Chapter 2

MATERIALS AND METHODS
CHAPTER II

MATERIALS AND METHODS

The patients attending the Sexually transmitted disease (STD) clinic of Civil Hospital Ahmedabad having complaint of genital ulcer, genital discharge or genital growths were studied in detail.

The patients were interviewed according to a standard Proforma which contained details about the demography - age, sex, education, occupation, marital status, socioeconomic status, domicile of the patient and other details.

Details were also taken regarding sexual behaviour of the patients i.e. homosexual, heterosexual, bisexual or others (exposures with Eunuchs). Information regarding the number of partners and whether they are of high risk activity (commercial sex workers and persons having multiple homosexual and heterosexual partners) was noted in detail. Information was also obtained regarding the last high risk exposure (other than spouse or regular partner) to the onset of STDs to get idea about the incubation period of each STDs and also about the use of condoms in the last outside exposure. The patients were also questioned about past history of genital ulcers, warts or discharges. Information regarding the personal history of the patient like history of alcohol or drug abuse, tobacco chewing, smoking or travelling job were taken into account.

Clinical examination of the patients

A detail clinical examination of the patient was done. Examination of the external genitalia, perianal and anal region, skin and oral mucosa was also done. Examination of
body lesions were carried out to rule out secondary syphilis. Submental, cervical, suboccipital, epitrochlear, and inguinal secondary nodes were also palpated. Their size, consistency and whether discrete or matted was noted.

The patients after clinically diagnosed as having sexually transmitted disease were then advised to give blood to perform the following tests.

Approximately 5 ml of venous blood was aspirated and the following tests were performed. Rapid Plasma Reagin (RPR) test was done to rule out syphilis followed by ELISA testing to rule out HIV. Those patients who were positive for HIV by ELISA method were again confirmed by Immunocomb or Comb-Aids method and also by Rapid Tridot method.

The cases having complaint of urethral discharge were subjected to Gram staining for gonococci. Tzanck smear was also carried out in patients with herpes progenitalis to look for multinucleated Giant cells.

The patients were counseled with the help of counsellor appointed for STD clinic about the disease, its modes of acquisition, transmission, knowledge regarding safe sexual practices (condom usage), and information about abstinence of sexual activity until subsidence of the symptoms. The patients were also taught about the use of condoms and were also provided freely. The patients were advised to come for a follow up visit after a week to see the response of treatment given and also to see the report of RPR and Elisa tests. The patients were persuaded to bring their partners on the follow up visit, so that they can also be clinically diagnosed and if necessary tested for STDs including HIV and also counselled.
The patients positive for HIV were counselled and depending on the signs of HIV illness (fever, weight loss, severe diarrhoea, lymphadenopathy) were subjected to CD4 cell count analysis to know the immune status of the patients. Depending on the CD4 counts the patients were referred to HIV medicine from where they are advised about initiating Antiretroviral therapy (ART).
**PROFORMA**

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Principle

Syphilis is a sexually transmitted disease caused by spirochete Treponema pallidum. After infection the host forms treponemal antibodies to Treponema pallidum, in addition the host also forms Non Treponemal antilipoidal antibodies in response to lipoidal material released from the damaged host cell. These antibodies are traditionally referred to as ‘Reagins’.

The Rapid Plasma Reagin (RPR)/ Carbon Antigen test is a macroscopic non treponemal flocculation test for detection and quantitation of antilipoidal antibodies. Non Treponemal tests like CARBOGEN are of great value when used for screening and follow up of therapy.

During the test procedure, the specimen, serum or plasma is mixed with CARBOGEN reagent and allowed to react for eight minutes. If antilipoidal antibodies are present in the specimen, they react with CARBOGEN reagent forming visible black floccules. If antilipoidal antibodies are not present in the specimen, there is no flocculation.

Reagents

1. CARBOGEN reagent: A particulate carbon suspension coated with lipid complexes.

2. Positive control, reactive with the CARBOGEN reagent.
3. Negative control, non-reactive with the CARBOGEN reagent.

CARBOGEN detects antilipoidal antibodies in serum or plasma. As against conventional V.D.R.L. reagents, test sample does not require heat inactivation. Other materials required are: Disposable slides with eight reaction circles, Disposable sample / control dispensing pipettes, Mixing sticks, Rubber teat, Reagent dropper for dispensing the carbon antigen.

Sample

Fresh serum or plasma should be used for testing. Samples not tested immediately should be stored at 2-8 °C.

Method

Bring reagent and sample to room temperature before testing. Thoroughly mix the Carbogen reagent suspension by gentle agitation before testing.

Qualitative method

1. Pipette one drop of test specimen, positive and negative controls onto separate reaction circles of the disposable slide using a slide dispensing pipette.

2. Add one drop of well mixed CARBOGEN reagent next to the test specimen, positive control and negative control by using a reagent dropper provided with the kit. Do not let the dropper tip touch the liquid on the slide.
3. Using a mixing stick, mix the test specimen and the CARBOGEN reagent thoroughly spreading uniformly over the entire reaction circle.

4. Immediately start a stopwatch. Rotate the slide gently and continuously either manually or on a mechanical rotor at 180 r.p.m.

5. Observe the flocculation macroscopically at 8 minutes.

6. Flocculation positive indicates the presence of antilipoidal antibodies in the test specimen.

Quantitative method

1. Using isotonic saline prepare serial dilutions of the test specimen positive in the qualitative method 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and so on.

2. Perform the qualitative test procedure using each dilution as a test specimen.

3. The titre is reported as the reciprocal of the highest dilution which shows a positive test result.

   The titre of antilipoidal antibodies is thus the highest dilution of the test specimen giving a positive test result.
Methods for detection of HIV

I. Enzaids HIV 1+2 ELISA TEST KIT (Span Diagnostics, Surat, India)

Principle

➢ The antigenic peptides/proteins are adsorbed onto the wells of the microplate. These antigens are selected from immunodominant regions of HIV-1 and HIV-2, so as to provide maximum level of sensitivity.

➢ A special sample diluent is developed using proprietary formulation to minimize non-specific binding.

➢ Test serum and controls, along with sample diluents are added to respective wells and incubated.

➢ If antibodies to the HIV type 1 and/or HIV type 2 envelope proteins are present in the specimen, they will bind to the antigens coated onto the solid surface. A wash step removes non-specifically bound material.

➢ A conjugate consisting of enzyme horse radish peroxidase, chemically coupled to anti-human immunoglobulin is added to each well and incubated. The conjugate binds to HIV antibodies that are already bound to immobilized antigens. A wash step removes nonspecifically bound conjugate.

➢ The substrate for the enzyme peroxidase and the chromogen 3,3',5,5' Tetramethylbenzidine (TMB) are added to all wells and incubated further, resulting in a blue coloured complex.

➢ The reaction is terminated by the addition of stopping solution yielding a stable yellow coloured end point.
The intensity of colour is proportional to the concentration of anti HIV-1 and/or HIV-2 present in the test specimen or control.

Reagents

Reagent: 1 Sample Diluent (1 x 22mL)
Contains Tris buffer, proteins and preservatives. (Ready to use)

Reagent: 2 Conjugate (1 x 5.5mL)
Peroxidase labelled anti-human immunoglobulin containing protein stabilizers and 0.01% Thimerosal as preservative. (Ready to use)

Reagent: 3 Washing Buffer (1 x 50mL)
Concentrated (10x) buffer containing Tween-20 and 0.01% Thimerosal as a preservative. Before use dilute by adding one volume of concentrate to 9 volumes of distill water. (Span product no. 23668A or equivalent)

Reagent: 4 Negative Control (1 x 0.5mL)
Anti-HIV negative human serum, negative for Anti HIV-1 and Anti HIV-2 and containing 0.01% Thimerosal as preservative. (Ready to use)

Reagent: 5 Positive control (1 x 0.5 ml)
Inactivated serum containing anti-HIV antibodies and 0.01% Thimerosal as preservative. (Ready to use)
Reagent: 6 Colour Reagent (1 x 11 ml)
Buffer containing hydrogen peroxide and 3,3',5,5' Tetramethylbenzidine (TMB) in solution. (Ready to use)

Reagent: 7 Stopping solution (1 x 12 ml)
Mineral Acid

Reagent: 8 Microwell strips (96 wells)
Coated with HIV 1+2 Recombinant and synthetic peptides.

Sample
Serum or plasma can be used. Specimens can be kept at 2-8°C for four weeks only. However, the specimens should be stored frozen (-20°C or lower) for long term storage. Grossly hemolysed or contaminated samples should not be used in the test.

Method
1. Bring all the reagents to room temperature before use, except colour reagent (to be stored at 2-8°C, till use). Remove the required number of Microwells / strips (Reagent 8) from the packet. Label the wells appropriately.
2. Leave the reaction blank well empty.
3. Dispense 200μl of Sample diluent (Reagent 1) to the rest of the required wells. Use three Negative (Reagent 4) and one Positive (Reagent 5) controls in each run.
Add 10 µl of Negative, Positive and test serum samples to the respective wells. Mix properly and cover the strips with adhesive strip cover. Incubate for 30 minutes at room temperature (20 °C-30 °C).

4. Remove and discard the adhesive strip cover. Decant the contents of the well into a waste container. Fill the wells with diluted Washing Buffer (approx. 350 µl) and allow a soak time of 30 seconds (program the auto washer for soak time of 30 seconds) and then decant it in a waste container. Repeat for 4 more times. Drain wells on a disposable absorbent pad or towel and tap firmly to remove excess of fluid. Take care not to scratch the inner surface of the well with pipette tips or tissue paper.

5. Add 50 µl of Conjugate (Reagent 2) to each well immediately except the one used for the reaction Blank control.

6. Mix the contents of the microwells by agitating the strips gently for 5-10 seconds.

7. Cover the strips with fresh adhesive strip cover and incubate for 30 minutes at room temperature (20 °C-30 °C).

8. Remove and discard the adhesive strip cover. Wash the strip as in step 4, five times with diluted washing Buffer.

9. Add 100 µl of Colour Reagent (Reagent 6) into each well including the blank well.

10. Leave at room temperature for 15 minutes in dark.

11. Stop the reaction by adding 100 µl of stopping solution (Reagent 7) in all the wells.

12. Mix the content of the microwells by agitating the strips gently for 5-10 seconds.
13. Using either monochromatic (450nm) or bichromatic (450+630nm) mode, absorbance reading must be taken after blanking with A1 (reagent blank) well.

Calculation for determining Cutoff value

1. **Negative Control Mean (NCX)**

   Determine the mean of Negative control by taking mean of absorbance of Negative control in triplicates. The absorbance of the individual Negative Control must be greater than -0.010 O.D. units and less than or equal to 0.200 O.D. units.

2. **Positive control**

   To consider the assay run to be valid, the absorbance value obtained from the positive control should be at least 1.00 otherwise repeated.

3. **Blank**

   The absorbance value (O.D.) of the reagent blank is read and it should fall between 0.000 to 0.100.

4. **Cutoff Value**

   The cutoff value is the mean O.D. of Negative Control and addition of Factor (0.225).

   For the assay run to be valid, the Positive and Negative Controls must be always within the acceptable range. If they are not, the assay should be repeated.
Interpretation of test results

All the samples with the absorbance less than the cutoff value should be considered Negative for anti-HIV antibodies and those with absorbance equal to or more than cutoff value must be considered Positive for anti-HIV antibodies.

The specificity and sensitivity of the test is 100%.

II (a) Immunocomb (R) II HIV 1 & 2 Bispot (Orgenics, Isreal; UN AIDS/WHO, 1998).

Principle of the test

The immunocomb II HIV1 and 2 Bispot is an indirect solid phase enzyme immunoassay (EIA). The solid phase is the comb with 12 projections ("teeth"). Each tooth is sensitized at three spots:

Upper spot - goat antibodies to human immunoglobulin (internal control)
Middle spot - HIV-2 synthetic peptides
Lower spot - HIV-1 synthetic peptides

The developing plate has 6 rows (A-F) of 12 wells, each row containing a reagent solution ready for use at different step in the assay. The test is performed stepwise by moving the comb from row to row, with incubation at each step.
Kit contents including reagents

Combs

The kit contains 3 plastic combs. Each comb has 12 teeth, one tooth for each test.

Each tooth is sensitized with 3 reactive areas:

Upper spot- goat antibodies to human immunoglobulin
Middle spot- HIV-2 synthetic peptides (derived from the env glycoprotein gp36)
Lower spot - HIV-1 synthetic peptide (derived from env glycoproteins gp41 and gp120)

The combs are provided in aluminium pouches containing discarding bags.

Developing plates

The kit contains three developing plates, covered by aluminium foil. Each developing plate contains all the reagents needed for the test. The developing plates consist of 6 rows (A-F) of 12 wells each. The contents of each rows are as follows:

Row A - specimen diluent
Row B - washing solution
Row C - Alkaline phosphatase-labeled goat anti-human Ig antibodies
Row D - Washing solution
Row E - Washing solution
Row F - Chromogenic substrate solution containing 5-bromo-4 chloro-3-indoyl phosphate (BCIP) and nitro blue tetrazolium (NBT)
Positive control - 1 vial (red coloured cap) of 1 ml diluted human plasma positive for anti-HIV-1 and anti-HIV-2 antibodies, inactivated by addition of β-propiolactone and by heat treatment.

Negative control - 1 vial (green coloured cap) of 1 ml diluted heat inactivated human plasma, negative for antibodies to HIV.

Perforator - for perforation of the aluminium foil, covering the wells of the developing plate.

Storage of the kit

Store the kit in its original box at 2°-8°C. Under these conditions, the kit will remain stable until the expiry date on the label. Do not freeze the kit.

Sample

Either serum or plasma can be used.

Specimens may be stored for 7 days at 2°-8°C before testing. To store for more than 7 days, freeze the specimens at -20°C or colder. After serum specimens have thawed, centrifuge them. Test the supernatant. Avoid repeated freezing and thawing.

Procedure

To start the test the serum or the plasma specimens are added to the diluent in the wells of row A of the developing plate. The comb is then inserted in the wells of row A.
Anti-HIV antibodies if present in the specimens, will specifically bind to the synthetic peptides, on the lower and/or middle spots on the teeth of the comb. Simultaneously immunoglobulins present in the specimens will be captured by anti-human immunoglobulin antibodies on the upper spot (Internal control).

Unbound components are washed away in row B. In row C, the IgG captured on the teeth will react with anti-human IgG antibodies labelled with alkaline phosphatase (AP). In the next two rows the unbound components are removed by washing. In row F, the bound alkaline phosphatase reacts with chromogenic components. The results are visible as gray blue spots on the surface of the teeth of the comb.

The kit includes a positive control (containing antibodies to HIV-1 and HIV-2) and a negative control to be included in each assay run. Upon completion of the test the tooth showing positive control show 3 gray blue spots and that used with negative control indicates solely the upper spot. The upper spot should also appear on all other teeth, to confirm that the specimen was added, that the kit functions properly and that the test was performed correctly.

Interpretation of the results

- The sole appearance on the upper spot (internal control) indicates that the specimen is non reactive for antibodies to HIV-1 and HIV-2.
- A circular, coloured middle spot indicates the presence of antibodies to HIV-2.
- A circular coloured lower spot indicates the presence of antibodies to HIV-1.
• Sometimes high concentration of either anti-HIV-1 or anti-HIV-2 antibodies has been found to produce a faint secondary spot, in addition to the more intense major spot obtained with homologous antigen.

• In case of HIV-1/HIV-2 coinfection, two spots of equal intensities have been observed.

This test has a sensitivity of 100 percent and specificity of 99.4 percent.

II (b) Comb - Aids- RS (Span Diagnostics Ltd, Surat, India; Constantine et al., 1994)

Combination of Genetically Engineered Recombinant Protein and Synthetic Peptides for Rapid and Visual Screening of HIV-1 and/or HIV-2 Antibodies

Principle

Dot immunoassay employs the binding of coloured particles of colloidal gold to visualize the immobilized immune complex. The circular spot on each tooth of the polystyrene comb is coated with critically standardized blend of HIV-1 & HIV-2 recombinant and synthetic peptides. When incubated with a specimen containing HIV 1 and/or 2 antibodies, these antibodies bind specifically to the peptide antigens. The antibody peptide complex is directly visualized after incubation with protein-colloidal gold signal reagent. A positive result is indicated by the presence of Pungent-red coloured dot on the surface of the comb where peptides have been spotted.
Reagents

Reagent 1: Washing Buffer (5X)
Reagent 2: Colloidal Gold signal Reagent
Reagent 3: Sample diluent
Reagent 4: Negative Control
Reagent 5: Positive Control
Reagent 6: Antigen Coated Combs

Accessories

Wash reservoir, disposable plastic droppers, clamp, micro test plates, rubber teats, seal bag, adhesive strip cover.

Sample

Serum or plasma can be used. For short term storage, specimens can be stored at 2-8°C. However, they should be stored frozen (-20°C or lower) for long term storage. Grossly haemolysed or contaminated samples should not be used.

Preparation of washing solution

Dilute the concentrated washing solution 1:5 with distill water by adding 15 ml concentrate to 60 ml distill water. Fill the wash tray with washing solution. Taking care to avoid foaming. Use 75 ml diluted washing solution to wash four combs.
Method

1. Bring all the reagents to room temperature.

2. Determine the number of arms required (Samples & Controls)

3. Dilute the washing buffer.

4. Add 2 drops (0.1 ml) of sample diluent in microtest wells.

5. Pipette 0.1 ml of samples and controls into each microtest well containing Sample diluent.

6. Place the antigen coated comb into the respective wells.

7. Incubate for 10 minutes at room temperature.

8. Add 4 drops (0.2 ml) of colloidal gold signal reagent in required number of microtest wells.

9. Wash the antigen coated comb by moving the comb forward and backward 10 times in washing solution.

10. Place the antigen coated comb into the microtest wells containing Colloidal Gold signal reagent.

11. Incubate for 10 minutes at room temperature.


13. Allow the comb to air dry and note the colour development on spotted area on the tip of arms of the comb.
**Interpretation of results**

A positive result is indicated by the presence of pungent red colour spot in the area of peptides. The absence of coloured spot indicates that the sample is free from HIV 1 & 2 antibodies.

When reading the combs, examine them in moderate light, preferably with comb against a white surface. The surface of comb should be perpendicular (at 90° angle) to the eye. Do not attempt to read the comb by viewing it at other angles, as a faint, uncoloured spot may be visible which does not represent true reactivity.

The test system has a sensitivity of 100 percent and specificity of 98.7 percent.

**III. HIV tridot method (J. Mitra and Co. Ltd., New Delhi, India)**

Rapid Visual Test for the Qualitative Detection of Antibodies to HIV-1 and HIV-2 in human serum/plasma or Seperate Dots for HIV-1, HIV-2 and Control.

**Principle of the test**

HIV antigens are immobilized on a porous immunofiltration membrane. Sample and reagents pass through the membrane and are absorbed into the underlying absorbent. As the patient's sample passes through the membrane, HIV antibodies, if present, bind to the immobilized antigens. Conjugate binds to the Fc portion of the HIV antibodies to give a distinct pinkish purple DOT(s) against the white background.
Materials required

HIV TRI-DOT Test device Packed individually. Device has membrane with 1 Control and 2 Test Dots, one each for HIV-1 and HIV-2.

Buffer Solution Buffer containing BSA and Sodium Azide.

Protein-A Conjugate Protein-A conjugate in liquid form containing Sodium Azide.

Negative Control - Human serum tested non reactive for HBsAg, HCV, HIV-1 and HIV-2 and contains sodium azide.

Positive Control - Human serum Positive for antibodies to HIV-1 and HIV-2, and contains sodium azide.

Sample Dropper Long plastic dropper provided for adding the sample.

Specimen /Sample collection

Collect the blood in a clean dry sterile vial and allow to clot or separate the serum by centrifugation at room temperature. It is recommended that fresh sample should be used if possible. If the serum is not to be assayed immediately it should be stored at 4-8°C or frozen at minus 20°C (-20°C). Only serum or plasma should be used for the test.

Method

1. Add 3 drops of Buffer Solution to the centre of the device.

2. Hold the dropper vertically and add 1 drop of patient’s sample (serum or plasma) using the sample dropper provided (use a separate sample dropper for each specimen to be tested).

3. Add 5 drops of Buffer Solution.
4. Add 2 drops of Liquid Conjugate directly from the conjugate vial.

5. Add 5 drops of Buffer Solution and read results.

Read the results immediately and discard the device considering it to be potentially infectious. It is important to allow each solution to soak in the test device before adding the next solution.

Interpretation of Results

NON-REACTIVE

If only One Dot (only the Control Dot) appears the specimen is non reactive for antibodies either to HIV-1 or HIV-2. Interpret sample as non-reactive.

REACTIVE

- If two DOTS, one for the control and the other for HIV-1 appears the specimen is reactive for antibodies to HIV-1.
- If two DOTS, one for the control and the other for HIV-2 appear the specimen is reactive for antibodies to HIV-2.
- If all the three DOTS, one each for control, HIV-1 and HIV-2 appeared the specimen is reactive for antibodies to HIV-1 and HIV-2.

Performance characteristics of the test

Performance of the test with reference to sensitivity and specificity has been evaluated by National HIV Reference Centres of Government of India and WHO, Geneva and both have shown the sensitivity and specificity of the test to be 100 percent.
CD4+ and CD8+ cells counts

The CD4+/CD8+ T cells were measured using Fluorescent activated cell sorter (FACS) machine by Becton Dickinson FACS Count Work station

Materials and Methods

Materials: Vacuette tubes coated with EDTA is used for collection of patient’s blood. Electronic micropipette (Single knob) with automatic adjustment for collection of patients sample.

Sample used - is unclotted blood with EDTA in Vacuette tubes.

Vortex Genie-2, used for the vortexing purpose.

FACS count coring station - It is used to break the seal of the vortex tube.

Measuring CD4 and CD8 counts in Becton Dickinson FACS cell counter.

Procedure

1. Vortex reagent pair inverted for 5 seconds.
2. Vortex reagent upright for 5 seconds.
3. Open the reagent pair with coring station.
4. Pipette 50μL of blood into each tube. Use a FACS count electronic pipette. Refer to FACS count system user guide.
5. Cap and vortex upright for 5 seconds. Incubate for 60-120 min.
6. Pipette 50 μL fixative solution into each tube.
7. Cap and vortex upright for 5 seconds.
8. Immediately before running on FACS count instrument, vortex upright for 5 seconds.

9. CD4 and CD8 are measured from the sample.

Staining techniques

A. Gram staining

The Gram staining was introduced by Hans Gram in 1884 (Dyck et al, 1999). It is useful for the diagnosis of gonococcal and non gonococcal urethritis.

Collection of specimen for gram staining

Specimens were collected with the help of sterile cotton wool swats.

Reagents required

Gram's Crystal violet
Gram's iodine
Gram's decolorizer
Safranin 0.5% w/v (Gram's counterstain)

Method It involves three main steps:

- Preparation of smear
- Fixation of smear
- Staining
Preparation of smear

A thin glass slide wiped with a gauze piece is taken and passed it through flame twice or thrice and wiped. Two vertical lines 2.5 cm apart with a glass marking pencil are drawn on the central part of the slide. The swab containing the specimen is rolled over the marked area and spread it to make a smear of 2 x 1 cm size. The smear is labelled on the corner of the slide.

Fixation of smear

Fixing kills the organism, fixes it to the slide, prevents autolytic changes and makes the organism permeable to the dye and harmless to the person handling the smear. The slide containing the smear is held facing upwards and passed through the flame of sprit lamp two to three times. Afterwards the smear is allowed to cool.

Staining

Staining was done by Gram stains kit of HiMedia laboratories. The fixed smear is stained with Gram's Crystal Violet for 1 minute and then rinsed under running tap water. The slide is flooded with gram's Iodine for 1 minute. Again washed with water and decolorised with Gram's decolorizer until no further violet colour comes off. After washing it is counterstained with (0.5%) safranin for 1 minute. Later on washed with water and blotted and observed under oil immersion objective.
Result

A smear is considered positive for gonorrhoea when gram negative diplococci with typical morphology are identified within or closely associated with polymorphonuclear leukocytes.

B. Tzanck smear for herpes genitalis

Tzanck smear for herpes genitalis was done by Giemsa stain. Giemsa stain is a simple bedside test which can be used in diagnosis of herpes genitalis.

Collection of specimen

The intact roof of the vesicle or blister is opened along one side and folded back. Scrape the undersurface of the roof of the vesicle and the floor of the ulcer with a curette or scalpel and smear the obtained material on a clean glass slide.

Reagents

Diluted Giemsa stain: 30 drops or (3 ml) of giemsa stain is diluted with distill water to 30 ml.

Procedure

A thin smear is prepared and dried in air. The smear is fixed by covering with methanol for 3-5 minutes. The slide is stained with diluted Giemsa stain for 30-40 minutes. Drain of the stain, wash the film with distill water allowing the preparation to differentiate for 1 to 3 minutes. Dry in air and examine under microscope.
Result

The smear is examined under low power and then under the oil immersion objective. Herpes progenitalis shows multinucleated giant keratinocytes and/or balloon cells.

Histopathology

Histology was performed in some cases of genital warts and molluscum contagiosum to confirm the diagnosis. Histology was carried out using the standard technique of haematoxylin-eosin (HE) staining.

The genital growths were removed with the help of punch biopsy. Blotted free of blood and fixed in alcoholic Bouins fixative. Fixation was carried out at room temperature for 18 hours after which they were transferred to 70% alcohol. A pinch of lithium carbonate was added to remove excess of picric acid. Several changes in 70% alcohol were given until yellow colour disappeared from the section. The sections were then dehydrated by passing through ascending grades of alcohol, cleared in xylene, embedded in paraffin wax (58 to 60 M.P.) and transverse sections (TS) were taken at 5 μm on a rotary microtome (ERMA, Japan). The sections were stained with Ehrlich's haematoxylin and eosin (sprit soluble), dehydrated, cleared in xylene and mounted in DPX. Stained sections were photographed using Nikon microscope.