Plague diagnostic recommendations

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Joint Action on Efficient response to highly dangerous and emerging pathogens at EU level
EMERGE

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Brief instructions for the diagnostic of specimens coming 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 pneumonic plague patients. Therefore, rapid diagnosis and treatment are of utmost importance 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).

The following information must be provided by the sender of specimens on the sample submission form in order to allow the laboratory appropriate sample procession:

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 the drugs applied, if applicable

8. Travel history (please provide dates when patient entered and left the endemic area of plague, if applicable)

Subject	Recommendations
Clinical manifestation	Plague can present in different clinical forms depending on the route of infection. Transmission to humans either results from flea bites resulting in bubonic plague or by direct exposure to infected tissues or respiratory droplets with pneumonic plague as outcome. Clinical forms of plague: • Plague pneumonia (pneumonic plague results from inhalation of infectious aerosols (primary plague pneumonia), which is also the case in human-to-human transmission. This is the dominant clinical form in the plague outbreak in Madagascar 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 ongoing with the expectoration of bloody or watery sputum. Pneumonic plague finally ends up 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). Bubos usually develop in the proximal lymph nodes with regard 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 remains untreated and results from hematogenous dissemination. Patients present with high fever, chills, extreme weakness, sometimes also gastrointestinal symptoms, which are followed in the later stages of the disease by disseminated intravascular coagulation and multi-organ failure. • In rare cases pharyngitis 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 presentation: • Pneumonic plague: Blood cultures should be taken and are usually culture-positive at this time; 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. Selective media are therefore required.

	• Bubonic plague: Lymph node aspirates should be taken from swollen lymph nodes. 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 plague infection.
Shipment of clinical specimens	Clinical specimens for primary diagnosis (from patients who have not yet been diagnosed as having plague, if not belonging to the group described below) can be labelled as Biological Substance Category B (UN 3373) and shipped according to the packaging guidelines for UN3373 and packing instruction P650 (road transport). http://www.un3373.com/info/regulations/ Clinical specimens from patients with a laboratory-confirmed diagnosis of plague or from patients who are very likely to suffer from plague without having been laboratory confirmed yet, e.g. close contacts of laboratory-confirmed cases revealing typical symptoms of plague as well as bacterial cultures must be labeled as Infectious Substance Affecting Humans (UN 2814) and shipped according to the packaging guidelines for UN2814 and packing instruction P620 (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 second means of methods, e.g. positive amplification results should be confirmed by culture (appropriate biosafety conditions required!), immunological test, or by amplifying a second genetic region, distinctive form the one that yielded already positive/negative test results. In case of early stages of diseases and negative results, 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 (RDT) 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, tests are not licensed for human diagnostics yet. Usually, the results of these assays are recommended to 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, or lymph node specimens. Organisms may be seen in blood smears presenting with a safety-pin-like appearance. Note: Blood smears taken from patients early in the course of illness are usually negative in microscopy, however can be positive by culture or PCR. Affected buboes contain numerous organisms and can be evaluated microscopically. Fluorescence in situ hybridization (FISH) can also be used. Visualization of bipolar-staining, ovoid, Gram-negative organisms with a “safety pin” appearance permits a rapid presumptive diagnosis of plague. Staining of tissue specimens and cultured bacteria (solid, liquid medium)

	Recommended 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) - gramnegative - single cells, pairs or short chains, safety pin structure
-Culture	*Y. pestis* can be cultivated from various specimens, depending on the clinical presentation (e.g. bubo aspirates, blood, sputum). 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.
-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 punctions (bubonic plague) or from biopsies of various inner organs (postmortem).
-AST	Antimicrobial susceptibility testing should be performed according to CLSI M45 3rd ed. for at least the following substances: gentamicin, streptomycin, ciprofloxacin, levofloxacin, doxycylin, trimethoprim/sulfamethoxazol, chloramphenicol;
- Antigen and Antibody Detection (Serology)	Y.-pestis-specific antigen detection can be done using rapid diagnostic tests (RDT) targeting the F1-capsule antigen of Y. pestis. See also note above. If cultures and PCR yield 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 not available, in-house tests are based on detection of antibodies against F1-capsule antigen and are reserved to reference laboratories.
Differential diagnostic	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. <u>Bubonic plague</u>: streptococcal and staphylococcal lymphadenitis, tularemia, infectious mononucleosis, cat-scratch disease, tuberculous adenitis, toxoplasmosis. <u>Pneumonic plague</u>: leptospirosis, anthrax, melioidosis, glanders, tularemia and other severe bacterial lung infections. <u>Septicemic plague</u>: 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 specimen collection 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.

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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

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