



RTI/STI/HIV Training Manual for Laboratory Technicians

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ENGENDERHEALTH
Improving Women's Health Worldwide

CONTENT

INTRODUCTION		2
SESSION I	LABORATORY DIAGNOSIS OF VAGINAL DISCHARGE	4
SESSION II	LABORATORY DIAGNOSIS OF URETHRAL/ENDOCERVICAL DISCHARGE	7
SESSION III	LABORATORY TEST FOR SYPHILIS	9
SESSION IV	MAITENANCE OF LABORATORY EQUIPMENT	11
ANNEXURE		

INTRODUCTION

Laboratory Technicians can play an important role in provision of laboratory support for diagnosis of reproductive tract infections at peripheral health facilities also. However, since the basic Laboratory Technicians training frequently does not significantly include investigations required for diagnosis of common RTIs/STIs, additional training is proposed to build up knowledge and skill in this area. This training would complement the medical officers training envisaged in the RTI/STI training and additional support for expendables and essential laboratory equipments, the district projects are proposed to operationalise RTIs/STIs services at BPHC and CHC level.

The use of these simple laboratory investigations among common asymptotic or poorly symptomatic patients can contribute to appropriate case management of reproductive and sexually transmitted infections with syndromic approach. This manual gives a compilation of background material and a suggested training design for adaptation as appropriate and feasible for training of Laboratory Technicians at these rural clinics.

TRAINING OBJECTIVE AND DESIGN

The objective of refresher training is to better equip the Laboratory Technicians practicing at BPHC/CHCs with the knowledge and skills in conducting simple laboratory test required for the diagnosis of common RTIs/STIs.

The training is suggested to be conducted at the level of district hospital where the clinical case load will be sufficient for a batch of few participants. The suggested duration of the training is one day.

One - Day Refresher Training of Technicians in Laboratory Investigations for Common Reproductive Tract Infections

Venue- District Hospital (Where clinic case load will be sufficient for a batch)

Duration of Training – One day

Training Schedule:

Timings	Session Topic	Method
900-1000	Introduction of training program, common RTIs/STIs and importance of lab. Support for diagnosis	Lecture cum Discussion
1000-1045	Session I Laboratory tests for Vaginal Discharge Vaginal pH test (method & interpretation) Amine test (method & interpretation) Wet mount (collection, transportation, microscopy and interpretation) Hydrogen Peroxide test (method and interpretation)	Lecture cum Discussion
1045-1115	Session II Lab diagnosis of urethral / endocervical discharge Collection of specimen- In male and female Microscopy-Preparation, fixing, staining examination and Interpretation	Lecture cum Discussion
1115-1130	Tea	
1130-1200	Session III Lab. Tests for serology of syphilis Collection of specimen Testing of sera RPR card test VDRL Interpretation of results	Lecture cum Discussion
1200-1415	Practical demonstration and practice session-Session I, II, and III	demonstration and practice
1415-1500	Lunch	
1500-1530	Session IV Maintenance of laboratory equipment	Lecture/discussion
1530-1600	Session V Registration and record keeping	Lecture/discussion
1600-1630	Follow-up actions and closing	

SESSION 1

LABORATORY DIAGNOSIS OF VAGINAL DISCHARGE

Vaginal discharge is the most common complaint amongst women suffering from RTIs/STIs. Following organisms are commonly responsible for these infections:

- *Gardenerella vaginalis*
- *Trichomonas vaginalis*
- *Candida albicans*

1) **Specimen Collection:** The specimen would be from the two sites

- Posterior vagina fornix: Discharge should be collected with the help of a sterile cotton wool swab soaked in normal saline.
- Endocervical: If origin of discharge is from cervix, an endocervical swab needs to be collected

2) **Methodology:** The following five simple tests are suggested for incorporation in the PHC setting to improve diagnosis. The methodology for the laboratory investigation is summarised below:

A) Vaginal pH test

- Take pH indicator paper strips with a range of ± 3.8 to ± 6.0
- Touch the specimen swab on the pH paper or
- Touch the pH paper to the tip of vaginal speculum after removing it from the vagina or touch the pH paper to the wall of vagina directly.

Do not allow the contact of pH paper with cervical secretions.

Reading

- ✓ Normal adult vagina has an acidic pH of ± 4.0
- ✓ In Bacterial vaginosis the pH is raised to 4.5
- ✓ In Candida infection pH is reduced to less than 4
- ✓ In Trichomoniasis pH is raised to more than 5.5

Presence of menstrual blood, cervical mucus semen may also raise the vaginal pH.

B) Wet mount (KOH wet mount):

- Place the specimen on a clean grease free microscope slide
- Mix two drops of 10 percent potassium hydroxide (KOH, 10 gms/100 ml) with the specimen.
- Put a clean cover slip over the specimen
- See that no air bubble is trapped under the cover slip
- Gently heat the slide over a flame for 10-20 sec. But do not allow to boil.
- Observe the slide under 40X objective of light microscope

Reading

- ✓ Round or oval yeast cells, about 5-7 μm in diameter - Presence of mycelia or pseudohyphae
- ✓ Saline wet mount can also be used but adding KOH makes it a better test.
- ✓ About 50-80% of patients (*Candida albicans*) can be diagnosed by this method.

C) Wet mount

- Take a clean grease free microscope slide, put a drop of vaginal fluid on it.
- Mix with one drop of normal saline and place a cover slip over it.
- Observe the slide first under 10X magnification. Any field, which shows the suspected organism, is then seen under 40X magnification of light microscope.

Reading

- ✓ Trichomonads are 15 μm in size.
- ✓ They have a pyriform shape with an anterior tuft of flagella and a lateral undulating membrane
- ✓ The parasite moves actively, showing jerky mobility, confirming diagnosis of Trichomoniasis
- ✓ Presence of clue cells suggests diagnosis of Bacterial Vaginosis. These cells do not have a well defined edge because of the presence of bacteria and disintegration of cell.

D) Amine test

- Take a drop of vaginal fluid on a clean grease free microscope slide.
- Put a drop of 10% Potassium hydroxide (KOH) on the vaginal fluid.
- Bring the slide close to the nose to smell the amine odour.

Reading

- ✓ An intense putrid fishy odor indicates a positive reaction
- ✓ Positive reaction indicates Bacterial Vaginosis

E) Hydrogen Peroxide test

This is a simple test and can be conducted at peripheral facilities. However, the test has not been widely used and would need to be validated.

Add a drop of 3% Hydrogen peroxide on to a slide having specimen of vaginal discharge. Mixing of a drop of hydrogen peroxide solution with vaginal secretions on a slide will immediately produce foaming bubbles in the presence of white blood cells typically found in the trichomonas infection., but will not react with Candidiasis Bacterial Vaginosis . in case of discharge being endo cervix (either Gonorrhoea or Chlamydia), a positive reaction will be noted.

SESSION II

LABORATORY DIAGNOSIS OF URETHRAL/ENDOCERVICAL DISCHARGE

1. Collection of specimen:

Collection of specimen from the proper site is essential for correct diagnosis of clients with suspected Gonorrhoea/Chlamydia. For collection of sample requirements includes: Sterile gloves, Sterile cotton wool swab sticks, Glass slides, Normal saline, Spirit lamp, Vaginal speculum.

2. Method of sample collection in Male:

- Collect the specimen while wearing the sterile gloves
- Collect the specimen at least 1 hour after the patient has urinated.
- Retract the prepuce, clean the tip of the meatus with normal saline and collect pus directly on the sterile cotton swab if purulent discharge (pus) from urethra is present.
- If no discharge is seen, milk the urethra towards the orifice and collect the discharge.
- If no discharge is obtained, insert a sterile thin cotton wool swab -3 cms into the urethra and rotate it for 5- 10 seconds to gently scrap the mucosa.

3. Method of sample collection in Female:

- Insert the sterile vaginal speculum in the vagina and inspect exocervix. Clean exocervix with sterile cotton wool swab.
- Do not apply any antiseptics, analgesics or lubricants. The speculum can be moistened with warm water.
- Insert another cotton wool swab for 2-cms in the cervical canal. Rotate it for 5-10 seconds and withdraw.

MICROSCOPY

1. PREPARATION OF SMEAR

- Take a clean, grease free microscope slide and wipe it with gauze piece.
- Pass it through the flame twice and again wipe it clean. This removes the excess grease on the slide.
- On the central part of the slide draw two vertical lines, 2.5 cms apart. The lines are drawn with the help of a glass marking pencil.
- Roll the cotton wool swab with the specimen on to this marked area.
- Spread it to an area of 2 cmsX1 cm
- Allow the smear to dry in air before heat fixing.
- Label the smear on the right or left hand corner of the slide.

Note: The cleanliness of the slide can be judged by placing a drop of water over it and spreading this drop. If the slide is clean and free of grease, this drop spreads into a thin even film; but if the slide is not properly cleaned, the water collects on the slide in form of fine droplets and the film cannot be made.

2. FIXING OF SMEAR:

Heat fixing:

- Hold the slide, film upwards
- Pass it over the flame of a burner or a spirit lamp twice or thrice.
- Feel the temperature on the back of the hand. When the slide is just hot enough to be tolerated, fixing is complete. Too much heating chars the smear and alters the morphology of the organisms. Less heating fails to fix the smear and it may be washed away during the staining procedure. Fixing kills the organism, fixes it to the slide, prevents autolytic changes, makes the organism permeable to the dye and harmless to the person handling the smear. After heat fixation the film is stained.

3. STAIN AND STAINING PROCEDURE

Gram's Staining: Reagents

1. Crystal violet

Solution A:	Crystal violet powder	20 gm
	Ethyl alcohol	200 ml
Solution B:	Ammonium oxalate	8 gm
	Distilled water	800 ml

Mix solutions A and B and filter.

2. Iodine	Resublimated iodine	20 gm
	1 N Sodium hydroxide sol (- 4 gm NaOH per 100 ml distilled water).	100 ml
3. Acetone alcohol	Acetone	100 ml
	Ethylene alcohol	500 ml

Other acetone alcohol ratios may also be used (1:4, 1:2 and 1:1). Higher the acetone content, more rapid is the decolorisation.

4. Safranin	Stock solution: Safranin	10 gm
	Ethyl alcohol	200 ml
	Working solution: Stock sol	100 ml
	Distilled water	900 ml

Procedure:

1. Cover the slide with crystal violet solution and allow the reagent to act for 20 sec.
2. Pour off the stain and pour iodine on the slide holding at an angle so that it washes away the crystal violet. Cover the slide with fresh iodine and allow it to act for 30 seconds

3. Wash off the iodine with acetone alcohol and add fresh acetone alcohol. Tilt the slide from side to side till violet colour ceases to come off the slide. This can be confirmed by holding the slide against a white background. Absolute alcohol (100% ethanol) can be used instead of acetone alcohol for decolorisation.
4. Wash the slide with water.
5. *Pour the safranin on the slide and leave for 1-2 minutes*
6. Wash with water, blot the slide dry between the tow blotting papers.
7. Put a drop of liquid paraffin on the smear and observe under oil immersion lens.

4. Examination

Examine the slide using a light microscope. Put a drop of liquid paraffin on the smear and focus under 100X objective. Push the condenser up and open the iris diaphragm so that maximum light passes through the slide.

5. Interpretation

Examine the smear for epithelial cells, polymorphonuclear leucocytes (pus cells), organisms and their location - whether extracellular or intracellular. The gonococci are intracellular, bean shaped and are usually arranged in pairs. $0.8\ \mu\text{m} \times 0.6\ \mu\text{m}$ in size. They are Gram negative in reaction and are stained pink along with the nuclei and protoplasm of pus cells.

SESSION III

LABORATORY TEST FOR SYPHILIS

1. Serological tests

Two types of serological tests are available for the diagnosis of late primary, secondary and tertiary syphilis. The non-treponemal or standard tests for syphilis detect the presence of non-specific antitreponemal antibodies (Reagin antibodies) in the serum. The other group of tests called the specific or treponemal test detect the antibodies for pathogenic treponemas in the serum.

A. Collection of blood sample for serology

Material

- Sterile gloves
- Disposable syringe and needles no 21 or 22 gauge
- Sodium hypochlorite 0.5 -1% in a discarding jar
- Tourniquet
- Spirit
- Sterile dry tubes
- Pasteur pipette
- Centrifuge

Method

- Apply tourniquet in the region of biceps.
- Ask the patient to close his/her fist with the thumb inside.
- Clear the antecubital fossa with a spirit swab.
- With gloved hands, feel the antecubital vein. It is prominent and can be seen in some clients.
- With the help of syringe and needle collect 3-5 ml of blood.
- Open the tourniquet, remove the needle from the vein and press the site with a spirit swab.
- Put the blood in the sterile test tube and allow it to clot (1-2 hrs)
- Discard the syringe and needle in the discarding jar with hypochlorite solution.
- With the help of pastier pipette remove the supernatant serum into another sterile test tube.
- Centrifuge the serum at 1500 RPM for 15 minutes. Any red cells transferred to this test tube settle down after centrifugation.
- Remove the serum with a Pasteur pipette into a sterile screw capped bottle.
- Label the vial and store in fridge at 4 C till further use and store in the freezer of the fridge if needed in future.

B. Rapid Plasma Reagin Test (RPR Test)

The RPR test is available as a commercial kit. It is simpler than the VDRL, and can be done even in peripheral facilities. The detail method of doing the test is given in each kit. The main steps are given in brief.

Material:

RPR card test kit. The following material is provided in the kit.

- 1 Plastic coated cards with circles on them.
- 2 Antigen suspension in an unbreakable container.
- 3 Needle 20 gauge without bevel
- 4 Plastic stirrer.

Other materials needed but not provided with the kit are:

- 1 Mechanical rotator (adjusted to rotate forming a circle of 2cm diameter). Speed of the rotator should be adjusted to the number or RPM (revolutions per minute) specified in the kit.
- 2 Normal saline
- 3 Pipettes- automatic or glass – 0.5 ml having 0.01ml division, 1ml and 2ml.

Test method:

- 1 Take one test card. With the pipette place 0.05 ml of unheated serum on one of the circles.
- 2 Spread the sample on the circle with the disposable plastic stirrer.
- 3 Gently shake the RPR card test antigen and put one drop (1/60 ml) of this antigen in the serum sample on the circle.
- 4 Rotate the card on the mechanical rotator after adjusting the speed. The duration of the rotation should be as per the instruction with the kit.
- 5 Remove the card from the rotator and see the result immediately with naked eye in the bright light.

Reading:

Small to large clumps	-	Reactive
No clumps or slight roughness	-	Non reactive

SESSION IV

MAINTENANCE OF LABORATORY EQUIPMENT

Maintenance of equipment in working condition is absolutely essential for the smooth functioning of a laboratory. Some of the common equipment and their maintenance is given below:

- 1 **Microscope:** (Light, Darkfield and Fluorescent) Every time the microscope is used, the lenses should be cleaned with a soft gauge piece soaked in xylene. Alcohol should not be used to clean the optical system because it dissolves the cement material and there by detaches the lenses. The whole microscope can be cleaned with a clean piece of gauge to get rid of the dust particles, every time it is used. Special care should be taken to avoid damage to the light source of darkfield and fluorescent microscope.
- 2 **Centrifuge:** It should always be placed in horizontal position, the safety cover should be closed when in use and there should an RPM check yearly by an experienced hand.
- 3 **Rotator:** The VDRL rotator should be checked every time when it is used by placing the finger vertically over it and counting the RPM. The RPM should be adjusted to the correct speed.

SESSION V

REGISTRATION AND RECORD KEEPING

Registration and record keeping is as important in the laboratory as it is in the clinic, although the kind of information recorded in the laboratory is different from that recorded in the clinic. In the clinic the records kept are of individual patients, with information of name, address, sex, age, medical history, finding on physical examination, laboratory result, diagnosis, treatment and follow up visits. This will allow the physician to provide quality medical care, and will also assist in the identification of repeat clients.

Confidentiality with regard to the laboratory test result is of the utmost importance. Not only the breach of confidence is punishable by law, it is also very damaging to provider-client relationship.

Laboratory registration and record keeping serves two purposes:

- a) To ensure that the result of test requested by physician is reported back to the physician, in such a way that there is no confusion as to which patient these result belong to.
- b) To record the work load in the laboratory, keeping track of number of tests performed.

The following steps should be taken in recording and registration:

- 1 Specimen tubes, slides and containers should be labelled with the name of patient, or with the serial number on the form, if patient identification is by number rather than by name.
- 2 All incoming request should be entered in the register, preferably using a different book for each kind of test or procedure.
- 3 Test results should be entered on request form as well as in the recording books.
- 4 The test result should then be sent back to the medical officer. A record should be kept of outgoing reports. This may be done in the same book where the test and its results are recorded.
- 5 Monthly tallies can be made of number of different tests or procedures performed and of the results.
- 6 Record of tests and results should be kept **confidential**, especially if these are recorded under the name of the individual clients, rather than under number. It is clear that records kept under number provide better guarantees for confidentiality.