



Details regarding laboratory diagnostics for suspected cases of Ebola virus disease

In the event of a probable case of Ebola virus disease, which is defined as having

- symptoms compatible with Ebola virus disease AND a corresponding travel history AND possible exposure to Ebola virus disease (www.rki.de/ebola-flussschema) or
- symptoms compatible with Ebola virus disease AND exposure in Germany (www.rki.de/ebola-uebersicht > [Differenzierung von Personen nach Expositionsrisiko \[in German\]](#)),

the patient should be transferred to a special isolation ward at a treatment center – after consultation with the health authorities responsible and possibly also a competency center – in order to initiate laboratory diagnostics.

For probable Ebola virus disease cases, routine laboratory diagnostics (e.g. clinical chemistry) and specific laboratory tests for differential diagnosis (e.g. for malaria) should be performed accordingly in a special isolation ward (point-of-care diagnostics, small series of diagnostic tests).

In case it is necessary to carry out diagnostics outside of the context of a special isolation ward, the procedure should be agreed upon with the competent health authorities and possibly also a center of competency and treatment.

For probable Ebola virus disease cases, laboratory diagnostics should be carried out in a BSL-4 laboratory (addresses see appendix). For asymptomatic contacts no laboratory investigations for Ebola virus are indicated.

The individual steps of performing Ebola virus laboratory diagnostics for probable cases of Ebola virus disease are described below.

- 1. Specimen collection**
- 2. Shipping/transport of specimen**
- 3. Laboratory investigations**
- 4. Acquisition of findings/assessment of findings**
- 5. Report of diagnosis**

Appendix

- 6. Addresses of laboratories for Ebola virus diagnostics**
- 7. Examples of packaging for the shipment of samples and shipping companies**
- 8. Information regarding preliminary diagnostics**



1. Specimen collection	<ul style="list-style-type: none"> • Documentation by a complete protocol of specimen collection (including persons and times). • Collection of patient samples in a case of probable Ebola virus disease or a confirmed case of Ebola virus disease (as applicable) is performed only by observing reasonable protective measures (www.abig.rki.de/abig/ebola-psa [in German]). • Specific requirements of the sampling technique and of hygiene standards necessitate, in addition to instructions on how to act, an advanced training in infectiology (including practical exercises) of physicians and nursing personnel who take samples. • In case pointed and sharp medical devices must be used in sampling, tools provided with safety features (safety tools) must be used (see TRBA 250 section 4.2.5 paragraph 3 ff. [in German]). • Sample materials: the preferred material for primary diagnostics is serum or whole blood (to document and confirm recovery, also urine, stools, sweat, swabs from conjunctiva or oral mucosa). • Remember to take a retained sample.
2. Shipping/transport of specimen	<ul style="list-style-type: none"> • Prior to shipping samples from a patient with a probable Ebola virus disease, contact the diagnostic laboratory (addresses of the laboratories see appendix) in order to announce the shipment of samples and to consult about shipment details. • Prepare a complete transport protocol (including persons, state of the samples and times). • Ship diagnostic samples from a case of probable Ebola virus disease as class 6.2 category A according to ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road); packing instruction P620 designated „Infectious substances affecting humans“ UN 2814. • Attach the biohazard symbol required for the dangerous goods class 6.2; in addition, the information “If packaging is damaged or contents released inform health authorities” can be placed. • The submitter is the person responsible for announcing the shipment of the sample, its proper classification and adhering to the transportation directives, in case of doubt it is the head of the institution of the submitter. • The laboratory acknowledges the arrival of the specimen by contacting the attending physician/medical officer and the submitter immediately after receipt of the specimen. • Means of transportation and location of the diagnostic laboratory should be selected in such a way that the sample will arrive at the laboratory within six hours. • Packaging <ul style="list-style-type: none"> ○ P620 for infectious substances according to UN 2814 ○ Triple packaging system, consisting of <ul style="list-style-type: none"> ▪ 1. Primary container (specimen container) ▪ 2. Secondary container (safety container) ▪ 3. Outer shipping package. • For liquid materials (e.g. blood) specimen container and safety container have to be impermeable and sufficiently absorbent material must be placed between the two containers. Packaging is available from specialist shops or will be provided by the courier.



3. Laboratory investigation	<ul style="list-style-type: none"> • Generally, diagnostics consists of real-time PCR for the detection of the virus genome in the blood (serum or plasma) during the acute phase of the disease. • Detection of specific antibodies (IgM, IgG) is possible using immunofluorescence, ELISA and neutralization test, but this is not meaningful for the purposes of primary diagnostics. • Confirmation diagnostics for a positive primary PCR result includes PCR amplification of an additional genome region with the same nucleic acid sample or by sequencing of the PCR product. Simultaneously to the confirmation tests, in the case of a positive first result, an aliquot of the sample is sent to the Consultant Laboratory for Filoviruses, Institute of Virology, Philipps University Clinics, Marburg, or to the National Reference Centre for Tropical Infectious Agents, Bernhard Nocht Institute for Tropical Medicine, Hamburg. For confirmation purposes it is not necessary to isolate the virus, however, this can be done in order to characterize the virus further. The isolation of Ebola viruses can only be done from non-inactivated patient material and requires a BSL-4 laboratory (in Germany, at present, only at the Consultant Laboratory for Filoviruses, Institute of Virology, Philipps University Clinics, Marburg, or at the National Reference Centre for Tropical Infectious Agents, Bernhard Nocht Institute for Tropical Medicine, Hamburg). In general, the isolation of Ebola viruses plays only a minor role in primary diagnostics. • If the confirmation test is negative, the investigation on Ebola virus is repeated using a retained sample. • Results can be expected within 6–8 hours after arrival of the sample at the diagnostic laboratory, provided that there are no unexpected technical problems.
4. Acquisition of findings/assessment of findings	<p>Depending on the time of specimen taking from a suspected case, the following algorithms lead to these results:</p> <ol style="list-style-type: none"> 1. The taking of a blood sample is done > 48 hours after the onset of symptoms consistent with Ebola virus disease: <ul style="list-style-type: none"> • Primary test and confirmation diagnostics are positive: → Confirmed case of Ebola virus disease. • Primary test is negative: → No case of Ebola virus disease. 2. The taking of a blood sample is done ≤ 48 hours after the onset of symptoms consistent with Ebola virus disease: <ul style="list-style-type: none"> • Primary test and confirmation diagnostics are positive: → Confirmed case of Ebola virus disease. • Primary test is negative and the patient meets the criteria of a probable case: → Comprehensive differential diagnostics should be performed. → For a period of 48 hours, the patient continues to be placed in isolation, but not necessarily in the special isolation ward. → Regardless of a confirmed differential diagnosis, an additional patient sample, taken at the earliest on the 3rd day (> 48 hours) after onset of symptoms consistent with Ebola virus disease, should be tested for Ebola virus. Primary test of this sample is negative in the PCR: → No case of Ebola virus disease. Primary test and confirmation diagnostics are positive: → Confirmed case of Ebola virus disease.
5. Report of diagnosis	<p>Immediate report of the laboratory results (negative and positive), because the results are relevant for further measures, to the following parties involved:</p> <ul style="list-style-type: none"> • to the submitter/attending physician(s). They in turn notify the competent local health authorities (Gesundheitsamt) according to section 6 IfSG paragraph 1 number 1g. • to the competent local health authorities (Gesundheitsamt) according to section 7 IfSG paragraph 1 number 12. They in turn notify the competent state health authorities (Landesbehörde) according to sections 11, 12 IfSG and then the RKI.

In consultation with the Consultant Laboratory for Filoviruses, Institute of Virology, Philipps University Clinics, Marburg, the National Reference Centre for Tropical Infectious Agents, Bernhard Nocht Institute for Tropical Medicine, Hamburg, and the Institute of Microbiology of the German Armed Forces, Munich.



Appendix

6. Addresses of laboratories for Ebola virus diagnostics

BSL-4 Laboratories

Consultant Laboratory for Filoviruses

Institute of Virology

Philipps University Clinics Marburg

Hans-Meerwein-Str. 2

35043 Marburg / Germany

Contacts: Prof. Dr. S. Becker, Dr. M. Eickmann

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E-Mail: becker@staff.uni-marburg.de ; eickmann@staff.uni-marburg.de

Homepage: http://www.ukgm.de/ugm_2/deu/umr_vir/index.html ,

<http://www.uni-marburg.de/fb20/virologie/diagnostik>

National Reference Centre for Tropical Infectious Agents

Bernhard Nocht Institute for Tropical Medicine

Bernhard-Nocht-Str. 74

20359 Hamburg / Germany

Ärztliche Leitung: Prof. Dr. med. Stephan Günther / Prof. Dr. med. Dennis Tappe

Partner in the Laboratory Network (NaLaDiBa)

Questions for diagnostic indication and procedures:

Phone: +49 40 285380-211; +49 40 285380-0 (telephone switchboard)

Telefax: +49 40 285380-252

E-Mail: Labordiagnostik@bni-hamburg.de

Questions regarding diagnostic indications and procedures: Microbiological Central Diagnostics

Phone: +49 40285380-219; +49 40 285380-0 (24/7 Zentrale)

Telefax: +49 40 285380-252

E-Mail: Labordiagnostik@bni-hamburg.de

Questions regarding treatment: Out-patient clinic of the clinical department

Tel.: Telefon: +49 40 285380-219; +49 40 285380-0 (24/7 Zentrale)

Homepage: <https://www.bnitm.de/labordiagnostik/uebersicht>

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Homepage:

<https://www.rki.de/EN/Content/infections/Diagnostics/SpecialLab/highlyPathogenicViruses.html>

Additional laboratories providing preliminary diagnostics for Ebola viruses

Institute of Microbiology of the German Armed Forces



Prof. DrR. Wölfel (Head of institute)
 Dr. S.Zange (Diagnostics)
 Prof. J. J. Bugert (Virology)
 Institute of Microbiology
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Institut für neue und neuartige Tierseuchenerreger

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http://www.baain.de/portal/a/baain/!ut/p/c4/04_SB8K8xLLM9MSSzPy8xBz9CP3I5EyrpHK9pMTEzDy9IMzUvOKSYr3yzGL9gmxHRQAPpsfO/

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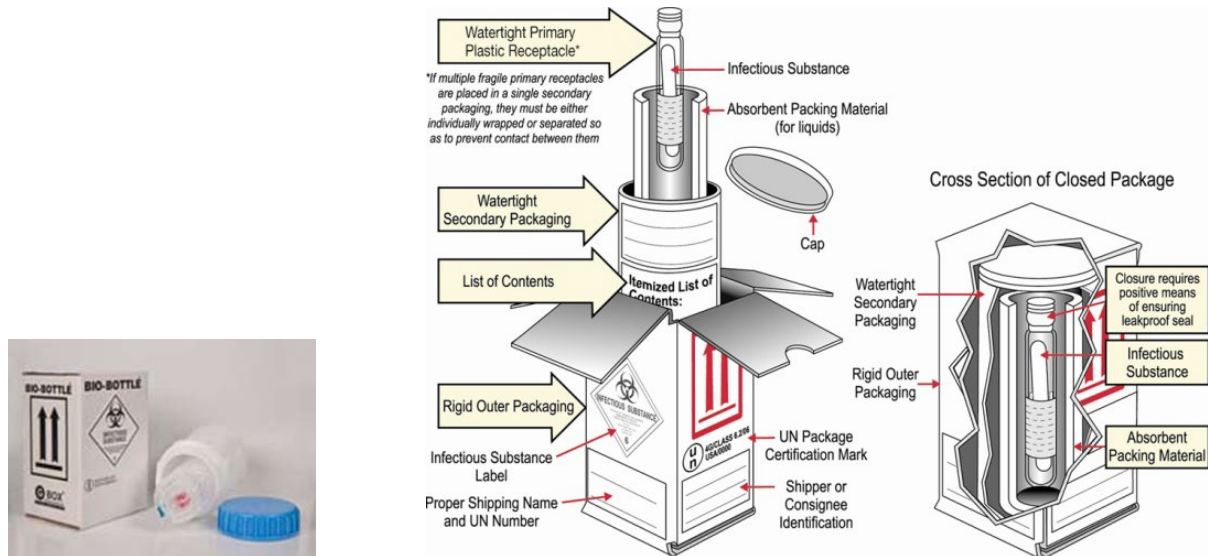
E-Mail: hendrik.streeck@ukbonn.de

Homepage: www.virology-bonn.de



Appendix

7. Examples of packaging for the shipment of samples and shipping companies



Source: <http://www.cdc.gov/vhf/ebola/hcp/packaging-diagram.html>

Shipping companies in Germany for infectious substances (UN 2814)

It is recommended to discuss the shipment of samples with the shipping company at an early stage. The following companies offer transportation according to UN 2814:

1. World Courier

<http://www.worldcourier.com/about/locations/europe#ch-germany>

2. CMK-Logistik Breisach

<http://www.cmk-logistik.de>



Appendix

8. Information regarding preliminary diagnostics

In case a preliminary diagnostics is required outside of the context of BSL-4 laboratories, this should be done after consultation with the competent health authority and competency center. Primary diagnostic investigations using PCR can be attributed to BSL 3 according to the Biological Agents Ordinance in conjunction with the Technical Rule for Biological Agents (TRBA) 100 “[Protective measures for activities involving biological agents in laboratories](http://www.baua.de/en/Topics-from-A-to-Z/Biological-Agents/TRBA/TRBA-100.html)” (<http://www.baua.de/en/Topics-from-A-to-Z/Biological-Agents/TRBA/TRBA-100.html>): “4.4.1 ... (4) If an infection with a biological agent of risk group 4 is suspected, all indicative examinations of the primary sample with material that has not been inactivated must be conducted at least under the conditions of **protection level 3.**”

Therefore preliminary diagnostics for suspected cases of Ebola virus disease can also be performed in BSL-3 laboratories in Germany, in addition to BSL-4 laboratories, provided that any risk to the personnel is eliminated as well as the further spreading of the virus. Suitable laboratories are those that fulfil the following conditions:

1. Operation of a virology BSL-3 laboratory ensuring the protection of the personnel.
2. Experience in molecular diagnostics of viral diseases.
3. Use of commercially available kits for the detection of Ebola virus RNA which contain an extraction and amplification control (e.g. from Altona Diagnostics, Roche Diagnostics etc.).
4. Successful participation in external quality assessment trials regarding Ebola virus PCR diagnostics.

Simultaneously to the confirmation tests, in the case of a positive preliminary result, the sample is sent to the Consultant Laboratory for Filoviruses, Institute of Virology, Philipps University Clinics, Marburg, or to the National Reference Centre for Tropical Infectious Agents, Bernhard Nocht Institute for Tropical Medicine, Hamburg.