samples to EMERGE laboratories (for primary diagnosis or confirmation) is organized well in advance and detailed information exchange by phone or by e-mail is taking place between the sender and the receiving Laboratory. If the Material Transfer Agreements (MTAs) are already in place, these should be used and disseminated. For further details about procedures for the transport of highly pathogenic infectious substances, please consult WHO guidelines: http://www.who.int/ihr/publications/who_hse_ihr_2012.12/en/index.html. The Coordinators of the Viral Network of the EU joint Action EMERGE, can support EU MS in facilitating the contact with the appropriate laboratory and with courier information, and may be contacted as follow: Giuseppe Ippolito, Scientific Director, e-mail: giuseppe.ippolito@inmi.it; Antonino Di Caro, Director Biocontainment labs , email: antonino.dicaro@inmi.it
Biosafety issues	– Biosafety and biosecurity procedures are essential for the safe and appropriate management of specimens from suspected/confirmed Lassa patients. We suggest that all laboratories refer to the documents "CWA 15793:2011 Laboratory Bio-risk Management" and "CWA 16393:2012 Laboratory bio-risk management - Guidelines for the implementation of CWA 15793:2008" for a complete guide, available at: ftp://ftp.cenorm.be/CEN/Sectors/TCandWorkshops/Workshops/CWA15793_September2011.pdf and – http://www.uab.cat/servlet/BlobServer?blobtable=Document&blobcol=urldocument&blobheader=application/pdf&blobkey=id&blobwhere=1345653753282&blobnocache=true, or to national guides if they provide an assessment for adequate implementation of CWA 15793:2008. Other relevant WHO laboratory biosafety and biosecurity guidelines are: – Laboratory biosafety manual, 2004, available at: http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/ – Biorisk management, Laboratory biosecurity guidance, 2006, available at: http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf – Integrated European Checklist for Laboratory Biorisk Management in Handling of High Consequence Risk Group 3 and 4 Agents (ECL-Biorisk), available at: http://www.emerge.rki.eu/Emerge/EN/Content/Topics/Rules/ECL_Biorisk.html

Additional resources	– WHO. Emergency preparedness and response available at http://www.who.int/csr/don/19-february-2016-lassa-fever-benin/en/ – ECDC: http://ecdc.europa.eu/en/publications/Publications/RRA-Lassa-fever-Germany-march-2016.pdf – http://ecdc.europa.eu/en/healthtopics/lassa_fever/Pages/index.aspx – Public Health Agency of Canada http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/lassa-eng.php – Germany http://www.rki.de/DE/Content/InfAZ/L/Lassa/Lassavirus-Infektion.html – Management of Hazard Group 4 viral haemorrhagic fevers and similar human infectious diseases of high consequence. Available at: https://www.gov.uk/government/publications/viral-haemorrhagic-fever-algorithm-and-guidance-on-management-of-patients – EURONHID Manual for Infection Control Management of HID patients. Available at: http://www.inmi.it/linee_guida/EuroNHID%20Manual%20for%20the%20management_of%20HIDs%20-%202011.pdf – Integrated European Checklist for Laboratory Biorisk Management in Handling of High Consequence Risk Group 3 and 4 Agents, QUANDHIP 2015 (available at http://www.emerge.rki.eu/Emerge/EN/Content/Topics/Rules/ECL_Biorisk.pdf?__blob=publicationFile – Efficient response to highly dangerous and emerging pathogens at the EU level (EMERGE project): **www.emerge.rki.eu**