STD SERIES
3

LABORATORY MANUAL FOR THE DIAGNOSIS OF SEXUALLY TRANSMITTED DISEASES (STD)

Ministry of Health
Malaysia

KEMENTERIAN KESIHATAN MALAYSIA

SERIES 3

LABORATORY MANUAL FOR THE DIAGNOSIS OF SEXUALLY TRANSMITTED DISEASES (STD)

AIDS/STDs Section
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CHAPTER 1

INTRODUCTION

Laboratory tests are very important in the diagnosis of sexually transmitted diseases (STD). Clinical diagnosis is often insufficient and requires microbiological tests for confirmation. Accurate diagnosis is essential for the management and control of STDs.

A manual of laboratory procedures is therefore helpful for medical personnel in the clinics and hospitals. The management of STDs is now being integrated into the primary health care clinics in Malaysia.

CHAPTER 2

GONOCOCCAL INFECTIONS

Aetiology: Neisseria gonorrhoeae

2.1 Collection of specimen

The primary site for specimen collection in women is the endocervical canal. Secondary sites are the urethra, vagina, rectum and orpharynx. In heterosexual men material should be collected from the urethra. The primary sites in homosexual men are the urethra, rectum and oropharynx.

2.1.1 Endocervix

Wipe the cervix clean of vaginal secretion and mucus. The use of antiseptics, analgesics and lubricants should be avoided. Insert a sterile speculum lubricated with sterile normal saline into the endocervical canal. Insert a sterile cotton, calcium alginate or dacron swab 2-3 cm into the cervical canal. Rotate the swab for 5-10 seconds to permit absorption of the exudate.

2.1.2 Urethra

Wipe the urethra clean with sterile gauze or swab. Collect the exudate with a sterile swab. (Collect the urethral secretion or free discharge 1 hour or more after urinating.)

If no discharge is evident, the male urethra is stripped towards the orifice to express the exudate. If no exudate is obtained, insert a sterile small loop, or a thin swab 2-3 cm into the urethra and rotate for 5 seconds. In women, massage the urethra against the pubic symphysis and use the same technique as for men.

2.1.3 Rectum

Insert a cotton swab 3 cm into the anal canal and rotate for a few seconds to sample exudate from the crypts inside the anal ring.

2.1.4 Vagina

Only recommended for women who have had a hysterectomy. Use speculum and swab the posterior fornix for a few seconds.

2.1.5 Oropharynx

Swab the tonsilar crypts and the posterior pharynx.

2.2 Transport of specimen

Before seeding the specimen directly on the growth media or before inserting them in a transport medium, make a smear for microscopy.

Obtain a thin homogeneous film by rolling the swab on to a clean slide, and allow smear to air dry.

If the specimen cannot be inoculated immediately, insert the wab into a non nutritive transport medium eg. Amies or Stuart or a nutritive transport medium eg Transgrow.

If plates are available, inoculate the swab immediately onto a modified Thayer-Martin plate in a Z fashion. Place the plate in a tin. Light a candle, place it in the tin and close the lid. Send the tin containing the plate as soon as possible to the laboratory or store in the 37°C incubator.

2.3 Microscopy

2.3.1 Gram stain

2.3.1.1 Preparation of slides and staining

Prepare slides and perform a gram stain as in appendix 1. Note: smears for detection of N. gonorrhoeae are best fixed using absolute methanol or ethanol.

2.3.1.2 Examination and reporting of smears

Use a bright light microscope, immersion oil of good quality and examine the slide with a 100x objective.

Describe what is seen on the smear: epithelial cells, polymorphonuclear leucocytes, type of bacteria, intracellular or extracellular position.

Neisseria gonorrheoea are gram negative diplococci with adjacent sides flattened, 0.6 - 1.5 um in diameter.

In smears from specimen, N. gonorrhoeae are usually found inside pus cells (intracellular) in groups.

Some organisms may be seen lying free outside the pus cells (extracellular) especially if cells have been damaged when spreading specimen on the slide.

2.4 **Culture**

Transport specimen to a central laboratory for culture and sensitivity

CHAPTER 3

CHLAMYDIA TRACHOMATIS INFECTION

Aetiology

a.	trachomatis Serovar Ll, L2, L3	-	Lymphogranuloma venereum
b.	trachomatis Serovar A, B. Ba, C	-	Hyperendemic blindness trachoma
c.	trachomatis Sorovar D, E, F, G, H, I, J, K	)	Inclusion conjunctivitis (adults & newborn), nongonococcal urethritis,cervicitis, salpingitis, proctitis, epididymitis, pneumonia of newborns

(Association of serovars with disease: predominant but not exclusive)

3.1 Microscopy

3.1.1 Giemsa stain

Direct microscopy using Giemsa stain is recommended for conjunctival specimens obtained from symptomatic patients (conjunctivitis or trachoma).

3.1.1.1 Preparation of slides and staining

The smear is air dried, fixed with absolute methanol for at least 5 min and again dried. it is then seeded with the working Giemsa solution (freshly prepared the same day) for 1 h. The slide is then rinsed in 95% ethyl alcohol and dried.

3.1.1.2 Examination and reporting of smears

Examined for the presence of typical intracytoplasmic inclusions in epithelial cells.

Elementary bodies stain purple, whereas initial bodies are slightly more basophilic and tend to stain blue. Cytoplasmic areas around the inclusion are grey and the nucleus of the cell stains pink.

The specimen should contain at least 1000 epithelial cells for adequate results. It is necessary to use the oil-immersion objective to ensure the detection of typical inclusions. Some microscopists prefer to examine slides using dark-field microscopy.

3.2 2 glass urine test

3.2.1 Collection and method

Ask the patient to hold urine for at least 4 hours before attending the clinic, to allow discharge to collect within the urethra. Ask the patient to pass about 60 - 120 ml into the first glass (first voided urine) and the remainder into the second glass.

3.2.2 Examination of urine

The presence of threads to specks of pus and a hazy appearance which is not due to phosphates (ie does not clear after addition of 5-10% of acetic acid) in the first glass confirms anterior urethritis.

This helps to differentiate an anterior from a posterior urethritis, cystitis or nephritis ie hazy urine with threads in both glasses.

3.3 Direct immunofluorescence (IF) test

Complete kits are available from different companies: Micro Trak, Syva, Imagen, Boots-Celltech, Chlamydia FA, Ortho Diagnostics.

Please follow procedures for collection of specimen from various sites ie endocervical, urethral, rectal, conjuncttival ets.

Prepare slides and fix smears as indicated in the brochure.

3.3.1 If Fluorescence microscopy and trained personnel are not available, proceed as follows:

When the slidc is dry, told into the collection packet, ensuring that the fixed specimen is not touched or disturbed.

Store and transport the sample either at room temperature range of 20tiC - 33t'C or refrigerated at 2°C - 80C. Stain sample within 7 days of collection of a stated in kit brochure.

If not stained within 7 days of collection, the fixed specimen should be stored at - 20"C.

Transport to a central laboratory.

3.3.2 If Fluorescence microscopy and trained personnel are available, proceed as follows (read kit brochures in use):

3.3.2.1 Preparation of smears

Smears are air dried on an 8mm well of a teflon covered glass slide and fixed with acetone.

Thirty microlitres of a fluorescein-labelled monoclonal antibody to all serovars of C. trachomatis is applied directly to the specimen. The slide is then incubated for 15 min at room temperature in a moist chamber. Then, the slide is rinsed gently with distilled water for 20 seconds to remove excess conjugate. Excess water is gently shaken off and the slide allowed to air dry. A cover slip is mounted over the well.

3.3.2.2 Examination and reporting of smears

Examine the specimen under a fluorescent microscope at 500x magnification.

Chlamydial elementary bodies appear as bright applegreen pinpoints. The cells are counterstained in red.

It is very important to incorporate positive and negative control slides into the test procedure to avoid erroneous results.

Inadequate smears are a major cause of low sensitivity. Any particles and artefacts showing yellow-green rather than apple-green fluorescence are also a source of false positive results.

3.4 Cell culture

C. trachomatis can be isolated in specimen from all sites eg bubo pus, genital ulcer, rectal tissues etc using tissue culture teachnique on cycloheximide treated McCoy's cells.

Refer to a reference laboratory where this method is available for further details eg on transport medium for specimen etc.

CHAPTER 4

SYPHILIS

Aetiology: Treponema pallidum

4.1 Darkfield microscopy

4.1.1 Principles and precautions

Darkfield microscopy is the sole-method which provides an instant and direct diagnosis of infection with T. pallidum. It is essential for the direct diagnosis of early acquired and early congential syphilis.

In darkfield microscopy only light rays striking organisms or particles at an oblique angle enter the microscope objective, giving a self-luminous appearance against a black background.

Darkfield microscopy must be performed by well trained and experienced personnel in order to adjust the microscope and to differentiate T. pallidum from nonpathogenic spiral organisms commonly found on genital and anal mucous membranes, darkfield examination of oral lesions is not recommended.

4.1.2 Collection and preparation of specimens

Primary and secondary lesions may be examined by darkfield microscopy.

The lesion should be cleansed carefully with gauze and saline, abraded gently with dry gauze and squeezed to produce a serous exudate.

Serous fluid or exudate from the lesion can be transferred to a microscope slide using a thin inox spatula, bacteriological loop, or by touching the slide directly to the fluid.

The material is suspended in a drop of saline on a slide and covered with a cover slip.

Examine the slide immediately.

4.1.3 Examination and reporting of smears

Place a few drops of immersion oil on the condenser of a previously adjusted darkfield microscope. Lower the condenser slightly so that the oil is below the level of the stage. Place the slide on the microscope and raise the condenser until there is good contact between the oil and the bottom of the slide. Carefully avoid trapping air bubbles in the oil. Do not place oil on top of the cover slip.

Using the low power objective (10x) bring the specimen into focus. Centre the light in the field by adjusting the centring screws located on the condenser, and focus the condenser by raising or lowering it until the smallest diameter of light is obtained. Recentre the light if necessary. Then use the dry 40x objective, bring the specimen into focus, an examine the slide carefully. The contrast will be better when the microscopy is done in the dark. Avoid bright daylight.

T. pallidum appears white luminated on a dark background. It is identified by its typical morphology, size and movement. It is a thin (0.25-0.3 um) organism with 8 - 14 regular, tightly winded deep spirals, and is 6 - 14 urn long. It exhibits quick and rather abrupt movements. It rotates relatively slowly about the longitudinal axis (like a corkscrew). This rotation is accompanied by bending (twisting) in the middle and is stiffly executed. Expanding and shortening (like an elastic expander spiral) may be seen. Distortion may occur in tortuous convolutions. When the organism is attached to or obstructed by heavier objects, vigorous struggling distorts the coils. Other atypical spirochetes may be loosely coiled, thick and coarse. The motility is different, unlike a corkscrew, but more a writhing motion with a marked flexion and frequent relaxation of coils.

The demonstration of treponemas with characteristic morphology and motility for T. pallidum constitutes a positive diagnosis in primary and secondary syphilis. Patients with a primary chancre which is darkfield positive may be serologically negative. They normally become serologically reactive within several days to a few weeks.

Failure to find the organism does not exclude a diagnosis of syphilis. Negative results may mean that (I) an insufficient number of organisms was present (a single darkfield examination has a sensitivity of no more than 50%), (2) the patient had already received antibiotics, (3) the lesion was approaching natural resolution, and (4) the lesion was not syphilitic. Whatever the result of the darkfield examination may be, blood should always be taken for serologic tests.

4.2 RPR Card Test

4.2.1 Collection and transport of specimen

Collect 5 ml of venous blood aseptically in a sterile plain bottle / tube. Label and despatch promptly.

4.2.2 Materials and reagents needed

1. RPR card kit: This contains card test antigen suspension, disposable calibrated 20-gauge needle, plastic antigen, dispensing bottle, plastic-coated cards with circles and disposable plastic sampling pipette spreaders, which deliver 50 ul of serum (HWD kit, Becton Dickinson). Kits of other brands contain the same material without sampling pipettes, wooden or plastic sticks are included for mixing serum and antigen.

2. Additional materials:

   - a mechanical rotator, circumscribing a circle of 2 cm in diameter at a speed of 100 rpm, with automatic timer.

   - saline (0.9%); - serum non-reactive to syphilis diluted at 2% in saline: required for further titration of sera giving a positive results at the 1:16 dilution.

4.2.3 Qualititative test procedure

a. Place 50 ul of unheated serum within a circle on the test card (using a sampling pipette delivered with the kit or an automatic pipetting device). Spread the drop to fill the

entire circle with the reverse end of the dispensing device or a toothpick. Do not allow the serum to spread beyond the circle. Use a clean spreader for each sample.

b. Add exactly one free-falling drop of the gently resuspended antigen suspension. Do not stir.

c. Place the card (10 sera can be tested on each card) on the mechanical rotator under a humidity cover and rotate for the time prescribed by the manufacturer.

d. Read the reactions with the naked eye under a light source immediately after removing the card from the rotator. A brief rotating and tilting of the card by hand could aid differentiating reactive serum from non-reactive.

e. Results are reported as reactive regardless of the degree of reactivity:

reactive: small to large clumps (flocculation)

non-reactive: no clumping or only very slight roughness specimens giving any degree of reactivity should be fully titrated.

Caution: Prozone effect may occur. Undiluted non-reactive specimen giving no flocculation but a certain roughness reaction or a slight granulation should also be titrated.

4.2.4 **Quantitative test procedure**

a. For each specimen to be tested use one row (five circles) of a test card. Place 50 ul of saline on circles 2 through 5. Do not spread the drops.

b. Place 50 ul of serum on to the circles numbers I and 2. Using a pipette mix the saline and the serum in circle 2 by drawing the mixture up and down the pipette five to six times. Avoid bubbles.

c. Transfer 50 ul from circle 2 (1:2 dilution) to circle 3 and mix. Transfer 50 ul of circle 3 (1:4) to number 4 and mix.

Transfer 50 ul of 4 (1:8) to circle 5 (1:16), mix and discard 50 ul.

d. Using the reverse of a clean dispensing device of a clean toothpick spread the highest serum dilution (circle 5) within the confines of the circle. Repeat this action with the same stirrer in circles 4, 3, 2, and 1 in that order.

e. Perform the test further as described for the qualitative reaction (see section 4.2b, c, d).

f. Report results in terms of the highest dilution giving any reactivity.

g. If the 1:16 dilution is still reactive, continue as follows:

- Prepare a 1:16 dilution by adding 100 ul of the positive serum to 1.5 ml of saline. Mix.

- Place 50 ul of the 2% non-reactive serum in saline (see Materials and reagents) circles 2 through 5 of a card). Measure 50 ul of the 1:16 serum dilution to circles I and 2 and make two fold dilutions and complete the test as described in section 4.3b - f.

4.3 **TPHA Testing Procedure (Micro Hemagglutination Assay Technique)**

4.3.1 **Qualitative Assay on Serum**

A) Outline of Qualitative Test

Cup No.	Test Serum ml	+	Serum Diluent = ml	Serum Dilution	Diluted Unsensitized Cells, ml	Diluted Sensitized Cells, ml	Final Serum Dilution
1	0.025		0.025	1:2			
2	0.025		0.1	1:10			
3	0.025		0.025	1:20	0.075		1:80
4	0.025		0.025	1:20		0.075	1:80
5	0.025		0.025	1:40		0.075	1:160

B) Reagent Controls

mCup No.			Serum Diluent ml	Control Serum Dilution	Diluted Unsensitized Cells, ml	Diluted Sensitized Cells, ml	Final Serum Dilution
	positive control serum, me						
1	0.025	(1:80)	0.025	1:160	-	0.075	1:640
2	0.025	(1:160)	0.025	1:320	-	0.075	1:1280
3	0.025	(1:320)	0.025	1:640	-	0.075	1:2560
4	0.025	(1:640)	0.025	1:1280	-	0.075	1:5120
5	0.025	(1:1280)	0.025	1:2560		0.075	1:10240
	Reagent Controls						
1	-		0.025	-	-	0.075	-
2	-		0.025	-	0.075	-	-

C) Shake the trays gently, cover with an empty tray

D) Incubate the trays at room temperature (15-25°C) for at least 2 hrs. The incubation period may be extended overnight with no change in patterns.

E) Read the pattems.

Degree of Hemagglutination	Reading Interpretation	
Smooth mat of cells covering entire bottom of well, edges sometimes folded	4+	Positive
Smooth mat of cells covering less area of well	3+	Positive
Smooth mat of cells surrounded by red circle	2+	Positive
Smooth mat of cells surrounded by smaller red circle	1+	Positive
Button of cells hacing small 'hole' in center		**Retest
Definite compact button in center of well or may have a very small 'hole' in the center	-	Negative

4.3.2 Quantitative Assay on Serum

A) Outline of Quantitative Test

Cup No.	Test Serum ml	+ Serum	Diluent = ml	Serum Dilution	Diluted Unsensitized Cells, ml	Diluted Sensitized Cells, ml	Final Serum Dilution
1	0.025		0.025	1:2			
2	0.025		0.1	1:10			
3	0.025		0.025	1:20	0.075		1:80
4	0.025		0.025	1:20		0.075	1:80
5	0.025		0.025	1:40		0.075	1:160
6	0.025		0.025	1:80		0.075	1:320
7	0.025		0.025	1:160		0.075	1:640
8	0.025		0.025	1:320		0.075	1:1280
9	0.025		0.025	1:640		0.075	1:2560
10	0.025		0.025	1:1280		0.075	1:5120

B) Set up Reagent Controls as in "Qualitative Assay on Serum".

C) Complete test and read as described in the Qualitative Assay

CHAPTER 5

GENITAL HERPES SIMPLEX INFECTION

Aetiology: Herpes simplex virus I and 2

5.1 Introduction

At present laboratory diagnosis of herpes simplex infection is available at a reference or central - level laboratory: for performance of virus cultures, direct immunofluorescence tests on smears etc.

5.2 Microscopy

5.2.1 Tzanck smear

5.2.1.1 Preparation of slide and staining

Rupture an intact vesicle with a scapel blade and blot the fluid with a gauze square.
Gently scrape base of lesion to remove cells-try to minimize or prevent bleeding.
Smear material onto a slide with the blade.
Air dry, then fix with 95% methanol.
Apply Giemsa stain or methylene blue for 3 minutes

5.2.1.2 Examination and reporting of smears

Observe the smear under low and high power for characteristic multinucleated giant cells ie multinucle ated giant cells showing margination of the nucleoplasm - nuclei with darker blue rims and centers of eighter blue (steel gray nuclei) Aggregates of normal keratinocytes can simulate multinucleation (but lack margination). Varicella zoster (chicken pox) and Herpes zoster also produce multinucleate giant cells.

5.3 Virus culture

5.3.1 Collection and transport of specimen

Vesicle fluid (90%) and pustules (70-80%) are most likely to be culture-positive. Early first episode ulcers yield virus in 80% of patients, but ulcers from recurrent infection are virus culture negative in 50% of patients. Only 25% of crusted lesions contain recoverable virus. Vesicles should be aspirated with a tuberculin syringe and 26-gauge needle, pre-filled with a small amount of viral transport medium, and immediately expelled into a vial containing transport medium, kept at 4°C until cultured and the delay before culture should be as short as possible. If a delay of more than 24 h is necessary, the specimen should be kept frozen at -70°C. Never store specimen a to - 20°C.

Specimen can also be collected with a cotton or dacron swab. After opening of the vesicles, fluid and cells from the base of the lesion are collected with a swab pre-moistened in the transport medium.

For sampling ulcers, swabs are vigorously rubbed against unhealed areas. Crusts should be removed first. Swabs from female genitalia should include a cervical specimen.

For further details please refer to the reference laboratory where the test is available.

5.4 Direct specimen identification and typing by immunofluorescence technique.

Complete kits are available. Please follow procedure as indicated in brochure.

5.4.1 Collection of specimen

Moisten a large or small dacron swab in sterile distilled water and vigorously swab the entire base of the lesion to obtain nonsuperficial epithelial cells for diagnosis.

5.4.2 Preparation of slides

Smear the scraping immediately into two wells of a teflon-coated slide.
Allow specimen to air dry completely.

Lay the slide flat and flood with 0.5 ml of acetone fixative and allow to evaporate completely.

5.4.3 Storage and Transport of slides

Store / transport either at room temperature of 20°C - 30°C or refrigerated at 2°C - 8°C.

For best results, stain slide immediately upon receipt in the laboratory.

If not stained within 3 days, the fixed specimen should be stored dessicated at -70°C.

5.4.4 Slide Examination

If test and trained personnel are available on site continue procedures as in kit brochure.

Examine slides under a fluorescent microscope and interpret accordingly.

CHAPTER 6

CHANCROID

Aetiology: Haemophilus ducreyi

6.1 Collection and transport of specimen

The ulcers should be cleansed with a dry gauze to remove crusts and superficial debris. Extensive cleaning is not required.

Using a cotton or dacron swab exudate and material from the ulcer base should be sampled.

Specimens from fluctuant buboes may be obtained by aspiration but are often negative on culture.

6.2 Microscopy

6.2.1 Gram stain

6.2.1.1 Preparation of slides and staining

Prepare slides and perform a gram stain as in appendix 1.

6.2.1.2 Examination and reperking of smears

Examination under high power with oil immersion, sometimes reveals pleomorphic Gram negative organisms in a 'school of fish' pattem ie clumps of string of bacteria along mucus strands, but these smears may be difficult to interpret because of the polymicrobic flown of most genital ulcers.

6.3 Culture

Please refer to a central laboratory for procedures on collection and transport for culture as H. ducreyi is a fastidious organism and have special requirements for growth.

CHAPTER 7

GRANULOMA INGUZALE (DONOVANOSIS)

Aetiology: Calymmatobacterium granulomatis

7.1 Microscopy

7.1.1 Giemsa stain

7.1.1.1 Preparation of tissue smear

Cleanse around the ulcerated area using a swab moistened with physiological saline.

Obtain a small piece of granulation tissue from the border of the lesion.

Remove the deeper portion of the specimen and crush between 2 slides.

Air dry and deliver the smear to the laboratory within 2 hours of collection.

If a delay is expected, fix the smear with methanol for 2 - 3 minutes prior to transporting.

7.1.1.2 Staining of smears

Upon receipt in the laboratory, fix in methanol for 2 - 3 minutes.

Cover the slide with Giemsa stain for 7 - 10 minutes. Wash with a stream of buffered water (pH 7.0 - 7.2).

Leave clean water on slide for 2 - 3 minutes. Allow slide to dry in a draining rack.

7.1.1.3 Examination and reading of smears.

The gram negative coccobacilli appearing in vacuoles in the cytoplasma of large histocytes and sometimes in plasma cells or polymorphonuclear leucoccytes are called Donovan bodies.

The intracellular organisms have a prominent clear capsule when mature. Stained bacteria may resemble a closed safety pin.

7.2 Histology

Histological study of sections requires experience in interpreting the findings and should be carried out in a central laboratory where skilled personnel are available.

7.2.1 Preparation and transport of biopsy material

Remove a piece of tissue, 3 - 5 mm thick, from the border of the lesion. Place the tissue in a bottle of formaldehyde saline or other suitable fixative. Label appropriately and despatch to the central laboratory.

CHAPTER 8

TRICHOMONAS VAGINALIS INFECTION

Aetiology: Trichomonas Vaginalis

8.1 Collection and transport of specimens

In females, specimens should be collected from the posterior vaginal fornix with cotton-tipped, dacron or calcium alginate swabs. A nonlubricated speculum should be use.

In males, 20ml of first voided urine should be collected.

After centrifugation, the sediment is examined microscopically or cultured. Specimens may be examined immediately by direct microscopy or after culture.

If transport of specimens is required, Amies transport medium can be used: T. vaginalis will survive at room temperature for at least 24 h.

8.2 Direct microscopy (saline wet mount)

Place a drop of 0.9% saline on a slide. make a suspension of vaginal fluid in the drop. Cover with a cover slip and examine with 400x magnification : T. vaginalis sppears as an ovoid globular protozoan exhibiting a characteristic jerky motility.

8.3 Culture

Please refer to a reference laboratory, when a culture is necessary.

CHAPTER 9

BACTERIAL VAGINOSIS

Aetiology: Gardnerella vaginalis / polymicrobial

9.1 Collection and transport of specimens

9.1.1 Vaginal specimens

Collect a sample of vaginal discharge on a sterile cotton wool swab and insert in a container of Amies transport medium.

Make a smear of the discharge.

9.2 Physiological tests

9.2.1 Vaginal fluid pH

The normal nature vagina has an acid pH of 4.0. In BV the pH is elevated to more than 4.5 (range 4.5 - 5.5). Collect vaginal fluid from the lateral and posterior fornices using a cotton tipped swab. Touch the swab on a pH indicator paperstrip (range 3.8 - 5.4 available from E. Merck) and read the colour change immediately. Alternatively, a pH paperstrip can be touched to the top of the withdrawn speculum.

An elevated pH may also be seen: a) in trichomonal vaginitis, b) if vaginal fluid is contaminated with menstrual blood, cervical mucus or semen.

9.2.2 Amine odour

Amines associated with BV are produced by anaerob bacteria, decarboxylating amino acids (lysine the caverdine and arginine to putrescine). By adding KOH, these amines become volatile producing the typical amine odour (fishy odour).

Mix the vaginal specimen with a drop of 10% KOH on a slide to detect the amine odour. A positive test will quickly become negative upon standing due to complete volatilization of the amines.

9.3 Microscopy

9.3.1 Wetmount

A suspension of vaginal fluid is made in a drop of saline on a slide, covered with a cover slip and microscopically examined (400 x magnification) for the presence of clue cells. Clue cells are squamous epithelial cells covered with small, coccobacillary organisms. The edges of the epithelial cells are not clearly defined due to the large number of bacteria present and the apparent disintegration of the cells. In most patients a mixture of clue cells and normal exfoliated epithelial cells will be seen. The adhering bacteria on the cells are predominantly G. Vaginalis, sometimes mixed with anaerobes.

Pus cells are usually absent or few in number.

9.3.2 Gram Stain

9.3.2.1 Preparation of slides and staining

Using a cotton tipped swab, a smear of the vaginal fluids is made on a slide, dried, heat fixed and gram stained as in appendix 1.

9.3.2.2 Examination and reporting of smears

Normal vaginal fluid contains predominantly Lactobacillus sp, with or without other bacteria such as streptococci, coryneform rods, G. vaginalis. In BV lactobacilli are replaced by a mixed flora dominated by anaerobes and G. vaginalis.

Large Gram positive rods with flat ends are the Lactobacillus morphotype. When they are present alone or predominantly mixed with other

morphotypes in combination with normal vaginal squamous epithelial cells, the smear is interpreted as normal.

The presence of clue cells combined with a mixed bacterial flora such as coryneform rods, Gram positive cocci, small Gram negative rods, curved rods is consistent with BV.

9.4 Culture

Routine culture is not recommended but could be done in a reference laboratory.

CHAPTER 10

CANDIDIASIS

Aetiology: Candida albicans / other Candida spp

10.1 Collection and transport of specimens

A specimen of vaginal fluid is collected using cotton, calcium alginate of dacron swabs. A transport medium (Amies) may be used, but is not necessary.

In males with balanitis, a swab premoistened in saline is used to rub the glands of the penis.

10.2 Microscopy

10.2.1 Wetmount

Make a suspension of the vaginal fluid in a drop of 10% KOH on a slide an cover with a cover slip. KOH will break up clumps of epithelial cells for easier visualization of the elements. A KOH wet mount is 10% more sensitive than a saline wet mount. Examine under low power (40x) for the typical morphology of budding yeast (blasto conidia), pseudohyphae may be present. In most cases, the quantity of yeast is higher in vaginal fluid from patients with vaginitis than in fluid frorn asymptomatic women colonized by Candida. Microscopy is less sensitive than culture but more specific for diagnosis of vaginal candidiasis.

10.2.2 Gram stain

Prepare slides and perform a gram stain as in appendix I Ezamine for budding yeast and pseudohyphae.

10.3 Culture

If culture facilities are available, inoculate Sabouraud dextrose agar, for the isolation of Candida species. Incubate plates at 37°C for two days (or at room temperature 25°C for three days).

10.3.1 Examination of plates and further test

Colonies are white to creamy; when confirmation is needed to differentiate them from bacteria, a colony should be suspended in a drop of saline on a slide and microscopically examined.

10.3.2 Germ Tube Test

An easy test which differentiates C. albicans form other yeast species is a subculture in serum, incubated at 37°C for 4h: C. albicans shows the production of germ tubes (short lateral hyphae filaments with no constrictions) while other species do not.

Further identification of yeasts is not recommended for routine work.

TABLE 1

LABORATORY DIAGNOSIS FOR SEXUALLY TRANSMITTED DISEASES

	ORGANISMS	TESTS*
1.	Neiseria gonorrhoeae	1. Gram smear 2. Culture
2.	Chlamydia trachomatis	1. 2 glass urine test 2. Urethral smear 3. Culture 4. Direct immunoflourescence
3.	Treponema Pallidum	1. Darkfield microscopy 2. VDRL or RPR card test 3. Treponema Pallidum Hae magglutination test (TPHA) 4. FTA-abs
4.	Herpes Simplex	1. Virus culture 2. Immunoflourescence techniques 3. Pap smear 4. Tzanck smear
5.	Haemophilus ducreyi	1. Gram/giemsa smear 2. Culture
6.	Calymmatobacterium granulomatis	1. Tissue smear (Giemsa) 2. Histology (biopsy)
7.	Trichomonas vaginalis	1. Saline wet mount 2. Pap smear
8.	Gardnerella vaginalis	1. Saline wet mount

		2. Gram smear 3. Vaginal fluid pH 4. Amine odour 5. Culture
9.	Candida albicans	1. Gram stain 2. Wet mount 3. Culture

* Some of the tests will need to be done in a central or reference laboratory

APPENDIX I

DIRECT MICROSCOPIC EXAMINATION

Preparation of Slides for Staining

Allow smears to air dry.

Fix the smear by passing the slide repidly 3 times through a flame, the film side uppermost.

Avoid overheating as this distorts the cellular morphology.

The slide should be merely warm when touched to the back of the wrist.

Gram Stain

a. Cover the fixed smear with crystal voilet for 30 - 60 seconds,. Wash rapidly in running water.
b. Tip off all the water and flood the smear with Lugol's iodine, leave for 30 60 seconds. Gently rinse with running water.
c. Decolourize rapidly with acetone-alcohol or acetone-iodine (usually about 10 - 20 seconds). Rinse quickly in running water to stop the decolouration. Drain off excess water.
d. Counterstain with safranin (neutral red or dilute carbol fuchsin) for 60 seconds.
e. Rinse with running water and gently blot dry or place in a draining rack for the smear to air dry.

Examination of Smears

Examine the smear microscopically, first with the 40X objective to check the staining and to see the distribution of material and then with the oil immersion objective to look for bacteria cells. Open fully the condenser iris when using the oil immersion lens.

Results

	Staining
Gram positive bacteria	Dark purple
Yeast cells	Dark purple
Gram negative bacteria	Pale to dark red
Nuclei of pus cells	Red
Epithelial cells	Pale red

Reporting of Gram Stain

The report should include the following information:

1. The number of bacteria present, whether many, moderate, few or scanty.
2. The Gram reaction of the bacteria, whether Gram positive or Gram negative (variability may occur)
3. The morphology of the bacteria, whether cocci, diplococci, rods or coccobacilli and whether they are intracellular.
4. The presence and number of pus cells.
5. Presence of yeast cells and epithelial cells.

Giemsa's staining method

1. Air dry smear
2. Fix in absolute methanol or 95% ethanol for 5 mins.
3. Prepare freshly a giemsa stain solution:

   (a) 9 parts of phospate buffered solution pH 6.8.

   (b) 1 part of Giemsa's stock stain. (N.B. one slide requires about 3 ml. of stain)

4. Cover the slide with Giemsa stain for 20 min.
5. Rinse slide rapidly with Buffered solution.
6. Repeat step 5.
7. Cover slide with Buffered solution for 5 mins.
8. Air dry slide.
9. Mount slide.

APPENDIX II

GIEMSA STAIN

a. **Stain:**

Dissolve 0.5g Giemsa powder in 33 ml acetone free absolute methanol

(Note:- methanol is toxic and flammable).

Mix and store at room temperature.

Prepare a working dilution of this stock solution using buffered water at a ratio of 1 ml of Giemsa solution to 40 ml diluent.

Buffered water:

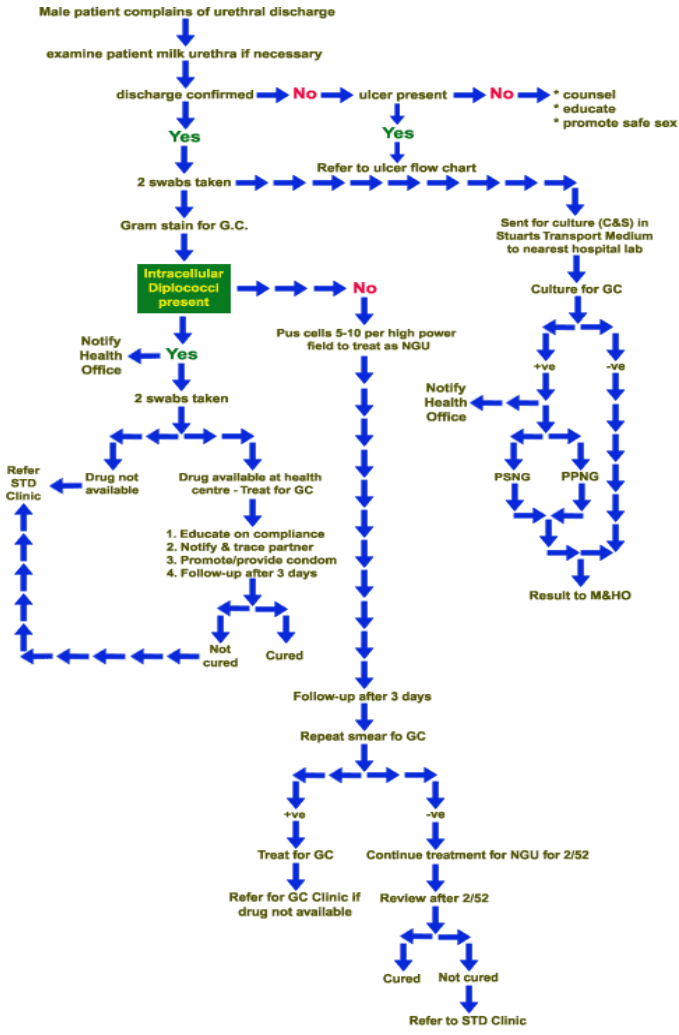
Prepare 1/15 M Na2HP04 using 9.5g of the anhydrous salt in 1 litre of distilled water (A) Prepare 1/15 M NaH-2PO4 using 9.2g of the salt in 1 litre of distilled water (B).

Mix 72 ml A with 28 ml B and add 900 ml of distilled water to obtain buffered water pH 7.2.

Note: - Refer staining techniques to relevant section where the method is used.

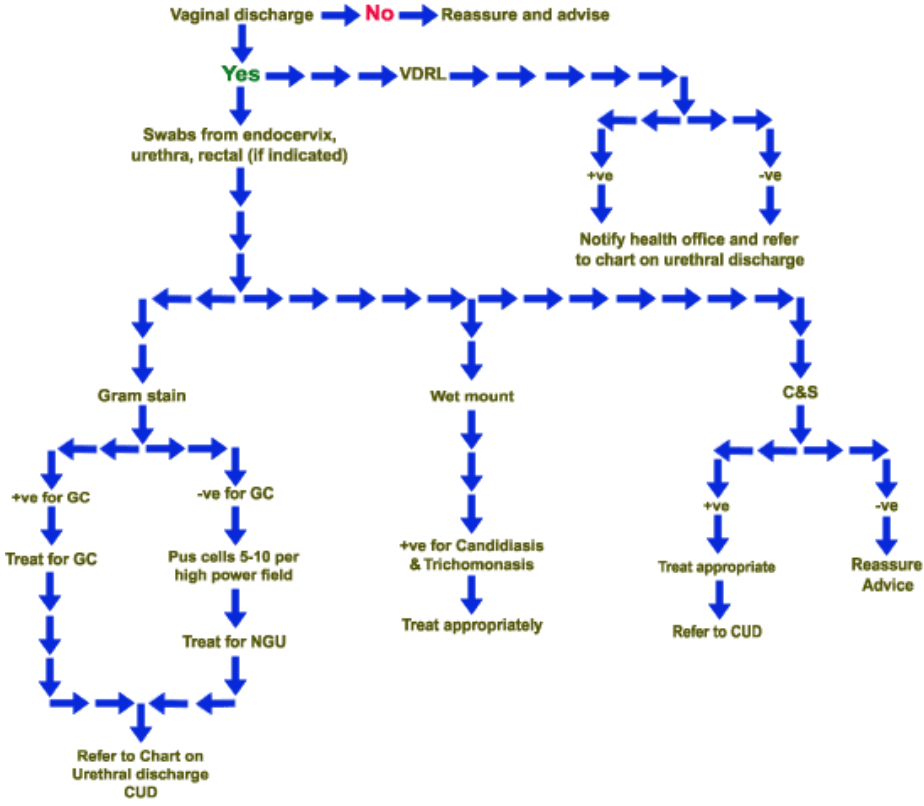
APPENDIX III

FLOW CHART FOR THE DIAGNOSIS OF URETHRAL DISCHARGE



APPENDIX IV

FLOW CHART FOR THE DIAGNOSIS OF VAGINAL DISCHARGE



APPENDIX V

FLOW CHART FOR THE DIAGNOSIS OF GENITAL ULCER