Examination of pus, ulcer material and skin specimens

PUS

Possible pathogens*

*It is impossible to list all the pathogens that may be found in pus. Those listed are the more commonly isolated pathogenes from wounds, abscesses, burns, and draining sinuses.

_ BACTERIA

Gram positive	***Gram negative***
Staphylococcus aureus	*Pseudonomas aeruginosa*
Streptococcus pyogenes	*Proteus* species
Enterococcus species	*Escherichia coli*
Anaerobic streptococci	*Bacteriodes* species
Other streptococci	*Klebsiella* species
Clostridium perfringens and other clostridia	*Pasteurella* species
Actinomycetes *Actinomyces israeli*	
Also *Mycobacterium tuberculosis*	

Commensals

Any commensal organisms found in pus are usually those that have contaminated the specimen from skin, clothing, soil, or from the air if an open wound .

ULCER MATERIAL AND SKIN SPECIMENS

Possible pathogens

_ BACTERIA

Gram positive	***Gram negative***
Staphylococcus aureus	*Escherichia coli*
Streptococcus pyogenes	*Proteus*
Enterococcus species	*Pseudomonas aeruginosa*
Anaerobic streptococci	*Yersinia pestis*
Bacillus anthracis	Vincent's organisms

Commensals

Commensal organisms that may be found on the skin include:

Gram positive	***Gram negative***
Staphylococci	*Escherichia coli*
Micrococci	and other coliforms
Anaerobic cocci	
Viridans streptococci	
Enterococci	
Diphtheroids	
Propionibacterium acnes	

Notes on pathogens

- *S. aureus* is the commonest pathogen isolated from subcutaneous abscesses and skin wounds. It also causes impetigo (small pustules that form yellow crusty sores, usually around the mouth). Penicillin and methicillin resistant strains of *S. aureus* are common causes of hospital-acquired wound infections.
- *P. aeruginosa* is associated with infected burns and hospital-acquired infections.
- E. *coli* , *Proteus* species , *P. aeruginosa*, and *Bacteroides* species are the pathogens most frequently isolated from abdominal abscesses and wounds.
- *C. perfringens* is found mainly in deep wounds where anaerobic conditions exist.

COLLECTION AND TRANSPORT OF PUS, ULCER MATERIAL, SKIN SPECIMENS

Specimens should be collected by a medical officer or an experienced nurse. Pus from an abscess is best collected at the time the abscess is incised and drained, or after it has ruptured naturally. When collecting pus from abscesses, wounds, or other sites, special care should be taken to avoid contaminating the specimen with commensal organisms from the skin. As far as possible, a specimen from a wound should be collected before an antiseptic dressing is applied.

When mycetoma is suspected : Obtain a specimen from a draining sinus tract using a sterile hypodermic needle to lift up the crusty surface over the sinus opening. This method of specimen collection has the advantages that the pus obtained is usually free from secondary organisms and the draining granules can usually be seen clearly and removed for microscopical examination. Transfer the pus to a sterile container.
When the tissue is deeply ulcerated and necrotic (full of dead cells): Aspirate a sample of infected material from the side wall of the ulcer using a sterile needle and syringe. Transfer to a sterile container.

LABORATORY EXAMINATION OF PUS, ULCER AND SKIN SPECIMENS

1 Describe the appearance of the specimen

When from a patient with suspected mycetoma or actinomycosis, report the appearance of the specimen and whether it contains granules.

Detection of granules

White, yellow, brown, red, or black granules of varying size, shape , and consistency may be found in pus draining from sinuses in mycetoma ,in actinomycosis . The granules are colonies of organisms. To free the granules from the pus, shake a portion of the specimen (or dressing) in sterile distilled water. Wait for a few minutes (to allow the granules to settle), remove the supernatant fluid, and transfer a few of the granules to a slide.

2 Examine the specimen microscopically

Note: When a swab has been used to collect the pus, inoculate the culture media *first* before using the swab to make smears.

Gram smear

Examine the smear for bacteria among the pus cells using the 40_ and 100_ objectives. Look especially
for:

- Gram positive cocci that could be *S. aureus* or streptococci that could be

S. pyogenes or other *beta*-haemolytic streptococci, anaerobic streptococci, or enterococci .

- Gram negative rods that could be *Proteus* species, *E. coli* or other coliforms, *P. aeruginosa* or *Bacteroides* species.
- Gram positive large rods with square ends that could be *C. perfringens* or *B. anthracis* .

ADDITIONAL

Ziehl-Neelsen smear when tuberculosis or* M. ulcerans *disease is suspected

3 Culture the specimen *Blood agar ,MacConkey agar, cooked meat medium (or thioglycollate broth)*

– Inoculate the specimen:

- On blood agar to isolate *S. aureus* and streptococci. Add a bacitracin disc if streptococci are seen in the Gram smear.
- On MacConkey agar to isolate Gram negative rods.
- Into cooked meat medium or thioglycollate broth

Cooked meat medium: This is an enrichment medium for aerobes and anaerobes. The glucose in the medium helps to produce a rapid growth of anaerobes (at the bottom of the medium).

Incubate the inoculated blood agar plate at 35–37_C in a carbon dioxide atmosphere (candle jar) and the MacConkey agar plate aerobically.

Incubate the inoculated cooked meat medium at 35–37 _C for up to 72 hours.

Subculture at 24 h, and if indicated at 48 h and 72 h.

Day 2 and Onwards

4 Examine and report the cultures *Blood agar and MacConkey agar cultures*

Look especially for colonies that could be:

_ *Staphylococcus aureus* ,_ *Streptococcus pyogenes* _ *Pseudomonas aeruginosa*

_ *Proteus* species _ *Escherichia coli* _ *Enterococcus* species _ *Klebsiella* species

Summary of Microbiological Examination of Pus, Ulcer Material and Skin Specimens

Day 1

			ADDITIONAL INVESTIGATIONS
1	**Describe Specimen**		■ ***Look for granules:*** When mycetoma or actinomycosis is suspected
2	**Culture Specimen**	■ ***Blood agar*** Incubate aerobically ■ ***MacConkey agar*** Incubate aerobically ■ ***Cooked meat medium*** Subculture at 24 h, 48 h, and 72 h as indicated ■ ***Neomycin blood agar when anaerobic infection is suspected*** Incubate anaerobically up to 48 h	■ ***Culture for M. tuberculosis or M. ulcerans*** Requires facilities of a Tuberculosis Reference Laboratory
3	**Examine Microscopically**	■ ***Gram smear*** For pus cells and bacteria	■ ***Ziehl-Neelsen smear:*** When tuberculosis or *M. ulcerans* disease is suspected ■ ***KOH preparation:*** When a fungal or actinomycete infection is suspected ■ ***Giemsa or Wayson's smear:*** When bubonic plague is suspected ■ ***Polychrome methylene blue:*** When cutaneous anthrax is suspected ■ ***Dark-field microscopy:*** To detect treponemes when yaws or pinta is suspected

Day 2 and Onwards

4	**Examine and Report Cultures**	■ ***Blood agar and MacConkey agar cultures*** Look particularly for: *S. aureus* *S. pyogenes* *P. aeruginosa* *Proteus species* *E. coli* *Enterococcus* species *Klebsiella* species Anaerobes: *C. perfringens* *Bacteroides fragilis* group *Peptostreptococcus* species	■ ***Antimicrobial susceptibility tests*** As indicated