



Public Health
England

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Plague: interim guidance for clinical laboratories in England for diagnosing potential plague cases

November 2017

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1. Scope, diagnostic process and risk assessment

Scope

This guidance is intended for use by clinical diagnostic laboratories in the context of suspected cases associated with a plague outbreak occurring outside the UK.

Diagnostic process

- appropriate clinical specimens (refer to clinical guidelines in the [plague guidance collection](#)) should be submitted to [Rare and Imported Pathogens Laboratory \(RIPL\)](#), PHE Porton Down, for detection of *Y. pestis* by PCR, as long as this has been agreed by prior consultation with the [Imported Fever Service \(IFS\)](#)
- concurrently, clinical laboratories should aim to identify *Yersinia pestis* in appropriate specimens (see clinical guidelines in the [plague guidance collection](#)), as long as laboratory work can be performed safely at Biosafety Level 3 (BSL3)
- microscopy and culture are the primary diagnostic methods
- local laboratories should also attempt antibiotic sensitivity testing if this can be done at BSL3
- all isolates suggestive of *Y. pestis* should be referred urgently to RIPL for PCR detection of *Y. pestis*
- RIPL will forward PCR-positive isolates to the [Gastrointestinal Bacteria Reference Unit \(GBRU\)](#), PHE Colindale, for confirmation of identification and antibiotic susceptibility testing
- *Yersinia pestis* is a [notifiable organism](#) under the Health Protection (Notification) Regulations 2010

Local risk assessment

Yersinia pestis is an ACDP Hazard Group 3 pathogen and therefore should be covered by existing risk assessments for handling such organisms in diagnostic laboratories. All laboratory procedures should be performed by experienced scientists in a BSL3 facility using a Class 1 protective cabinet.

Receipt of samples

Samples should have been labelled as 'high risk' or the possibility of plague should have been highlighted by the submitting staff, and samples should be handled according to local relevant protocols.

Protection of clinical laboratory staff

All laboratory procedures must be performed in a BSL3 facility using a Class 1 biological safety cabinet. Under these circumstances antibiotic prophylaxis is not recommended unless there is an inoculation injury or a spillage causing aerosols (i.e. blood, sputum, liquid culture or bacterial suspension).

Prophylaxis may be required for staff who handled samples in the open laboratory from patients subsequently shown to have plague. The **PHE Health Protection Team** (HPT) (local to the individual's usual address) should be informed if post-exposure prophylaxis is commenced, because appropriate follow-up will need to be arranged.

Any member of laboratory staff working with specimens or cultures from plague patients, who develops a febrile/respiratory illness within 8 days of handling specimens or cultures, should seek urgent medical attention and the **local HPT** should be informed.

2. Isolation and identification

Laboratory images relevant to plague are available via an [archived HPA webpage](#).

PCR testing of clinical specimens (other than isolates)

Clinicians/laboratories are advised to discuss urgently any suspected cases with the Imported Fever Service, which will advise on the need for direct testing of clinical specimens by PCR at RIPL. RIPL will only accept clinical specimens from cases that have been discussed with the [Imported Fever Service](#) in advance. Direct testing of clinical specimens by PCR may provide more rapid detection of *Y. pestis*, but it does not replace the requirement for concurrent local microscopy, culture and sensitivity testing.

Microscopy

Direct smears from clinical samples, as well as from cultures, may be stained with Gram, Giemsa or Wayson's (if available) stains to demonstrate bipolar staining coccobacilli. Additionally, clinicians are advised to obtain blood in EDTA tubes for direct microscopy. Visualisation of bipolar-staining, ovoid, Gram-negative organisms with a “safety pin” appearance permits a rapid presumptive diagnosis of plague, if present.

Culture

Y. pestis is a small Gram-negative coccobacillus, which commonly exhibits bipolar staining and pleomorphism, particularly in clinical specimens. The diagnosis of plague must be confirmed by culture. Specimens should be inoculated onto blood and MacConkey agars, and CIN agar if available, and incubated aerobically at 28°C-30°C for optimal growth. Aspirates and CSF should also be placed into an enrichment broth (eg brain heart infusion, trypticase soy or nutrient broth) with subculture after 24-48 hours.

One or more sets of blood cultures should be taken. If plague is considered likely, an additional broth bottle should be taken and incubated at 22-28°C as *Yersinia pestis* will grow more rapidly at this temperature. Note that subcultures from blood culture bottles may take 48 hours to grow.

On blood agar the organism forms tiny, translucent colonies after 24 hours. After 48 hrs incubation colonies range between 1-2mm in diameter and grey-white to slightly yellow in colour; there is no haemolysis. On MacConkey agar it appears as pinpoint non-lactose fermenting colonies, which disappear after 2-3 days, presumably due to autolysis. *Y. pestis* is catalase positive and oxidase negative.

Biochemical identification can be obtained using API 20E or other commercial test strips, although these may not be reliable, so suspected isolates should always be referred to RIPL for further testing - in the first instance, for PCR testing.

Automated culture identification systems have been known to misidentify *Y. pestis*. Therefore, in a patient with clinical features consistent with plague or microbiological findings of an organism with bipolar staining, the possibility of *Y. pestis* should not be discounted if these other organisms are detected by such systems. Refer isolates urgently to RIPL for *Y. pestis* detection by PCR.

Antibiotic sensitivity testing

Antimicrobial susceptibility tests should be set up as early as possible. Susceptibility testing for the following antibiotics is recommended: gentamicin; doxycycline /tetracycline; ciprofloxacin; chloramphenicol; streptomycin. Use standard laboratory methods at BSL3.

GBRU can provide advice on breakpoints and cut-offs, if required. Isolates submitted to RIPL and confirmed positive by PCR for *Y. pestis* will be forwarded to GBRU for further testing.

Susceptibility to beta-lactams in vitro does not predict clinical efficacy.

Confirmation

All suspect isolates must be sent to RIPL, which must be notified prior to the dispatch of samples. RIPL will perform a detection PCR assay for *Y. pestis* and will forward PCR-positive isolates to GBRU for further testing.

Serology

Confirmation of infection using serological assays is not currently offered by PHE. The principal method of laboratory diagnosis is isolation of *Y. pestis*.

Packaging and transport of samples for specialist and reference testing

All isolates from suspected plague cases and all confirmed isolates should be packaged, labelled and transported in accordance with Category A transportation regulations. UN 2814 packaging must be used for sample transport.

Clinical specimens (not isolates) from suspected or confirmed cases may be packaged, labelled and transported in accordance with Category B transportation regulations. UN 3373 packaging must be used for sample transport.

PHE follows the [guidance on regulations for the transport of infectious substances 2017-2018](#), published by the World Health Organization.

Handling and processing of routine clinical specimens from suspected and confirmed cases

Other essential investigations should not be postponed pending results of *Y. pestis* identification, as long as investigations can be performed safely.

Routine laboratory tests, such as commonly requested haematology and biochemistry tests, should be carried out where possible in auto-analysers using standard practices and procedures at biosafety level 2 (BSL2).

Any procedure that involves potentially infectious material and is associated with a risk of generating aerosols, droplets or splashes, should always be performed within a biological safety cabinet at biosafety level 3 (BSL3).

Work that should be conducted at BSL3

It is recommended that the following work is conducted in a biological safety cabinet at BSL3:

- division, aliquoting, or diluting of respiratory tract specimens and tissue specimens that have not been inactivated
- inoculation of bacterial or fungal culture media
- reading culture plates
- direct microscopy of blood for *Y. pestis*
- preparation of tissue or respiratory tract specimens for molecular testing (eg respiratory virus PCR) prior to sample inactivation
- rapid antigen tests of respiratory tract specimens
- processing of any non-inactivated respiratory tract or tissue specimen that might result in the generation of aerosols
- preparation and fixing (chemical or heat) of smears for microscopy

Work that may be conducted at BSL2

The following work may be conducted at BSL2 following standard laboratory precautions, as long as it is consistent with the terms of the local risk assessment:

- diagnostic assays using whole blood, serum and plasma, including routine biochemistry and haematology, unless there is a risk of generating aerosols
- assays using bacteria-inactivated specimens, including molecular testing of inactivated specimens

- staining and microscopy of heat-fixed or chemically-fixed smears
- rapid diagnostic tests for malaria parasites, as long as they are performed within a biological safety cabinet at BSL2

3. Contact details for PHE Specialist and Reference Laboratories

Rare and imported pathogens laboratory (RIPL)

Public Health England
Manor Farm Road
Porton Down
Wiltshire
SP4 0JG

Email (09:00 to 17:00 hours, weekdays): ripl@phe.gov.uk

Telephone (09:00 to 17:00 hours, weekdays): 01980 612 348

Telephone if out-of-hours and urgent: 01980 612100

DX address: DX 6930400, Salisbury 92 SP

Webpage: <https://www.gov.uk/government/collections/rare-and-imported-pathogens-laboratory-ripl>

Gastrointestinal Bacteria Reference Unit

Public Health England
61 Colindale Avenue
London
NW9 5EQ

Email (09:00 to 17:00 hours, weekdays): gbru@phe.gov.uk

Telephone (09:00 to 17:00 hours, weekdays): 020 8327 7887

If out-of-hours and urgent, contact the Colindale Duty Doctor on 0208 200 4400.

DX address: PHE Colindale Bacteriology, DX 6530002

Webpage: <https://www.gov.uk/guidance/gbru-reference-and-diagnostic-services>