Health Protection Scotland

NHS National Services Scotland

LABORATORY INVESTIGATION OF POSSIBLE ANTHRAX IN DRUG USERS

Case presentations

- Any injecting drug user who presents with severe soft tissue infection, including necrotising fasciitis or cellulitis/abscess particularly if associated with tissue oedema (often marked)
- Any drug user who presents with signs of severe sepsis **even without** evidence of soft tissue infection
- Any drug user who presents with **meningitis** (particularly haemorrhagic meningitis). Also be suspicious if IDU/DUs present/ have CT evidence suggestive of subarachnoid haemorrhage/intracranial bleed)
- Any drug user who presents with signs and symptoms of inhalational anthrax. The signs and symptoms of inhalational anthrax include:
    - Flu-like illness, progressing to severe respiratory difficulties and shock
    - Chest x-ray signs (pleural effusions, mediastinal widening, paratracheal fullness, hilar fullness, parenchymal infiltrates)
    - **Progressively enlarging haemorrhagic pleural effusions are a consistent feature**
    - The disease is often biphasic, with a prodrome of general malaise for 2-3 days, followed by a day or two of apparent remission before the full blown picture develops
    - Respiratory symptoms may also be accompanied by signs and symptoms suggesting meningitis or intracranial bleeding in the rapidly advancing stages of the disease process due to haematogenous spread.

- It is also possible that a drug user might present with classical cutaneous anthrax

Collection of samples

Despite its reputation, anthrax is not contagious, and humans are not highly susceptible to the disease. While theoretically it only takes one spore to initiate a cutaneous infection, *B. anthracis* is not 'invasive' and usually requires a portal of entry through the skin, although in many cases this may be so small as to be unnoticed. **Standard Infection Control Precautions apply.** That is wearing personal protective equipment (PPE) sufficient to prevent exposure to blood or body fluids (e.g gloves, plastic apron, and a visor if risk of splash). This must be adopted for all clinical procedures including taking samples. Hands should be washed in soap and water after removal of PPE.

Precautions for sampling

The samples outlined below should be taken to confirm the diagnosis. These must be taken using Standard Infection Control Precautions (as above) and with the utmost care to avoid inoculation injuries. The procedures for transporting samples to the laboratory are outlined below. The receiving laboratory should be telephoned to expect arrival. Specimen (s) must be labelled as High Risk and ?Anthrax.

Samples to be taken from acutely ill patients

- Blood for culture (taken before antibiotic treatment).
- CSF
- Tissue samples (NOT simply a swab whenever possible)

- Respiratory samples including lung aspirates, pleural fluids, or sputum samples
- Whole blood EDTA
- Serum

Post-mortem specimens
- Blood from a vein (the blood is non-clotting at death in anthrax).
- Respiratory tract samples.
- Haemorrhagic exudate from orifices.
- Other body fluids if appropriate.
- Lung, spleen or lymph node tissue

LABORATORY PROCEDURES IN MICROBIOLOGY

Risk assessment
B. anthracis is a Hazard Group 3 pathogen, and should thus be covered by existing risk assessments for handling such organisms in diagnostic microbiological laboratories.

Receipt of samples in microbiology
Samples should have been labelled as 'High risk'? Anthrax by the submitting staff, and should be handled according to local protocols for such samples. All laboratory procedures should be performed in a containment level 3 facility using a Class 1 protective safety cabinet.

Isolation and identification
Two smears should be made on microscope slides and fixed by immersion in absolute ethanol for 1 minute. Slide 1 should be stained with Giemsa or Gram's stain, and the typical capsulated short chains of "box-car" bacilli looked for under oil immersion. Their presence is highly suggestive of anthrax. If numerous bacilli in short chains are visible, dispatch the second slide to the reference laboratory for confirmation. The specimens should also be cultured on to blood agar for incubation at 37°C in air/CO_2, plus your routine culture media as appropriate for the sample.

Antimicrobial susceptibility tests must be set up as soon as possible.

Culture
B. anthracis is a non-motile, Gram-positive, aerobic bacillus 1.2 to 10μm in length, capable of forming central and terminal spores.

Gram stain of Bacillus anthracis

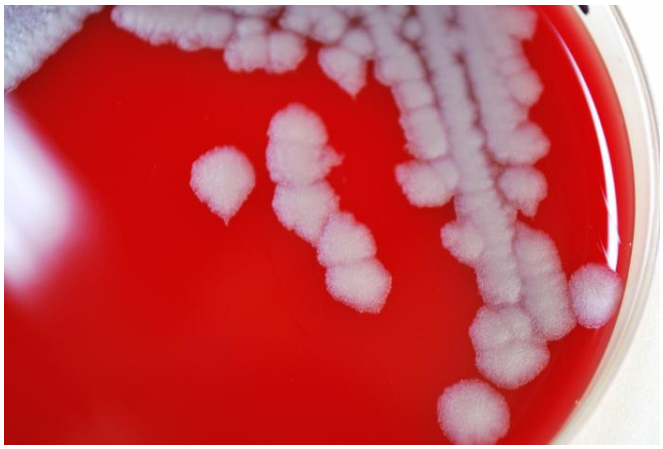
After 24h incubation, colonies are typically:
- white and non-haemolytic
- medusa-head (comma shaped) or oval
- irregular edge
- ground-glass appearance

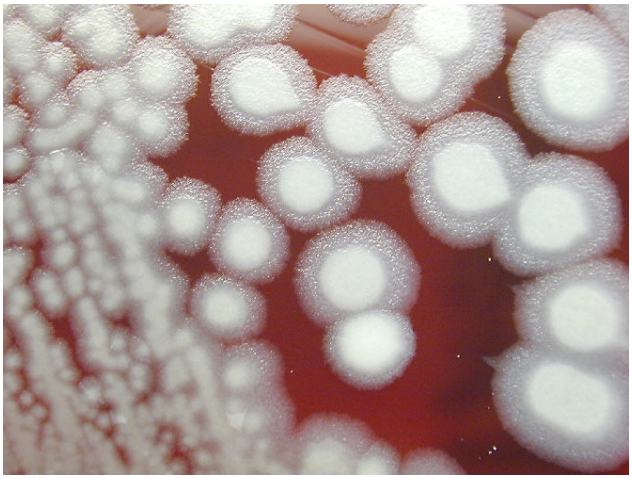
- slightly granular but not dry
- about 2mm diameter
- characteristically tacky on teasing with a loop
- catalase positive (DO NOT UNDERTAKE THIS TEST OUTSIDE A SAFETY CABINET)

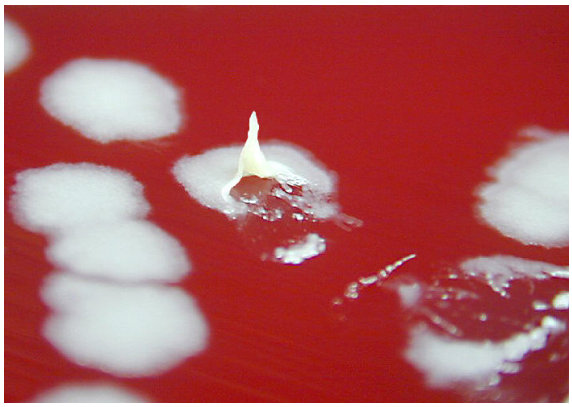
Using a Class 1 protective cabinet within a containment level 3 facility, suspicious colonies can be subcultured to an agar slope in a bijoux bottle, and then be sent to the reference laboratory for confirmation.

See below for photographs of colony appearance.

***Bacillus anthracis* on blood agar after 24hours incubation**







Further pictures of *Bacillus anthracis* are available HPA website at:
http://www.hpa.org.uk/HPA/Topics/InfectiousDiseases/InfectionsAZ/1204619483853/

Antibiotic susceptibility
Organisms should be tested for sensitivity to antibiotics including ciprofloxacin, penicillin, clindamycin, doxycycline and gentamicin.

Confirmation
Clinical microbiology laboratories should take care not to regard all isolates of *Bacillus* species as contaminants, especially if isolated from sterile sites (blood, cerebrospinal fluid) and/or multiple cultures are positive from the same patient.

All sterile site *Bacillus* isolates should be further evaluated, and if non-motile or non-haemolytic (particularly if they form short chains), and/or if the clinical syndrome is suggestive of anthrax, the isolates should be immediately referred to the reference laboratory.

Waste disposal
In the laboratory, hypochlorite (10,000ppm) disinfection should be used with a minimum of 10mins contact time. All other waste containers should be autoclaved. Samples when ready for disposal should also be autoclaved and then incinerated.

REFERENCE LABORATORY
All positive isolates and cultures should be sent to the reference laboratory for confirmation. In addition, samples may be sent there directly if local laboratories lack the facilities for dealing with them. All samples and cultures must be packaged appropriately, taking care to observe the procedures outlined below. The sender's name and address should be clearly marked. The reference laboratory should be telephoned prior to sending to expect the sample. Samples should be forwarded urgently to:

Dr Tim Brooks
HPA Centre for Emergency Preparedness and Response
Special Pathogens Reference Unit
Porton Down
Salisbury SP4 0JG
Tel: (+44) 01980 612100 (24hours)

Further information on SPRU and referral of specimens and samples:
http://www.hpa.org.uk/servlet/Satellite?c=Page&cid=1197637055828&pagename=HPAweb%2FPage%2FHPAwebAutoListName

Particular medical problems can be discussed with Dr Tim Brooks (07766 775149; tim.brooks@hpa.org.uk) or Dr Bob Spencer (07885 434000 or Blackberry bob.spencer@hpa.org.uk)

Transportation of samples with suspicion of *B. anthracis*
Strict procedures apply for transport of samples to the laboratory. Biological agents, or materials that contain or may contain them, are allocated to UN Division 6.2 – infectious substances. Infectious substances are divided into Category A or Category B.

- **Cultures of *B. anthracis*** are Category A infectious substances capable of causing disease in humans or animals and are therefore assigned to UN2814 and must be packaged in accordance with UN Packaging Instructions PI620 (road/rail) /PI602 (air). Category A transfers should be individually requested through an approved courier. The service will be a next day, tracked door-to-door delivery, which must be signed for at collection and receipt.

- **Clinical samples** are generally classified as Category B and are assigned to UN3373 ("Biological Substances, Category B) and should be packaged in accordance with UN PI650. Clinical samples may be posted.

Guidance on transport arrangements is available here
http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1202487028723

PROTECTION OF LABORATORY STAFF

All laboratory procedures must be performed in a Containment Level 3 facility using a Class 1 biological safety cabinet. Under these circumstances there is no indication for antibiotic prophylaxis for laboratory staff unless there is an inoculation injury or a spillage releasing aerosols containing spores. Anthrax vaccine is only indicated for laboratory staff routinely working with the organism.

Any member of laboratory staff, working with specimens or cultures of anthrax, who develops a febrile/ respiratory illness, should seek urgent medical attention.

SPECIFIC GUIDELINES FOR BLOOD SCIENCE LABORATORIES

Note that blood/body fluid samples from anthrax patients in clinical chemistry and haematology etc can be handled according to the standard procedures that should already be in place to prevent the acquisition of blood borne viruses. However the utmost care should be taken to prevent inoculation injury and any splashes/spillages must be promptly and effectively dealt with i.e. 10,000ppm hypochlorite for 10mins (see HPS Infection Control Guidelines).

Any glass slides/sharps generated should either be incinerated or autoclaved (prior to disposal).

Blood or body fluid samples sent to blood sciences from confirmed cases **must be autoclaved prior to disposal/or incinerated.** It is suggested that locally the microbiology laboratory should liaise with its colleagues in blood sciences re - possible, probable and confirmed cases. Samples from possible/probable cases should be held by each lab until known to be either confirmed or not and then they should be disposed of appropriately. If this approach is not possible, then all samples from possible, probable or confirmed should be incinerated or autoclaved prior to disposal.