Plague diagnostic recommendations

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Brief instructions for the diagnostic of specimens from suspected plague cases and exposed contacts, including recommendations for diagnostic confirmation

Plague is a severe, rapidly progressing and life threatening bacterial disease caused by the gram negative bacterium *Yersinia pestis*. The case fatality rate is high and can reach up to 100% among untreated patients suffering from pneumonic plague. Therefore, rapid diagnosis and treatment are highly important and should be initiated immediately when plague is suspected. Plague should be considered in any patient with clinical symptoms of plague and a recent history of travel to a plague endemic area (https://www.cdc.gov/plague/maps/index.html). In most European countries, plague is a notifiable disease.

The following information must be provided on the sample submission form by the sender of specimens in order to allow an appropriate sample analyzing procedure at the responsible diagnostic laboratory:

1. Patient name or unique specimen identification number
2. Type of specimen (e.g. sputum, lymph node aspiration liquid, etc.)
3. Suspected etiology
4. Date of onset of symptoms
5. Brief description of symptoms
6. Date of specimen collection
7. History of antibiotic treatment, date of therapy start, name and dosage of drugs applied, including Malaria prophylaxis (tetracyclines, e.g. doxycycline), if applicable
8. Travel history (please, provide dates when patient entered and left the endemic area of plague, if applicable)

The sending of specimens should be announced to the diagnostic laboratory before shipment.

Subject	Recommendations
Clinical manifestation	Plague can appear in different clinical forms depending on the route of infection: - Bubonic plague results from flea bites, whereas - pneumonic plague results from direct exposure to infected tissues or respiratory droplets. Clinical forms of plague:

	• Pneumonic plague (plague pneumonia) results from inhalation of infectious aerosols (primary plague pneumonia), which is also the case in human-to-human transmission. This clinical form is the dominant form seen in the plague outbreak in Madagascar in 2017. In addition, pneumonic plague can also be the result of hematogenous spread in bubonic or septicemic cases (secondary plague pneumonia). The incubation period of primary pneumonic plague is very short ranging from less than 24 hours to four days. Patients rapidly develop high fever, headache, weakness, and a severe pneumonia with shortness of breath, chest pain, and cough, sometimes accompanied by an expectoration of bloody or watery sputum. Pneumonic plague finally ends with respiratory failure and shock. Untreated pneumonic plague usually has a fatal outcome. • Bubonic plague presenting as painful regional lymphadenitis after bite of an infected flea (overall predominant form). Buboes usually develop in the lymph nodes proximal to the flea bite, e.g. in the groin, axilla or cervical lymph nodes. Patients usually suffer from fever, chills, headache and weakness. The incubation period for bubonic plague is 1 to 7 days. • Septicemia without an evident bubo (septicemic plague) may develop when bubonic plague, resulting from hematogenous dissemination, remains untreated. This form can also cause infections in other organs (e.g. liver, spleen). Patients present with high fever, chills, extreme weakness, sometimes also gastrointestinal symptoms, which are followed by disseminated intravascular coagulation and multi-organ failure in the later stages of the disease. • In rare cases, pharyngitis, meningeal plague, and cervical lymphadenitis, resulting from exposure to larger infectious droplets or ingestion of infected tissues (pharyngeal plague), may develop. Note: If plague is suspected (by applying pre-defined case definition criteria), antibiotic treatment must be initiated immediately, but appropriate specimens for laboratory diagnostics must be taken before applying the first dose, if possible. Local and state health departments must be notified immediately.
Clinical specimens	**Preferred specimens:** Appropriate sites for specimen collection depend on the clinical manifestation: • Pneumonic plague: Blood cultures should be taken and are usually culture-positive at early and later stages of the disease; sputum can also be used for nucleic acid extraction followed by PCR detection. Bronchial/tracheal lavage may be taken from suspected pneumonic plague patients. However, throat specimens are not ideal for isolation of *Y. pestis* since they often contain other bacteria that can mask the presence of plague. • Bubonic plague: Lymph node aspirates should be taken from swollen lymph nodes; it is proposed to inject 1-2 ml of saline prior to aspirate. Note that this procedure is painful for the patient. • Septicemic plague: Blood cultures are preferred.

	• Postmortem specimens: Lymphoid tissue, spleen, lung fluid, lung tissue, and liver tissue or bone marrow samples may yield evidence of a plague infection.
Shipment of clinical specimens	Clinical specimens for primary diagnosis (from patients who have not yet been diagnosed for plague can be labelled as "Biological Substance Category B" (UN 3373) and shipped according to packing instruction P650 (air and road transport). http://www.un3373.com/info/regulations/ Clinical specimens from patients with a laboratory-confirmed diagnosis of plague or from patients who are showing typical symptoms of plague and are very likely to suffer from plague without laboratory confirmation, e.g. close contacts of laboratory-confirmed cases, as well as bacterial cultures must be labeled as "Infectious Substance Affecting Humans, Category A" (UN 2814) and shipped according to the packaging instruction P620 (air and road transport). WHO Link: http://www.who.int/ihr/publications/WHO-WHE-CPI-2017.8/en/
Diagnostic procedures	**NOTE:** It is recommended to confirm a positive/ negative test result by a further method, e.g. positive amplification results by PCR should be confirmed by amplification of a second target (usually done in parallel), by culture (appropriate biosafety conditions required!), immunological test etc. In case of an early stage of disease and negative result, additional laboratory investigations should be considered when the clinical suspicion of plague is still in place and clinical symptoms are progressing. Note: Rapid Antigen detection tests targeting the F1 capsule are commercially available and can be directly applied to bubo aspirates or sputum samples. Positive reaction strongly suggests plague. Negative results are not meaningful. Furthermore, usually, tests are not yet licensed for human diagnostics. It is recommended that results of these assays should be confirmed by other established laboratory methods specific for plague. Important: Enrichment cultures or pure cultures of *Y. pestis* must be incubated at 37 °C in order to allow the bacteria to express the capsule antigen, because otherwise the results may not be reliable.
- Microscopy	*Y. pestis* may be identified microscopically by examination of stained smears from peripheral blood, sputum, lymph node specimens, blood cultures, isolates. Organisms may be seen in blood smears presenting with a safety-pin-like appearance. Note: Blood smears taken from patients at an early stage of illness are usually negative in microscopy whereas culture or PCR may be positive. Affected buboes contain numerous organisms and can be examined microscopically. Immunofluorescence and "Fluorescence in situ hybridization" (FISH) can also be used although these are only available in a few specialized laboratories. Observation of bipolar-staining, ovoid, gram negative organisms with a "safety pin" appearance permits a rapid presumptive diagnosis of plague. Recommended conventional staining procedures: - Gram stain - Wright stain - Giemsa stain - Waysons's stain - Methylene blue stain - Fluorescent labeled antibody against the F1 capsule antigen

	Bacterial characteristics: - small bacilli (1 to 2µm by 0.5µm rods) - gram negative - single cells, pairs or short chains, safety pin structure
-Culture	*Y. pestis* can be cultivated from various specimens, depending on the clinical manifestation (e.g. bubo aspirates, blood, sputum). Growth can usually not be observed before 24 h of cultivation. However, *Y. pestis* is often overgrown by other bacteria, particularly from bubo aspirates and respiratory secretions. In these cases, semi-selective media (e.g. CIN agar) should be used. Best growth occurs at 28°C. Cultures of *Y. pestis* must be handled under BSL3 conditions!
-Molecular	PCR usually targets the genes of plasminogen activator (pla) and the F1 capsule antigen (caf), located on two different plasmids (Riehm et al 2011). Targets are specific for *Y. pestis*. PCR can be applied to nucleic acid extracted from cultivated bacteria and various specimens, like EDTA-blood (septicemia, pneumonic plague), sputum/ respiratory secretions (pneumonic plague), aspirates or puncture (bubonic plague) or from biopsy of various inner organs (postmortem).
-AST	Antimicrobial susceptibility testing should be performed according to CLSI M45 3rd ed. at least for the following substances: gentamicin, streptomycin, ciprofloxacin, levofloxacin, doxycycline, trimethoprim/sulfamethoxazole, chloramphenicol; Recommendations from EUCAST (European Committee on Antimicrobial Susceptibility Testing) are currently not available.
- Antigen and Antibody Detection (Serology)	Y.-pestis-specific antigen detection can be done using rapid diagnostic tests targeting the F1-capsule antigen of *Y. pestis*. For limitations, see also note above. If culture and PCR show negative results and plague is still suspected, serologic testing is possible to confirm the diagnosis. One serum specimen should be taken as early as possible, followed by a convalescent sample taken 4-6 weeks or more after disease onset. Commercial tests are currently not available, in-house tests are based on detection of antibodies against F1-capsule antigen and are reserved to reference laboratories.
Differential diagnoses	There are a number of differential diagnoses to be considered in suspected cases of plague, the causative pathogens of which should be included in the laboratory diagnostic procedures, if appropriate. Bubonic plague: streptococcal and staphylococcal lymphadenitis, tularemia, infectious mononucleosis, cat-scratch disease, tuberculous adenitis, toxoplasmosis. Pneumonic plague: leptospirosis, anthrax, melioidosis, glanders, tularemia and other severe bacterial lung infections. Septicemic plague: Malaria, meningococcal infections, sepsis or meningitis due to other severe bacterial infection, Rocky Mountain spotted fever, purpura anaphylactoides. Flue-like symptoms, like high fever, body pain and headache might mimic influenza.
Biosafety Biosecurity	National and international regulations are to be respected. Patients particularly presenting with pneumonic symptoms should be isolated for at least 48 hours and specifically managed under conditions preventing respiratory droplets transmission. Specimen collection from plague patients should be carried out using protective equipment (protective gown, glasses, gloves and FFP3 filter masks). Clinical specimens coming from suspected plague cases could be handled in BSL 2

	environments. Appropriated biosafety and biosecurity measurements should be in place including *inter alia*: gloves, lab coat, respiratory masks, class II biosafety cabinet. Respective waste management plans, including autoclaves, should be in place. Confirmed clinical specimens, cultures or enrichments should be handled in BSL 3 environments or appropriate level of safety. Appropriated biosafety and biosecurity measurements should be in place including *inter alia*: FFP3 respiratory masks, gloves, lab coat, hairnet, and class III biosafety cabinet. Respective waste management plans, including autoclaves, should be in place. WHO: *Laboratory biosafety manual*. 3rd ed. 2004, Geneva: World Health Organization. 178. http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/ CDC: Biosafety in Microbiological and Biomedical Laboratories. https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

References:

Clinical Laboratory Standards Institute (CLSI). Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, 3rd Edition, M45.

Riehm JM, Rahalison L, Scholz HC, Thoma B, Pfeffer M, Razanakoto LM, Al Dahouk S, Neubauer H, Tomaso H. Detection of Yersinia pestis using real-time PCR in patients with suspected bubonic plague. Mol Cell Probes. 2011 Feb;25(1):8-12.

Manual of Clinical Microbiology, 10th Edition. Editors: James Versalovic, Karen C. Carroll, Guido Funke, James H. Jorgensen, Marie L. Landry, David W. Warnock.

Zoonoses. Infectious Diseases Transmissible from Animals to Humans. 3rd Edition. Editors: Hartmut Krauss, Albert Weber, Max Appel, Burkhard Enders, Henry D. Isenberg, Hans G. Schiefer, Werner Slenczka, Alexander von Graevenitz, Horst Zahner.

Additional Links

WHO: Plague, Emergencies preparedness, response
http://www.who.int/csr/disease/plague/en/

WHO: Plague- Madagascar (02.10.17) http://www.who.int/csr/don/02-october-2017-plague-madagascar/en/

CDC: Resource for clinicians, plague: https://www.cdc.gov/plague/healthcare/clinicians.html

Bundesamt für Bevölkerungsschutz und Katastrophenhilfe: Biologische Gefahren II (in German). https://www.bbk.bund.de/SharedDocs/Downloads/BBK/DE/Publikationen/PublikationenForschung/BioGefahren-II-MedVers.pdf?__blob=publicationFile

European Centre for Disease Prevention and Control: Risk assessments https://ecdc.europa.eu/en/threats-and-outbreaks/reports-and-data/risk-assessments

European Centre for Disease Prevention and Control: Outbreak of plague in Madagascar, 2017 (09.10.17) https://ecdc.europa.eu/sites/portal/files/documents/Plague-Madagascar-Oct-2017.pdf

European Centre for Disease Prevention and Control: Outbreak of pneumonic plague in Madagascar: recent introduction in the Seychelles (13 October 2017) https://ecdc.europa.eu/sites/portal/files/documents/plague-madagascar-seychelles-rapid-risk-assessment-october-2017.pdf