Lab.N0 - 3

Stool collection procedure

A clean technique should be used to collect the stool sample to avoid contamination, which may result in inappropriate treatment. Some patients may be able to collect their own specimen, but it is important to explain the steps clearly, emphasise the need for good hand hygiene and explain how to avoid contaminating the specimen.

The procedure:

1. Discuss the procedure with the patient, explaining why the sample is being taken and when the results are expected.
2. Explain to the patient why the sample is taken and when the results will be available.
3. Ensure privacy and dignity as patients may find the procedure embarrassing.
4. Wash hands with soap and water (may be risk of cross infection).
5. Ask the patient to pass urine before taking the stool sample in order to avoid urine mixing with faeces and contaminating the sample.
6. Ask the patient to defecate into the sterile container.
7. If the patient is incontinent, a sample can be taken from the bed linen but contamination with urine should be avoided.
8. If segments of tapeworm are seen, send these to the laboratory. Tapeworm segments can vary from the size of rice grains to a ribbon
9. Use the integral spoon (or wood stick) in the sample pot to collect enough faeces to fill around a quarter of the specimen pot.
10. Secure. the top of the container – this will prevent leakage.
11. Wash. hands with soap and water to reduce the risk of cross infection.
12. Examine. the specimen Macroscopically.
13. Label. the sample and record some information such as recent antibiotic treatment, foreign travel, and suspected food poisoning – this will assist with accurate laboratory testing.
14. Send. the sample to the laboratory as soon as possible.
15. Document. the procedure in the patient's notes.

Stool specimen storage and transport

1) The patient should be asked to deliver the specimen to the clinic immediately after collection.
2) If it is not possible for the specimen to be delivered to the laboratory within 2 hours of its collection, a small amount of the stool specimen (together with mucus, blood and epithelial threads, if present) should be collected on two or three swabs and placed in a container with transport medium **(Cary–Blair**,**Stuartor Amies**, **PVA or 33 mmol/l of glycerol–phosphate**. **buffer)**
3) Pathogens may survive in such media for up to 1 week, even at room temperature, although refrigeration is preferable.

Cary-Blair semisolid transport medium

Lab- 4 Trichomonas

Trichomonas differs from other flagellates, as they exist only in trophozoite stage. Cystic stage is not seen.
*Genus *Trichomonas* has 3 species, which occur in humans.
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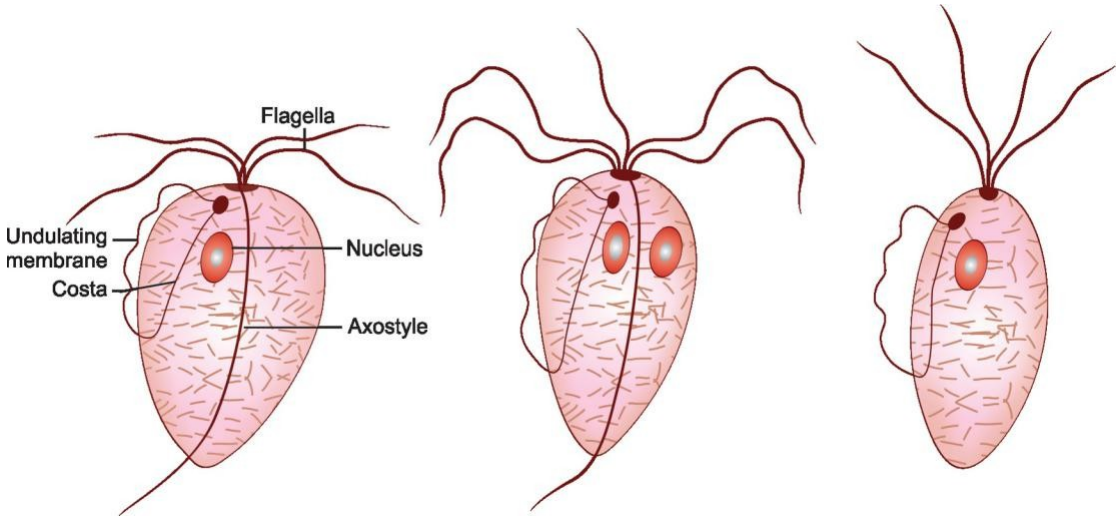

1- *T. vaginalis*
2- *T. hominis*
3- *T. tenax*

Trichomonas Vaginalis

History and Distribution

T. vaginalis was first observed by Donne (1836) in vaginal secretion.
*Prevalence of trichomoniasis varies from 5% patients at hospitals to 75% in sexual workers.

Morphology

It is **pear-shaped** or **ovoid** and measures 10–30 µm in length and 5–10 µm in breadth with a short undulating mem brane reaching upto the middle of the body (Fig. 4.4A).
*t has four anterior flagella and fifth running along the outer margin of the undulating membrane, which is supported at its base by a fl exible rod, **costa**.
* A prominent **axostyle** runs throughout the length of the body and projects posteriorly like a tail.
*The cytoplasm shows prominent siderophillic granules, which are most numerous alongside the axostyle and costa.
*It is motile with a rapid **jerky or twitching** type movement.

Habitat

In females, it lives in vagina and cevix and may also be found in Bartholin's glands, urethra, and urinary bladder. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate and preputial sac.

Life Cycle

Life cycle of *T. vaginalis* is completed in a single host either male or female.

Mode of transmission:

The trophozoite cannot survive outside and so infection has to be transmitted directly from personto- person. Sexual transmission is the usual mode of infection.

€*Trichomoniasis often coexists with other sexually transmitted diseases; like candidiasis, gonorrhea, syphillis, or human immunodeficiency virus (HIV).

€*Babies may get infected during birth.

€*Fomites such as towels have been implicated in transmission.

*Trophozoites divide by **binary fission**.

*As cysts are not formed, the **trophozite** itself is the **Infective form**.

*Incubation period is roughly 10 days.

Protozoan's transmitted by sexual contact

••*Trichomonas vaginalis* ••*Giardia lamblia*

••*Entamoeba histolytica*

Pathogenesis

T. vaginalis particularly infects squamous epithelium and not columnar epithelium. It secretes cystine proteases, lactic acid, and acetic acid, which disrupt the glycogen levels and lower the pH of the vaginal fluid.

*It is an **obligate parasite** and cannot live without close association with the vaginal, urethral, or prostatic tissues.

*Parasite causes petechial hemorrhage (**strawberry mucosa**), metaplastic changes, and desquamation of the vaginal epithelium.

*Intracellular edema and so called **chicken-like epithelium**, is the most characteristic feature of trichomoniasis.

Clinical Features

Infection is often asymptomatic, particularly in males, although some may develop urethritis, epididymitis, and prostatitis.

*In females, it may produce severe pruritic vaginitis with an offensive, yellowish green, often frothy discharge, dysuria, and dyspareunia. Cervical erosion is common. Endometritis and pyosalpingitis are infrequent complications.
*Rarely, neonatal pneumonia and conjunctivitis havebeen reported in infants born to infected mothers.
*The incubation period of trichomoniasis is 4 days to 4 weeks.

Laboratory Diagnosis

Microscopic examination

*Vaginal or urethral discharge is examined microscopically in saline wet mount preparation for characteristic jerky and twitching motility and shape. In males, trophozoites may be found in urine or prostatic secretions.
*Fixed smears may be stained with acridine orange, papanicolaou, and Giemsa stains.
*Direct fluroscent antibody (DFA) is another method of detection of parasite and is more sensitive than the wet mount.

Culture

Culture is recommended when direct microscopy isnegative and is considered as a '**gold standard**' as well as the most sensitive (95%) method for the diagnosis of *T. vaginalis* infection.
*It grows best at 35°–37°C under anaerobic conditions. The optimal pH for growth is 5.5–6.0.
*It can be grown in a variety of solid or liquid media, tissue culture, and eggs. Cysteine-peptone- liver-maltose (CPLM) medium and plastic envelope medium (PEM) are often used.

Serology

ELISA is used for demonstration of *T. vaginalis* antigen in vaginal smear using a monoclonal antibody for 65-KDA surface polypeptide of *T.vaginalis.*

Molecular method

DNA hybridization and PCR are also highly sensitive (97%) and specific (98%) tests for the diagnosis of trichomoniasis.

Treatment

Simultaneous treatment of both partners is recommended.
*Metronidazole 2 g orally as a single dose or 500 mg orally twice a day for 7 days is the drug of choice.