

COLLECTION, TRANSPORT, AND EXAMINATION OF ASCITIC FLUID

Possible pathogens

Gram positive

Enterococci
Streptococcus pneumoniae
Staphylococcus aureus
Streptococcus pyogenes
Streptococcus agalactiae
Viridans streptococci
Clostridium perfringens

Acid fast bacilli

Mycobacterium tuberculosis

Gram negative

Escherichia coli
Klebsiella strains
Other enterobacteria
Pseudomonas aeruginosa
Bacteroides species

Fungi

Candida

Collection and Transport of Effusions

Sample collection in a hospital

1. After aspiration, aseptically
 - Dispense 5 ml fluid into a bottle of sterile thioglycollate broth and mix.
 - Dispense 3 ml fluid into a dry, sterile, screw- cap tube (Use this to perform cell count and protein estimation).
2. Label each bottle with the data (patient's name, sample type, and medical world name).

3. Your medical secretary then sends the samples with a request form to the microbiology infection control collecting room (patient's {name, age, names of used antibiotics, diagnosis, and date of admission}, sample type, and medical ward name).

4. Infection control technician will take the samples to the microbiology laboratory within a few hours. The inoculated thioglycollate broth should be kept in a warm environment, but not over 37 °C or in direct sunlight.

Sample Processing in a microbiology laboratory

Day I

I. Describe the Appearance of the Specimen

Report:

- Color of the effusion
- Whether it is clear, cloudy, or purulent (like pus)
- Whether it contains blood

	Transudate	Exudate
Appearance	pale yellow, clear	purulent or cloudy or a blood-stained
Clotting	unclotted	Often clot
Cells	Few cells Less than 100/ mm ³	Purulent: many cells, mostly neutrophils. Non purulent: few or many cells, mostly lymphocytes More than 1000/ mm ³
Protein	Less than 2.5 g /dl	More than 3 g /dl
Culture	sterile	positive

Note:

- *Purulent or Blood-stained effusion:* examine and preliminary report will be send to the medical world after direct Gram stained smear as soon as possible. Proceed to examine the specimen as for *pus*.

II. Perform a Cell Count

Count the number of white cells in the effusion and report whether the cells are mainly polymorphonuclear neutrophils (pus cells) or lymphocytes.

A transudate contains Less than 100/ mm³cells, whereas an exudate usually contains more than1000/ mm³ cells.

If Cells count between 100 and 1000 / mm³ access other fluid parameters.

III. Estimate the Protein

A transudate usually contains less than 2.5 g/dl o protein whereas an exudate contains more than 3g/dl.

IV. Examine the Specimen Microscopically

Routine:

- **Gram smear**

Make a thin evenly spread smear of a *purulent effusion* or sediment from the centrifuged *non purulent sample*, when dry, fix the smear with methanol for 2 minutes and stain by Gram technique.

Examine the smear for pus cells and bacteria using 40x and 100x objectives.

- **Ziehl-Neelsen smear**

Make a smear on a slide using several drops of sediment from the centrifuged fluid. When dry, fix with methanol for 2-3 minutes. Stain by the Ziehl-Neelsen method I.

Examine the smear first with the 40 x objective to see the distribution of material and then with the 100x objective to detect the acid fast bacilli.

The AFB: usually few and therefore a careful search of the smear is required.

V. Culture the Specimen

Culture the effusion if it Exudate.

Centrifuge the sample in a sterile tube a high speed for about 20 minutes to sediment the bacteria. Remove the supernatant fluid (do not discard) and resuspend the sediment.

Routine:

Blood agar (aerobic and anaerobic), Chocolate agar and MacConkey agar.

- Incubate the chocolate agar plate in a carbon dioxide enriched atmosphere at 35-37 °C for up to 48hours, checking for growth after overnight incubation.
- Incubate the blood agar plate anaerobically at 35-37 °C for up to 72 hours, examining for growth after overnight incubation.
- Incubate the MacConkey and blood agar plates aerobically at 35-37 °C overnight.

Additional:

Lowenstein Jensen medium if tuberculosis is suspected.

Day 2 and Onwards

Examine and Report the Cultures

Routine:

Chocolate agar, blood agar, and MacConkey agar cultures

Additional:

Lowenstein Jensen culture for Mycobacterium tuberculosis

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