

***BASIC LABORATORY  
PROCEDURE MANUAL***

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## **PREFACE**

The purpose of this manual is to be a concise guide to basic laboratory tests that can be done with minimal equipment, training and little or no electricity. The manual can be used to train basic laboratory personnel and/or used as a reference for those already operating in the field. Included in this book are both the standard manual field methods and more updated methods that can be incorporated in a basic laboratory which will allow the lab worker to become more efficient. The manual was designed to be used in conjunction with the Comprehensive Laboratory Atlas which should alleviate uncertainty in laboratory diagnosis.

This version of the manual should be viewed as a draft effort. It was developed in consultation with various organizations concerned with health, education, and lab practices in both Pakistan and Afghanistan. If this manual is found useful for laboratory workers and for a teaching reference, translated versions can be considered.

Many thanks to G. Mohyuddin and M. Yaqub for the many hours spent typing the manuscript.

**Carol L. Phillips**

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# **GENERAL INFORMATION**

## BASIC EQUIPMENT

Binocular Microscope (10X/40X/100X lenses)

Hand centrifuge

Spirit lamp/bunsen burner

Microscope slides

Coverslips

Staining dishes with lids

Cells counter

WBC pipette

Hemacytometer

Sahli hemoglobinometer

Sahli pipette

Tubing/mouthpiece

Disposable lancets

Cotton wool

Immersion Oil

Lens paper/air brush

Iodine (lugol's)

Sodium chloride

Reagent Containers w/Lids

Xylene

Wash Bottles

Drying Rack for Slides

Timer

Test Tube Stand

Test Tube Holder

Beakers

Measuring Cylinders

5 and 10 ml Pipettes

Conical Centrifuge Tubes

Specimen Containers

Wooden Applicator Sticks

Matches

Reagents/Stains-depend on size of lab, preference of the medical officer, and skills/training of lab worker.

Note: This is only a basic list. Quantity and quality will depend on the individual facility.

## **THE RESPONSIBILITY OF THE LABORATORY PERSONNEL**

The laboratory worker is responsible to perform laboratory tests according to their own capabilities and those of the facility in which they are working. These tests should be precise as well as accurate to provide the doctor (or their representative) proper information in order to benefit the patients. The lab personnel, like the doctor, must regard the information gained from the course of their daily work, as strictly confidential. Only the doctor or his representative should receive the test results, this includes the patients themselves.

## LABORATORY RULES

1. Always use the *proper* test methods; do not make up your own.
2. Refer to the manual for procedure questions.
3. Say when you don't know something.
4. When in doubt, ask for a second opinion.
5. *Always* confirm irregular results (repeat the test if necessary).
6. Always maintain your equipment.
7. Learn to work *quickly* and *accurately*.
8. Keep your work area clean.
  
9. Never give a patient results. Results are given only to the doctor or nurse requesting the tests.
10. Keep your registers neat and simple.
11. Always follow manufacturer's instructions when using equipment, reagents, etc.
12. All reagents should be clearly labeled.
13. When making new reagents, label should include:
  - name of reagent
  - date made
  - date expires
  - your initials
14. Do not disregard expiration dates.



## SAFETY RULES

1. Always add acid to water, slowly.
2. Keep acids and alkalis on the lower shelves of cupboard. Always hold the bottle upright when removing or storing. Your hands should always be dry.
3. Never pipette by mouth.
4. Never heat the bottom of test tube. Heat the middle of the tube, shaking gently with the mouth of the tube pointed away from you.
5. Never place inflammable liquids (acetone, ethanol benzene, toluene, ether) near on open flame. (Bunsen burner, spirit lamp, etc).
6. Always light the match and hold it to the burner before turning on the gas.
7. Always turn gas off at the end of a workday or when you are leaving the area for a long period of time (lunch, prayer, etc).
8. Wash your hands after drawing blood or handling specimens. (stools, urine, sputum).
9. Never eat or drink in the laboratory area.
10. Keep all chemicals out of the reach of children.
11. Be familiar with first aid practices for laboratory accidents.
12. Know where first aid supplies are kept.
13. Label all hazardous chemicals.
14. Work cautiously around an open flame.
15. Always use proper methods to dispose of laboratory waste such as specimen containers, used needles and lancets, used gauze, etc.

## FIRST AID FOR THE LABORATORY

Most often, accidents in the laboratory are caused by carelessness, rushing and untrained personnel.

Items that incur injury are:

1. Acids (nitric, sulfuric, hydrochloric, etc).
2. Alkalis (sodium, potassium, ammonium hydroxide, lye, etc).
3. Toxic substances, inhaling toxic fumes (gases), or accidental swallowing by pipetting.
4. Heat (flammable solutions, hot water, bunsen burners, etc).
5. Broken Glass (pipettes, beakers, slides).
6. Electricity (faulty equipment, wet hands etc).

To minimize injury, the following items should always be found in the laboratory.

1. Wash bottles containing clean water.
2. Fire blanket.
3. Tincture of iodine.
4. Adhesive bandages.
5. Cotton wool and gauze.

This equipment should always be readily available. **DO NOT** lock in a cupboard.

Refer to your physician in charge for first aid measures he wishes you to employ to minimize damage to the accident victim.

## LABELING AND LABORATORY RECORDS

All specimens that come to the lab must have a request form and be given a number or some other form of identification.

Numbering the specimens:

- This should be done immediately.
  - (a) on the specimen
  - (b) request form
  - (c) slide
  - (d) any test tubes that will be used

This will prevent mistakes.

*Record all results* in the appropriate register.

Hematology (Hgb)  
Parasitology (Malaria, stools)  
Urinalysis (Urine)  
Bacteriology (TB)

## CLEANING GLASSWARE

Rinse all used glassware in cold or lukewarm water *before* residue dries in the glass. Never rinse blood stained tubes in hot water.

Soak glassware in water mixed with soap. (Wash powder or liquid detergent) for 2-3 hours. Be sure to clean the insides with a test tube brush.

Remove individually and rinse thoroughly with tap water. Make sure no soap residue is left that will interfere with testing.

Place containers (beakers, flasks, etc) on a draining rack to drip dry. Place tubes upside down in a wire basket. Place the basket in a hot air oven at 60°C or cover with a clean cloth and place in a sunny place to dry.

All clean glassware should be stored in a cupboard to protect it from dust. If possible, all beakers and flask tops should be plugged or covered.

### Pipettes

- Rinse immediately after use in a stream of cold water.
- Soak in soapy water.
- Rinse with tap water to remove soap and then rinse with acetone and blow dry.

## DISPOSAL OF SPECIMENS

All specimens that are brought to the lab should be considered "infected material".

Specimens may be:

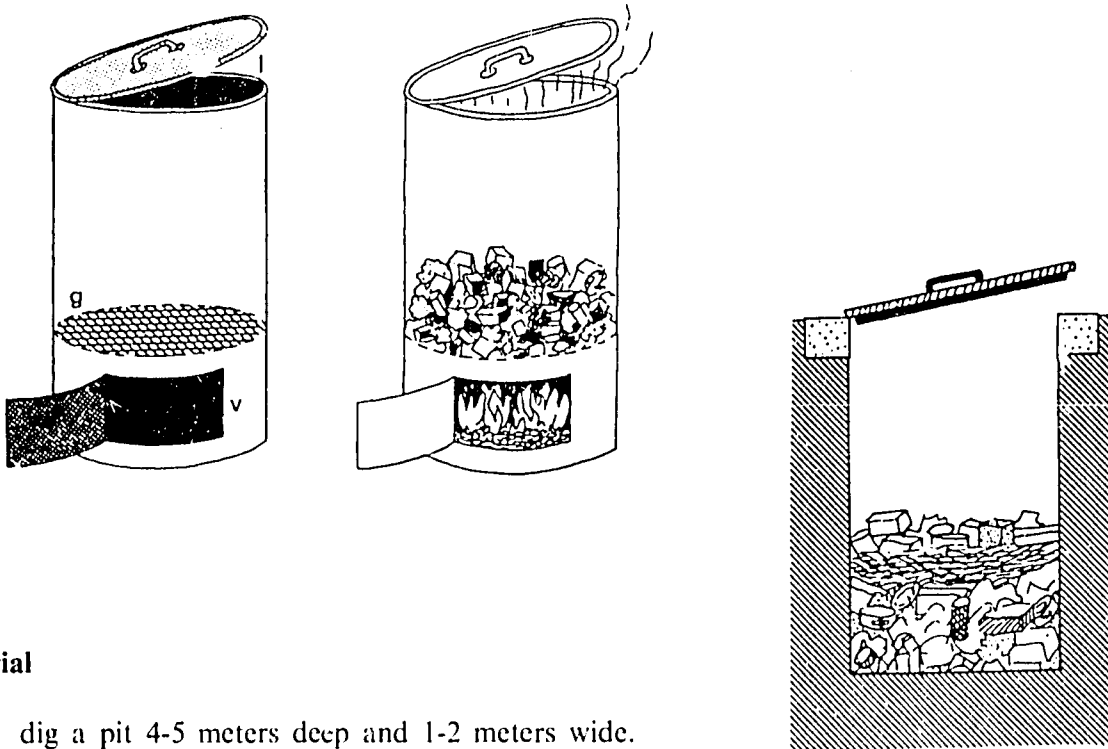
- burned (incinerated)
- buried
- boiled

Depending on the container (disposable) or methods at your lab.

Incineration is the easier and most effective method.

- use an old metal drum.
- removable lid.
- fix a metal grate about  $\frac{1}{3}$  the way up to drum.
- cut a vent in drum below the grate.

The incinerator should be closed (lid and vent) when not in use.



### Burial

- dig a pit 4-5 meters deep and 1-2 meters wide.
- make a lid that fits tightly over the pit.
- strengthen the upper rim using bricks or stones.
- throw infected material into the pit and replace the lid.
- at least once per week cover the refuse with a layer of dried leaves or quicklime (if available).

## NON-DISPOSABLE CONTAINERS

### Stool

- fill the containers with 5% phenol.
- leave for 24 hrs.
- empty into lavatory (toilet).
- clean with soap and water.

### Sputum

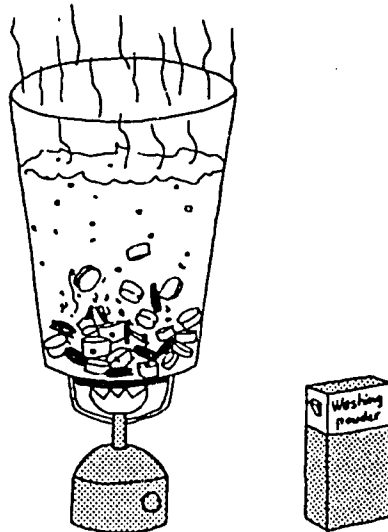
- Pour into each container 10 ml of 10% formalin solution *or* 5 ml of 5% phenol.
  
- leave for 24 hours.

### Boiling in detergent

- keep a pan *only for this purpose*.
- *boil for 30 minutes.*
- *water should be a strong solution of detergent.*

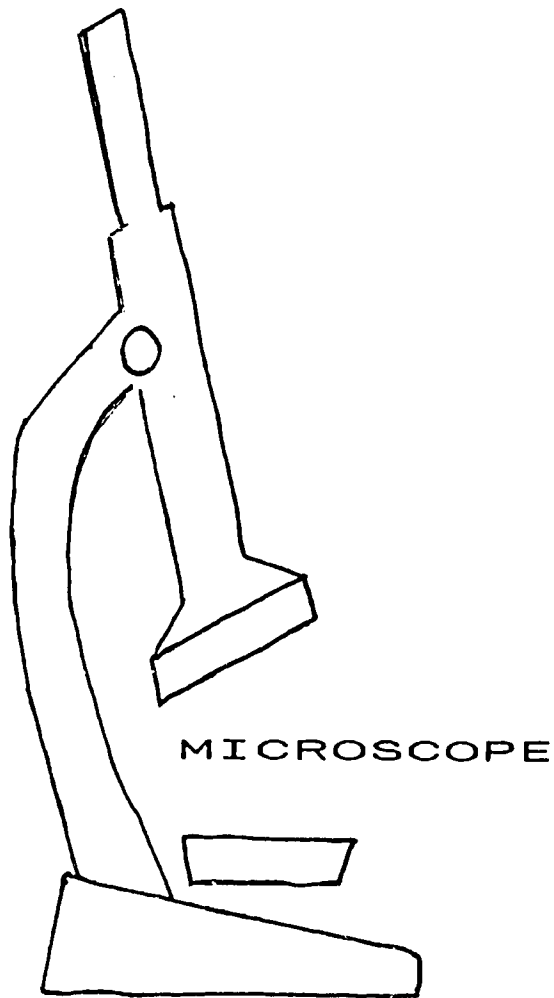
### Urine

- empty bottles in lavatory.
- fill with 10% solution of bleach.
- leave for 24 hrs.



Note: Disposable containers require less work and are easier to get rid of.

Are the above containers always cleaned properly?



## MICROSCOPE USE AND MAINTENANCE

A clinical laboratory without a microscope or one that is not properly maintained is not really a laboratory.

Most of the basic test procedures discussed in this manual include a microscope at one point or another.

Learn your microscope well and keep it properly maintained and your clinical laboratory will be efficient.

### Components of the Microscope

1. The support system:

- (a) the base
- (b) the arm
- (c) the stage
- (d) the nosepiece

2. The magnification system:

- (a) The objectives
  - (1) X10 objective (low power)
  - (2) X40 objective (high power)
  - (3) X100 objective (oil immersion)
- (b) The eyepieces — the magnifying power is marked.
  - (1) X4 eyepiece — magnifies 4 times.
  - (2) X6 eyepiece — magnifies 6 times.
  - (3) X10 eyepiece — magnifies 10 times.

3. The illumination system:

- (a) The mirror reflects rays from the sun.
  - (1) Plane surface
  - (2) Concave side — forms a low power objective — not used if a condenser is present.
- (b) The condenser — brings the rays of light to a common focus on the object.
  - (1) found between the mirror and stage.
  - (2) *raised* for maximum light.
  - (3) *lowered* for minimum light.
- (c) The diaphragm
  - (1) within the condenser.
  - (2) used to reduce or increase the angle and amount of light that passes into the condenser.

4. The adjustment system:

- (a) Coarse adjustment — used first to get an approximate focus.
- (b) Fine adjustment — brings object into perfect focus.
- (c) Condenser adjustment — used to bring in more or less light.
- (d) Iris diaphragm — small lever on the condenser used to open or close the diaphragm.

Note: There is an instruction manual that comes with each microscope. Use it to identify the 4 different systems. Always keep book and microscope together.

**Focusing**

1. Low Power (X10 objective)

- Condenser is down.
- Lower objective until it's just above the slide.
- Use coarse adjustment to find the object.
- Fine adjustment for perfect focus.

2. High Power (X40 objective)

- Condenser should be halfway down.
- Use coarse adjustment till blurred image appears.
- Bring into focus with fine adjustment.

Note: If microscope has no condenser, use the concave side of the mirror.

3. Oil Immersion (X100 objective)

- Place a drop of immersion oil on slide.
- Condenser is up and diaphragm is fully open.
- Lower X100 until it comes into contact with oil.
- Use fine adjustment *slowly* until object is in focus.
- Use a blue filter for better resolution.

Images seen using the microscope are seen in a circle of light known as the “microscopic field”.

To establish the position of the object, use the hands of a clock.

For example, a parasite is at “6 O'clock”.

Before changing objectives, be sure object is in the *middle* of the field.



## **Maintenance**

Your microscope needs daily attention to keep it in good working order.

## **Materials**

1. Lens paper or tissue paper
2. Soft *clean* cloth
3. Xylene
4. Microscope cover (plastic)
5. Fine paintbrush or rubber bulb to clean lenses.

## **Cleaning Objectives**

X10 — Wipe with lens paper or clean cloth. Wipe across not in a circle.

X40 — Same as above.

X100 — Remove oil with lens paper dampened slightly with Xylene. Wipe again with clean paper. This should be done *daily*.

*In hot dry climates* — objectives may need cleaning throughout the day. Use a fine brush or bulb to clean.

## **Cleaning Eyepieces**

*Only* clean the surface where your eye is placed.

Cleaning inside the eyepiece only allows more dust to enter.

Use lens paper and/or a fine brush.

## **Cleaning Condenser/Mirror**

The mirror is cleaned with a soft cloth moistened with alcohol.

The condenser is cleaned the same as the objectives.

## **Cleaning Painted Surfaces**

Support and Stage — Use soft cloth. No alcohol or Xylene.

Use a damp cloth with plain water if needed and be sure surface is dry before putting the microscope away.

### **DO's**

1. Keep the microscope covered when not in use.
2. Clean oil immersion lens daily.
3. Clean oculars (eyepieces) with soft lint free cloth or lens paper.
4. Follow manufacturer's instructions when a question or problem arises.
5. Always carry a microscope with 2 hands.
6. Keep fingers away from objectives and eyepieces.

### **DON'Ts**

1. Don't use alcohol to clean painted surfaces.
2. Don't dismantle microscope.
3. Don't exchange lenses with other microscopes.
4. Don't store in direct sunlight.
5. Never store microscope with condenser up and oil immersion object in center.
6. Don't use microscope on an uneven or wobbly counter.
7. Never keep microscope in a wooden box in hot, humid climates.

# URINALYSIS

## URINALYSIS

In order for urinalysis to be meaningful, the urine must be properly collected. Improper collection may invalidate the results of the laboratory tests no matter how carefully the tests are performed. There are many types of containers that can be used for collecting urine. Regardless of the type, they must be capable of being *cleaned and thoroughly dried* before specimens are collected.

### Methods of obtaining Specimens:

#### (a) Freshly voided (random)

- Adequate for most tests except for microbiological cultures.
- The patient should be instructed to void directly into a clean, dry container or bedpan (transfer to appropriate containers).
- Most common.

#### (b) Midstream Clean Catch

- Suitable for microbiological cultures and chemical testing.
- Avoid contamination with vaginal discharge or menstrual blood.
- The female patient should be instructed to clean the vaginal area using a front to back motion.
- A small amount of urine is then passed into the toilet or bedpan to be discarded.
- The urine is then collected in a clean, dry container, covered and taken to the laboratory.

#### (c) Postprandial

- Used for glucose testing.
- The patient is instructed to have a meal, 2 hours later they return to the laboratory and give a specimen.

#### (d) First Morning Void

- Most concentrated.
- Best used for protein, nitrite, pregnancy testing, and microscopic exam.
- The patient is given a container or told to obtain one proper for urine collection. They are then told to collect the first urine upon waking.

Urine specimens should be examined within one (1) hour. When the examination is delayed:

Bacteria will use the glucose and multiply, accordingly:

- the glucose reading may be inaccurate
- the urine may turn alkaline
- casts may decompose
- red blood cells may lyse

If refrigeration is available, place the specimen there until the examination can be done. If not, the patient should be asked for another urine specimen.

## PHYSICAL PROPERTIES OF URINE

### 1. Appearance:

This is the first observation made on a urine specimen. Most facilities use the standard terms of:

- Clear
- Hazy
- Cloudy

### 2. Colour:

The colour of urine is affected by many elements: concentration, food, blood, disease and chemicals. The intensity of the colour of normal urine depends on the concentration.

Normal Colour:

- Colourless (very dilute urine)
- Straw
- Yellow

Abnormal Colour:

- Amber — hepatitis
- Brown — old blood, bile
- Red — menstrual blood (can be normal if red beets (beetroot) are eaten)
- Black — “Blackwater fever” *P. falciparum*

### 3. Turbidity:

Normal freshly voided urine is usually clear or transparent. If the urine is alkaline (pH greater than 7), it may appear turbid due to the presence of phosphates or carbonates. Abnormal turbidity may occur with urinary tract infections.

### 4. Volume:

The normal volume of urine voided by an adult in a 24 hour period ranges from 750 to 2,000 ml. The average is about 1,500 ml. The amount voided is directly related to the person's fluid intake, temperature and climate, and the amount of perspiration that occurs.

## CHEMICAL PROPERTIES OF URINE

Specific Gravity	Bilirubin
pH	Nitrite
Protein	Ketones
Glucose	Urobilinogen
Blood	Leukocyte Esterase

Chemically — impregnated reagent strips are available for rapid determination of the urine chemical properties as stated above. These strips are used in basic urinalysis and have virtually replaced older, more cumbersome and time consuming methods.

### Specific Gravity: (SpG):

- measures the concentrating and diluting abilities of the kidney.
- is needed to interpret the results of most tests performed in routine urinalysis.
- highest in the morning (greater than 1.020).
- normal values usually range from 1.010 to 1.025.

### Clinical Significance:

#### Low:

- Diabetes insipidus (most severe example) SpG usually ranges from 1.001 to 1.003.
- glomerulonephritis
- pyelonephritis

#### High:

- adrenal insufficiency
- hepatic disease
- congestive cardiac failure
- excessive loss of water (diarrhea, sweating, fever, etc)

#### pH:

- regulates acid — base balance of body
- helps in identification of crystals
- acid urine needed for case formation

### Clinical Significance:

#### Acid urine (lower than 6.0)

- acidosis and/or uncontrolled diabetes mellitus
- patients on high protein diets

**Alkaline Urine:**

- normal after meals due to HCL in gastric juices
- renal tubular acidosis
- urinary tract infections
- renal stone formation (phosphate, calcium carbonate)

**Glucose:**

- most common sugar found in urine
- glucosuria occurs when blood glucose level exceeds the reabsorption level of the renal tubules (renal threshold)

**Clinical Significance:**

- after heavy meals
- high emotional stress

**Pathological glucosuria:**

- diabetes mellitus — most common cause.

**Ketones:**

- improper fatty acid metabolism

**Clinical Significance:**

- diabetes mellitus
- restricted carbohydrate intake (fever, anorexia, fasting, starvation, diets)

**Protein:**

- Proteinuria is an increased amount of protein in the urine.
- important indication of renal disease.
- can be found in association with extreme physical exertion.

**Clinical Significance:**

- nephrotic syndrome
- lupus
- congestive heart failure
- multiple myeloma

**Functional:**

- fever
- exposure to heat or cold
- emotional stress

**Blood:**

- indicates damage to kidney or urinary tract
- may appear as intact cells or hemoglobin
- normal in women during menses

**Clinical Significance:**

- infectious disease
- transfusion reaction
- hemolytic anemia
- following severe burns
- various poisonings

**Bilirubin:**

- indicates hepatocellular disease or biliary obstruction.

**Clinical Significance:**

- hepatitis
- jaundice
- hemolytic anemia
- malaria

**Urobilinogen:**

- small amounts normally found in urine.

**Clinical Significance:**

- malaria
- hemolytic anemia
- pernicious anemia
- hepatitis
- congestive heart failure

**Nitrite:**

- If found in urine always indicates bacteriuria.

**Clinical Significance:**

- indicates infection in persons that experience no symptoms
- early warning useful for:
  - pregnant women
  - diabetes
  - elderly patients



## MULTIPLE-REAGENT STRIPS

### Advantages:

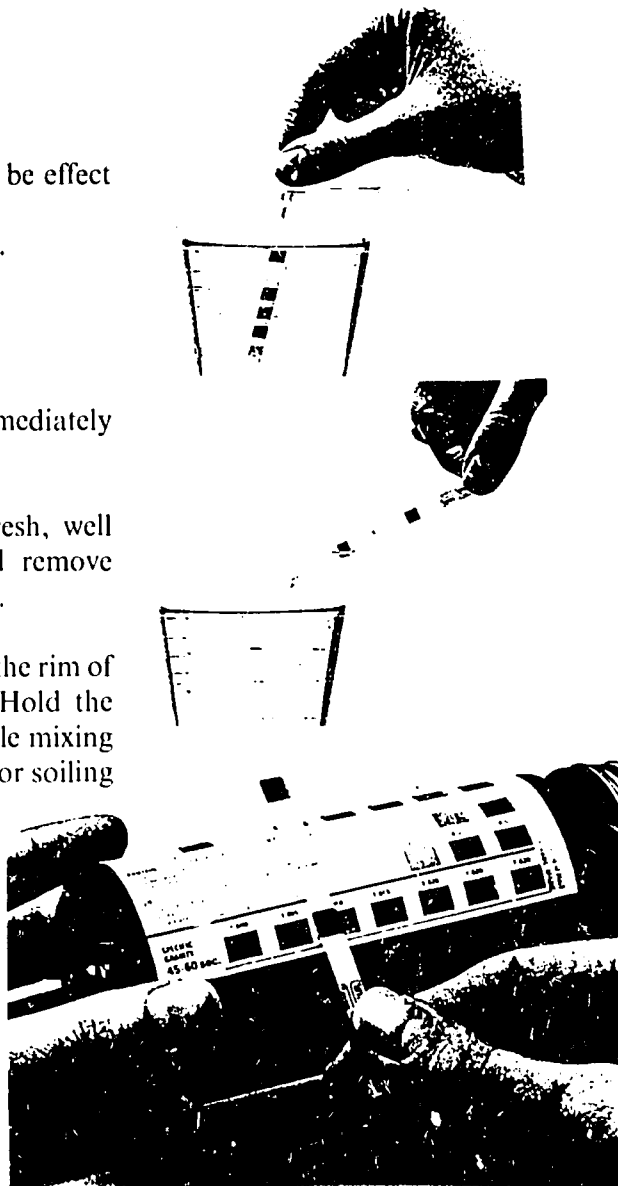
- Little specimen is needed.
- 1 minute to complete all tests.
- All reactions are a definite color change.
- No reagents to maintain.
- No equipment needed except the container the strips are stored in.
- No refrigeration.
- No temperature correction (SpG).

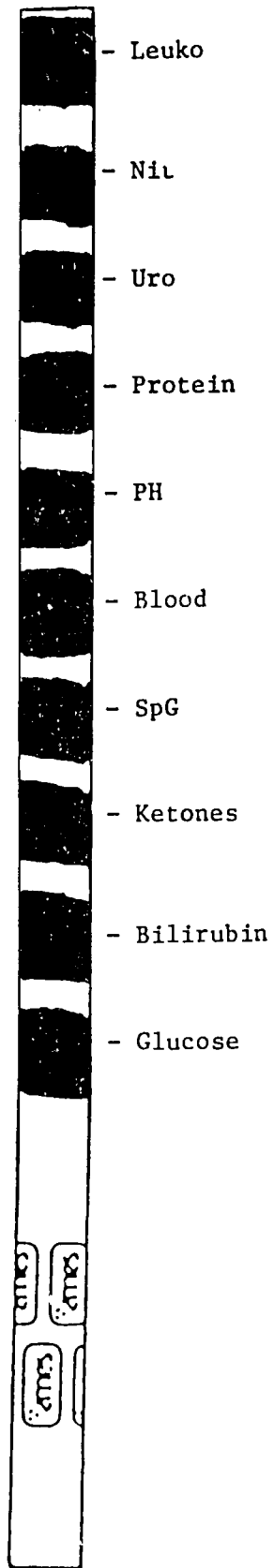
### Disadvantages:

- container must be air tight, moisture will be effect the chemical pads.
- Expiration date is sometimes a problem.
- Colorblindness.

### Procedure:

1. Remove a strip from the container and immediately close the lid.
2. Completely immerse all reagent areas into fresh, well mixed, uncentrifuged urine. Dip briefly and remove immediately to avoid dissolving out reagents.
3. While removing the strip, run the edge against the rim of the urine container to remove excess urine. Hold the strip in a horizontal position to prevent possible mixing of chemicals from adjacent reagent areas and/or soiling of hands with urine.
4. Compare test areas closely with corresponding colour chart on the bottle label at the time specified. Hold strip close to colour blocks and match carefully.
5. You are ready to spin the urine for the microscopic exam.





## **PRECAUTIONS USING REAGENT STRIPS**

Reagent test strips are sensitive chemical tests and need to be kept in good condition. Incorrect storage and handling will cause deterioration; strips in poor condition will give unreliable results.

Do read package insert carefully before.

Do replace cap immediately and close tightly.

Do check expiry date on bottle before use.

Do ensure that containers are absolutely clean and free from contaminants, e.g. antiseptics or detergents.

Do Use only freshly voided, uncentrifuged urine.

Do store reagents in cool, dry place. Do not store in a refrigerator.

Do Not remove descant from bottle.

Do Not touch test areas of the strip.

Do Not take out more strips than are required for immediate use.

Do Not transfer strips from one bottle to another.

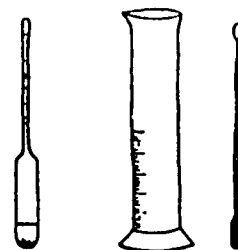
Do Not acidify, centrifuge, or filter specimens prior to testing.

## SPECIFIC GRAVITY (URINOMETER)

### Materials:

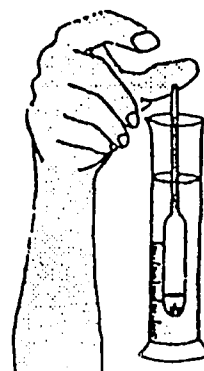
Urinometer  
measuring cylinder (50 ml)  
thermometer

At least 40 ml of urine is required. (If this method is used, inform the patient of the lab's requirement).

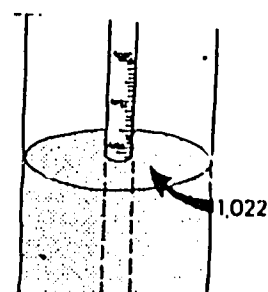


### Procedure:

1. Pour 40 ml of urine into the cylinder.
2. Lower the urinometer gently into the urine and release.
3. Wait for it to settle. It must not be touching the sides or bottom of cylinder.
4. Read the SpG given on the scale at the surface of the urine.



5. Remove the urinometer and take the temperature of the urine at once with the thermometer.
6. Temperature correction.
  - urinometer is calibrated at 20°C.
  - add 0.001 to the reading for each 3°C above the standard (20°C).
  - subtract 0.001 for each 3°C below 20°C.
  - glucose-subtract 0.004 from reading for each 1% of glucose.
  - protein-subtract 0.003 from reading for each 1% of protein.



Note: The above correction are necessary only when SpG is critical, as in urine concentration tests.

**Results:**

Range of equipment	1.000 — 1.035
Adult and child range (normal fluid intake)	1.015 — 1.025
Infant range	1.012 — 1.025

A low figure is not significant if the patient drank a large amount of fluid prior to testing.

**MEASUREMENT OF pH**

Normal freshly passed urine is slightly acid (around 6.0). When urine stands at room temperature or warmer for sometime it may be alkaline. Following a meal, urine becomes more alkaline due to gastric acid being secreted. Diets high in fruit and vegetables and low in meat protein may produce alkaline urine. High meat diets cause persistently acid urine.

<u>acid</u>	<u>neutral</u>	<u>alkaline</u>
0	7	14

**Procedure (Indicator Paper)**

1. Tear off a strip of the indicator paper.
2. Dip one end in and out of the urine.
3. Remove excess urine.
4. Compare the colour change according to the standard chart.

**Multiple reagent Strip (5.0-8.5)**

1. Remove strip and immediately close container.
2. Dip in and out of urine and remove excess on absorbent paper.
3. Compare to colour chart.
4. Record results.

Normal pH 5.0-7.0 (usually around 6.0)

## PROTEIN DETECTION IN URINE

### Method:

30% Sulfosalicylic Acid.

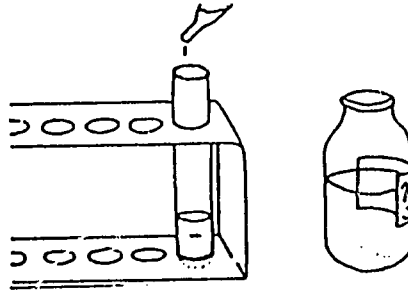
When sulfosalicylic acid is added to urine containing protein, a white precipitate forms. The urine must be clear. If it is cloudy, it should be filtered or use the supernatant fluid from a centrifuged specimen.

### Materials:

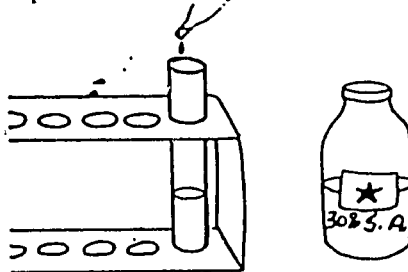
- Test tubes
- Test tube rack
- Graduated 5 ml pipettes
- 30% sulfosalicylic acid

### Procedure:

1. Pipette 5 ml of urine into a clear test tube.



2. Using a dropper, add 2 drops of sulfosalicylic acid solution to the urine.

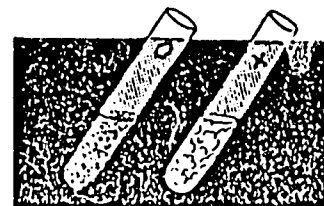


3. Compare with a tube of untreated urine against a black background.

### Positive Result

A white precipitate forms on the addition of reagent.

- |      |   |                       |
|------|---|-----------------------|
| +    | = | Trace                 |
| ++   | = | Small amount          |
| +++  | = | Moderate amount       |
| ++++ | = | Large amount (opaque) |



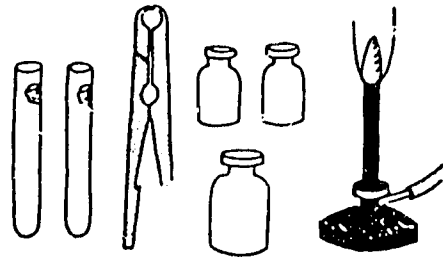
### Negative Result

No white precipitate forms. Solution remains clear.

## GLUCOSE DETERMINATION URINE Benedict's Method

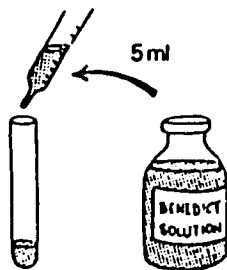
### Materials:

- Pyrex test tubes (for heating)
- Test tube holder
- Beaker
- Bunsen burner/spirit lamp
- Bottles
- Pipette
- Benedict's solution

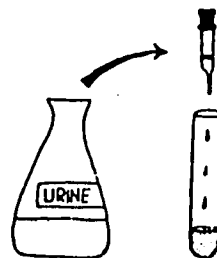


### Procedure:

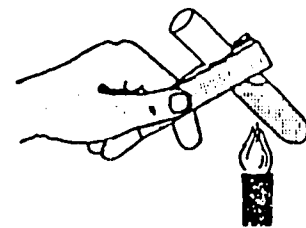
1. Pipette 5 ml of Benedict solution into clean test tube.
2. Add 8 drops of urine and mix well.
3. Boil over a bunsen burner or spirit lamp for 2 minutes. Keep the test tube at an angle. A can of boiling water can be used. The test tube is put in it for 5 minutes.



No. 1



No. 2



No. 3

4. Allow mixture to cool to room temperature.

<u>Colour</u>	<u>Result</u>	<u>Concentration (mmol/L)</u>
Blue	Neg	0
Green	Trace	14
Green/Yellow	1 +	28
Yellow/DR/Green	2 +	56
Brown	3 +	83
Orange/Brick Red	4 +	111 or greater

## URINE SEDIMENT

Urinary sediment is microscopic elements contained in urine suspension. These elements are collected upon standing or light centrifugation. The sediment should be examined within 3 hours.

### Elements

WBCs	Yeast
RBCs	Bacteria
CASTS: Hyaline	Parasites
Granular	Crystals
WBC	Foreign Debris
RBC	Spermatozoa
Epithelial	Mucus
Waxy and Broad	
Fatty	

### Urine Collection

Mid-stream urine and cath specimens are best for sediment detection because most of the contamination is gone. Patients should be instructed on the mid-stream method. A sterile container is the one of choice.

### Materials

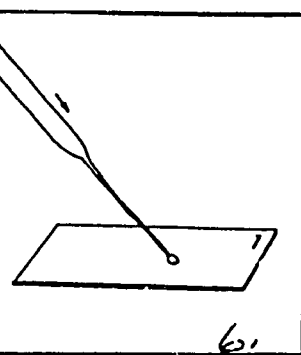
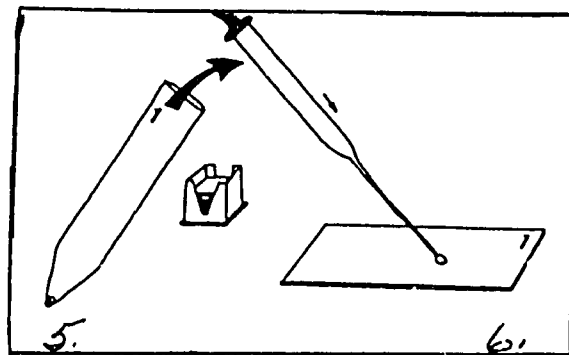
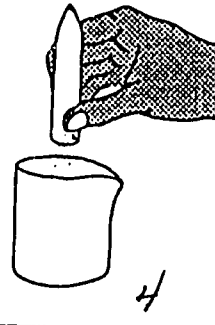
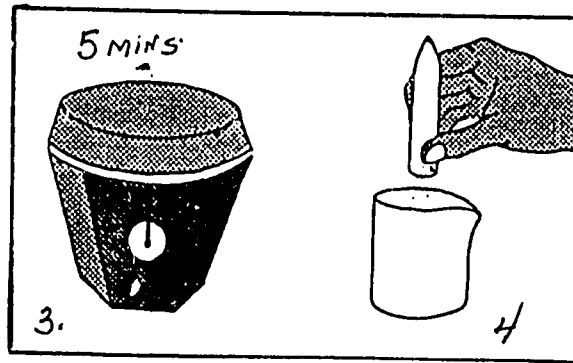
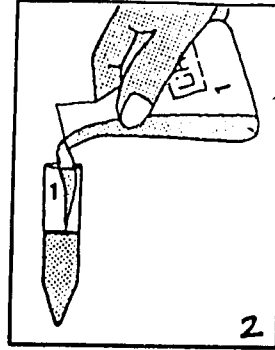
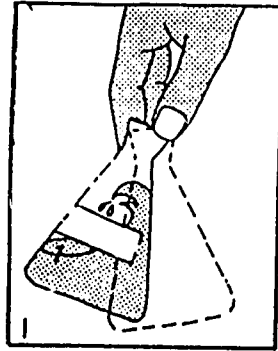
- electric or hand centrifuge.
- 15 ml conical centrifuge tubes.
- dropping pipette.
- Slides and coverslip 20 × 20 mm
- Microscope

### Procedure

1. Mix urine gently
2. Pour approximately 12 ml of urine into conical tube.
3. Centrifuge at medium speed for 5 minutes.
4. Pour off supernatant by inverting the tube quickly. Do not shake.  
Note: the supernatant can be used for chemical testing.
5. Shake the tube to resuspend the sediment.
6. Using a pipette, place one drop on the slide and cover with a coverslip.
7. Examine at once. Drying will distort the elements in the sediment.
8. Use the X10 objective first, using low light (condenser down), scan the sediment for casts, cells, mucus; etc.
9. Use the X40 objective to identify cells, yeast, presence of bacteria and crystals and to quantitate these elements. When using the X40 objective, remove the colored filter but do not raise the condenser.

Note: Report all elements seen.





## RBCs

- (a) Intact — small yellow/green disc darker at the edges.
- (b) Crenated — spikey edges usually smaller in diameter than intact.
- (c) Ghost — Swollen RBC's with a thin edge, may be very hard to see.

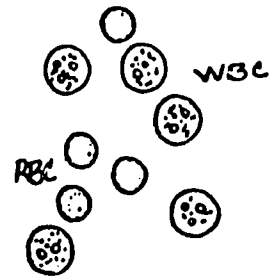
There are normally no red cells in the urine, they may be found in the urine of women if specimen is taken during the menstrual period.

RBC's may be confused with other elements.

- Only RBC's appear coloured
- Yeast cells bud
- Urate crystals — vary in size
- Air droplets — vary in size and are refractile

## Indicate

- Renal tumors
- Kidney stones
- Tuberculosis
- Prostatic conditions
- Bleeding and clotting disorders
- Tremendous stress to Kidney (excessive running)



## WBC's

- (a) Intact — fine granular disc — nucleus may be visible.
- (b) Degenerated — distorted, shrink
- (c) Pus — Clumps of degenerated WBC's

A few WBC's are normal. The presence of many leukocytes indicate infection.

## Indicate

- Pyelonephritis (protein present)
- Lower urinary tract infection (protein absent)
- Present without bacteria but with blood — tuberculosis of the kidney

## Squamous Epi Cells

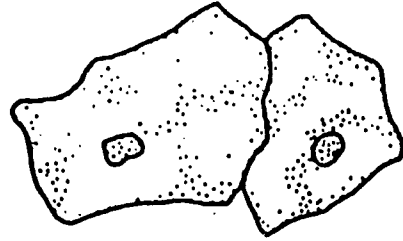
- Large, irregularly shaped cells
- Fine granulation
- Nucelus usually visible
- Come from the normal shedding of the epithelial lining of the urinary tract and organs (Ureter or vagina)

## Indicate

- Improper specimen collection
- Urine not clean catch

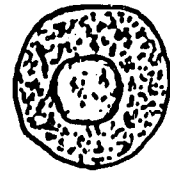
## Bladder Cells

- Large granular cells
- Often have pointed ends
- Distinct nucleus



## Renal Cells

- Smallest of epithelial cells
- Twice as large as WBC
- Granular with a distinct, sometimes refractile nucleus
- Usually protein in urine when these cells are present



Note: Most epithelial cells do not need to be identified other than “epi cells”. However, if renal cells are present, this should be noted for the doctors information.

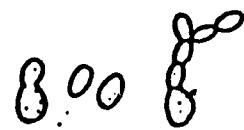
## Yeast

Do not confuse with RBC.

- Small, round to oval bodies that can be seen budding
- Colourless
- Red cells do not bud
- Not soluble in acetic acid, RBCs are

## Indicate

- Possible yeast infection (usually female)
- Old specimen
- Sometimes present when glucose is detected



## Bacteria

- Significant only in properly collected specimen that is examined promptly-probably urinary tract infection

## Spermatozoa

- Can be found in both male and female urine (most often male)
- Very small round to oval head, long flagella
- Motile in very fresh specimens



## Indicate

- Improper specimen collection
- Prostrate problems
- Recent sexual activity

## Trichomonas (Vaginalis)

- Protozoan that causes a genitourinary discharge
- Usually found in women but can be found in men
- Size of a white cell
- Motility is a rapid jerky motion
- 4 flagella
- Undulating membrane that seems to vibrate
- Nucleus at the flagella end, looks like an “eye”



## Casts

- Formed in the distal and collecting tubules
- Protein necessary for their formation
- Urinary pH (must be acid to agglutinate the protein)
- Time for urine to concentrate (high SpG)
- Parallel sides with round to blunt ends
- Formed during renal disease

## Hyaline Casts

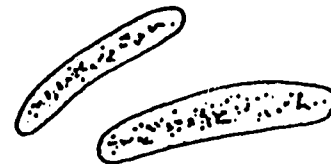
- Almost transparent and slightly refractile
- Must use low light or they will not be seen
- Disintegrate in alkaline solution
- May contain a few cells or debris that was present during formation. (less than 1/3 full)
- *Can be found* in normal people after exercise



## Granular Casts

### (a) Course granular

- Short casts filled with large granules
- Sometimes yellowish in colour
- Easy to locate on slide



### (b) Fine granular

- Short casts with small granules
- Not as refractive
- Not always filled with granules
- Do not confuse with hyaline casts

Both types are probably a degeneration product of epi cells, RBC, or WBC. Further degeneration may result in a waxy cast. Significant if found in large numbers.

### Waxy Casts (Rare)

- Smooth, homogeneous and opaque
- High refractive index
- May have a yellow colour
- Frequently short, broad and appears to be breaking



### Indicate

- Severe kidney disease
- Advanced renal failure

### Cellular Casts

Always clinically significant



### WBC Casts

- Originates only in renal tubules
- Do not confuse with clumps of WBCs
- Parallel sides round to blunt ends and *at least*  $\frac{1}{3}$  -  $\frac{1}{2}$  full of WBC's
- Must identify using X40 objective
- Sometimes hard to distinguish from mixed cell casts

### Indicates

- Pyelonephritis
- Glomerular disease

### RBC Cast (Rare)

- May appear colourless if only a few RBC's are present
- Degenerated RBC's will give the cast an orange-red colour
- If no cell margins are seen it is sometimes referred to as a hemoglobin or true blood cast

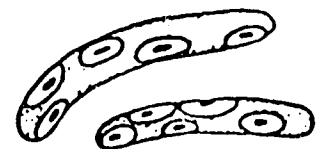
### Indicates

- Glomerulonephritis
- Vasculitis
- Rule out collagen disease in young females



### Epithelial Cell Cast (Rare)

- Larger than WBC Casts
- Cells are usually in 2 rows
- Cells generally more degenerated than WBC's
- Sometimes casts appear to be pale yellow



(a drop of 10% acetic acid will make cells more visible)

## Indicates

- definite evidence of tubular disease
- acute tubular necrosis

## Fatty Casts (Rare)

- usually large
- very refractile (black globules)
- associated with moderate to heavy proteinuria

## Indicate

- glomerular disease-nephrotic syndrome

## Casts

- scan on X10 objective using low light (condenser down) and coloured filter (if available)
- report all casts seen as number per low power field. Example = 0-2 hyaline/LPF
- Switch to X40 objective for positive identification

## Mucus threads

- thread-like, transparent forms of protein that precipitated from tubules
- low refractive index
- report amount as light, moderate, heavy
- usually associated with proteinuria
- can be seen after exercise



## Foreign Substances

- from dirty slides and/or coverslips
- specimen exposed to air you find the following:

Cotton fibers  
hair  
pollen grains  
oil droplets  
air bubbles  
starch granules (talc powder)

These substances cause problems for lab workers because at times they can be easily confused with casts, crystals and cells. Experience will eliminate most of this.

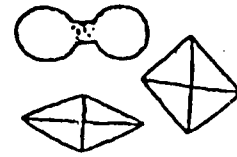
## Crystals

Crystals can be divided into 2 groups, acid crystals and alkaline crystals. All crystals except amorphous sediment have a somewhat regular geometric shape.

## Acid Crystals

### (a) Calcium Oxalate

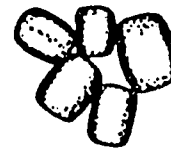
- envelope shaped
- square with refractive "X"
- can be peanut-shaped
- most common constituent of kidney stones



\* Can be found in alkaline urine

### (b) Uric Acid

- yellowish brown in colour
- football-shaped (diamond)
- can have a rosette form or barrel form
- outside edges usually darker in colour
- seen in gout patients



### (c) Calcium sulfate

- long prisms or flat blades
- can be separate or in bundles

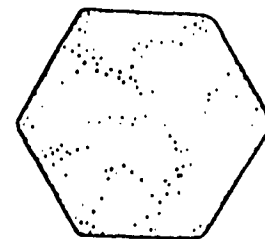
### (d) Amorphous Urates (granules)

- appear light pink in urine
- look yellow under the microscope
- will precipitate if urine is refrigerated
- can interfere with microscopic exam
- will dissolve if urine is gently heated



### (e) Cystine (Very rare)

- hexagonal plate (6 sides)
- colourless and very refractile
- found only in fresh urine (soluble in ammonia)



### Indicate

- cystinuria, a hereditary metabolic disease

### (f) Cholesterol (rare)

- plates that appear to have a stairstep shape or a corner missing
- colourless and refractile
- soluble in ether



**(g) Leucine (rare)**

- yellow brown in colour
- looks like a wheel or sofa pillow
- seen in severe liver disease

**(h) Tyrosine (rare)**

- yellow brown in colour
- sheaves of fine needles
- seen in severe liver disease

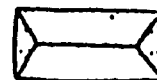
**(i) Bilirubin (rare)**

- small crystals, squares, beads or needles
- brown in colour
- about  $\frac{1}{2}$  the size of an RBC

**Alkaline Crystals**

**(a) Triple Phosphate**

- “coffin lid” appearance
- can be found in either pH
- “feathery forms” found only in alkaline urine
- colourless & refractile



**(b) Ammonium Urates (Biurate)**

- “thornapple” crystal
- yellow & refractile
- can be seen in bundles of needles
- often found with phosphates



**(c) Calcium Phosphate**

- prisims, star-shaped
- colourless

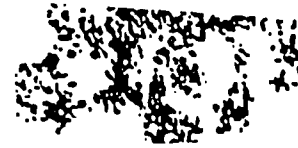
**(d) Calcium Carbonate**

- dumbbell-shaped, small
- colourless
- soluble in acetic acid-gives off  $\text{CO}_2$



**(e) Amorphous Phosphate**

- gives urine sediment a white colour
- small granules
- soluble in acetic acid



Report any crystals seen as rare, few moderate, many or heavy. Crystals should always be identified using the X40 objective.

# HEMATOLOGY

34a

## HEMATOLOGY

Hematology is the study of the blood, which includes the blood cells and the fluid make up of the blood.

There are 3 different types of blood cells:

1. Red blood cells (RBC) or erythrocytes
2. White blood cells (WBC) or leukocytes
3. Platelets or thrombocytes

### 1. RBC's

Appearance	Round cells with a central pallor (inner halo) no nucleus or granulation (smooth appearance)
Size	7-8 microns
Normals	Adults: Male 4.6-6.2 million/u/l Female 4.2-5.4 million/u/l
Function	Carries oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs.

### 2. WBC's

Appearance	Round to irregular shaped granulated cells containing a large nucleus.
Size	9-20 microns
Normals	5-10 thousand (adults)
Function	Body's defence against infection.

### 3. Platelets

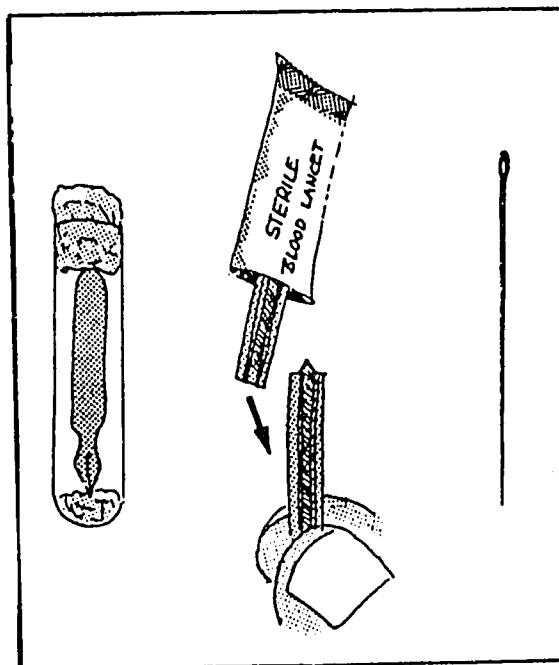
Appearance	Fragments of cells that look like large sand granules.
Size	2-5 microns
Normals	150,000-400,000
Function	Helps maintain the clotting action of blood.

## COLLECTION OF BLOOD

For the purpose of basic laboratory technicians, there are 2 methods of collecting blood, Venous and Capillary. Due to the methods used in this manual only the capillary method will be discussed.

The following equipment may be used:

- Disposable blood lancets (preferable)
- Sterile lancets
- Hagedarn needle (No. 7 or 8)



### Capillary Method

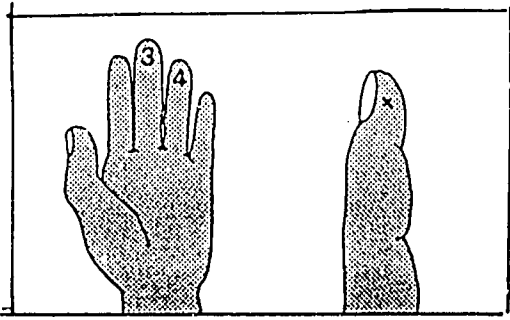
1. Find a spot on a finger tip (not calloused).
2. Use the side if possible-less sensitive.
3. Clean the site with cotton wool swab dipped in alcohol. (70% isopropal, ethanol, etc).
4. With a clean swab, dry the site.
5. Prick the finger with a firm rapid motion.
6. Wipe away the first drop of blood with dry cotton wool.

Use the Heel or Big toe for infants — usually under 6 months. If the infant is older than 6 months but small, the heel or toe may be used.

- Use outside edge of the heel.
- Usually 2 quick pricks are necessary to obtain the steady flow of blood.
- When using the toe, the outside edge is again less sensitive.
- Use the same procedure as the finger stick.

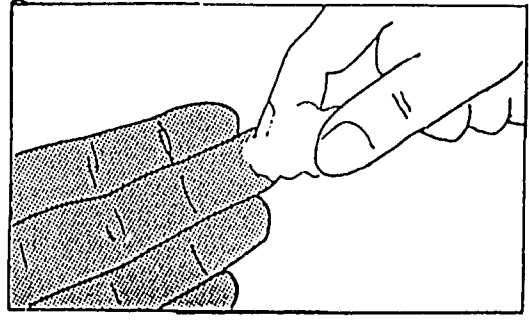
A few drops of blood taken from the finger, toe or heel are enough for certain laboratory tests used in this manual such as:

- White Blood Cell Count
- Blood differential
- Hemoglobin determination
- Detection of blood parasites

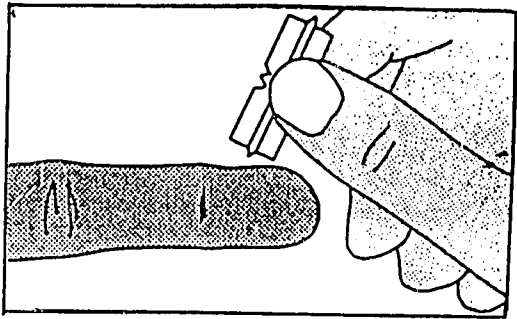


1.

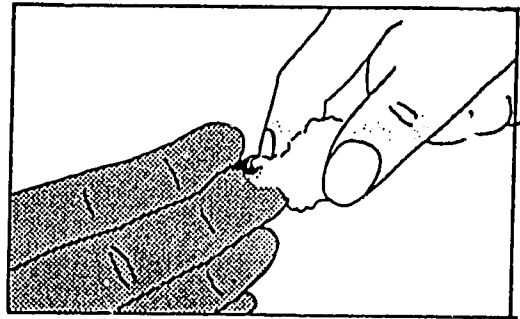
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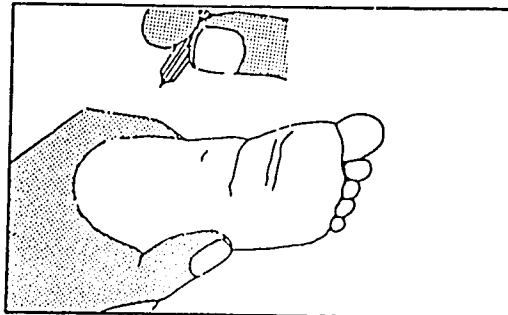
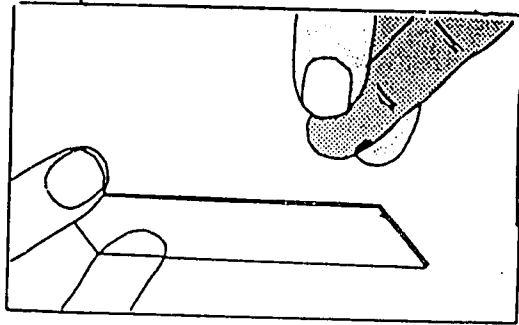
3.



5.



6.



## HEMOGLOBIN - SAHLI METHOD

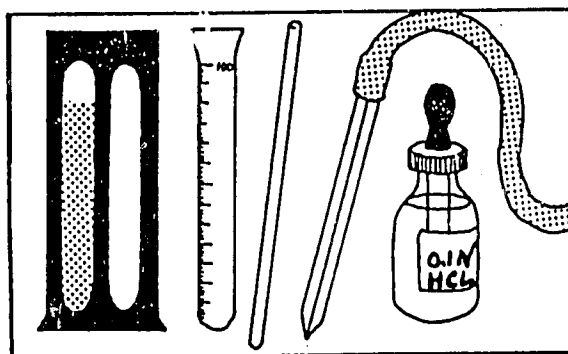
### Principle

The blood is diluted with an acid solution to acid hematin and the test solution is matched against a coloured glass reference.

**Note:** This is not an accurate way to estimate hemoglobin because not all hemoglobin is changed into acid hematin. The colour changes are not great and the brown colour of the standard is not a true match for acid hematin. The results, therefore are very subjective to each individual doing the test.

### Materials:

- Sahli hemoglobinometer
- Sahli Pipette
- Small glass rod
- Dropping pipette
- Absorbent paper
- 0.1N hydrochloric acid (HCL)
- Tubing and mouth piece



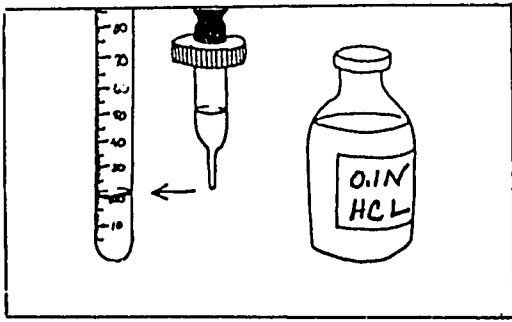
### Method:

1. Fill the graduated tube to the 20 mark with 0.1N HCL.
2. Draw capillary blood to the 0.02 ml mark of the sahli pipette - no air bubbles. Do not take the first drop of blood.
3. Carefully wipe the outside of the pipette, then check that the blood is still on the mark.
4. Blow the blood from the pipette into the acid solution and rinse 3 times. The mixture should be a brown colour.
5. Allow to stand for 5 minutes.
6. Place the tube in the hemoglobinometer and compare the colour. A good light source is needed so face a window. If the colour is the same or greater than the reference, hemoglobin is 40 g/d or less.
7. If colour is darker than reference, continue to dilute by adding 0.1N HCL drop by drop. Stir tube after each drop, stop when colour matches.
8. Note the mark reached on the tube. Depending on the type of hemoglobinometer, the concentration is given in g/100 ml or as a percentage of "Normal" (This type is not recommended).
9. To convert g/100 ml to g/l, multiply by 10. To convert percentages to g/l, multiply by 1.46.

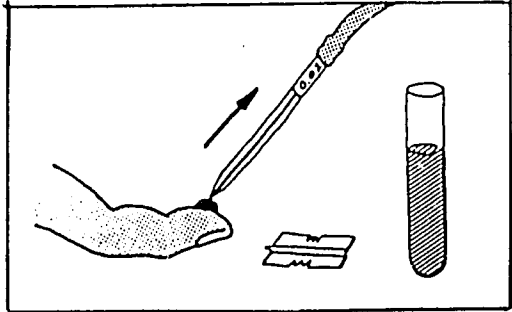
Examples:

- (a)  $14.8 \text{ g/100 ml} \times 10 = 148 \text{ g/l}$
- (b)  $85\% \times 1.46 = 124 \text{ g/l}$

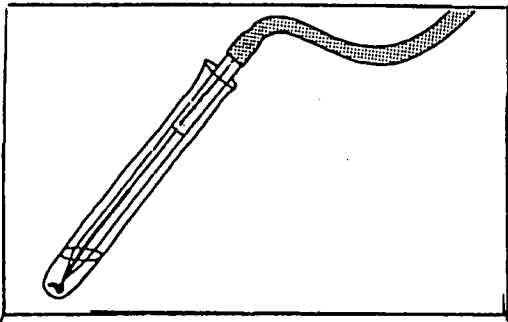
**Note:** This method has too much room for error.



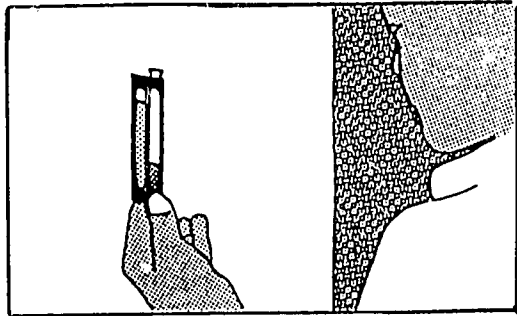
1.



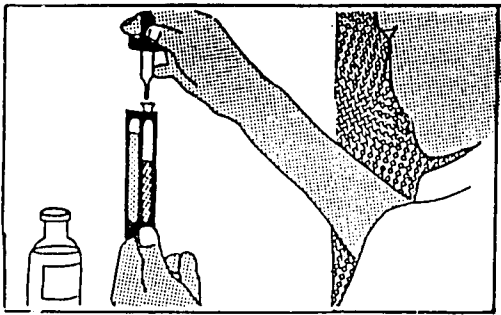
2.



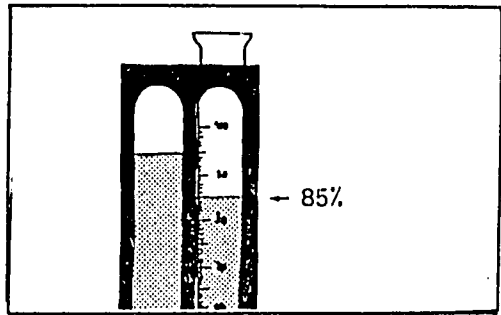
4.



6.



7.



8.

## HEMOGLOBIN ESTIMATION

### Copper Sulfate Method

This method is based on specific gravity. A drop of blood dropped into the solution is incased in a sac, which prevents only change in specific gravity for about 15 seconds. If the drop of blood has a satisfactory specific gravity it will sink within 15 seconds. If not the drop will hesitate, remain suspended, or rise to the top of the solution in 15 seconds. This is not a quantitative test and will show only that the hemoglobin is equal to, below, or above acceptable limits.

### Materials

- 2 containers of at least 30 ml with a screw cap.
- Copper sulfate solution with SpG of 1.053 (females)
- Copper sulfate solution with SpG of 1.055 (males)
- Capillary tubes
- Blood lancet
- Cotton wool swabs
- Alcohol

### Method

1. Clean the site for the finger or toe stick.
2. Puncture the site and allow blood to flow freely.
3. Do not Squeeze
4. Collect blood in capillary tube without allowing air to enter tube.
5. Let one drop of blood flow gently from the tube (1 cm from top) to the correct container (1.053 females, 1.055 males).
6. Observe for 15 seconds.
7. Record results:
  - greater than or less than 12.5 gm/dl: (Females)
  - greater than or less than 13.5 gm/dl: (Males)

Solution is stored at room temperature and tightly capped. For routine use, dispense 30 ml of solution into a correctly labeled, clean, dry tube or bottle. Change daily or after 25 tests.

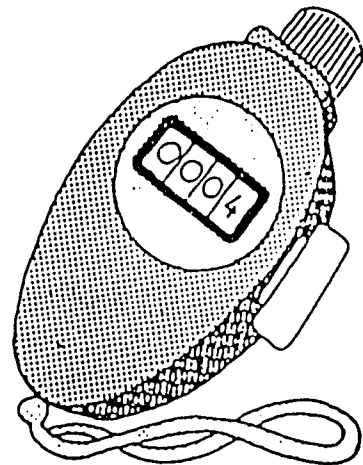
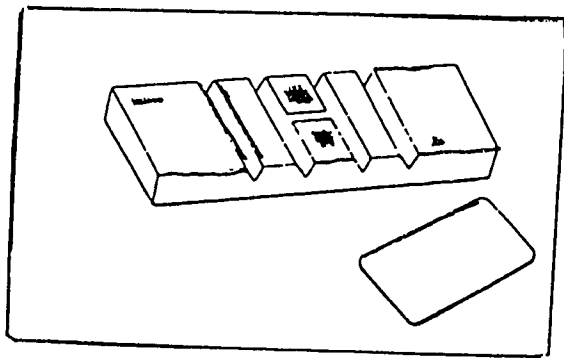
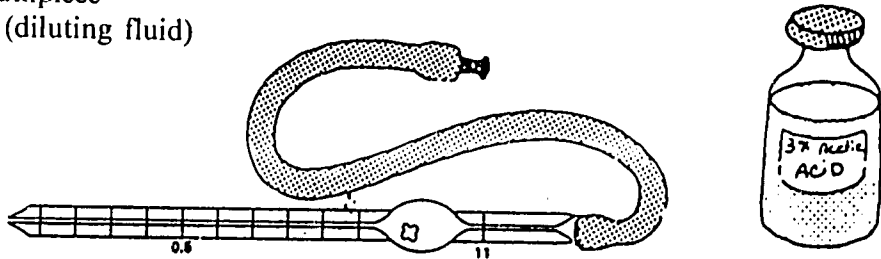
Note: Test results that indicate satisfactory hemoglobin levels are usually accurate but some results that indicate low hemoglobin levels are false.



## WBC COUNT

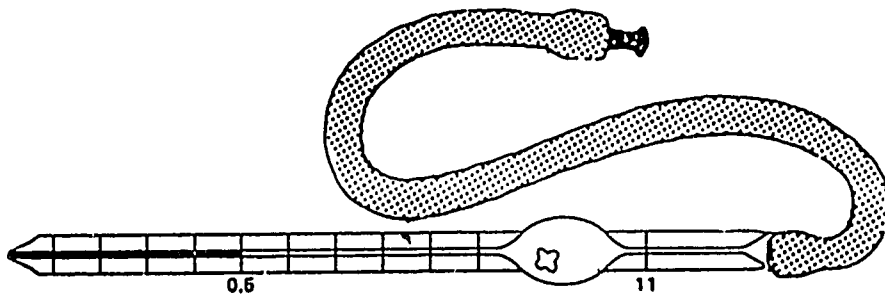
### Materials

WBC pipet  
Tubing and mouthpiece  
3% Acetic acid (diluting fluid)  
Lancet  
Cotton wool  
Alcohol  
Hemacytometer  
Microscope  
Counter

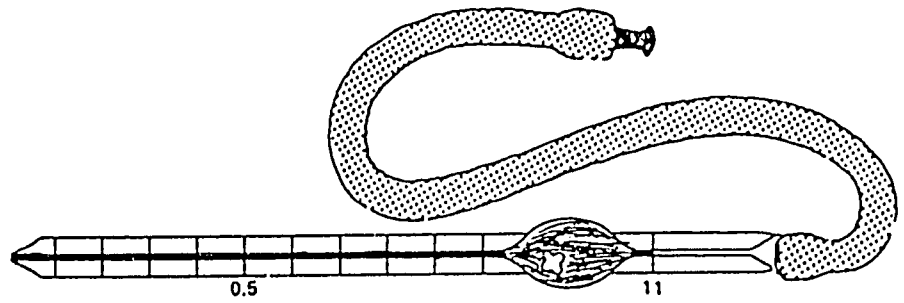


### Procedure

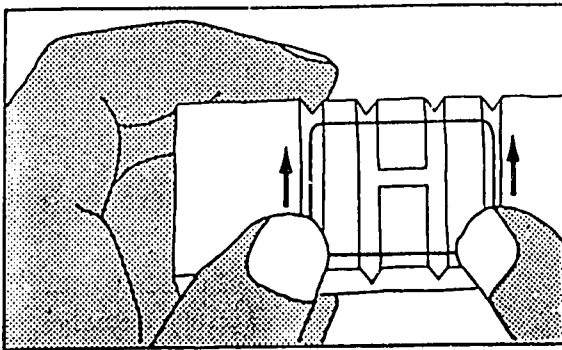
1. Place tubing on WBC pipet.
2. Prick finger and wipe away first drop of blood.
3. Place tip of pipet at a slight angle to the blood flow and fill pipette to the "0.5" mark.
4. Wipe excess blood off pipette and draw diluting fluid to the "11" mark.
5. Hold the ends of the pipette between the thumb and middle finger and mix in a figure 8 motion for 2 minutes.
6. Place pipette on a flat surface and allow mixture to settle for 5 minutes.
7. Make sure counting chamber and cover glass are clean. Place cover glass in position and you are ready to fill the counting chamber.
8. Discard several drops (2-3) onto absorbent paper and using the WBC pipette fill the counting area of the hemacytometer. Do not overfill.
9. Allow the fluid to sit in the chamber for 1-2 minutes to allow it to settle.
10. Place the chamber on the stage of the microscope, use the X10 objective (low power) and low light. Focus the rulings of the chamber.



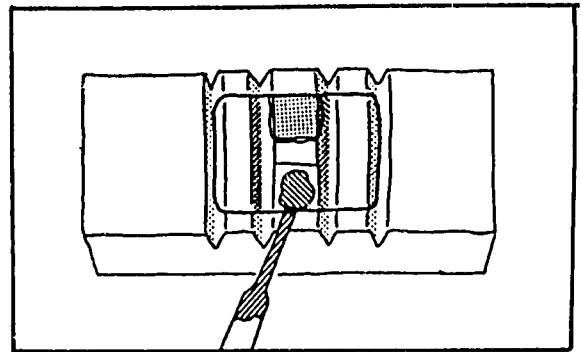
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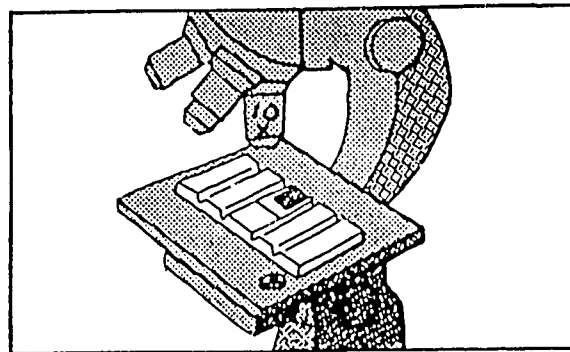
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7.

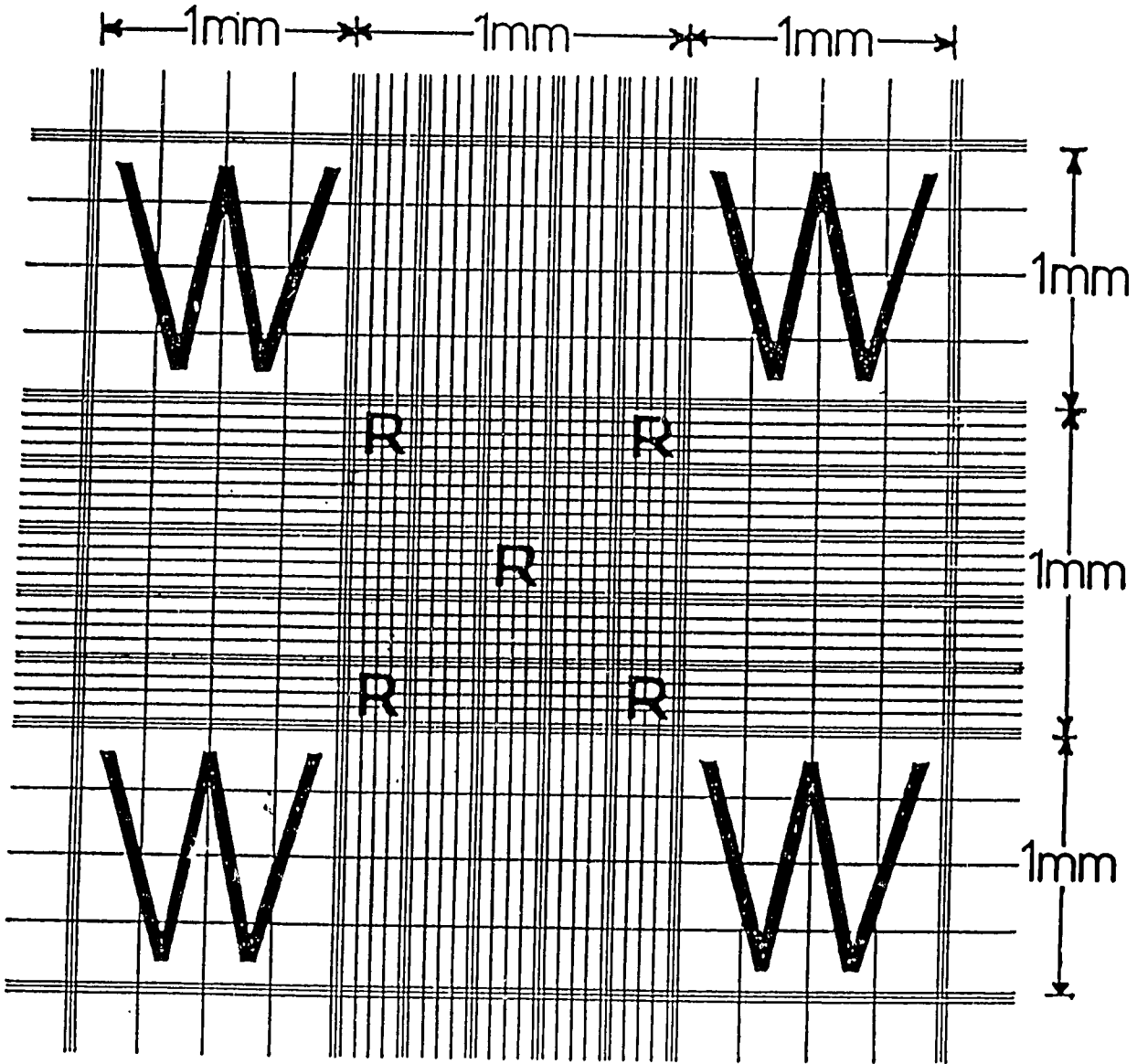


8.



10.

11. Count the cells in the 4 large outside squares (the four corners). Count the cells seen on the lines of 2 sides of each square counted.
12. Multiply the total number count by 50 to obtain the number of leukocytes per Cumm ( $\text{mm}^3$ ) of blood.



Cell Counting Area on One Side of Hemacytometer with Improved Neubauer Ruling

Area for wbc count: 4 large squares marked "W" (total area = 4 sq mm)  
 Area for rbc count: 5 small squares marked "R" (total area = 0.2 sq mm)

Example:

Number of cells counted = 176.  
 Cells in  $1 \text{ mm}^3 = 176 \times 50 = 8800$   
 Results reported =  $8,800/\text{mm}^3$ .

## **Normal Values**

Adults = 5 — 10,000/mm<sup>3</sup>

## **Pediatric Range**

Newborn = 9,000 — 30,000/mm<sup>3</sup>  
2 weeks = 5,000 — 20,000/mm<sup>3</sup>  
1 year = 6,000 — 18,000/mm<sup>3</sup>  
4 years = 5,500 — 17,500/mm<sup>3</sup>  
10 years = 4,500 — 13,500/mm<sup>3</sup>

## WBC COUNT UNOPETTE METHOD

### Material:

Unopettes  
Cotton wool  
Alcohol  
Lancet  
Microscope  
Counter  
Petri dish  
Filter paper

### Storage Instructions

If count cannot be performed immediately after blood is diluted, store reservoir at room temperature. Perform count with three (3) hours of making dilution.

### Procedure:

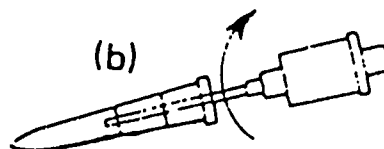
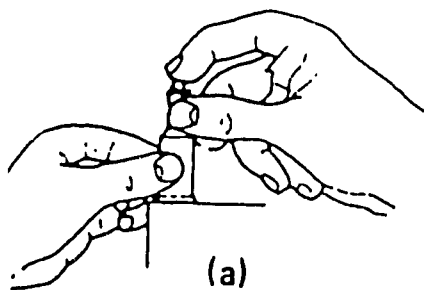
1. *Puncture Diaphragm:* Using the protective shield on the capillary pipette, puncture the diaphragm of the reservoir as follows:
  - (a) Place reservoir on a flat surface. Grasping reservoir in one hand, take pipette assembly in other hand and push tip of pipette shield firmly through diaphragm in neck of reservoir, then remove.
  - (b) Remove shield from pipette assembly with a twist.
2. *Add Sample:* Fill capillary with whole blood and transfer to reservoir as follows:
  - (a) Holding pipette almost horizontally, touch tip of pipette to blood. Pipette will fill by capillary action. Filling is complete and will stop automatically when blood reaches end of capillary bore in neck of pipette.
  - (b) Wipe excess blood from outside of capillary pipette, making certain that no sample is removed from capillary bore.
  - (c) Squeeze reservoir slightly to force out some air. Do not expel any liquid. Maintain pressure on reservoir.
  - (d) Cover opening of overflow chamber of pipette with index finger and seat pipette securely in reservoir neck.
  - (e) Release pressure on reservoir. Then remove finger from pipette opening. Negative pressure will draw blood into diluent.

- (f) Squeeze reservoir gently two or three times to rinse capillary bore, forcing diluent up into, but *not out of overflow chamber*, releasing pressure each time to return mixture to reservoir.
  - (g) Place index finger over upper opening and gently invert several times to thoroughly mix blood with diluent.
  - (h) Let stand for ten (10) minutes to allow red cells to hemolyze. Leukocyte counts should then be performed within three (3) Hours.
3. *Charge Hemacytometer:* Mix diluted blood thoroughly by inverting reservoir to resuspend cells.
- (a) Convert to dropper assembly by withdrawing pipette from reservoir and resetting securely in reverse position.
  - (b) To clean capillary bore, invert reservoir, gently squeeze sides and discard first three or four drops.
  - (c) Carefully charge hemacytometer with diluted blood by gently squeezing sides of reservoir to expel contents until chamber is properly filled.
  - (d) Place hemacytometer on moistened filter paper in petri dish. Cover petri dish and allow to stand ten (10) minutes to permit cells to settle. (moistened filter paper retards evaporation of diluted specimen while standing).
4. *Count and Calculate:* A leukocyte count is performed with a Neubauer hemacytometer as follows:
- (a) Under X40 magnification, leukocytes are counted in all nine (9) large squares of counting chamber.
  - (b) Add 10 percent of count to total number of cells counted.
  - (c) Multiply this figure by 100 to get total leukocyte count.

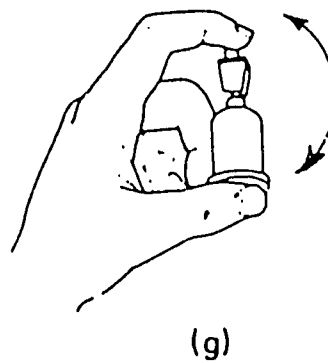
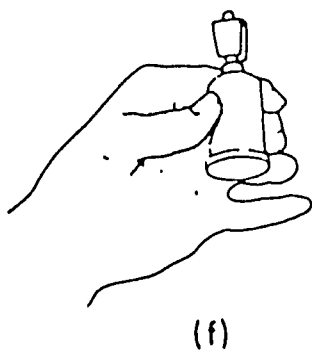
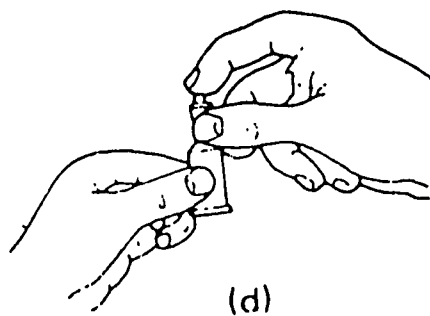
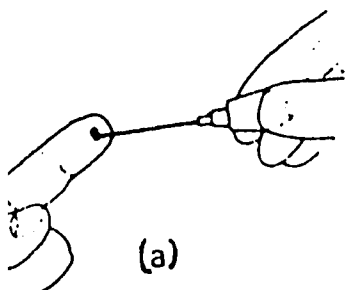
Example:

If 70 cells are counted, total count is:  $(70 + 7) \times 100 = 7700$  leukocytes/cu mm.

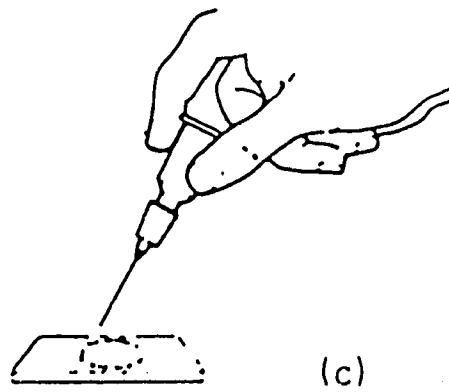
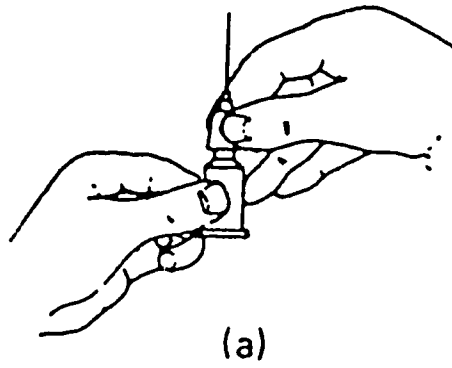
# 1. PUNCTURE DIAPHRAGM



# 2. ADD SAMPLE



### 3. CHARGE HEMACYTOMETER





## LEUKOCYTE DIFFERENTIAL

### Preparing the Slide:

#### Material:

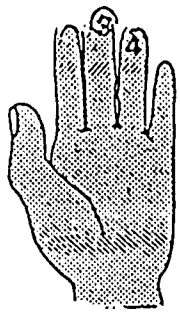
- Clean glass slides
- Sterile lancet
- Methanol
- Grease pencil
- Microscope
- A means of counting the different cells.
  - (a) Machine with key board
  - (b) Counting box with beans or beads
  - (c) Pencil and paper

#### Procedure

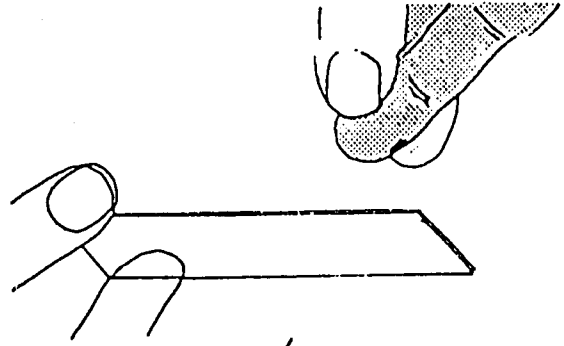
1. Use clean dry slides. If necessary clean them with ethanol and use a soft cloth.
2. Take a drop of blood from the side of the 3rd or 4th finger. Do not take blood from Index finger or thumb, infected finger or ear (too many monocytes).
3. Let blood flow freely and collect for WBC count first if requested.
4. Hold the slide by the edges and collect a drop of blood on one end.
5. Using a second slide as a spreader, place it just in front of the drop.
6. Draw spreader back until it touches the drop and let the blood run along the edge of the spreader.
7. Using a smooth movement, push the spreader to the end of the slide. All the blood should be spread out before reaching the end.
8. Check that the blood film is satisfactory:
  - no lines thru the film
  - no ragged edges at the end
  - film not too thick
  - no holes in film due to using a greasy slide
9. Dry the film adequately before staining.
10. Mark the dry film before staining with the patient's identification.
11. While the film is drying, protect it from flies and other insects that could distort the film.

#### Staining

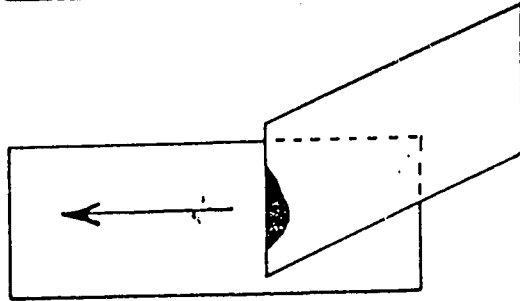
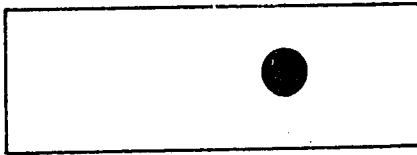
1. All glassware must be clean. Use methanol to remove stain deposits.
2. Make sure water is correct pH. (6.8-7.2).
3. Always allow slide to dry completely.
4. Always have each slide properly labeled.



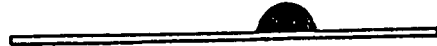
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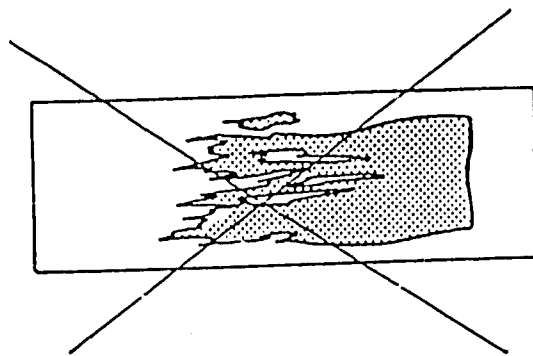
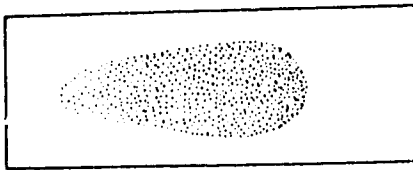
4.



5.



6.



## STAINING THE FILM

Leishman's and Wright's stain give similar results and are used as individual stains.

### Wright's Quick Stain is stain of choice

- No preparation time for stain
- Less equipment
- Least amount of staining time

### Staining with quick stain (Wright's)

#### Materials:

- 2 rods over a sink or staining rack
- Timer clock
- Wash bottle
- Drying rack for slides
- Pipette or syringe

#### Procedure:

1. After slide is completely dry, place on staining rack.
2. Using a syringe or pipette flood the slide with quick stain.
3. Set timer for ten seconds or just count to ten.
4. Using a wash bottle, rinse stain off slide (gently).
5. With forceps, stand slide upright and gently rinse again.
6. Allow slide to dry (air dry) and it is ready to read.

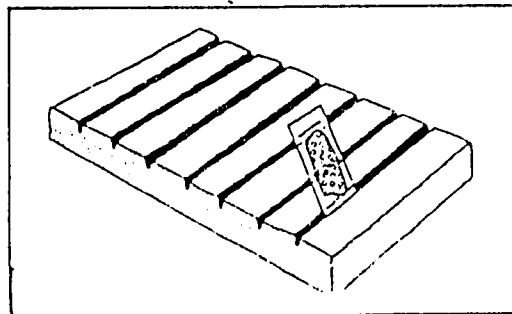
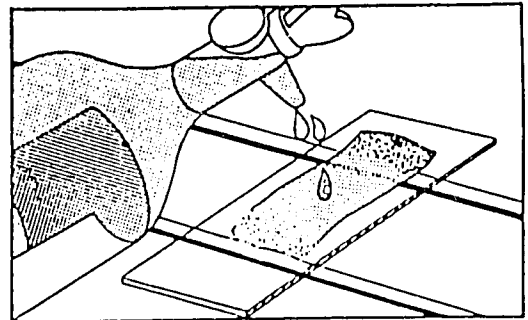
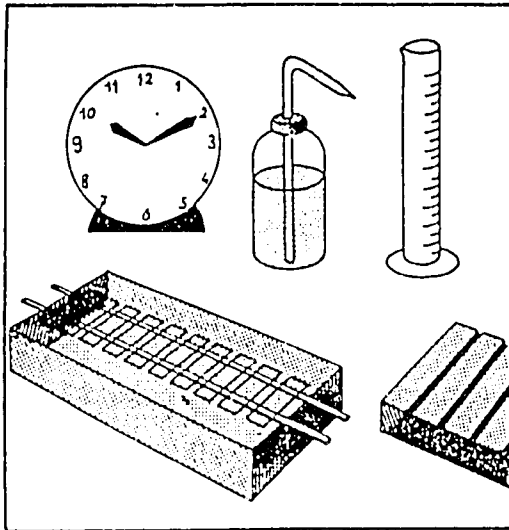
## LEISHMAN STAIN

### Materials:

- Staining rack or 2 glass rods over a sink
- Measuring cylinder
- Wash bottle containing buffered water
- Leishman stain
- Timer clock
- Methanol

### Procedure:

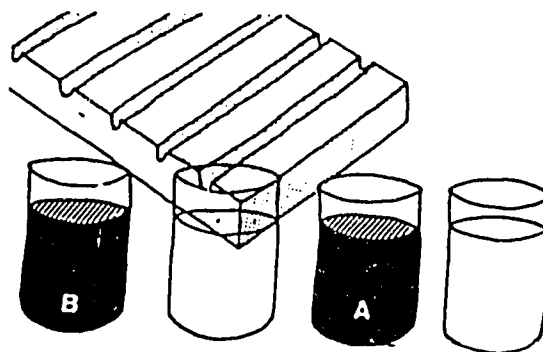
1. Fix slide with methanol for 2-3 minutes.
2. Prepare 1 in 3 dilution of stain using 1 part stain and 2 parts buffered water, Mix well. Note: Prepare enough stain for 1 day's use. New stain should be made daily.
3. Cover slide with stain for 7-10 minutes. (times may need changing depending on freshness of stain).
4. Wash stain off using a steady stream of buffered water. Do not tip stain off.
5. Leave clean water on the slide for 2-3 minutes to differentiate the film. The pH of the water is important to get correct results (6.8 to 7.2).
6. Tip water off and place in rack to dry.



## STAINING WITH FIELD STAIN

### Materials:

- Field stains A and B
- Wash bottle
- Beakers
- Methanol
- Timer clock



### Procedure:

1. Fix the slide with methanol for 2-3 minutes.
2. Dip slide into field stain B and count to 5.
3. Drain and Wash in 1st container of water.
4. Drain and dip into field stain A and count to 10.
5. Drain and wash in second container of water.
6. Examine colour of stain. It should appear neither too blue nor too pink. (mauve)
7. If colour is not correct return either to A or B stain for a few more seconds.
8. Place in rack and allow to dry.

## LEUKOCYTE DIFFERENTIAL

There are 5 main types of leukocytes (WBCs) which are normally found in the blood: neutrophils, lymphocytes, monocytes, basophils and eosinophils. Under pathologic conditions, other types are found such as immature forms of those mentioned above.

The differential WBC count directs the attention of the doctor to a particular disease or condition. It can also be used to monitor the patient's progress under a particular therapy.

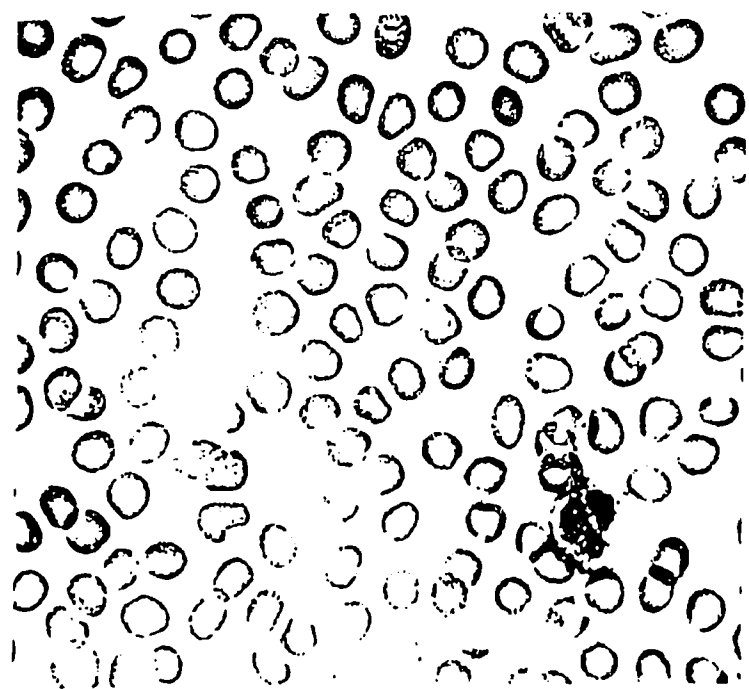
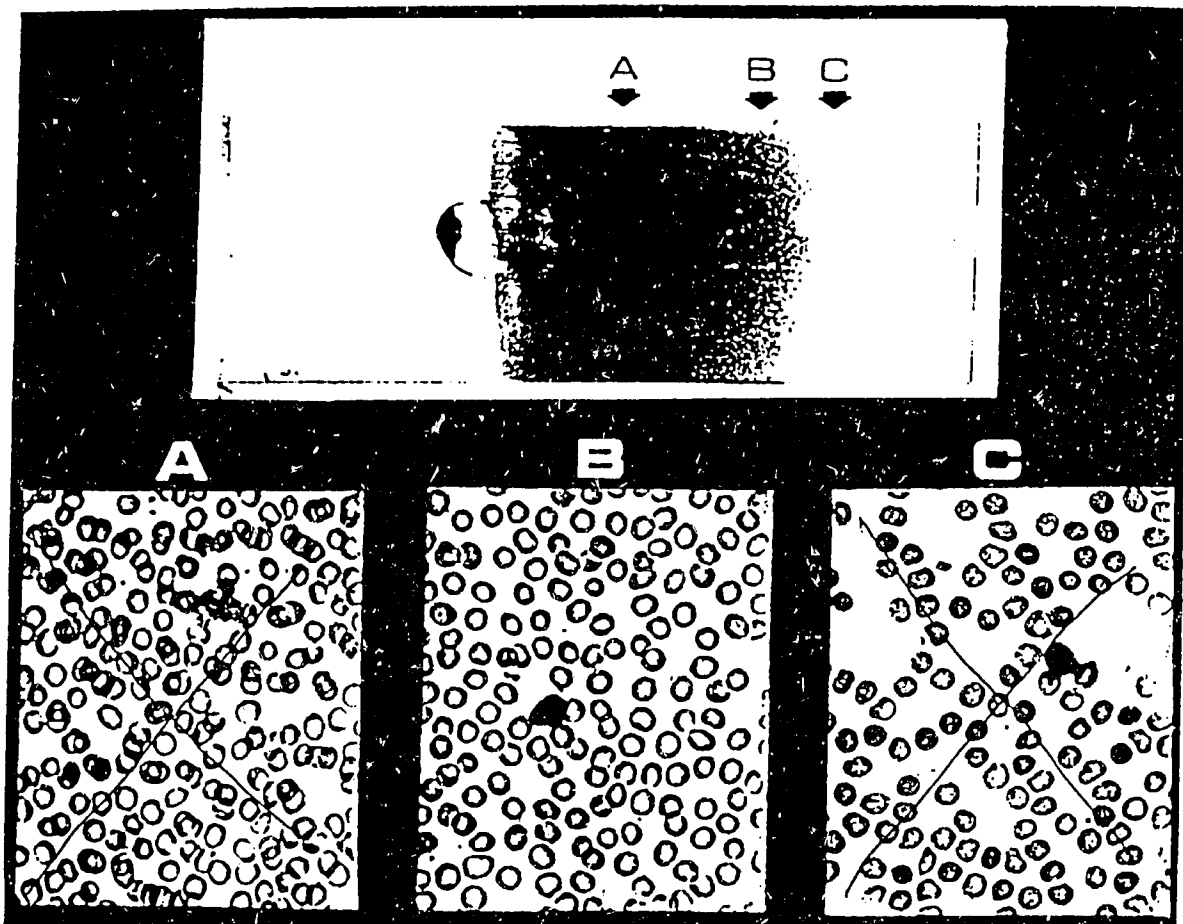
100 WBCs are counted and the number of each is recorded. The numbers should always add up to 100.

### Materials:

- Microscope with X100 and X10 objectives
- Immersion oil
- Well stained slide
- Counter system (keyboard, Bead counter, pencil and paper).

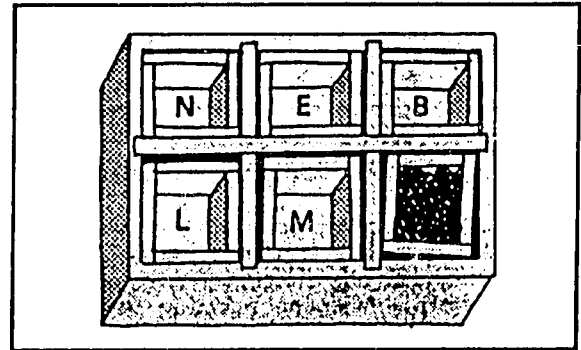
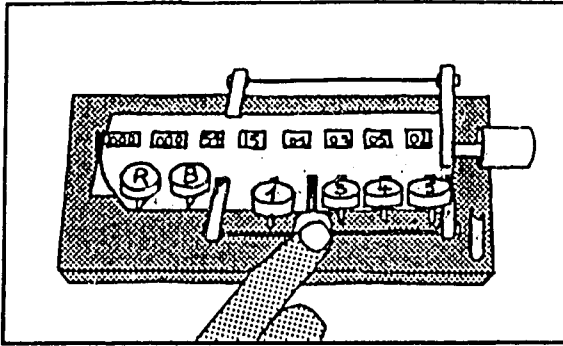
### Method:

1. Place slide on microscope, using X10 objective (low power) find correct area for counting. The area counted should be where the RBCs are barely touching or slightly overlapping (area B).
2. Move the X10 objective and place a drop of immersion oil in the area you have chosen to count.
3. Using the X100 objective, count 100 WBC's systematically moving from one field to the next.
4. Make sure you do not move into a field that is too thick. If you do, move in the opposite direction.
5. While you are counting, take note of the RBC morphology and platelet estimation.
6. Learn to identify normal cells correctly so you are certain to recognize abnormal cells.
7. Refer "Abnormal" smears to a doctor or for a second opinion. Most serious error — calling abnormal cells "NORMAL".



Normal RBC morphology. RBCs are round with little variation in size. A central pale area is seen in most cells because the normal RBC is a biconcave disc.

# COUNTING METHODS



	N	E	B	L	M
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
Total	59	8	1	28	4
Frac.	0.59	0.08	0.01	0.28	0.04



## IDENTIFICATION OF LEUKOCYTES

### In a well stained film:

- Neutrophils — Cytoplasm is salmon to faint pink and contains pink to mauve granules.
- Eosinophils — Large red to orange granules.
- Monocytes — Grey-blue cytoplasm — sometimes has vacuoles and may contain a few red granules.
- Lymphocytes — Cytoplasm stains a clear blue, (sm cells) cytoplasm stains a dark blue. Nucleus takes up most of the cell, may contain a few red granules.
- Basophils — Many dark blue-purple granules fill the cell.
- RBCs — Tan to pink.
- Platelets — Stain a dark blue-pink.

You are now ready to begin your count.

### To identify the types of WBCs:

1. Note the size of WBC compared to the red cells.
2. Note the shape of the nucleus and its size to the total area of the cells.
3. Appearance of cytoplasm. Colour pink, salmon, blue, dark/blue, purple, any granules in cytoplasm. (basophil, eosinophil, etc).
4. Check the nucleus (chromatin) whether it is dense or not and place of nucleus. (central or eccentric).
5. Vacuoles and nucleoli are round or oval areas that are distinct in the cell (holes). They may or may not stain.

## Normal Cells:

### A. *Polymorphonuclear Neutrophilic granulocyte* ("poly" or "Seg")

1. The nucleus has many forms, segmented or divided into lobes.
2. 2-5 lobes connected by a filament of nuclear membrane.
3. Nuclear chromatin highly clumped. (deep-staining).
4. Cytoplasm is abundant.

### B. "Band form" or "Stab cell"

1. Immature form of "poly".
2. Cytoplasm abundant — nucleus not yet formed a filament which divides the nuclear lobes.
3. Criteria to classify as "band"
  - (a) curved, band-shaped nucleus, without a thread-like filament.
  - (b) 2 sides of the nucleus run parallel for any distance.
  - (c) only slight indentation.

### C. *Eosinophil*

1. Large round cells
2. Numerous red-orange granules that are tightly packed.
3. Nucleus usually has 2 lobes.

### D. *Basophil*

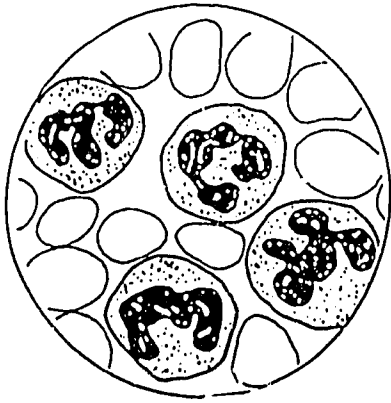
1. round cells with very large blue-black granules that cover all of the cell.
2. sometimes cytoplasm has vacuoles.
3. nucleus is hard to see because of granules.

### E. *Lymphocyte*

1. Small cell with consistent appearance.
2. Large, dark blue, dense nucleus that covers most of the cell.
3. Very little cytoplasm visible — blue with usually no granules seen.

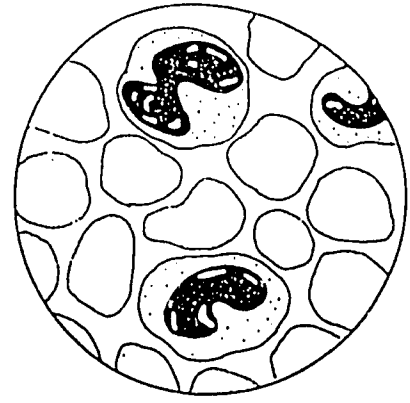
### F. *Monocyte*

1. Largest of normal leukocytes.
2. Cytoplasm is grey-blue, has a cloud-like appearance, often has pseudopods.
3. Nucleus lobulated (kidney bean or horseshoe shaped) chromatin less dense — appears "raked".
4. vacuoles may be seen, some are large.

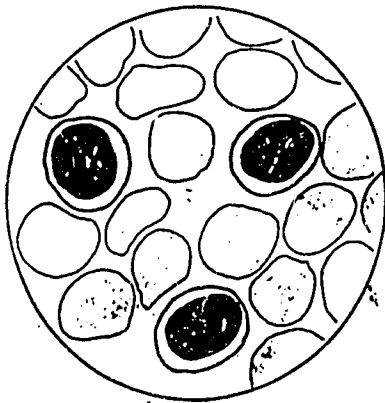


**POLYMORPHONUCLEAR NEUTROPHIL CELL**

**Size** 12-15  $\mu\text{m}$   
**Shape** rounded, well defined  
**Cytoplasm** abundant, pinkish  
**Granules** mauve and very small, numerous but separate  
**Nucleus** several (2-5) lobes, linked by strands of chromatin. The chromatin appears as a uniform deep purple mass.

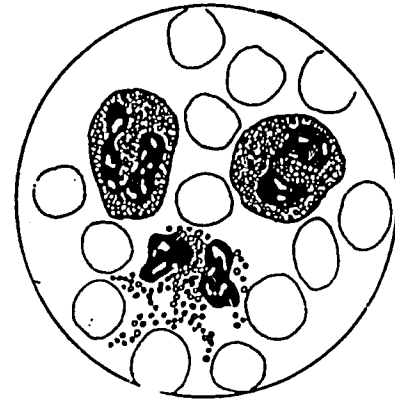


**IMMATURE POLYMORPHONUCLEAR NEUTROPHIL CELL ("BAND FORM" OR "STAB CELL")**



**SMALL LYMPHOCYTE**

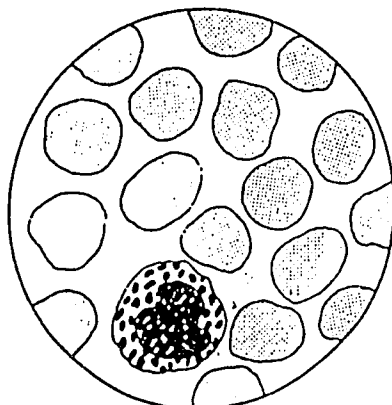
**Size** 7-10  $\mu\text{m}$   
**Shape** round  
**Nucleus** large, occupying most of the cell, chromatin dark purple, dense  
**Cytoplasm** very little visible; blue with no granules.



**POLYMORPHONUCLEAR EOSINOPHIL CELL**

**Size** 12-15  $\mu\text{m}$   
**Granules** large, round, orange-red, numerous and closely packed  
**Nucleus** usually 2 lobes.

Sometimes the cell appears damaged, with scattered granules.



**POLYMORPHONUCLEAR BASOPHIL CELL**

The rarest type of granulocyte.

**Size** 11-13  $\mu\text{m}$   
**Shape** round  
**Granules** very large, round, deep purple, numerous but less closely packed than those of the eosinophils  
**Nucleus** difficult to see because covered by the granules  
**Vacuoles** occasional small colourless vacuoles in the cytoplasm.

## NORMAL VALUES FOR THE DIFFERENTIAL LEUKOCYTE COUNT

Cells	At Birth	4 Weeks	4 Years	6 Years	Adult
Neutrophil	37-57%	25-35%	25-45%	45-50%	50-65%
Band	0-1%	0-1%	0-1%	0-1%	0-1%
Eosinophil	1-3%	1-3%	1-3%	1-3%	1-3%
Basophil	0-1%	0-1%	0-1%	0-1%	0-1%
Monocyte	4-8%	5-9%	4-8%	4-8%	4-10%
Lymphocyte	25-35%	45-65%	40-60%	40-45%	25-40%

(Compiled from data from Miale, J.B.: *Laboratory Medicine, Hematology*, 3rd ed., The C.V. Mosby Company, St. Louis, 1967, and Davidsohn, I. and Nelson, D.A.: *Blood*. In: *Clinical Diagnosis by Laboratory Methods*, Davidsohn, I. and Henry, J.B., Eds., W.B. Saunders Company, Philadelphia, 1969.)

## MORE COMMON ABNORMAL WBC's

### A. *"Atypical Lymphocyte"*

1. Size is variable — usually larger than normal lymph.
2. Cytoplasm is darker blue than normal lymph and have a dark edge.
3. Nucleus is eccentric, deeply cleft (indented) to lobulated distinct nuclear membrane. Chromatin densely packed.
4. Numerous vacuoles are sometimes present.

Indicative in viral infections — infectious mononucleosis. Can also be found in tuberculosis and severe malaria.

### B. *Plasma Cell*

1. Round or oval cell with round eccentric nucleus.
2. Chromatin is clumped and often seen in a "wheel" arrangement.
3. Dark blue cytoplasm with a pale staining area around the nucleus.

May be seen in measles, tuberculosis and multiple myeloma.

### C. *Hypersegmented Neutrophil*

1. usually larger than normal.
2. nuclei have 5-10 lobes.

Usually associated with folic acid or B-12 deficiency.

### D. *Blast Cell*

1. Most immature of all leukocytes.
2. Large round to oval cell.
3. Large round nucleus that contains 1-5 nucleoli and takes up most of the cell.
4. Cytoplasm is dark blue with a clear area around the nucleus. Indicative of leukemia.

## ABNORMAL DIFFERENTIAL

“*Shift to the left*” — Increase in band form. Indicates severe bacterial disease.

“*Shift to the right*” — Increase in lymphs. Indicates a viral disease.

*Hypersegmentation* — B-12 or folic acid deficiency.

*Atypical Lymphs* — Infections Mononucleosis.

*Eosinophilia* — increase of eosinophils-allergic reaction or parasitic infection.

*Blast forms* -- acute leukemia.

1. Absolute increase in the number of neutrophils (neutrophilia):
  - A. Appendicitis
  - B. Myelogenous leukemia
  - C. Bacterial infections
  
2. Absolute increase in the number of eosinophils (eosinophilia):
  - A. Allergenic reactions
  - B. Allergies
  - C. Scarlet fever
  - D. Parasitic infestations
  - E. Eosinophilic leukemia
  
3. Absolute increase in the number of lymphocytes (lymphocytosis):
  - A. Viral infections
  - B. Whooping cough
  - C. Infectious Mononucleosis
  - D. Lymphocytic leukemia
  
4. Absolute increase in the number of monocytes (monocytosis):
  - A. Brucellosis
  - B. Tuberculosis
  - C. Monocytic leukemia
  - D. Subacute bacterial endocarditis
  - E. Typhoid
  - F. Rickettsial infections
  - G. Collagen disease

## RBC MORPHOLOGY

- Size:** 6-8 um in diameter
- Shape:** Biconcave disks with donut-shaped appearance, edges stain darker than center because there is more hemoglobin there.
- Colour:** Lt pink to reddish-orange — deeper colour around the edge.
- Structure:** No nuclei, nuclear remnants or cellular inclusions.

Anisocytosis — Variety of sizes

Poikilocytosis — Variety of shapes

Sickle cell — Sickle shaped or crescent

Ovalocytes — Oval shaped (various anemias)

Acanthocytis — Irregular spaced Projections

Burr cells — Uniforme spaced Pointed Projections (Uremia, acute blood loss, cancer of Stomach)

Schistocytes — red cell fragments (DIC)

Basophilic stippling — Purple granules in RBC (lead Poisoning)

Microcytes — decrease in size (Thalassemia and anemias)

Macrocytes — Increase in size (liver disease)

Hypochromia — red cells with large central pallor (Iron deficiency)

Spherocytes — no central pallor. (hemalytic anemia)

Target cells — central stained area (hemoglolopathies)

Stomatocytes — Oval or rectangular area of central pallor. (Electrolyte imbalance)

Siderocytes — iron deposits (Prussion blue)

Howell-Jolly Bodies — purple staining nuclear fragments in the RBC. Often seen after Spleen Removal.

Cabot Rings — Purple staining rings in RBC. (Pernicious anemia and lead posioning)

Polychromatophilia — gray colour

Teardrop-shaped RBC's found in the thalassemia and some anemias.

Parasites — malaria is most common — parasite and cell must focus in same plane.

# **BACTERIOLOGY**



## BACTERIOLOGY

### Collection of Sputum

1. Using a sterile container with a tight lid, collect the first sputum of the morning.  
A nurse or lab person should be present to ensure the procedure is done right.
2. The patient should stand if possible and take a deep breath.
3. Empty his lungs in one breath, coughing as hard and deeply as he can.
4. He should spit what he brings up into the container.
5. Label the container with name and date.

Note: Liquid frothy saliva and secretions from the nose and pharynx are not acceptable. Sputum comes from the lungs and another specimen should be produced.

### Infected sputum can contain:

- thick mucus with air bubbles
- patches of pus and/or blood
- threads of fibrin

*Mycobacterium tuberculosis* (TB) is a very infectious disease. It is spread from person to person by inhalation of airborne bacteria and respiratory secretions (cough, sneezing or talking).

In the laboratory, special care should be taken when handling suspected specimens.

Disposable surgical masks should be used when handling sputum specimens.

### Preparations of Smears

#### Materials:

- 5% solution of bleach
- wooden applicator sticks
- 15 ml conical centrifuge tube
- clean slides
- cotton-tipped applicator

#### Procedure:

1. Mix the sputum with an equal part of 5% bleach.
2. Stir with wooden stick for even mixing.
3. Make sure lid is ON tight and shake for 2-3 minutes.

4. Allow to stand for 10 minutes at room temperature.
5. Pour into 15 ml conical centrifuge tube and spin for 10 minutes at low to med.speed.
6. Decant supernatant -- you now have a sterile creamy white sediment.
7. Using a cotton tipped applicator, transfer some sediment onto the slide.
8. Spread evenly and allow to air dry.

*No fixation with methanol is necessary.*

9. You are now ready to stain.

### **KINYOUN'S COLD ACID FAST STAIN**

#### **Procedure:**

1. Pass slide through the flame 3 times.
2. Place slides on a staining rack or 2 rods over a sink.
3. Flood with Kinyoun's carbol fuchsin and allow to stand for 5 mins. (Room temperature).
4. Rinse with distilled water.
5. Decolorize with acid-alcohol for 3 minutes.
6. Rinse again with distilled water.
7. Redecolorize with acid-alcohol for 1-2 minutes till no more red colour runs.
8. Rinse with water and drain water by tipping the slide to the side.
9. Flood slide with methylene blue and allow to stand for 4 minutes.
10. Rinse with water and allow to dry.
11. Examine under high power (X40) and confirm acid fast structures under oil immersion.

## ZIEHL - NEELSEN STAIN (HOT)

### Materials and Reagents:

- New glass slides
- Inoculating loop
- Cotton wool plug on metal wire for flaming
- Timer
- Carbol-Fuchsin
- Acid-ethanol
- Methylene blue
- Methylated spirit (for burning)
- Wash bottle of water

### Procedure:

1. Make the smear as thin as possible and use as much area as you can.

Note: If inoculating loop is used, put it into liquid detergent solution and shake. Then pass thru a flame until dry. This prevents airborne bacteria.

2. Allow smear to air dry then fix by passing thru the flame at least 3 times.
3. Place slides on staining rack.
4. Flood slides with carbol fuchsin (filter before use).
5. Dip cotton wool swab in the methylated spirit, light it and pass slowly under the slides.
6. When steam begins to rise, set timer for 5 minutes.
7. Continue to heat so that steam continues, without boiling, for 5 minutes.
8. If stain starts to dry, slowly add more.
9. Cool slides, then wash with distilled water till the run-off is colourless.
10. Decolorize with acid-ethanol and leave on for 3 minutes.
11. Wash slides and drain off water.
12. In thick smears, fuchsin may still be seen. Decolorize again for 1 minute and wash.
13. Cover slide with methylene blue for 30 seconds.
14. Wash with tap water for 1 minute.
15. Drain and place in rack to air dry.

16. Examine slide.

Do not mistake for tubercle bacilli.

1. Yeast-stained more or less red. When heated they break into groups of large red granules.
2. Spots of stain deposit — the slide is improperly decolorized.

**Principle of the stains**

Carbol fuchsin

- Stains all things in the sputum red.

Acid-alcohol (ethanol)

- Takes the red out of all organisms except acid-fast bacilli (TB).

Methylene blue

- Stains all organisms and elements blue.
- The acid-fast bacilli remain red.

**Slide Examination:**

- Scan on high power (X40) (3 minutes).
- Identify using oil immersion (10 minutes).

Tubercle bacilli are:

- red, slender, delicate
- 1-4 um long
- beaded or banded appearance
- may be curved
- may appear to have tapered ends
- often arranged in groups (bits of string or forked shaped)

**Results:**

Positive

- If 2 slides are made, and bacilli are found on the first, examine the second slide before reporting a positive result.

Negative

Examine both slides for 10 minutes each before reporting a negative result.

## RECORDING OF RESULTS

Number of acid-fast bacilli (AFB) found	Report	Alternatively
0	Negative for AFB	—
1-2/3000 fields	Numbers seen	±
1-9/100 fields	Number/100 fields	1 +
1-9/10 fields	Number/10 fields	2 +
1-9/field	Number/field	3 +
9/field	9/field	4 +

### **Pathogenicity**

The bacilli may enter the body through inhalation, producing the local lesions of pulmonary tuberculosis; by ingestion, producing primary lesions in the mouth or tonsils or in the wall of the intestine; or directly through the skin, producing a local ulceration. Pulmonary tuberculosis is the form of the disease with the highest rate of morbidity and mortality.

# **PARASITOLOGY**

## PARASITOLOGY

Parasitology is the study of parasites that cause disease in man. A parasite is an organism that lives in or on another living organism of a different species and obtains food from it.

Parasites can be found in the following types of specimens:

- Fecal (stool)
- Blood
- Urine
- Sputum
- CSF
- Tissue samples

For purposes of this manual we will discuss only those parasites commonly found in stool and blood.

### STOOL EXAMINATION

#### Specimen Collection

1. Stool will be collected into a clean, wide-mouthed container and should not be contaminated with urine, water or soil.
2. The stools should be brought to the laboratory as soon as possible.
3. Liquid specimens should be examined within 30 minutes if possible.
4. Formed specimens may be kept at room temperature for a few hours but should be examined the same day.
5. To be kept longer, the specimen should be refrigerated and some should be put into a preservative (10% formalin).
6. Multiple specimens collected over a period of time may be needed to diagnosis some parasitic infections.
7. Sufficient quantity of specimen is need to:
  1. prevent drying of stool
  2. find parasites in low concentration
  3. do a good microscopic exam
8. Formed stools should at least be the size of a walnut. Liquid stools should be at least 5 tablespoons ( $\frac{1}{2}$  teacup).
9. If many specimens are received at the same time, examine the watery stools first.
10. Never leave stools exposed to air (in containers without a lid).
11. All specimens must be properly labeled.

## MACROSCOPIC EXAMINATION

### Procedure:

1. Check label of specimen to be sure it matches the request form.
2. Carefully remove the lid from the container. Only one specimen at a time.
3. Check for:

- Colour
- Consistency
- Blood
- Mucus (quantitate)
- Worms
- Record the results

4. Colour

- Brown
- Black
- Dark brown
- Green
- Yellow

5. Consistency

- Watery
- Loose
- Soft
- Formed
- Semiformed
- Mucoid

#### Example:

1. Dark brown, loose, trace of mucus
2. Mucoid, Brown, 2 + blood
3. Black, Watery, 2 + mucus

6. Replace lid and move to next sample or do the wet mount immediately.



## WET MOUNT SLIDE PREPARATION

### Materials:

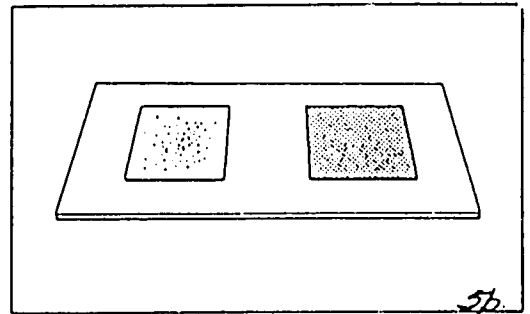
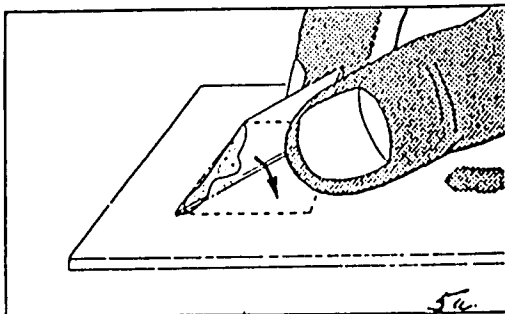
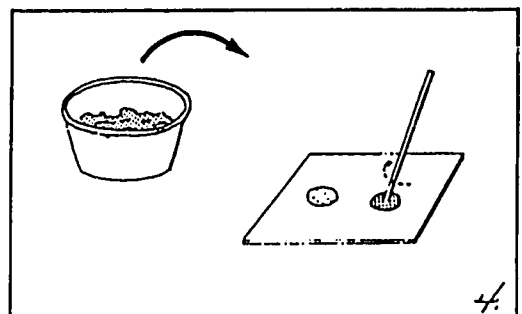
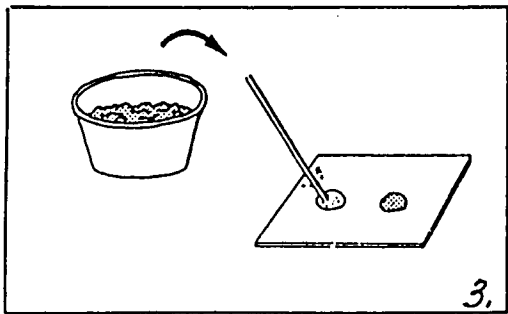
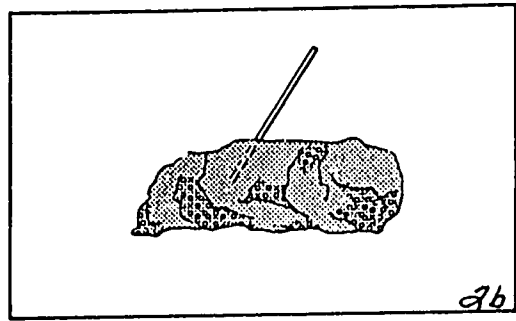
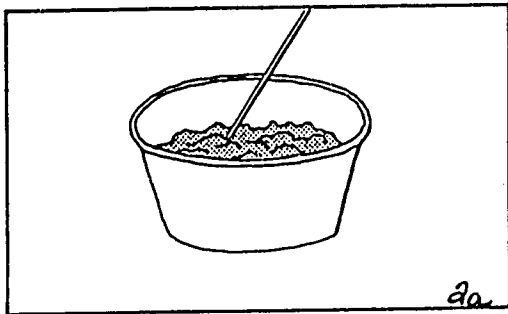
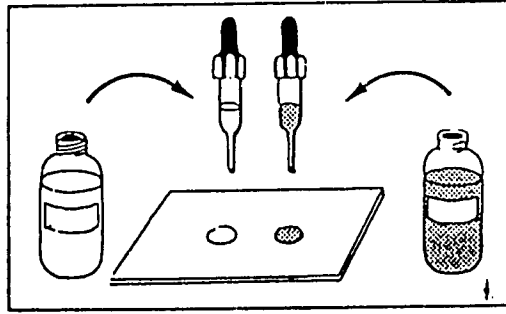
- Microscope
- Slides and coverslips
- Wooden applicator sticks
- Sodium chloride (saline)
- Lugol's iodine (diluted)

### Procedure:

1. Using a clean slide, place 1 drop saline on right half of slide and 1 drop lugol's on left half of slide.
2. Using an applicator, take a small portion of stool.
  - if stool is formed, take sample from the middle and the surface.
  - if stool has mucus or blood, take sample from the surface.
3. Mix the sample with the drop of saline first.
4. Take another sample and mix with the drop of iodine.
5. Place a coverslip over each drop as shown. (Avoid air bubbles).
6. Place the slide on the microscopic, using the low power X10 objective and low light, scan the saline side first.
7. Look for any motile forms, eggs or worms.
8. Identify all organisms on high power (X40).
9. Move to iodine side and using X40 and more light, look for cyst forms, eggs etc.

Note: The iodine will:

- destroy troph forms
  - stain cytoplasm of cysts (yellow-brown)
  - nucleus becomes distinct and can be counted
10. Record all organisms found. If no parasites are found report NPF.



## PINWORM TECHNIQUE

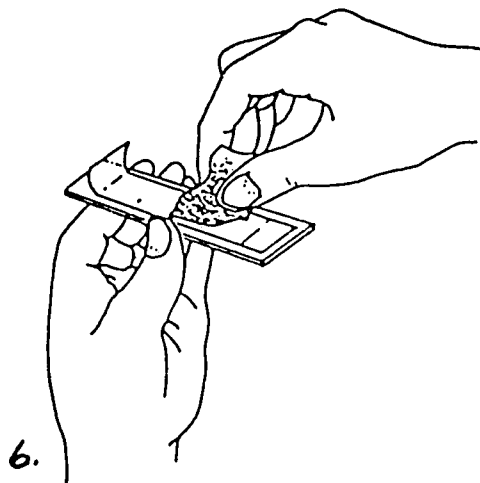
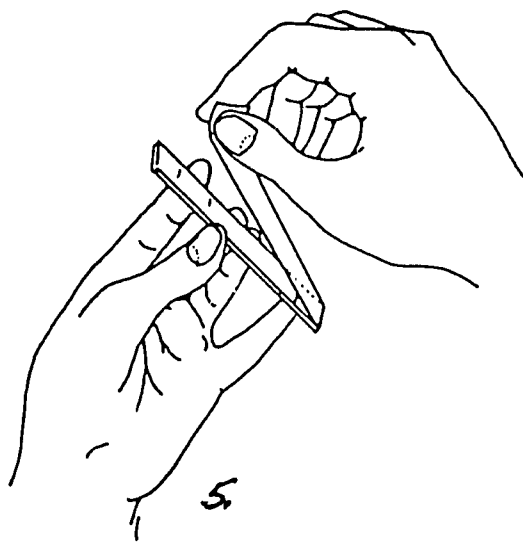
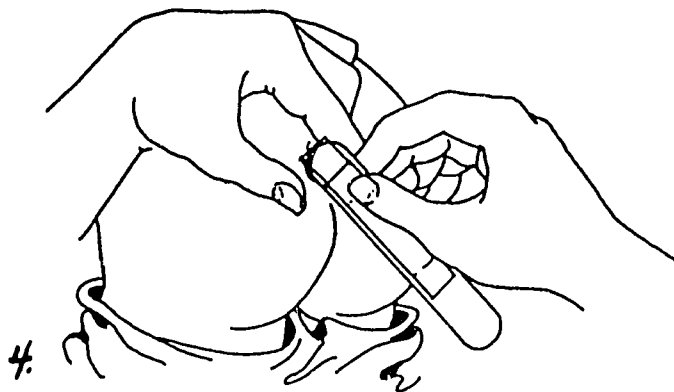
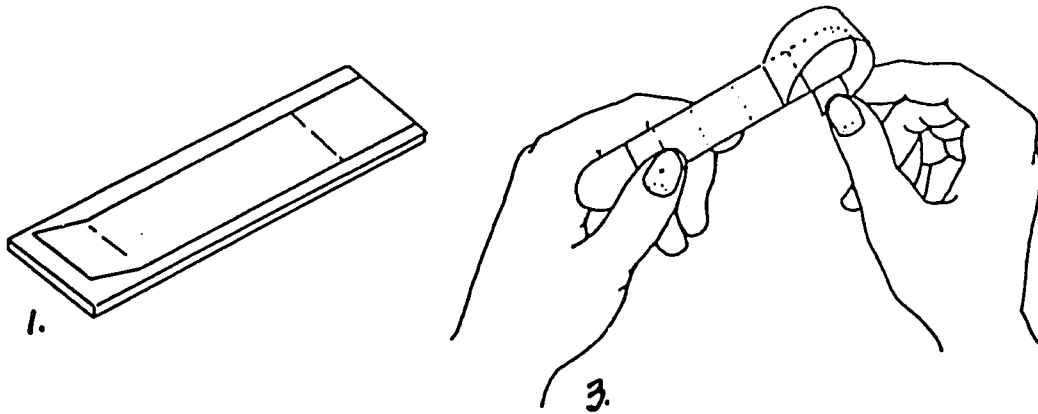
The eggs of the pinworm are usually collected (particularly in children) in the folds of skin around the anus. They are rarely found in stools.

### Materials:

- Cellophane tape
- Wooden tongue depressor
- Slide

### Procedure:

1. Place tape, sticky side down on a slide.
2. Place tongue depressor (or spoon handle) flat against underside of slide.
3. Gently pull the tape and loop it over the end of the tongue depressor (or spoon handle), hold firmly together.
4. Separate patient's buttocks and touch the tape (sticky side) against the skin in several areas.
5. Spread the tape back over the slide, sticky side down.
6. Press firmly and examine.
  - If no tape is available a cotton swab and saline can be used.
  - Wipe swab around outside of anus and dip into about 10 drops of saline.
  - Mix well and place a drop of mixture on slide, coverslip and examine.



## PARASITE IDENTIFICATION

Identification of parasites in humans depends mainly on the recognition of their cyst and/or trophozoite stage.

### Trophs

- Characteristically has a thin membrane and shows considerable variation in size and shape. They are also moving in some cases.

### Cysts

- Spherical, round, or elongate and have less size variation and usually have a uniform, smooth wall.

Characteristics that are used for identification:

- (a) Size — measured, by a micrometer, can vary depending on the age of the specimen and if it is in a preservative.
- (b) Motility — trophs may have some characteristic movement in freshly passed, liquid or soft stools.
- (c) Nuclei — depending on the species, 1 to 8 nuclei can be found with a fixed number in mature forms. Size, location, and karyosome all aid in identification.
- (d) Cytoplasm — coarse or fine granular, presence of nucleus, fibrils and ingested material (RBC's, WBC's etc) aid identification.

## PROTOZOA

Identification of intestinal protozoa is based on the morphology of trophs and cysts seen in the stool specimen. The trophozoites (trophs) are motile:

- slow, progressive movement of a cell. (amoeba)
- or because they have rapidly moving flagella (long whip-like threads) or cilia (many short hairs).

### Classification:

1. amoeba — without flagella or cilia
2. flagellates — with flagella
3. ciliates — with cilia

Some protozoa are pathogenic (harmful); others are less so or are non-pathogenic (harmless). However, the presence of some of the non-pathogenic organisms are “markers” that indicate the patient has ingested fecally contaminated food or water. Another specimen is needed to see if additional organisms are present.

### Amoeba

1. Entamoeba histolytica:
  - may cause dysentery, prolonged sickness can cause abscesses.
  - only amoeba that is always pathogenic.
2. Entamoeba coli:
  - non-pathogenic
  - indicates fecal contamination ingested
  - usually a reliable “marker”
3. Entamoeba hartmani:
  - non-pathogenic, however, this status is questioned by some.
  - important because it has to be distinguished from E. histolytica.
4. Endolimax nana:
  - smallest of the amoeba
  - non pathogenic
5. Iodamoeba butschlii:
  - non-pathogenic
  - large glycogen mass

6. *Dientamoeba fragilis*:

- considered by most to be non-pathogenic, however, it apparently has been the main cause of diarrhea and discomfort in some patients.
- no cyst stage.

**Flagellates**

1. *Giardia lamblia*:

- pathogenic (giardiasis)
- not difficult to diagnosis

2. *Trichomonas hominis*:

- non-pathogenic
- distinguish from giardia

3. *Chilomastix mesnili*:

- controversy on whether it's pathogenic
- flagella sometimes hard to see, can be confused with *E. histolytica*.

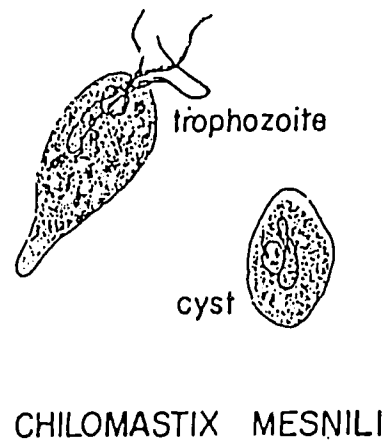
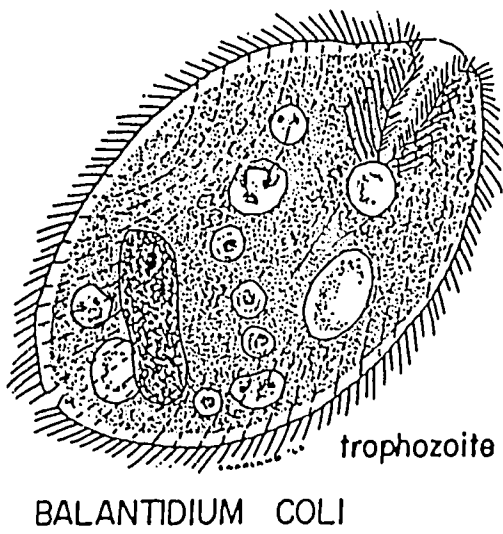
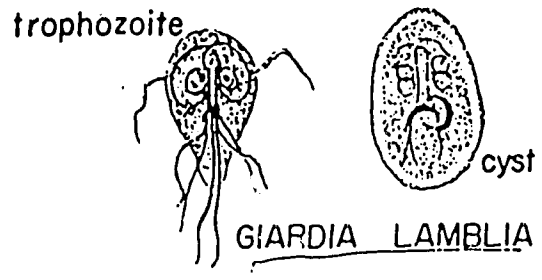
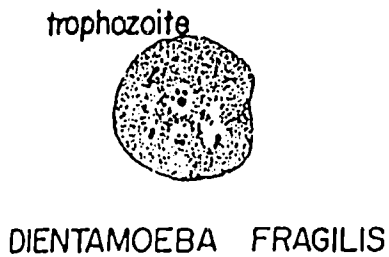
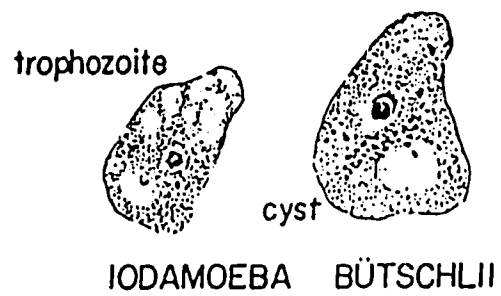
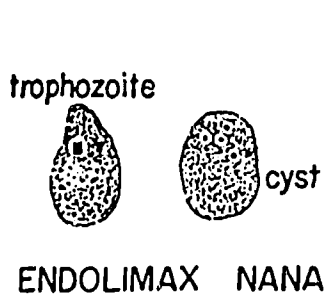
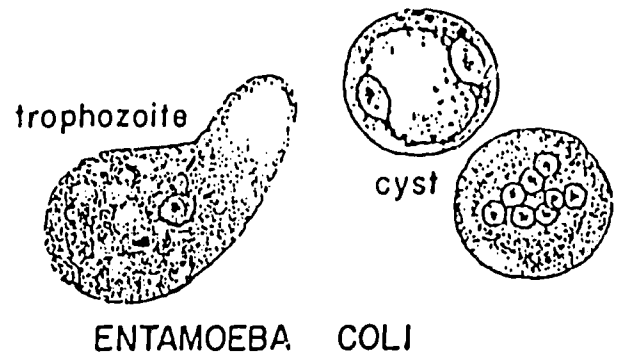
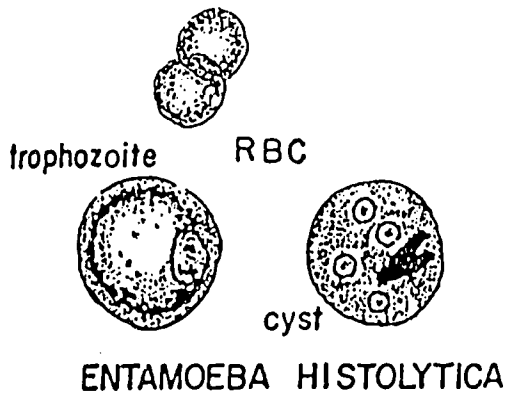
**Ciliates**

1. *Balantidium coli*:

- pathogenic
- largest of the protozoans
- only ciliate parasite found in humans

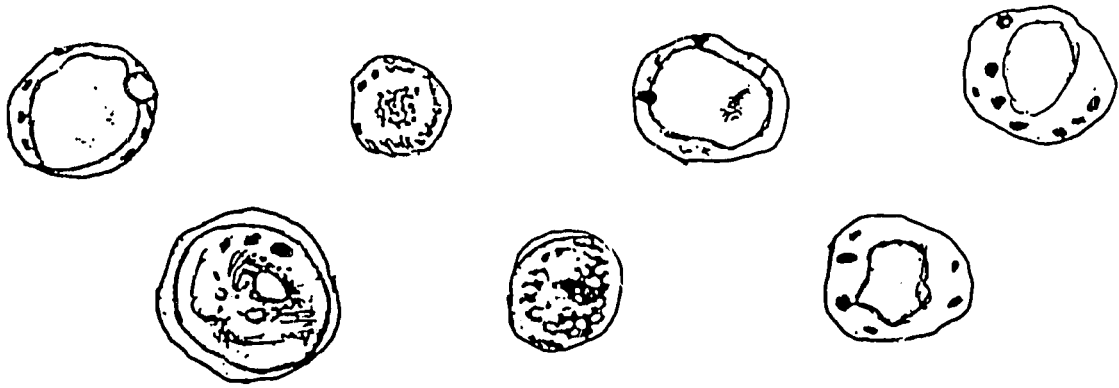
2. *Blastocystis hominis*:

- protozoan of uncertain classification
- in large numbers can cause prolonged, mild diarrhea.
- found in small numbers in stools, usually indicates the presence of other organisms.



**INTESTINAL PROTOZOA OF MAN**

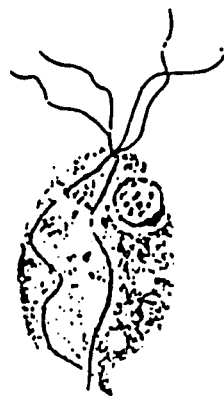




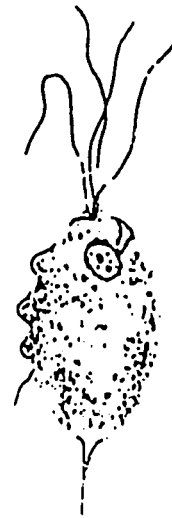
**Blastocystis hominis**



**Chilomastix mesnili**



**Trichomonas hominis**



**Trichomonas vaginalis**

**INTESTINAL PROTOZOA OF MAN**

## HELMINTHS

Helminth infections are diagnosed by finding characteristic eggs, larvae, or proglottids in stools. For the most part, the eggs are uniform in shape, colour and stage of development.

### Identification Criteria:

#### Size

- length range = 25 um to 150 um or longer
- diameter range = 12-14 um to 90 um
- assess according to microscopic field ( $\frac{1}{2}$  the field,  $\frac{1}{3}$  the field)

#### Shape

- from spherical to elongate is typical.

#### Eggshell

- most usually have a smooth shell that vary in thickness
- colour varies from colourless to yellow/brown
- structures can modify i.e.:
  - plugs (Trichuris)
  - ruffled (Ascaris)
  - striations (Taenia)
  - spines (Schistosoma)
  - knobs (Clonorchis)
  - operculum (Diphyllobothrium)

#### Stage of Development

Freshly passed eggs:

- characteristic for each species
- usually unembryonated
- if allowed to stand for a day or so can proceed to hatch (hookworm eggs)

Note: All findings are pathogenic.

#### 1. Enterobius vermicularis (pinworm):

- most common human infection (children)
- not found in feces but at the anal opening
- eggs are oval and flattened on one side with thick, colourless shell
- 50-60 um
- granular mass or embryo of worm inside

2. *Ascaris lumbricoides* (roundworm):

- largest roundworm of man
- Fertile egg — round to oval 45-65  $\mu\text{m}$ , “ruffled” edge with a thick inner hyaline shell.
- Decorticated egg — no “ruffles” with thick shell, inner content usually round and does not fill whole shell.

All eggs are brown/yellow in colour.

3. *Trichuris trichiura* (whipworm):

- barrel shaped 50-55  $\mu\text{m}$  with smooth thick shell
- round, transparent plugs at both ends

4. *Ancylostoma duodenale* (hookworm):

- thin shell, colourless 60-75  $\mu\text{m}$
- 4-8 granular cells — sometimes 16 depending on maturity
- eggs shed in feces in soil

5. *Strogylodes stercoralis* (threadworm):

- thin shell, resemble hookworm eggs
- rarely seen because eggs hatch in the intestine and the worm passes
- human infection by skin penetration (from soil)
- patient often asymptomatic (no diarrhea)

6. *Taenia saginata* (beef tapeworm):

- spherical, yellow-brown 30-45  $\mu\text{m}$
- thick shell with radial striations
- eggs not infective to humans
- six hooked embryo
- do not confuse with pollen
- look for proglottids

7. *Taenia solium* (pork tapeworm):

- identical to *saginata*
- eggs very infective to humans
- look for proglottids

8. *Hymenolepis nana* (dwarf tapeworm):

- spherical with thin hyaline shell 30-47  $\mu\text{m}$
- 4-8 filaments arranged like a fan
- common in children

9. *Hymenolepis diminuta* (rat tapeworm)

- large (70-85  $\mu\text{m}$ ) spherical
- yellow-brown thick shell
- 6 hooklets in a fan shape
- rat fleas are carriers

Note: Quantitate and note all eggs seen.

## WORMS

1. Large roundworm (*Ascaris*):

- lt. pink-salmon colour
- 15 cm to 25 cm long
- males have curved tails, female straight

2. Small roundworm (pinworm):

- white and motile
- female — long pointed tail (most frequent)
- male — blunt tail
- found in stools or around the anus

3. Whipworm:

- Males 30-45 um long coiled tail, females 35-50 um straight tail, thread — like bottom with thicker short heads.

4. Hookworm:

- long buccal cavity
- pointed tail
- 8-14 mm
- sheath around larvae

5. Threadworm:

- only female infective 2-3 mm
- short buccal cavity
- notched tail
- no sheath

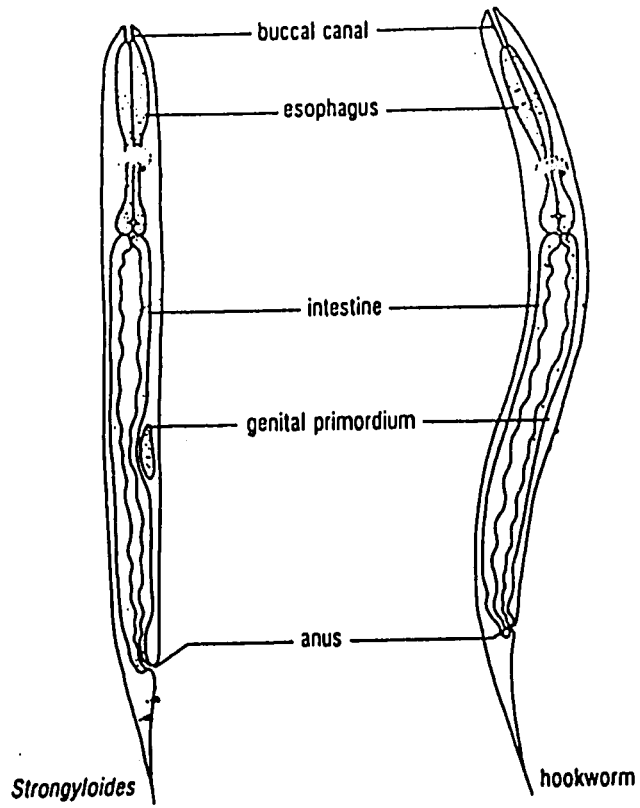
6. *Taenia* species (proglottids):

- *saginata* — scolex with 4 suckers, no hooklets, proglottids — longer than wide, over 15 branches per side, passed singly.
- *solium* — 2 rows of hooklets, proglottids passed in short chains, 7-13 branches on each side.

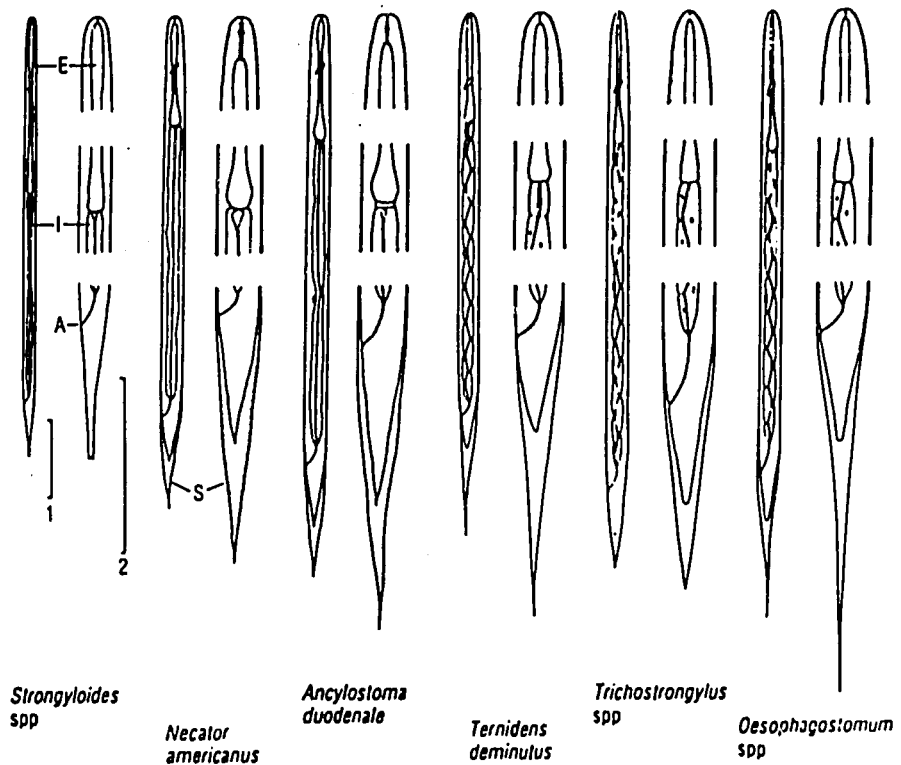
7. Examination of Worms and Proglottids:


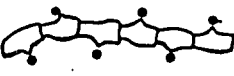
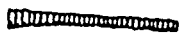


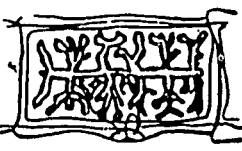
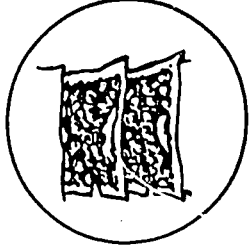

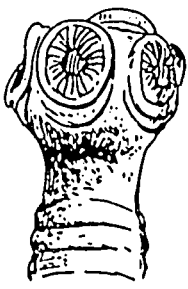

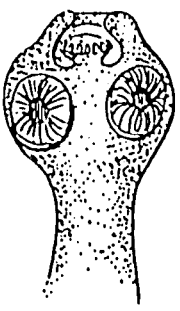
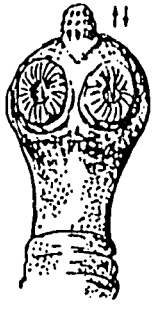
- examine chain or single proglottid
- place between two slides and hold up to light — you can see the branches

Note: If proglottids become dry and roll up, moisten with water. If whole worm is found, use forceps to carefully untangle, look for the head (rarely found) to look for the scolex. Use magnifying glass or microscope with X10 objective.



Morphology of first-stage rhabditoid larvae of *Strongyloides* and hookworm.



Most common	Less common		
<b>BEEF TAPEWORM</b> ( <i>Taenia saginata</i> )	<b>PORK TAPEWORM</b> ( <i>Taenia solium</i> )	<b>DWARF TAPEWORM</b> ( <i>Hymenolepis nana</i> )	<b>DOG TAPEWORM</b> ( <i>Dipylidium caninum</i> )
Single rectangular segments found in underclothes and in the bed; they pass through the anus independently of the stools	Small chains of 3-4 rectangular segments found in the stools	A small worm 2-4 cm long	A worm 5-30 cm long
They are part of an intestinal worm 3-5 m long			
			
Pores arranged in irregular alternation	Pores generally arranged in regular alternation	Pores all on the same side	Two pores on opposite sides of each segment
Ivory white segment of 1-2 cm	Pale blue segment of 0.5-1.5 cm	1 mm wide	Reddish segment of 0.3-0.5 cm
			
About 20 uterine branches	About 10 uterine branches	Uterine branches hardly visible	Two bunches of uterine branches
			
4 suckers (2 mm diameter) Very thin neck	2 crowns of hooklets - 4 suckers (1 mm diameter)	1 invaginated crown of hooklets - 4 suckers (0.5 mm diameter)	4 external crowns of hooklets - 4 suckers (0.5 mm diameter)

**OTHER TAPEWORMS:**

*Dibothriocephalus* is found mainly in cold climates.

*Echinococcus* is found in dogs; only the hydatid cyst is found in man.

## MALARIA PARASITES

Malaria parasites are found in the blood. Anopheline mosquitoes are responsible for the transmission to humans. There are 4 species that are found in humans.

*Plasmodium falciparum*  
*Plasmodium vivax*  
*Plasmodium malariae*  
*Plasmodium ovale*

*Falciparum* and *vivax* are most common in Pakistan and Afghanistan. Diagnosis of malaria is made by finding the parasite on a thin and/or thick blood slide stained with Giemsa or Field stain.

### Prepare the Slide:

Blood — parasites most numerous at the end of a fever cycle, take blood then. If the slide is negative, make another slide 10-12 hours later. Always collect blood before antimalarial drugs are taken. Capillary blood is best for the detection of malaria.

*Thick film* — used to detect parasites.

*Thin film* — used to identify species for treatment.

Always prepare both thick and thin smear.

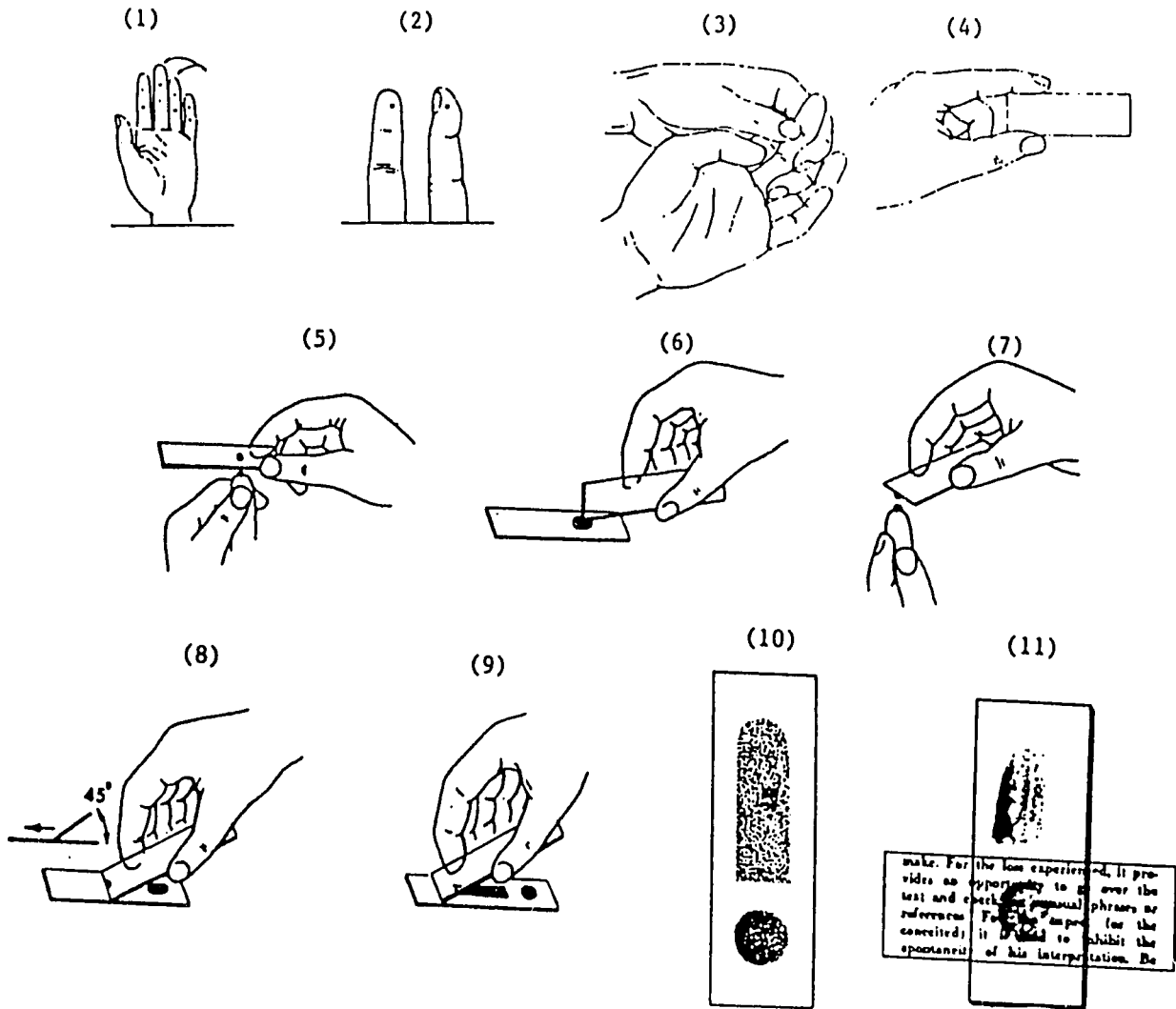
*Giemsa* is the recommended stain.

### Procedure:

1. Wipe finger with ethanol and dry well.
2. Using a sterile lancet, prick side of finger.
3. Collect the first drop of blood on slide and make film.
4. Spread drop using the edge of another slide. Too thick or thin will not stain well. (see print thru).
5. After thick slide is spread, collect drop for thin film.
6. Label slides properly and allow to dry.

Do not keep unstained slides for more than 4 days. Thick and thin smears may be on same slide (less staining time) or they may be done individually.





**Preparation of thick thin blood smear on same slide:**

1. Selection of 2nd or 3rd finger on either hand is suggested.
2. Puncture finger at side of ball of finger but not too close to nailbed.
3. Gentle squeeze of the finger may be needed.
4. Grasp slide by its edge (first slide).
5. Touch the slide with drop of blood from below.
6. Spread drop of blood to about 1 cm<sup>2</sup>.
7. Touch a new drop of blood with edge of another slide.
8. Bring edge of slide with the blood to touch surface of first slide, let blood spread along edge.

9. Push slide at about 45°, forward with steady rapid motion.
10. Write with pencil the slide identification number and date. Place slide on flat surface to allow thick smear to air-dry, keeping insects such as flies from lapping up the wet blood.
11. Thick blood smears are of the correct thickness when printing may be easily seen through it.

### **Staining of thick and thin films:**

#### **Fixation**

Prior to staining, fix thin film only in methanol and allow to air dry. If both thick and thin are on same slide do not touch thick film with methanol.

#### **Giemsa Stain (Dr. Chin's method)**

1. Fix thin smear and allow to air dry.
2. Stain for 1-3 slides.
  - using a syringe, draw up 1 ml of giemsa and 9 ml of water.
  - pull plunger back some and mix solution by slowly inverting.
  - place slide on a plastic convex rack face down, and flood slide.
  - allow slide to stain for 10 minutes.
  - carefully rinse in 2 changes of water.
  - place in rack and allow to air dry (stain side down to protect from dust).
  - examine under oil immersion.

Note: Dr. Chin developed this method for this area. Water pH is already around 7.2 and no buffer salts are required.

#### **Multiple Slide Staining (giemsa)**

- A 1 in 10 dilution of stain may be made in a graduated cylinder for many slides.
- mix gently with a glass rod.
- flood with stain and let stand for 10 minutes.
- rinse in 2 changes of water.
- allow to air dry and read.

## **Field Stain (Thick film only).**

### **Procedure**

1. Dip the dried thick smear into field stain A.
2. Count to 10, remove slide and drain.
3. Wash in container of tapwater — dipping until no more stain comes off.
4. Dip into field stain B and count to 10.
5. Rinse slide gently in tap water.
6. Dry in rack with film facing down.

### **IF:**

Smear too blue — stain longer in B  
Smear too pink — stain longer in A

### **Thin films:**

#### **Infected red cells may:**

- remain the same size
- stain more deeply
- become enlarged
- may contain pinkish dots

### **Thick films**

- reds cells disappear
- WBCs remain unchanged
- parasite forms remain
- report density (number per field)

Do not confuse platelets with malaria parasites.

Patients may have more than one type of malaria infection. After antimalarial drugs, parasites stain poorly and look distorted. When numerous parasites are found, the patient needs urgent treatment.

## FALCIPARUM

### Most pathogenic:

#### Host red cell:

- Not enlarged.
- Usually only trophozoites and gametocytes seen.
- May contain several parasites.
- No schuffners dots.

#### Trophs:

- Small delicate rings.
- May contain 2 chromatin dots.
- Heavy infections are common.

#### Gametocytes:

- Crescent (banana) shaped with round ends.

#### Schizonts:

- Very rarely seen -- their presence indicates severe infections.

## OVALE

Found mainly in West Africa (low prevalence).

#### Host Red Cells:

- May become oval.
- Schuffner's dots are present.

#### Trophs:

- Regular dense blue ring
- 1 Red dot.

#### Gametocyte:

- Large oval
- Has brown pigment.

#### Schizont:

- 8-14 merozoites in rosette.

## VIVAX

Reoccurrence if not treated properly.

#### Host Red Cell:

- Enlarged and irregular shape.
- Schuffner's dots are present.
- Pale staining.

#### Trophs:

- Large ring irregular in form.
- 1 Large red dot.

#### Gametocytes:

- Oval or rounded and dense blue.
- Contains scattered pigment.
- Often at one end of cell.

#### Schizonts:

- Large round
- Mature forms contain 18-24 merozoites and a small amount of pigment.

## MALARIA

Found in tropics and subtropic (low prevalence).

#### Host Red Cell:

- Normal size.
- No dots present.

#### Trophs:

- Thick dense ring.
- 1 Dot.
- Some granules of black pigment.

#### Gametocyte:

- Large, round to oval.
- Blue in colour.
- Red chromatin dot against one edge.

#### Schizont:

- Small compact neatly arranged merozoites.
- Yellow/brown pigment always present.

## REFERENCES

1. Lawrence R. Ash, Thomas C. Orihel: *Parasites: A Guide to Laboratory Procedures and Identification*; Chicago, Illinois, ASCP Press, 1987.
2. World Health Organization: *Manual of Basic Techniques for A Health Laboratory*; Geneva Switzerland, 1980.
3. Lawrence R. Ash, Thomas C. Orihel: *Atlas of Human Parasitology*, 2nd ed. Chicago, Illinois, ASCP Press, 1984.
4. Samuel I. Rapaport: *Introduction to Hematology*, 2nd Ed. Philadelphia, Penn, J.B. Lippincott Company, 1987.
5. Sarko M. Tilkian, M.D., Mary Boudreau Conover, R.N., B.S., ARA G. Tilkian, M.D., F.A.C.C.: *Clinical Implications of Laboratory Tests*. C.V. Mosby Company, St. Louis, Missouri, 1987.
6. S. Stanley Schneierson, M.D.: *Atlas of Diagnostic Microbiology*. Abbott Laboratories, North Chicago, Illinois.
7. L.W. Diggs, M.D., Dorothy Sturn, Ann Bell, M.S.: *The Morphology of Human Blood Cells*. Abbott Laboratories, North Chicago, Illinois, 1978.
8. *Modern Urine Chemistry*, Ames Division, Miles Laboratory, Inc.; Elkhart, Indiana, 1987.

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95644

PIISHTO

**COMPREHENSIVE ATLAS  
FOR THE  
LABORATORY**

دلا براتوار اڀاره مڪمل اڀلس

Text by  
Carol L. Phillips  
Medical Technologist, B.S., M.A.  
American Society of Medical Technologist

# COMPREHENSIVE ATLAS FOR THE LABORATORY

دلابراتوار لپساره مکمل اطللس

متن :- کارل، ل، فلیپس  
طبی تکنالوژیست  
د امریکا د طبی تکنالوژیستانو د تولنی غری  
ژبارونکی : داکتر غلام ربانی (( وردک ))

Text by  
Carol L. Phillips  
Medical Technologist, B.S., M.A.  
American Society of Medical Technologist

## PREFACE

The purpose of this atlas is to be a comprehensive reference to aid the microscopist in identification of blood cells, parasites, and urine sediment. The atlas can also be used as a training aid for instructors and students. This atlas is basic and by no means contains all the organisms or cells that can be seen. Use your knowledge and experience, this atlas, and then ask for a second opinion of those better qualified when identification is uncertain.

بسم الله الرحمن الرحيم

سريـزه

ددغه اطللس د جوړيدو مقصد د دى چه د مايكروسكوپيستانو له پاره يو پوره او مكممل ماء خذدى. او د نوموړى اطللس په مرسته دوى وكولى شي چه دوينى حجرى، پرازيتونه او دادرار ( بولى ) رسوبونه شه وپيژنى او تشخيصى كړى ددغه اطللس څخه شونكى ا و زده كونكى د زده كړى دمرستندوى په توگه گټه اخيستى شي څرنگه چه دا اطللس د پير ابتدائى دى نو ځكه د ټولو اورگانيزمونو او حجرو څخه وړيكيڼى يادونه نده شوى. كه چيرى په تشخيص باندې داده نه وي لمړى له خپلى پوهى تجربى او ددغه اطللس څخه كار واخلى ، كه چيرى بيا هم په تشخيص بريالى نشوى نو ديوه پوه او تجربه كار ه سرى څخه غوښتنه وكړى تر څو په دى هكله خپل نظر څرگند كړى.

Translations typed by Abdul Fateh and proof read by Rahim Ghaznawi.

Translated by WHO Peshawar,  
Printed with the cooperation of:

ترجمه : داکتر غلام ربانى وردک

Office of AID Representative and USAID  
Primary Health Care Project  
Islamabad, Pakistan

چاپ ټى د )

د AID د نمايندگى دفتر او USAID

په مرسته

لمړى روغتياى مراقبت پروژه

سرت ه رسيدلى دى

اسلام اباد - پاكستان



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# **HEMATOLOGY**



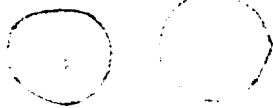
Normal (discocyte)  
سورماله حجره  
(دیسکوسیت)



Crenated (berry)  
کمبره داره حجره  
(دوباره سان حجره)



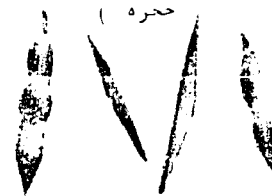
Burr (echinocyte)  
اغزی (اغزی لرونکی  
حجره)



Target (codocyte)  
سارکیت حجره  
(کودوسایت)



Spherocyte  
کروی حجره  
(سفروسایت)



Oval



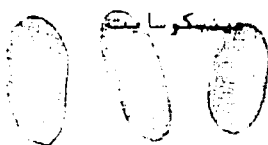
Sickled  
(drepanocyte, meniscocyte)  
دلوربه سان



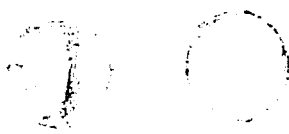
SC crystal



CC crystal



Ovalocyte  
(elliptocyte)  
بیضوی حجره  
(بیضوی حجره)



Stomatocyte



Folded



Marginal achromic  
(blast)  
خنده لرونکی  
سیریکه حجره (سماکی به سان حجره)



Helmet  
دحری حولی به سان  
حجره



Pinched (pinched)



Pointed (pear, teardrop, dacryocyte)  
تیره حوکه لرونکی  
حجره (دساک په دول، دآ و سکی دحا حکی  
به دول او دکروسایت



Filamented حجره  
تار لرونکی (تار لرونکی)



Triangular  
(مثلثه ورته حجره)



Poikilocyte (small, dark, irregular)  
سویکیلو سیروسایت  
(کوچنی، تیاره، غیر منظم)

Acanthocyte (thorn, spur, spicule)  
اکانتوسایت (تیلغ لرونکی  
سار اغزی لرونکی حجره  
(اغزی، مهمیز، سپیخک)

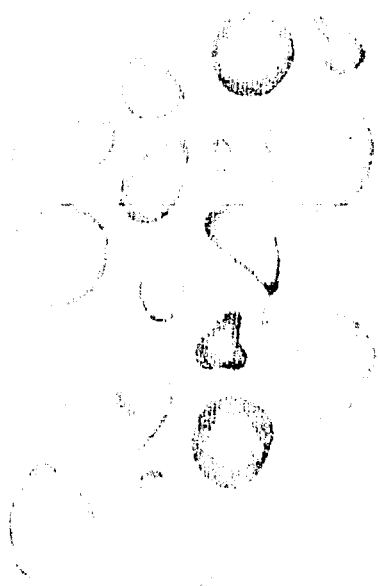
Schistocyte (schistocyte)  
شیزوسایت  
(سستوسایت)

Crescent

دمیاشتی به دول حجره (هلالی حجره)  
دنیمی میاشتی به دول  
(نیمه هلالی حجره)

### SHAPES OF RED CELLS

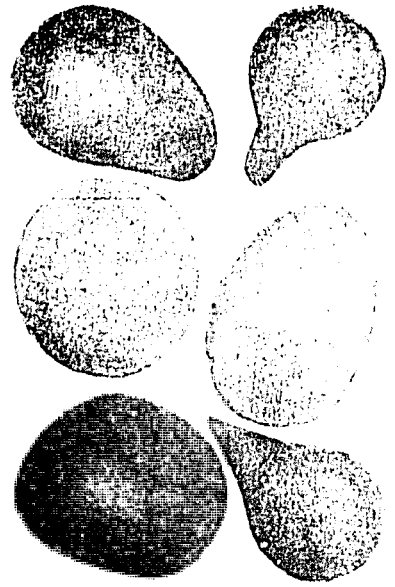
دسترو حجره و شکلونه



Iron Deficiency Anemia



Normal



Pernicious Anemia

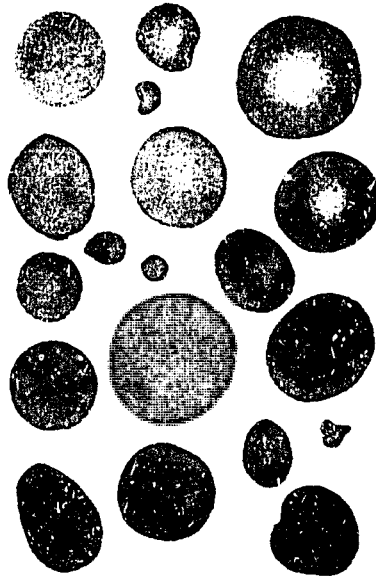
The cells portrayed in this plate do not represent actual fields but are cells selected to reveal the characteristic morphological variants. Magnification 2000 X

حسنة کمخونی  
 دا حجری چه به دغه کلیته اودریونورور اتلونکوکلیشو  
 کی نورمال رسمشوی دی دمیکروکوب داملی ساجی خخه نمابندگی  
 به کوی بلکه هغه حجری دی چه دسترو حجرو دثاکرو دولونو  
 لپاره شودل کیبری ( به ۲۰۰۰ مکنیفیکشن )



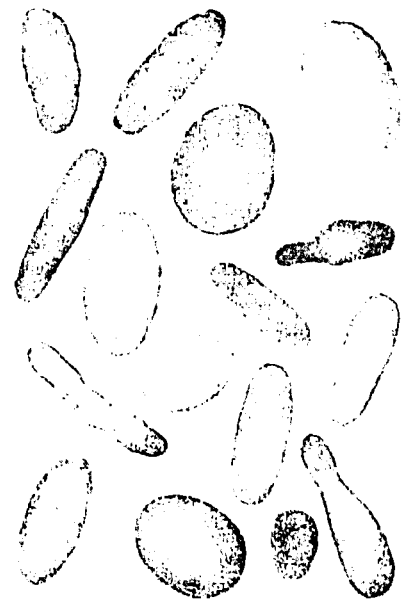
Thalassemia Major

لوبه تلسیمیا



Hereditary Spherocytosis

ارشی کروی دوله  
 حجری



Ovalocytosis

بیسوی حجری

لوی لمفوسیت چه سری بنفش  
دوله دانی (اوزوروفلیک  
آودشا وخوا حروپه ذریع  
عبر منظم شویدی .

ایزونوفیل

مونوسیت چه اسی حررگی  
سایتوپلازم لری، کروما ت  
شی خطی سری او حیره -  
معلومیری اوکا ذسی بیید

سوتروفیل چه دبا سده شکل  
هسته لری .

دوینی ستره حجره

سیگمنتی نیتروفیل

سیگمنتی نیتروفیل

لمفوسایت

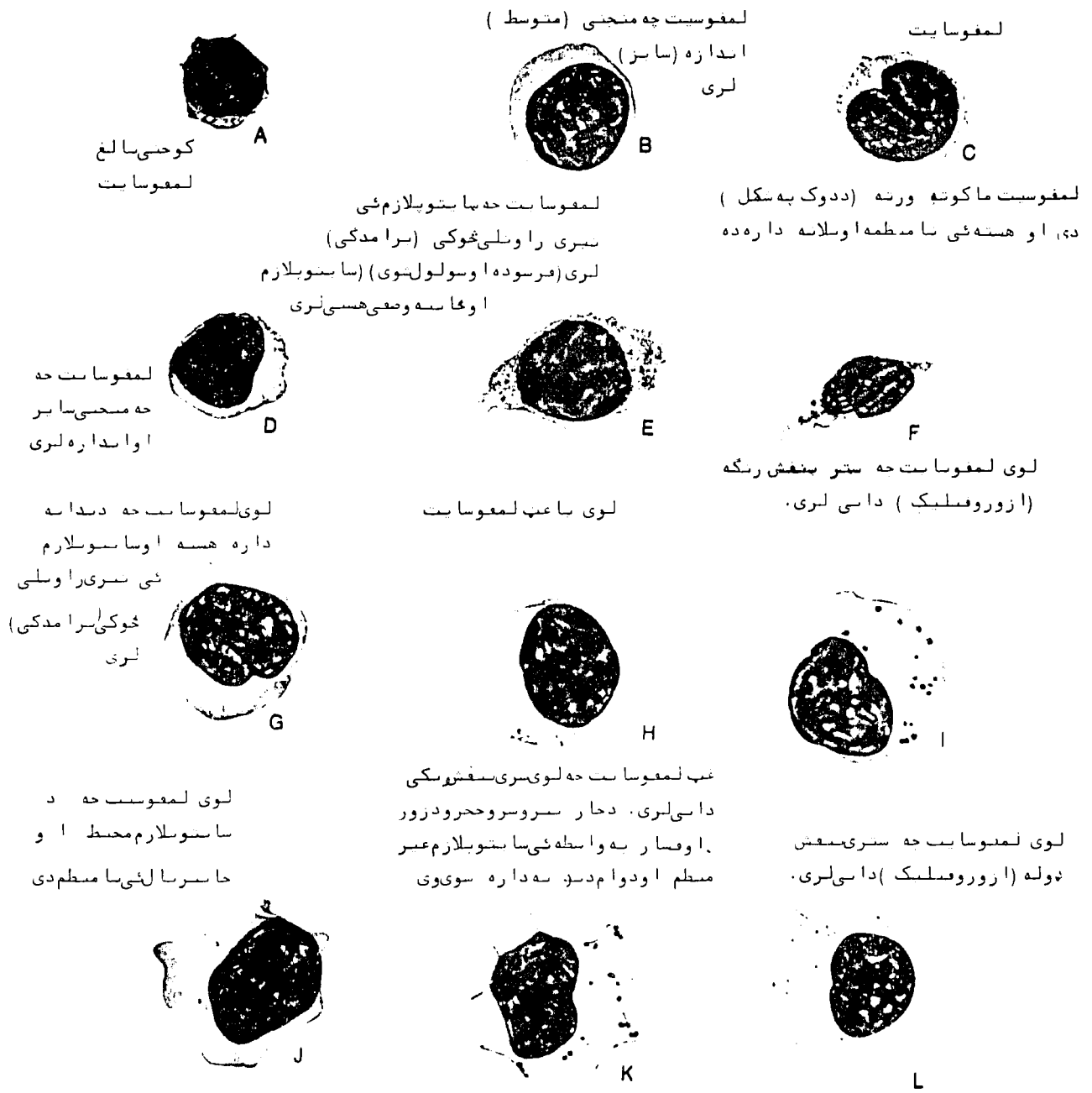
پروموسیت  
(دمویه صفحات)

سزولفیل

**CELL TYPES FOUND IN SMEARS OF PERIPHERAL BLOOD FROM NORMAL INDIVIDUALS**

- |  |   |
|--|---|
| A Erythrocytes   | F Monocyte with blue gray cytoplasm, coarse linear chromatin and blunt pseudopods |
| B Large lymphocyte with purplish-red (azurophilic) granules and deeply indented by adjacent erythrocytes | G Thrombocytes  |
| C Neutrophil segmented   | H Lymphocyte  |
| D Eosinophil   | I Neutrophilic band   |
| E Neutrophilic segmented   | J Basophil  |

*The arrangement is arbitrary and the number of leukocytes in relation to erythrocytes and thrombocytes is greater than would occur in an actual microscopic field.*



**LYMPHOCYTES** لمفوسایتونه

- |   |  |
|---|--|
| A Small mature lymphocyte   | H Large lymphocyte   |
| B Lymphocyte of intermediate size   | I Large lymphocyte with purplish-red (azurophilic) granules  |
| C Lymphocyte with indented nucleus  | J Large lymphocyte with irregular cytoplasmic contours   |
| D Lymphocyte of intermediate size   | K Large lymphocyte with purplish-red (azurophilic) granules and with indentations caused by pressure of erythrocytes |
| E Lymphocyte with pointed cytoplasmic projections (frayed cytoplasm); typical nucleus | L Large lymphocyte with purplish-red (azurophilic) granules  |
| F Spindle-shaped lymphocyte with indented nucleus                                     |  |
| G Large lymphocyte with indented nucleus and pointed cytoplasmic projections          |  |

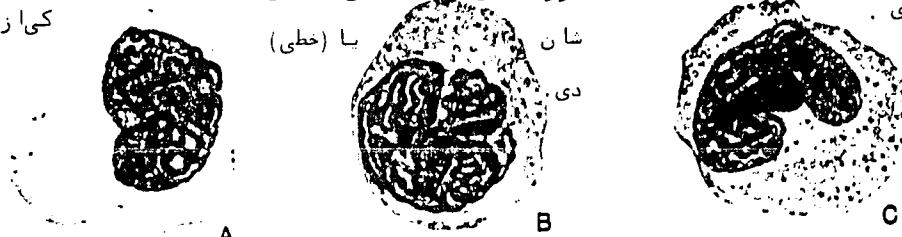
104

مونوسیت چه دمنوشویو -

شهو بنه لری او وریوری  
دانی به منظم دولیکی تورع  
شویدی کله کله ئی به سانبولارم  
کی ازوروفیلیک دانی او  
واکیولونه موجودوی

مونوسیت چه سابتوپلازم ئی دانی  
لرونکی وایی خاککی دوله رنگ لری  
هسته ئی خوبصمه (برخی) او  
کروماتین ئی دگرینی به  
شان دی  
یا (خطی)

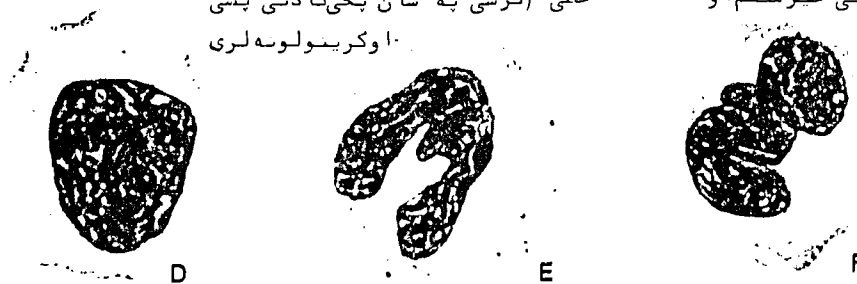
مونوسیت چه خرگنده او برجسته  
دانی ویا منطه او رندا داره .  
هسته لری .



مونوسیت چه هسته ئی منطه  
او دندانیه لری .

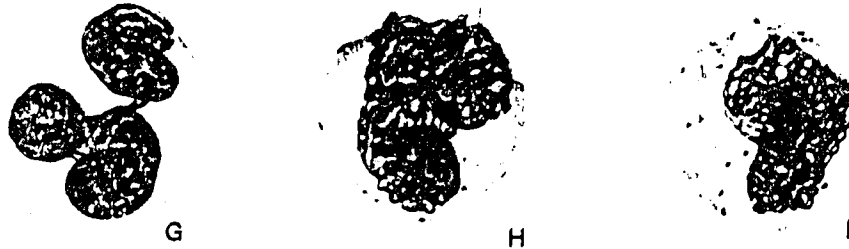
مونوسیت چه ایی خررنگ لری او هسته  
ئی تسمی ته ورته ده کروماتین ئی -  
خطی (کرسی به شان پخی کا ذبی پیسی  
او کریبولونه لری

نوسیت چه ایی خررنگ لری  
دهستی شکلئی غیر منظم او  
فصیصی دی .



مونوسیت چه هسته ئی پرخو  
برجوبشیل شوی ده .

مونوسیت چه واکیولونه لری او  
اکتوپلازم ئی بی دندانیه لری -  
ازوروپلازم ئی دندانیه لری .



مونوسیت چه خوبخی کا ذبی پیسی  
هسته ئی دندانیه داره نا منظمه  
والتوار داره (قائشوی) معلومی پی

MONOCYTES

مونوسیتونه

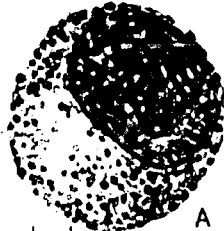
- A Monocyte with "ground glass" appearance, evenly distributed fine granules, occasional azurophilic granules and vacuoles in cytoplasm
- B Monocyte with blue gray cytoplasm and granules and with lobulation of nucleus and linear chromatin
- C Monocyte with prominent granules and deeply indented nucleus
- D Monocyte without nuclear indentations
- E Monocyte with blue gray color, band type of nucleus, linear chromatin, blunt pseudopods and granules
- F Monocyte with blue gray color, irregular shape and multilobulated nucleus
- G Monocyte with segmented nucleus
- H Monocyte with multiple blunt nongranular pseudopods, nuclear indentations and folds
- I Monocyte with vacuoles and with nongranular ectoplasm and granular endoplasm



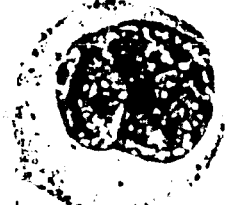
د ملوسیت چه د  
سوروفلیکودا بودمحلوط  
سره سوخای لمدل کسری ا و  
تساره سفین پورنگ لری

مونوسیت چه هستی شی  
سا و حورلی دی . سعی .  
هسه فاب سوی ده .

لوی لمفوسیت چه حلزونه ورتنه دی  
او هسته شی اسوانا نا ونوی هده  
(فات سوی نه ده .)



A د تیتا ملوسیت چه  
سا بتویلازم شی روشانه گلایی  
معلومیبری اونیوتروفلیکی  
داتی لری



B مونسیت چه سا بتویلازم  
ایی حررگی دی سرخته  
او واضح دایی وهسته شو  
قصیده کروماتین شی خطی



C لوی لمفوسیت چه سا بتویلازم شی دانی  
نه لری



D (کرسه لرونکی  
تارونه)



G میلوسیت



H وصفی مونسیت چه هسته شی خو  
فصونه لری . رنگی ایی حررنگ  
اوپخی کاذبی بشی لری .



I لوی لمفوسیت چه سری  
بنفشورنگه دانی  
لری وهسته شی  
موج داره ساختمان  
لری .

دمورفولوژی اندول یا مقایسه

نیروفیلیک کریبولوسا یتونه

ا ولمفوسا یتونه

**COMPARATIVE MORPHOLOGY:  
NEUTROPHILIC GRANULOCYTES, MONOCYTES, LYMPHOCYTES**

- |   |  |
|---|--|
| <p>A N. myelocyte with mixture of neutrophilic and dark reddish purple granules</p> <p>B Monocyte with nuclear fold</p> <p>C Large lymphocyte with scalloped shape and absence of folds in nucleus</p> <p>D N. metamyelocyte with light pink cytoplasm color and neutrophilic granules</p> <p>E Monocyte with blue gray cytoplasm, prominent granules and multilobulated nucleus (brain-like convolutions) and linear chromatin strands</p> | <p>F Large lymphocyte with nongranular cytoplasm</p> <p>G N. myelocyte</p> <p>H Typical monocyte with lobulated nucleus, blue gray color and blunt pseudopods</p> <p>I Large lymphocyte with purplish-red (azurophilic) granules and limpy nuclear structure</p> |
|---|--|

لمفوبلاست



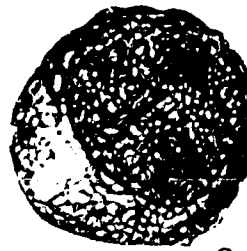
A

مونوبلاست



B

بلازموبلاست



C

پرولمفوبلاست



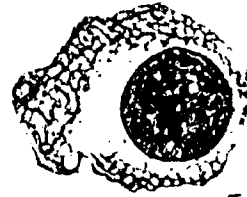
D

پرومونوسیت



E

پروپلاسموسیت



F

لمفوسیتجه در کروماتین



G

مونوسایت



H

پلازموسایت



I

لمفوسایتیک، مونوسایتیک، و بلازموسایتیک سیستمونه

**LYMPHOCYTIC, MONOCYTIC, AND PLASMOCYTIC SYSTEMS**

- A Lymphoblast
- B Monoblast
- C Plasmoblast
- D Prolymphocyte
- E Promonocyte

- F Proplasmocyte
- G Lymphocyte with clumped chromatin
- H Monocyte
- I Plasmocyte

# **PARASITOLOGY**

**PLATE 8**  
**Plasmodium vivax**

- Figures 1-3 Ring forms and young trophozoites.  
Figure 4 Trophozoites with ameboid appearance.  
Figure 5 Mature trophozoites with schuffner's dots.  
Figure 6 Early schizont, gametocyte and trophozoite.  
Figure 7 Mature schizont  
Figures 8-9 Mature schizont that fills red cell.  
Figures 10-12 Mature gametocytes.

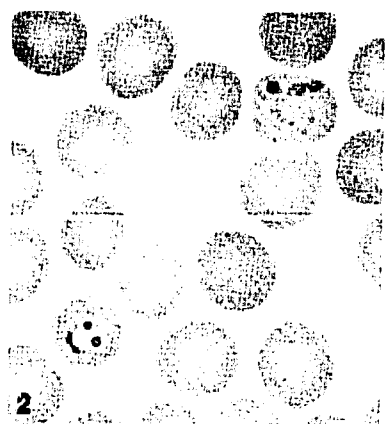
اتم تصویر

پلازمودیوم وایواکس

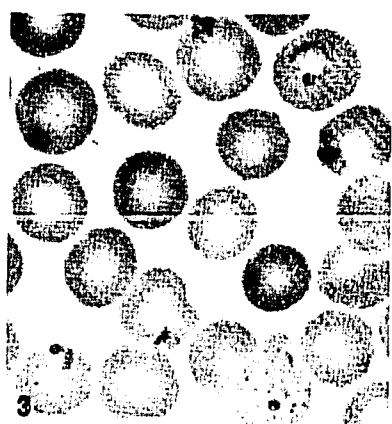
- ۳-۱ شکلونه :- دحلقی وصفی او مشخص شکلونه او محوان تروفوزایتونه .  
۴ شکل :- تروفوزایتونه چه امیب ډوله بڼه او خیره لری .  
۵ شکل :- بالغ تروفوزایتونه چه دشوښر دانی لری .  
۶ شکل :- لمړنی شیزانت کامیتوسایت او تروفوزایت .  
۷ شکل :- بالغ شیزانت .  
۸-۹ شکلونه :- بالغ شیزانت چه دوینی سری حجری ډکی کړی دی .  
۱۰-۱۲ شکلونه :- بالغ کامیتو سایتونه .



1



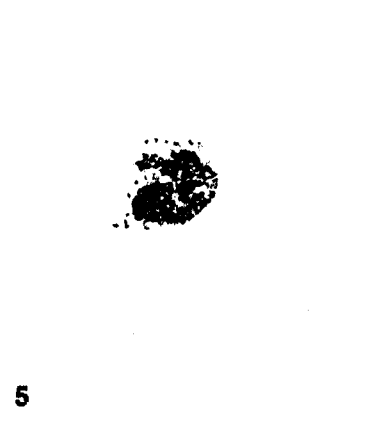
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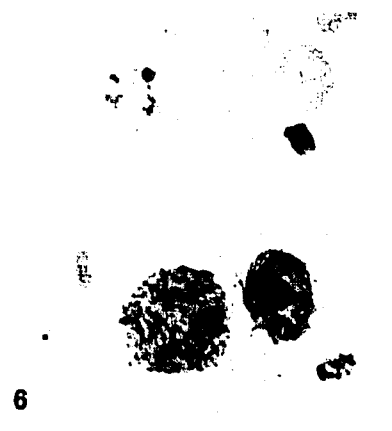
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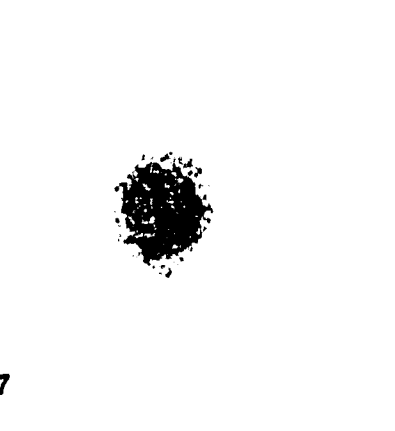
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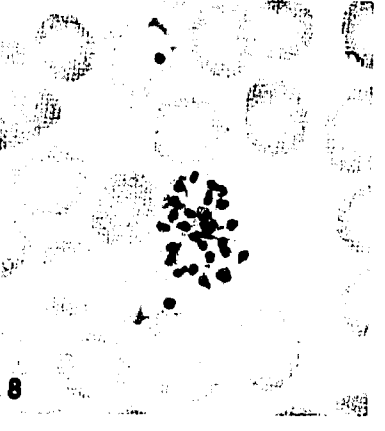
5



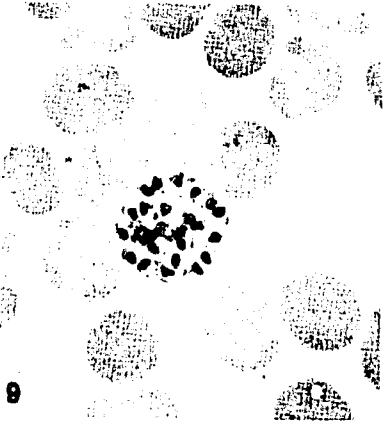
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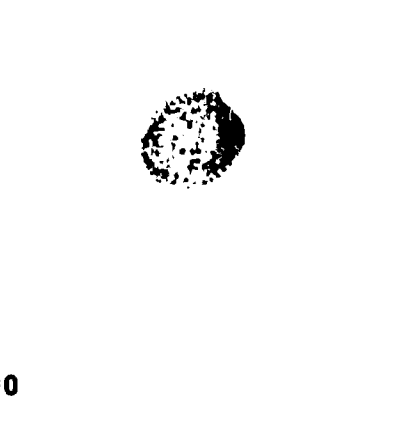
7



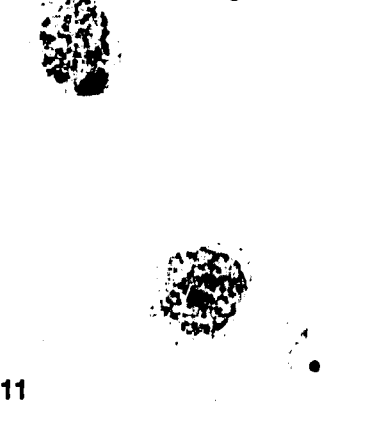
8



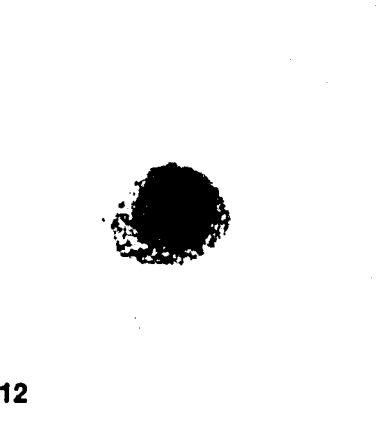
9



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**PLATE 9**  
**Plasmodium falciparum**

Figures 1-3 Ring forms.

Figures 4-5 Mature gametocytes.

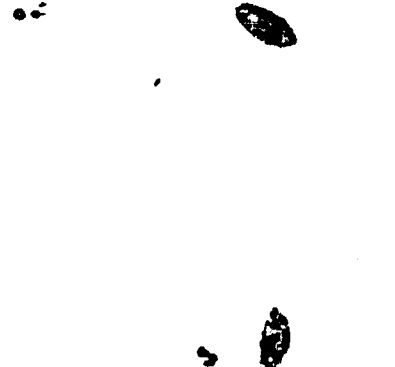
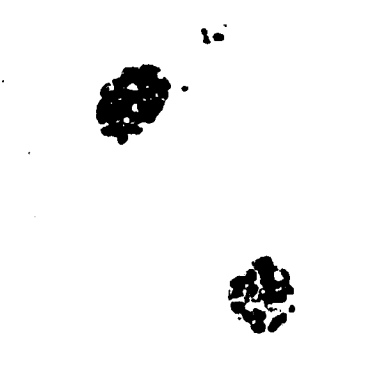
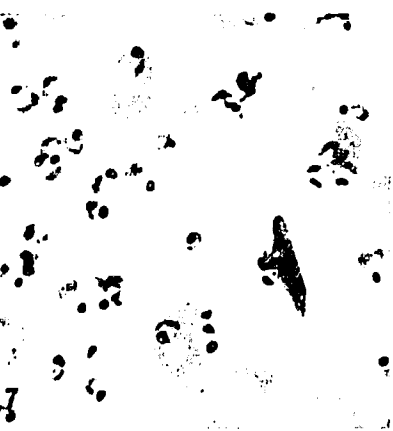
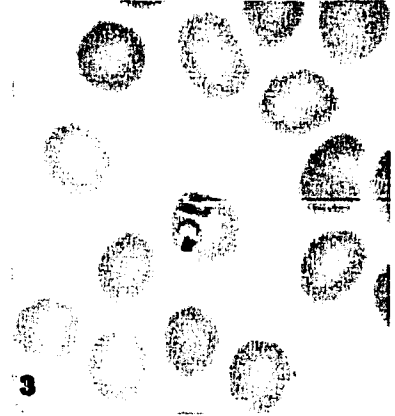
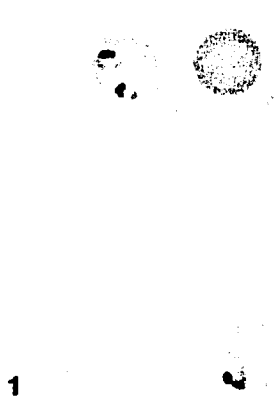
Figures 6-9 Heavy infections that are indicative of falciparum.

Figures 10-12 Developing gametocytes.

نہم تصویر

فلیسپارم پلازموڈیم

- |              |  |
|--------------|--|
| ۳-۱ شکلونه   | :- دحلقی شکلونه                              |
| ۵-۴ شکلونه   | :- بالغ گامیتوسایتونه .                      |
| ۹-۶ شکلونه   | :- شدیدانتانات چہ فلیسپارم پہ کوتہ کوی .     |
| ۱۲-۱۰ شکلونه | :- گامیتوسایتونه دپیشرفت اوانکشاف پہ حالکی . |



**PLATE 10**  
**Plasmodium malariae**

- Figures 1-2 Typical ring stages.  
Figures 3-6 Growing forms of trophozoites both band and basket forms.  
Figure 7 Immature schizont.  
Figures 8-9 Mature schizont.  
Figures 10-12 Gametocytes.

لسم تصویر

پلازموډيم ملاريسی

- ۲-۱ شکلونه - د حلقی وصفی مرحلی .  
۶-۳ شکلونه - تروفوزایتونه درشد به حال کیوی چه دفیتی (باند) اوتکوری  
(سبد) شکلونه لری .  
۷ شکل - نابالغ شیزانت .  
۹-۸ شکلونه - بالغ شیزانت .  
۱۲-۱۰ شکلونه - گامیتوسایتونه .

**GEOGRAPHIC DISTRIBUTION.** It occurs primarily in tropical Africa, but can be found in New Guinea and the Philippines. Rarely will it be found in Pakistan or Afghanistan.

د جغرافیائی تقسیمات. معمولاً د افریقا په استوایی قسمتونو، د هند بعضی قسمتونو، برما، سریلانکا، مالیزیا او انډونیزیا کېږي لیدل کېږي. ددی دانتاریدلو خطر له *P. vivax* او *P. falciparum* څخه کم  
(۵).





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## PLATE 11 Plasmodium ovale

- Figures 1-2 Typical ring forms.  
Figures 3-5 Growing trophozoites.  
Figures 6-8 Immature schizonts.  
Figure 9 Mature schizont.  
Figure 10 Gametocyte with black pigment granules.  
Figures 11-12 Gametocytes with compact pigment.

GEOGRAPHIC DISTRIBUTION. It occurs primarily in tropical Africa, but can be found in New Guinea and the Philippines. Rarely will it be found in Pakistan or Afghanistan.

### يولسم تصوير

#### پلازموډيم اوو ال

- ۲-۱ شکلونه :- دحلقی وصفی شکلونه .  
۵-۳ شکلونه :- تروفوزایتونه درشد په حال کی .  
۸-۶ شکلونه :- نابالغ شیزانتونه .  
۹ شکل :- بالغ شیزانت .  
۱۰ شکل :- گامیتوسایت چه توری صباغی دانی لری .  
۱۱-۱۲ شکلونه :- گامیتوسایتونه چه متراکم صباغ لری .

د جغرافیائی تعقیبات . معمولاً د افریقا په استوایی قسمتونو کښی لیدل کیږی ، مگر په نوی گینیا او  
فلیپین کښی هم لیدل کیږی . بعضاً په افغانستان او پاکستان کښی هم امکان د لیدلو شوی .



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## PLATE 12 Thick Films

- Figures 1-4 *P. vivax*. Rings and trophozoites (fig. 2 arrow) gametocytes (arrow figure 3).
- Figures 5-6 *P. ovale*. Rings, trophs and gametocytes.
- Figures 7-9 *P. malaria*. (fig. 7), 5 trophs and 2 WBCS (fig. 8), Central troph with 4 schizonts (fig. 9) gametocyte.
- Figures 10-12 *P. falciparum*, (fig. 10) rings (fig. 11), ring and gametocyte (fig. 12) gametocytes and platelets.

دولسم تصوير

ضخيم سلايد (فلم)

- ۱-۴ شکلونه : پلازموديم وايواکس :- حلقی او تروفوزايتونه ئی د علامی په ذریعه په شکل ۲ کی شودل شويدي
- گامیتوسايتونه :- په دريم شکل کی په علامه شودل شويدي .
- ۵-۶ شکلونه :- پلازموديم اووال :- حلقی آتروفوزايتونه او گامیتوسايتونه
- ۷-۹ شکلونه :- پلازموديم ملاریا :- ( شکل ۷ ) پنځه دانې تروفوزايتونه او دوه دانې د وینی سپینی حجری ( شکل ۸ )
- په مرکزی تروفوزايت د خلوروشیزانتونو سره شکل ۹ :- گامیتوسايت .
- ۱۰-۱۲ شکلونه :- پلازموديم فالسیپارم :- ( شکل ۱۰ ) حلقی ( شکل ۱۱ ) حلقه او کامیتو- سايت ( شکل ۱۲ ) گامیتوسايتونه او ترومبوسايتونه (دمويه صفحات)



## PLATE 13

Malarial parasites of the 4 plasmodium species showing a comparison in the stages of development.

ديارلسم تصوير  
دملاريا دڅلورو ډولونو پلازموډيمو دانكشاف او تكامل په اندوليزه توگه سره  
كتل كيري ( مقايصوي )

*Plasmodium falciparum*

*Plasmodium vivax*

*Plasmodium malariae*

*Plasmodium ovale*

پلازمودیم فلیسپارم

پلازمودیم وایواکس

پلازمودیم ملاریا ئی

پلازمودیم اووال

Early ring  
لمرئی حلقه

Late ring  
اخرئی حلقه

Early intermediate stage  
لمرئی منحنی مرحله

Late intermediate stage  
خرئی منحنی مرحله

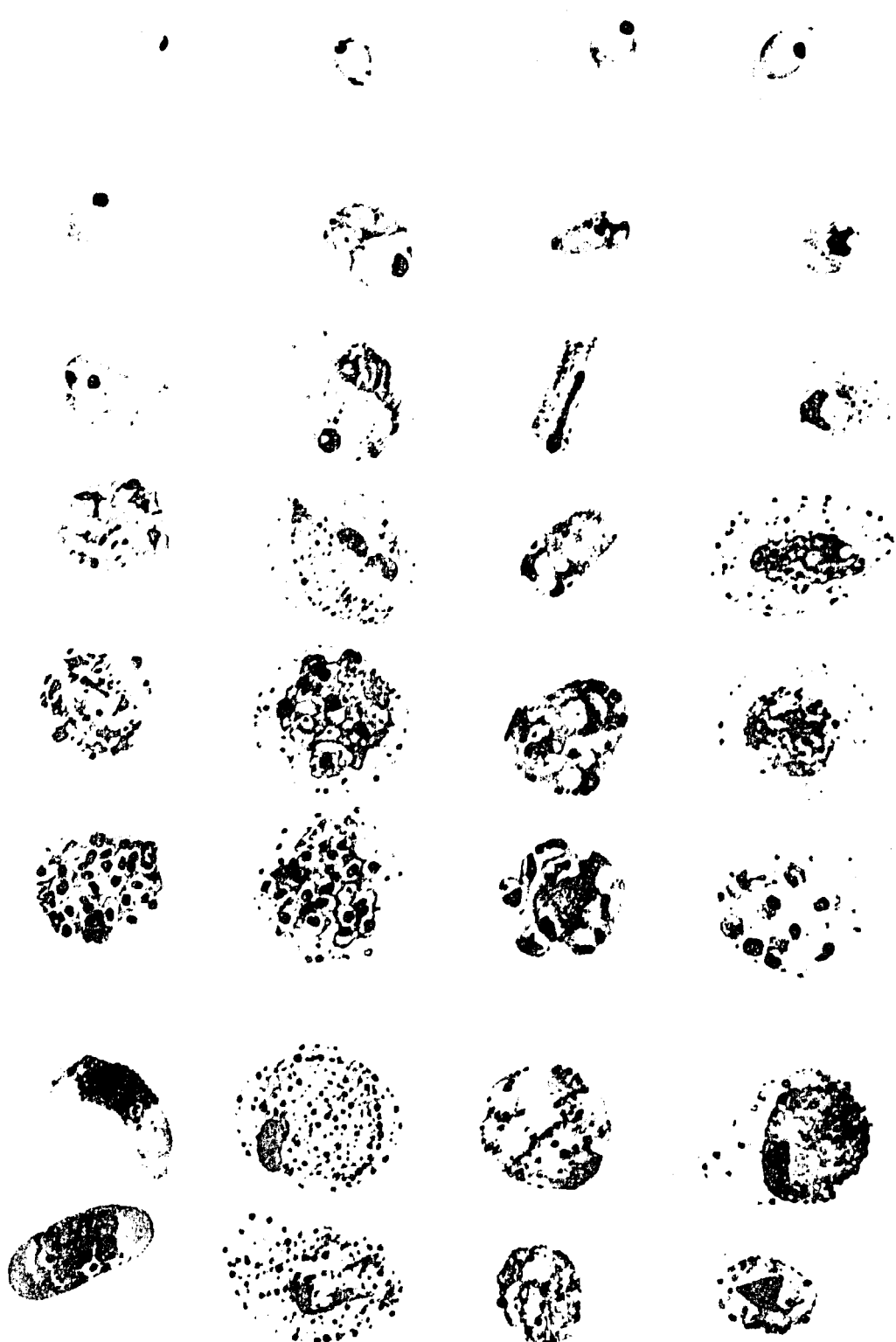
Presegmented  
دتوک ، توک کیدوئج  
مخکی مرحله

Segmented (schizont)  
دشیزانت توک ، نو  
شوی مرحله

Macrogametocyte  
میکروگا میتوساید

Microgametocyte  
میکروگا میتوساید

MALARIAL PARASITES  
دملاریا پرازیتونه



# **PROTOZOA**



**PLATE 14**  
**Entamoeba histolytica**

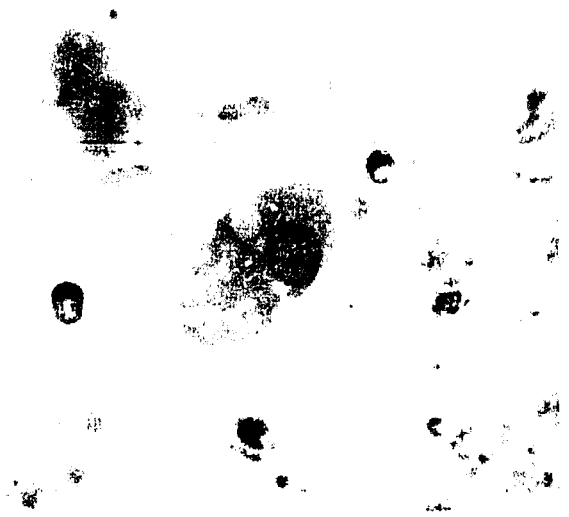
- Figures 1-3 Trophozoites with distinct karyosomes. Fig. 3., contains ingested RBCs.
- Figure 4 Iodine-stained cyst with 2 nuclei and a chromatoid body.
- Figure 5 Mature iodine-stained cyst with 4 distinct nuclei.
- Figure 6. Iodine-stained cyst that contains 1 nucleus and a distinct glycogen mass.

خوارلسم تصویر

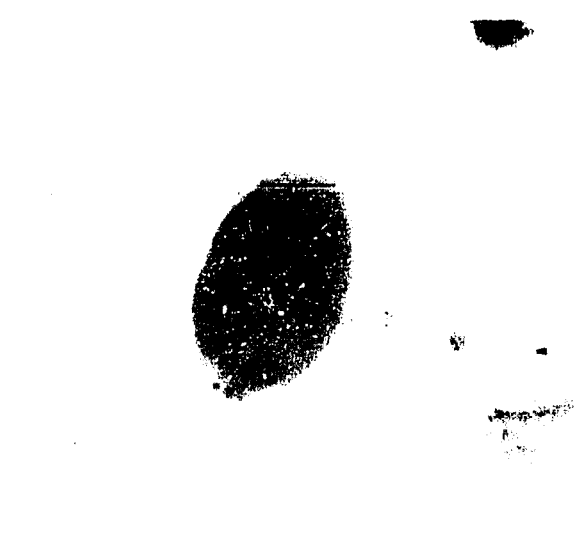
انتامیبا هستولیتسیکا

- ۱ - ۳ شکلونه :- تروفوزایتونه دواضح اوروشانه کاریوزوم سره (۳ شکل) سری  
هضمشوی حجرینسی .
- ۴ شکل :- دآیودین په واسطه رنگشوی (تلوینشوی) سیست چه دوه دانی  
هستی او په داخل کیئی کروماتید جسم لیدل کییری
- ۵ شکل :- دآیودین په واسطه رنگشوی (تلوینشوی) بالغ سیست چه برجسته  
او واضح هستی پکسی لیدل کییری
- ۶ شکل :- دآیودین په ذریعه رنگشوی (تلوینشوی) سیست چه یوه هسته لری  
او دگلایکوجن کتله په هغه کی واضحاً "لیدل کییری" .

1



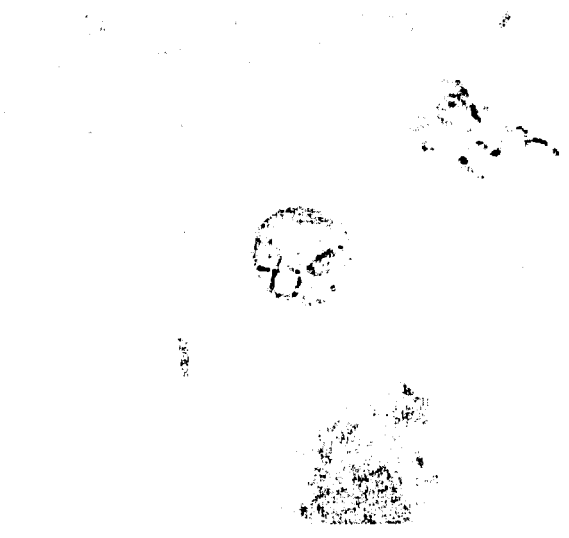
2



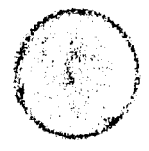
3



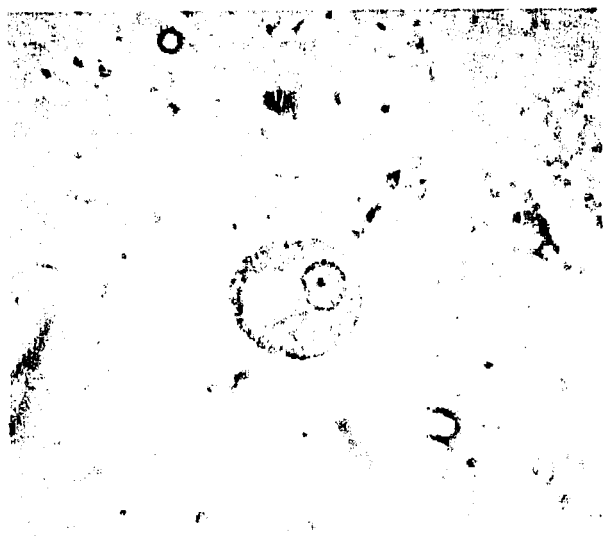
4



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12''

## Entamoeba coli

- Figures 1-2 E. coli trophozoites, trichrome stained with eccentric nuclei and karyosome.
- Figure 3 Typical mature cyst, iodine-stained.
- Figure 4 Size comparison of E. coli (larger) and E. histolytica (small) cysts.
- Figure 5 Unstained, formalin - preserved cyst.

### پنجاسم تصویر

#### انتامو باکولای

- ۲-۱ شکلونه :- د انتامیبا کولای تروفوزایتونه چه ددریورنگونوپه په ذریعه تلوین شوی او هستی د مرکز څخه محیط ته تمبول شوی دی .
- ۳ شکل :- وصفی بالغ سیست چه د آیودین په ذریعه تلوین شوی دی .
- ۴ شکل :- دای کولای دسیستونو د جسامت اندول (مقایسه کول )
- دای کولای سیست لوی اود انتمیبا هستولایتیکا سیست کوچنی دی .
- ۵ شکل :- هغه سیستونه چه تلوین شوی نه دی (نه دی رنگه شوی ) او په فورملین کی ساتل شوی .



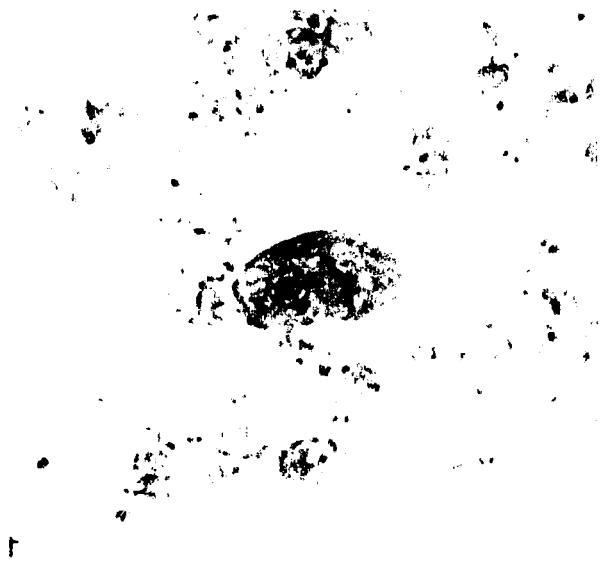
**PLATE 16**  
**Iodamoeba butschlii and Dientamoeba fragilis**

- Figure 1 I.B. trophozoite stained with trichrome that shows a dark staining karyosome.
- Figures 2-3 Typical iodine-stained cysts with dark glucogen mass. Fig. 2, is high power, Fig. 3 is low power.
- Figure 4-6 D. fragilis trophozoites that are typical of the parasite, each have 2 nuclei.

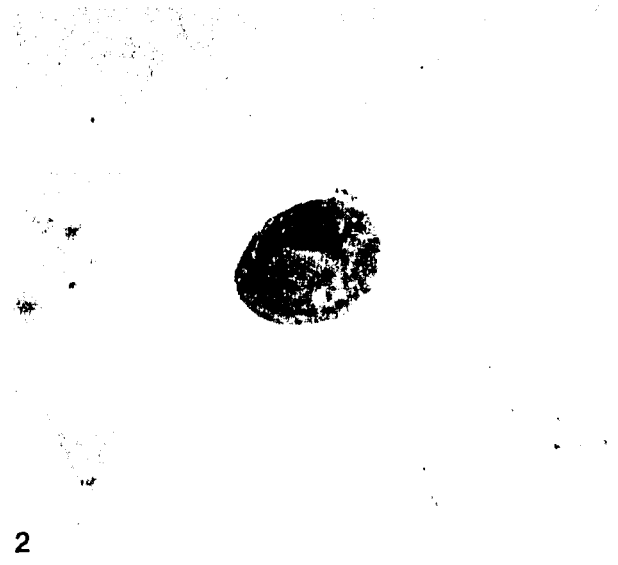
شپا رسم تصویر

ایودامیبا بشلی ودای انتامیبا فرجیلیس

- شکل (۱) :- دایودامیبا بشلی تروفوزایتونه چه ددریو رنگونو په ذریعه تلوین شوی او کاربیوزوم ئی په تیاره رنگ سره څرگندیږی.
- ۳-۲ شکلونه :- دایودین په واسطه تلوین شوی وصفی سیستونه . اودگلایکوجن کتله ئی په تیاره رنگ سره ښکاره کیږی.
- ۲ شکل :- دمیکروسکوپ په لوی قدرت او ۳ شکل دمیکروسکوپ په کوچنی قدرت - لیدل شوی دی.
- ۴-۶ شکلونه :- د دای انتامیبا فرجیلیس تروفوزایتونه چه دپرازیت لپاره بلکلوصفی دی. هریو ئی دوی هسته چی لری.



1



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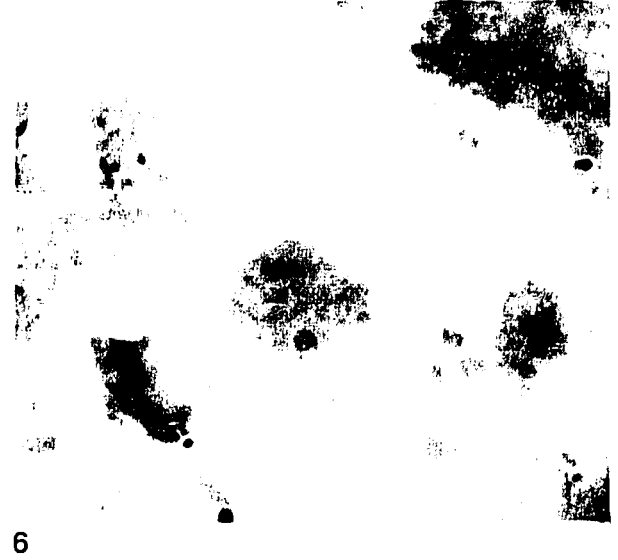
3



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## PLATE 17

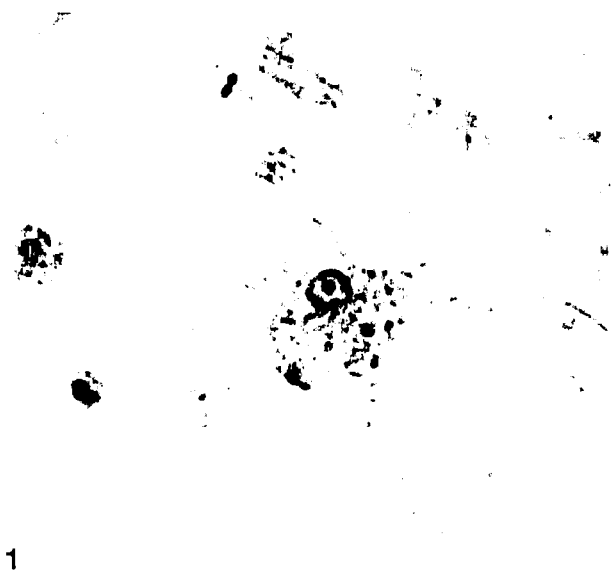
### **Entamoeba hartmanni, Endolimax nana, Chilomastix mesnili**

- Figure 1 E. hartmanni trophozoite.  
Figure 2 E. hartmanni cyst.  
Figure 3. E. nana trophozoite.  
Figure 4. E. nana cyst.  
Figure 5. C. mesnili troph.  
Figure 6. C. mesnili cyst (notice lemon shape)

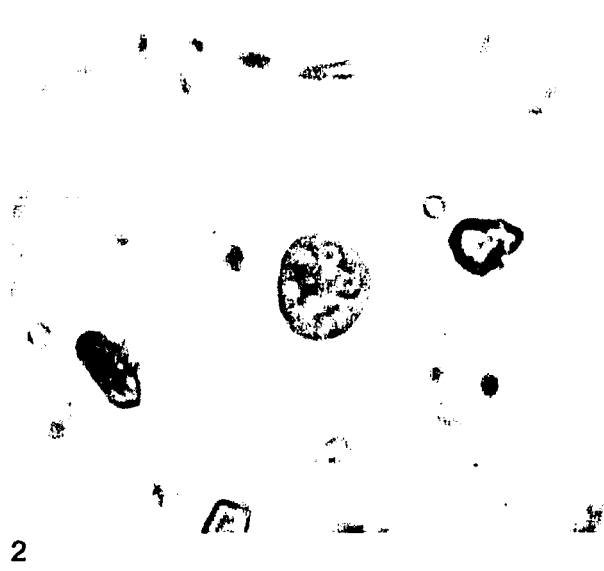
#### ا ولسم تصوير

انتيميا هاتمانی ، اندولیماکس ، کیلاما ستيکس ميسنيلي

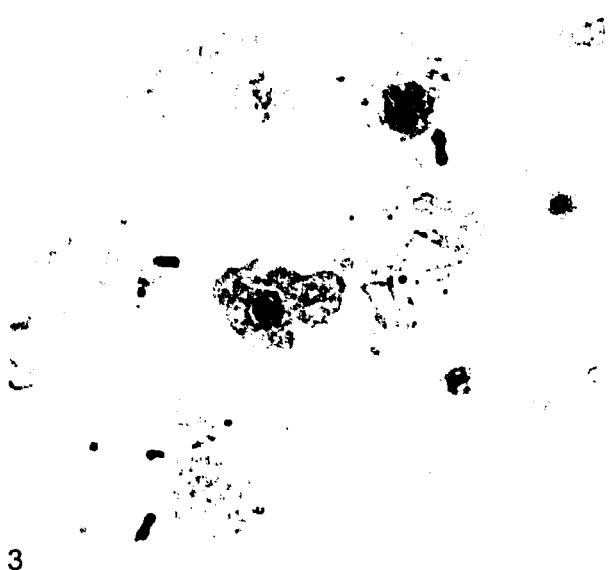
- ۱- شکل :- دانتامیبا هارتمانی تروفوزایت  
۲- شکل :- دانتامیبا هارتمانی سیست .  
۳- شکل :- داندوسیماکس ناتا تروفوزویت .  
۴- شکل :- داندو سیماکس ناتا سیست .  
۵- شکل :- دکیلما ستيکس ميسنيلي تروفوزویت .  
۶- شکل :- دکیلما ستيکس ميسنيلي سیست ( دلیموپه شان شکل ته پاملر نه وکری).



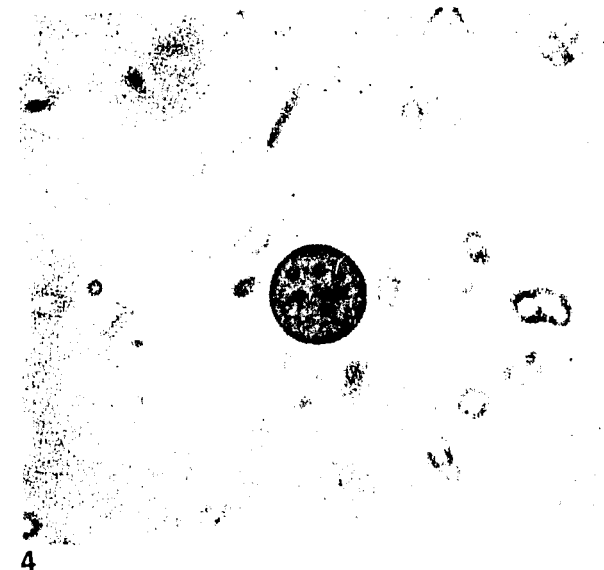
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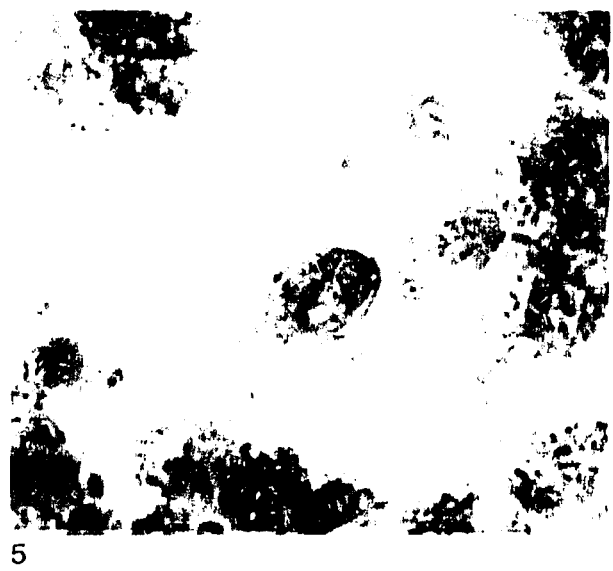
2



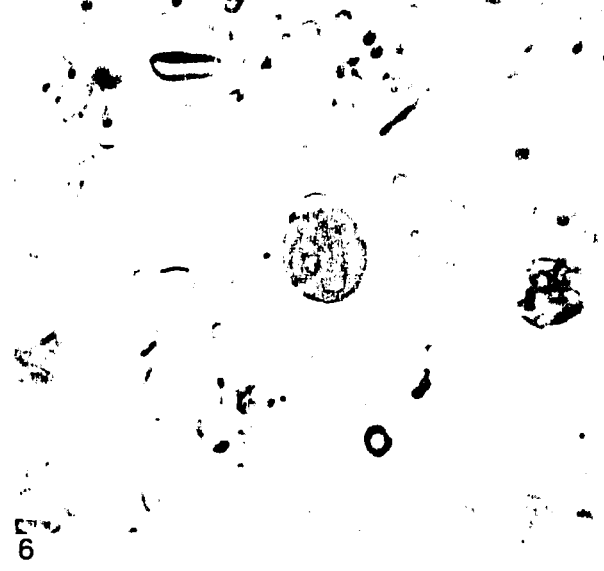
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**PLATE 18**  
**Giardia lamblia**

Figures 1-3 Giardia trophozoites with typical pear shape and 2 nuclei.

Figures 4-6 Giardia cysts. Figures 5 and 6 are smaller due to the shrinkage of the organisms during fixation.

اتلسم تصویر

جیاردیالمبیا

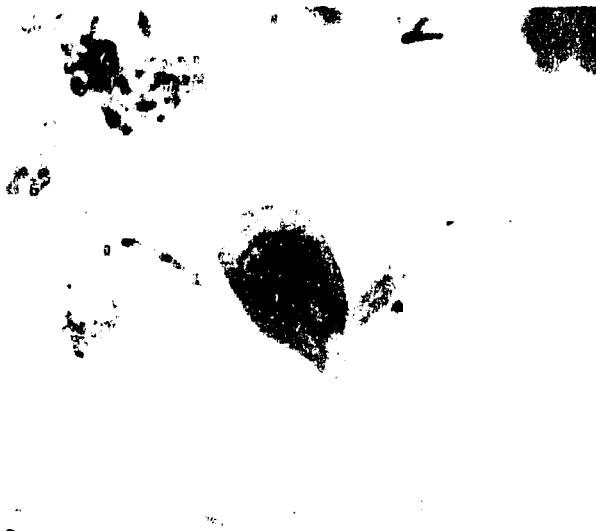
- ۳-۱ شکلونه :- دجیاردیا تروفوزایتونه چه (وصفی) کتومتناک ته ورته دی دوه هسته لری.
- ۴-۶ شکلونه :- دجیاردیا سیستونه (۵-۶ شکلونه) کوچنی دی خکه کله چه ارگانیزم تشبیت کیری حان را تولوی او چملک کیری (تکمش کوی).



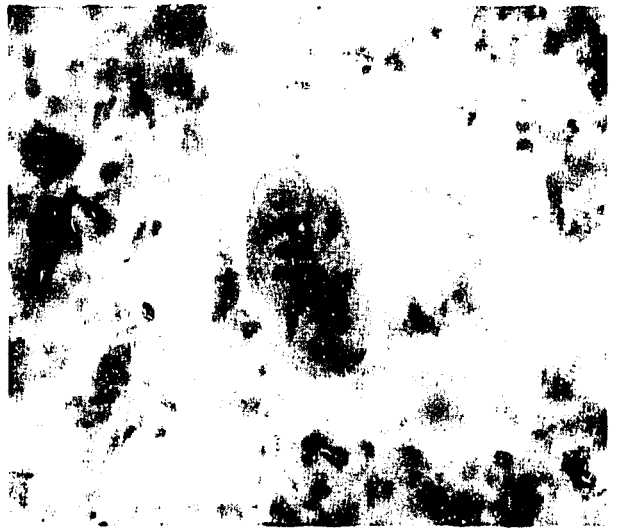
1



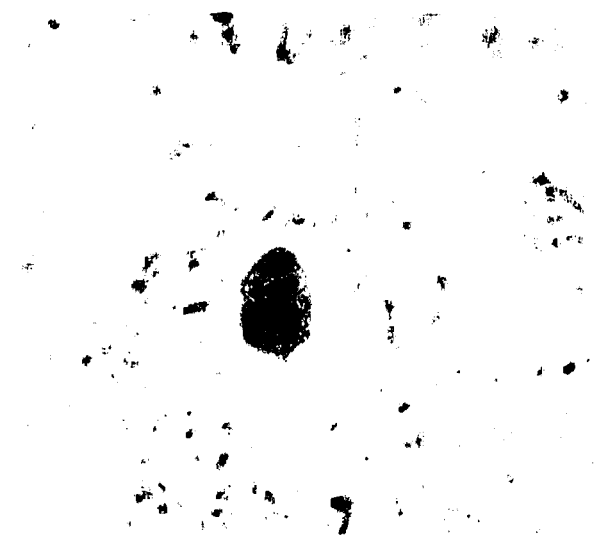
2



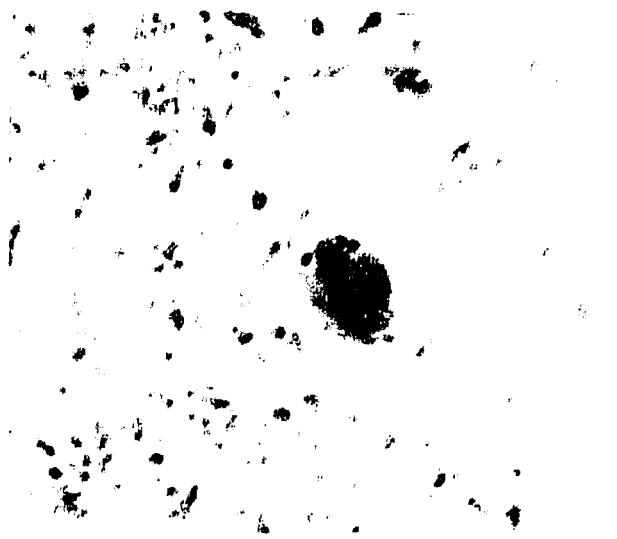
3



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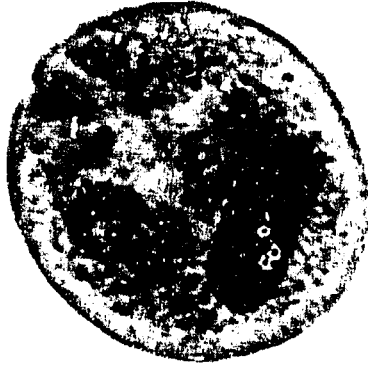
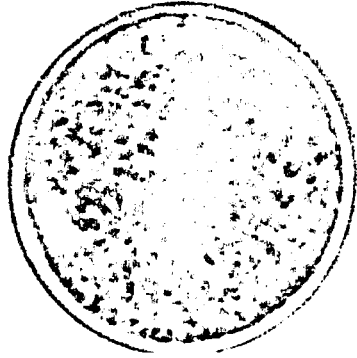
**PLATE 19**  
**Balantidium coli**

- Figures 1 and 3      B. coli trophozoites, easy to recognize because of their size.
- Figure 2              B. coli cyst with clear refractile wall.
- Figure 4              Trichrome - stained trophozoite that shows the key features of B. coli.

نولسم تصویر

بلانیدیم یوم کولای

- ۳-۱ شکلونه      :- دبلانیدیم کولای تروفوزایتونه داندازی له رویه ژرپیژندل کیږی.
- ۲ شکل              :- د بلانیدیم کولای سیست چه انکشاف ورکونکی قشر ئی په ډیر - روښانه توگه لیدل کیږی .
- ۳ شکل              :- هغه تروفوزویت چه ددریو رنگو په ذریعه تلوین شوی اود - بلانیدیم کولای دواضحو (نسونسانو ) مشخصاتو څخه ده .



# HELMINTHS

**PLATE 20**  
**Enterobius vermicularis (pinworm)**

- Figure 1 Pinworm eggs found in a cellophane tape preparation.
- Figure 2 Pinworm eggs that you can't see the flat surface.
- Figure 3 Adult female worm, notice the pointed tail.
- Figure 4 Adult male worm, notice the blunt tail.

شلم تصویر

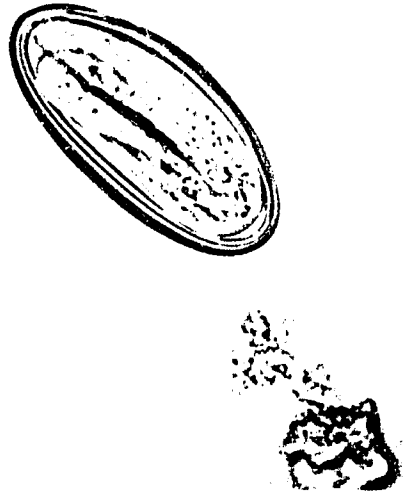
انتیروبیوس ویرمیکولاریس ( سجا قی چینجیا ن )

- |       |   |
|-------|---|
| ۱ شکل | :- دسجا قی چینجیا نو هگی چه دسلوفان دمستحضر په فیته که موجودی، دی |
| ۲ شکل | :- دسینجا قی چینجیا نو هگی چه هواره سطحه ئی نه لیدل کیبری .       |
| ۳ شکل | :- مونث بالغ چینجی چه لکی ئی تیره او په خرگنده ډول معلومیږی       |
| ۴ شکل | :- مذکر چینجی چه لکی ئی پخه ده .                                  |

۱۲۱



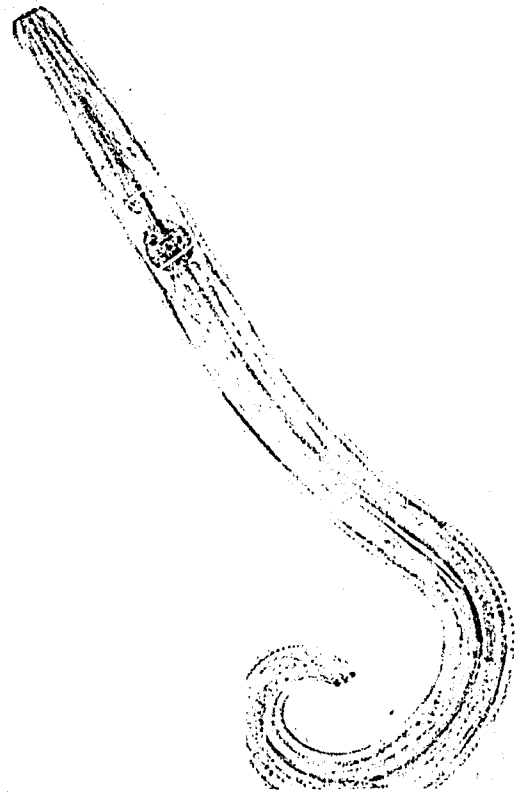
1



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**PLATE 21**  
**Ascaris lumbricoides**

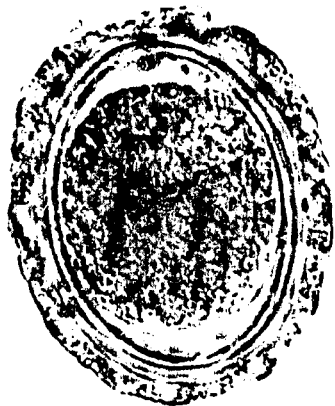
- Figure 1 Typical fertilized egg found in fresh stools.
- Figures 2-4 Eggs that demonstrate considerable variation of the external "ruffles".
- Figure 5 Unfertilized egg that is decorticated.
- Figure 6 Infertile egg that has a poorly developed "ruffle".

يوويشتم تصوير

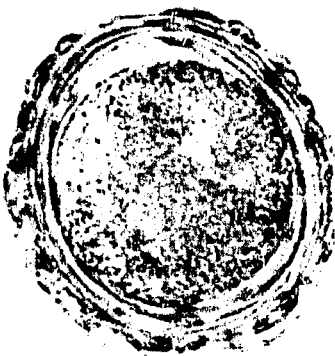
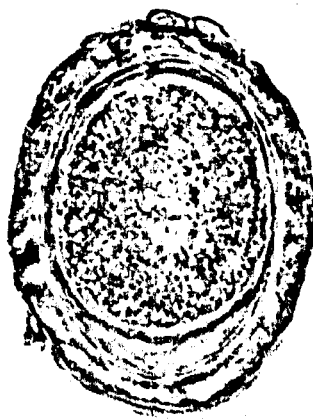
اسكريس لمبريكويدس

- ۱ شکل - داسکریس القاح شوی هگی په تازه موادغایطه کی لیدل کیږی .
- ۲-۴ شکلونه :- دځینو هگیو په خارجی قشرکی ډولراوتلی څوکی (برامدگی ) موجود دی
- ۵ شکل :- د غیرالقاح شویو هگیو بانندی قشرله منځه تللی دی .
- ۶ شکل :- عقیمه هگی چه بانندی قشر ئی لږ راوتلی څوکی ښی (برامدگی )

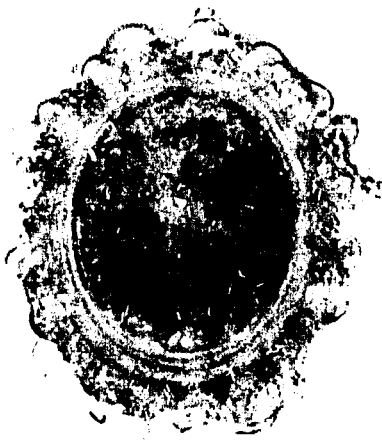




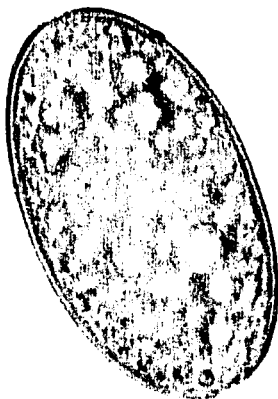
1



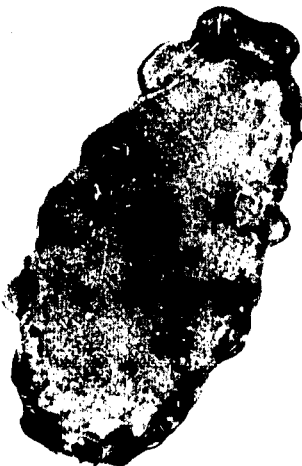
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**PLATE 22**  
**Trichuris trichiura and Hookworm**

Figures 1-2 Hookworm eggs with typical hyaline shell.

Figure 3 Male and female whipworms. The male usually has a coiled tail. Rare, not found in feces except following treatment.

Figures 4-5 *T. trichiura* eggs. Fig. 4 is an embryonated egg and Fig. 5 is an unembryonated egg. Both show the typical bipolar plugs.

دوه ويشتم تصوير

تريکوريس تريکوريا او چنگگ، دوله چنجيان

۲.۱ شکلونه :- دچنگگی چنجيان نوہگی پر خانگری هیالینی قشر لری .

۳ شکل :- دمتروکی په شان نارینه (مذکر) پسخینه ( مونث ) چنجيان .

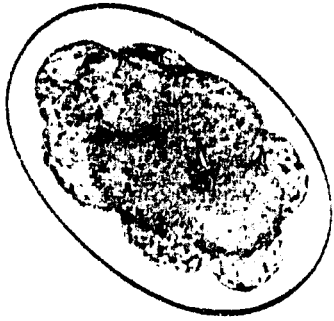
مذکر چنجيان مارپیچی لکی لری . په غایطه موادوکی ډیر لیدل کیږی . اما پس لدتداوی

ثخه هیخ نه لیدل کیږی .

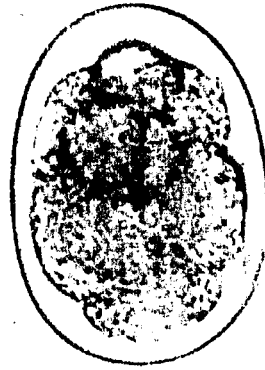
۴-۵ شکلونه :- دتريکوريس تريکوريا . په ۴ شکل کی القاح شوی هگی (جنین-ونکی)

لیدل کیږی . ۵ شکل القاح شوی هگی جنین هگی نه لری . اما دواړی نوعی هگی په اخرکی

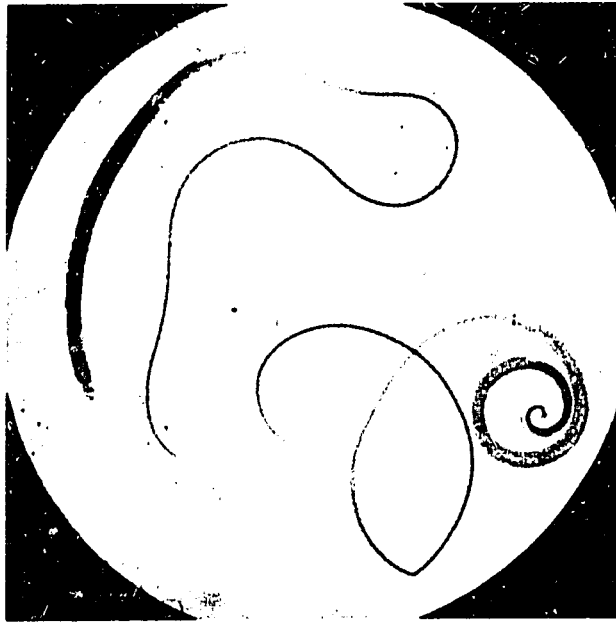
دپطنوس په شکل یوه دسته لری .



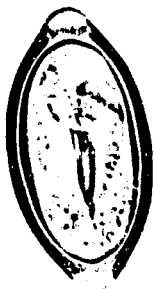
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**PLATE 23**  
**Hookworm and Strongyloides larvae**

- Figures 1 and 3      Hookworm larvae with a long buccal canal and the genital primordium cannot be seen.
- Figures 2 and 4      *S. stercoralis* larvae with a short buccal canal and the genital primordium can be seen (arrow).

درویشتم تصویر

چنگگی چنجیان او دسترانگیلایدز لاروا

۱-۲-۳- شکلونه :- دچنگگی چنجیانو لارواگان چه اوردفمی کاخال لری او ابتدائی

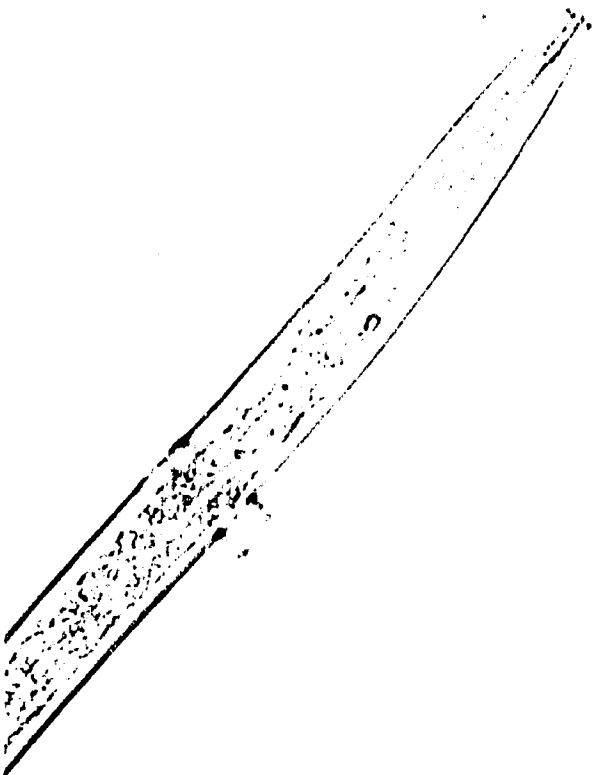
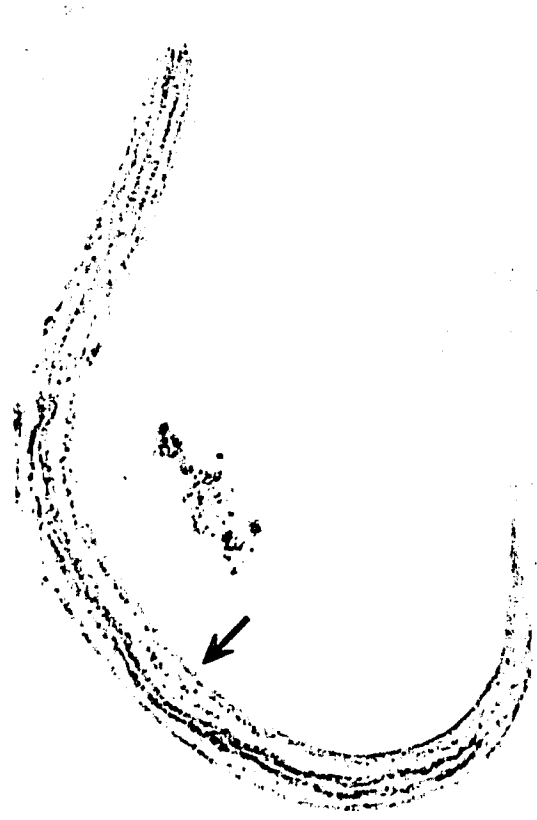
تناسلی غری ئی نه معلومیری .

۲-۴ شکلونه :- دسترانگیلایدز سترکورالیسر لارواگان چه فمی کانال لری او ابتدائی

تناسلی غری ئی لیدل کیپی. ( چه په عکس ورته اشاره شوی ده )

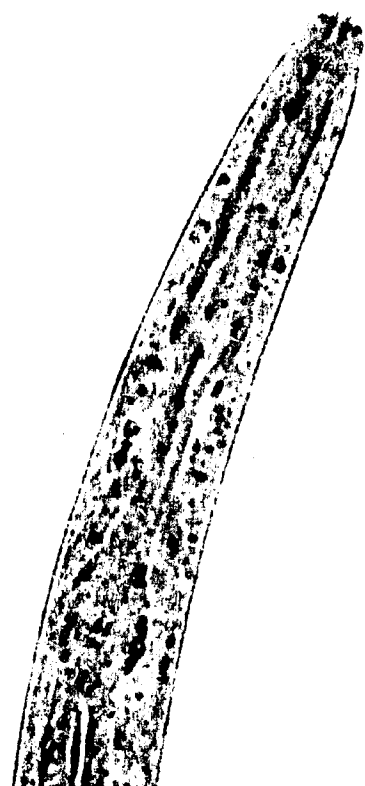


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**PLATE 24**  
**Hymenolepis nana**  
**Hymenolepis diminuta**

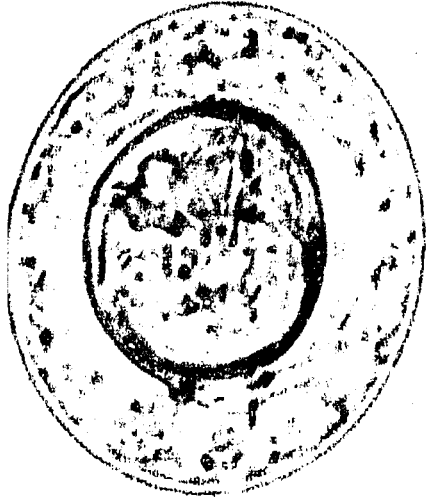
- Figures 1-2 Clear, thin - shelled eggs of *H. nana* that contain a six - hooked embryo.
- Figure 3 *H. nana* and *H. diminuta* eggs that show a clear size difference. *H. nana* is the smaller egg.
- Figure 4 *H. diminuta* egg. It is larger than *H. nana* and has a thicker shell.

څلور ویشتم تصویر

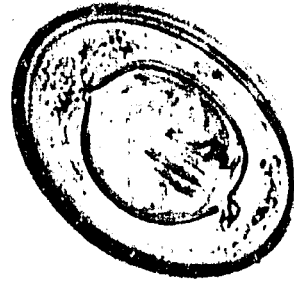
هیمینو لیپس نانا و

هیمینو لیپس دیمینوتا

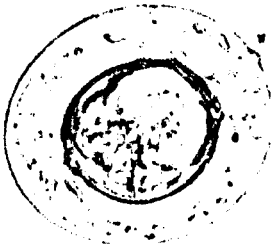
- ۱-۲ شکلونه: د هیمینولیسس هگی چه نازک او روښانه قشر ( پوستکی ) لری .  
او جنین ئی شپړ چنگکه لری .
- ۳- شکل: د نانا او دیمینوتا هگی چه دسایز او جسامت له کبله ډیر واضح توپیر لری د نانا هگی د دیمینوتا له هگی څخه کوچینی دی .
- ۴- شکل: د دیمینوتا هگی چه د نانا دهگو په پرته لوی دی او ډیر بند قشر لری .



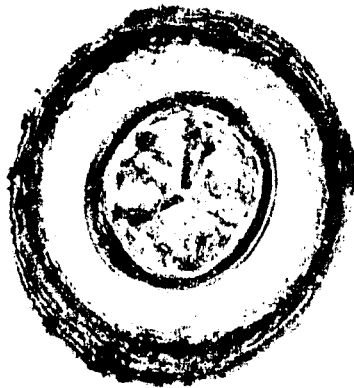
1



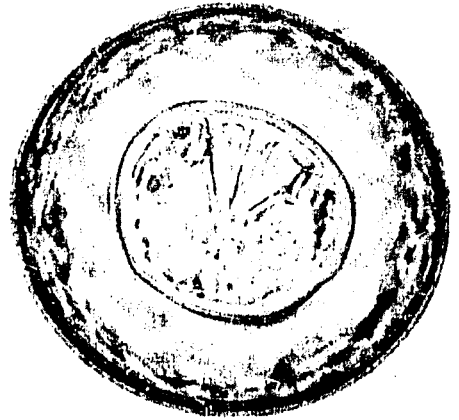
2



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**PLATE 25**  
**Taenia saginata and Taenia solium**

- Figure 1      T. solium scolex with 4 suckers and 2 rows of hooks.
- Figure 2      T. solium proglottid (less than 13 branches)
- Figure 3      Taenia species eggs with radially striated shell and 6-hooked embryo.
- Figure 4.     T. saginata scolex with 4 suckers and no hooks.
- Figure 5      T. saginata proglottid (more than 15 branches).

پنځه ویشتم تصویر

تینا سا جیناتا اوسینا سولیوم

اول شکل :- دتینا سولیوم سر ( راس ) څلور دانی پپوشکه لری او دوه قطاره چنگگونه لری.

دوهم شکل :- دتینا سولیوم یو پروگلوتید ۱۳ پناخی لری .

درېم شکل :- دتینا شپو ( نوعی ) هگی چه قشری شاعی او مخطط دی . او جنین ئی

۶ چنگگونه لری .

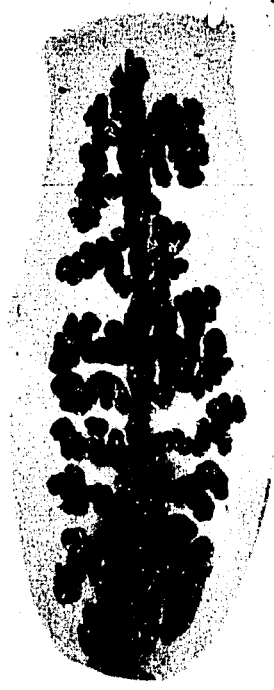
حلورم شکل :- دتینا ساجینا سر چه څلور دانی چوشکونه لری اما چنگگ نه لری .

پنجم شکل :- دتینا ساجیناتا یو پروگلوتید چه له ۱۵ څخه ریائی برخی او پناخی لری.

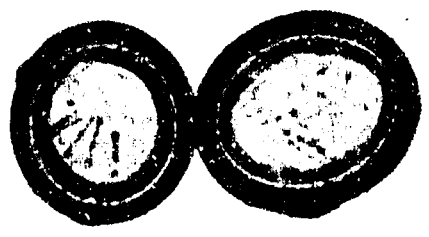




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## PLATE 26

### **Schistosoma mansoni, S. japonicum, and S. haematobium**

- Figures 1-2 *S. mansoni* eggs have a thin shell and prominent lateral spine.
- Figure 3 *S. japonicum* egg is the smallest of the schistosoma eggs. It has a thin shell and frequently has fecal debris attached, making identification difficult.
- Figures 4-5 *S. haematobium* eggs are found in the urine. They are large eggs with a terminal spine.

#### شپرویشتم تصویر

شیستوسوما مانسونی ، شیتوسوما جاپانیکم او شیتوسوما هیما توبیوم .

۱-۲ شکلونه :- دشیستوسوما مانسونی هگی یو نازک قشر لری او یو طرف ته راوتلی

خوکه ( برامدگی ) لری .

۳- شکل :- دشیستوسوما جاپانیکوم هگی چه دتولو شیتوسوما گانوله دولو خخه کوچینی دی

قشر (پوستکی ) ئی نازک او ( غایطمواد ) دکومیتازو بقایاوی پاته شونی ور

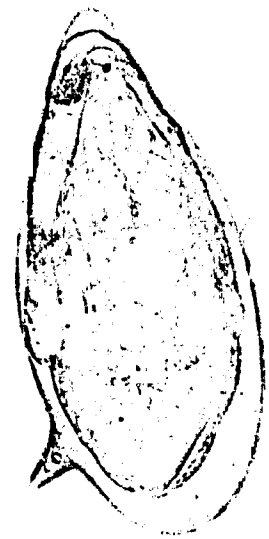
باندی نستی دی . له دی خاطره ئی تشخیص لری . شخه سخت او مشکل دی .

۴- شکل :- دشیستوسوما هیموتوبیوم هگی ادرار ( لول ) ئی لیدل کییری . او هگی ئی لویی

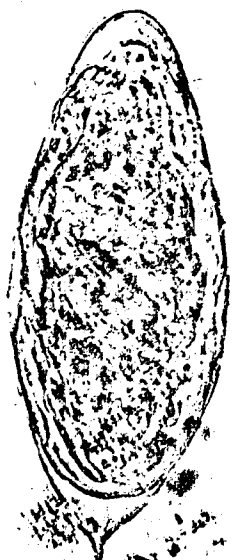
او په آخر سرکی ئی تیری ، نری راوتلی خوکی لری .



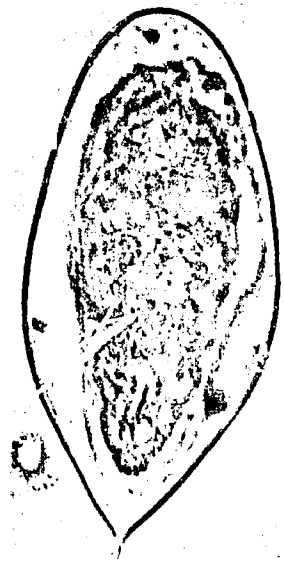
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## PLATE 27 Fecal Debris

- Figure 1 Plant fiber frequently confused with Strongyloides.  
Figure 2 Vegetable cell sometimes confused with helminth eggs.  
Figure 3 Charcot-leyden crystals frequently found in people with parasitic infections.  
Figure 4 Vegetable spiral.

### اوویشتم تصویر

(دگومیتازو) دغایط مواد بقایاوی ( یعنی له پرازیت خخه پرتة نورشیان )

۱- شکل :- بنائی رشتی ( تارونه ) چه عموماً له سترانگیلایدز سره غلطیبری

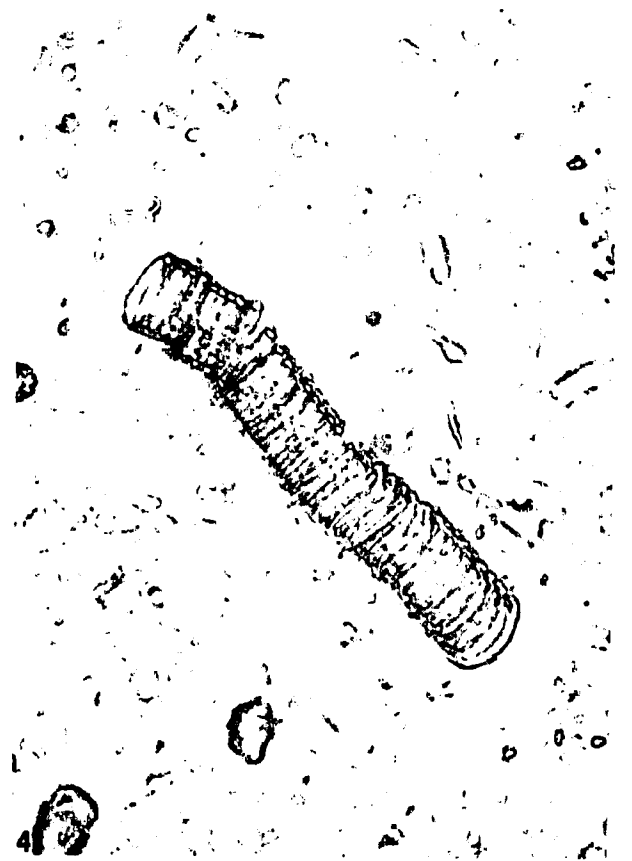
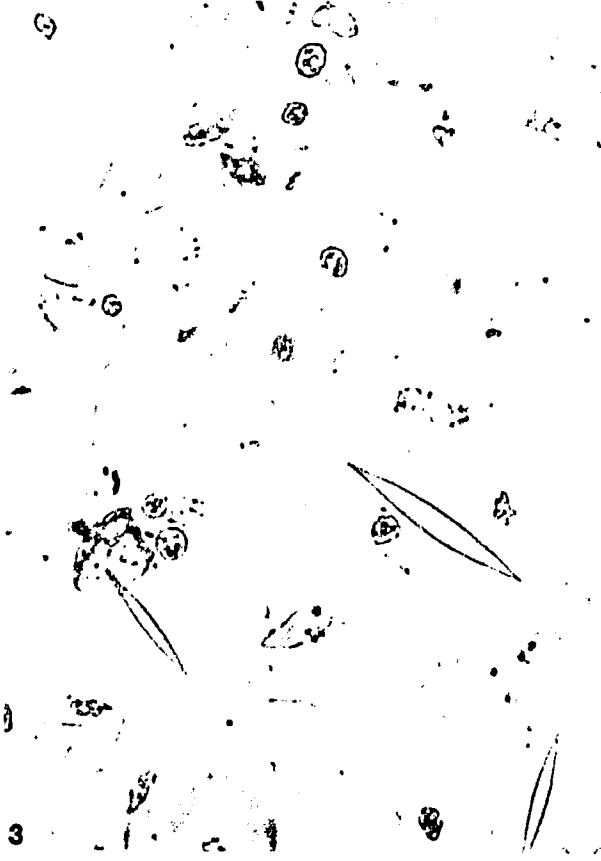
۲- شکل :- بنائی حجری چه کله کله د چینجیانو سره غلطیبری .

۳- شکل :-

کرسنالونه چه عموماً دهغو کسانو په دکومیتازوکی

چه پرازیتونه لری .

۴- شکل :- فنرته ورته بنائی الیاف ( تارونه )



## PLATE 28 Fecal Debris

Figures 1-2 Plant material.

Figures 3,5,6 Pollen grains

Figure 4 Diatoms occur in water. When found in fecal samples, this usually indicates water contamination.

اته ویشتم تصویر

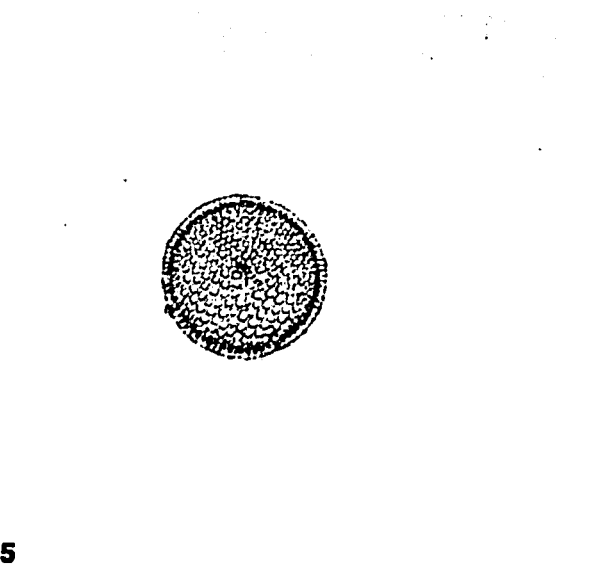
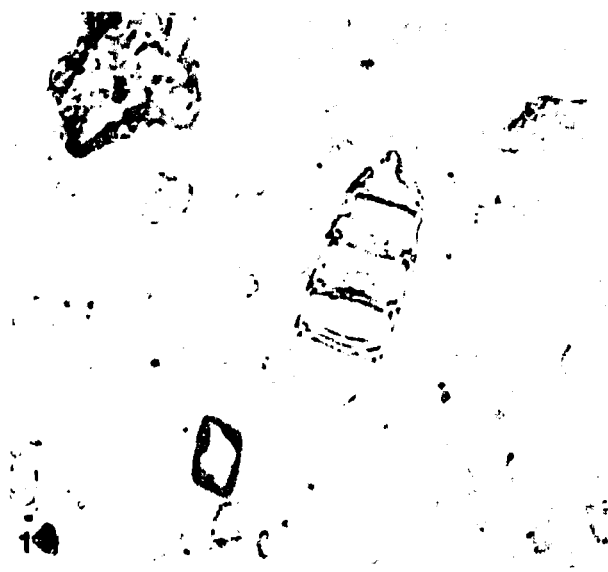
(دگومیتازو) دغایط موادوبقا یاوی ( غیر له پرازیتونو خخه دنورو شیاوشته والی ) بنائی مواد

۲-۱ شکلونه

۳-۵-۶ شکلونه :- دنباتاتو دگردی دانی (

۴ - شکل :- دیاتومونه په اوبوکی معلومیږی. کله چه په اوبو ولیدل شی نو هرومرو په

ملوثی(ناپاکی) وی .



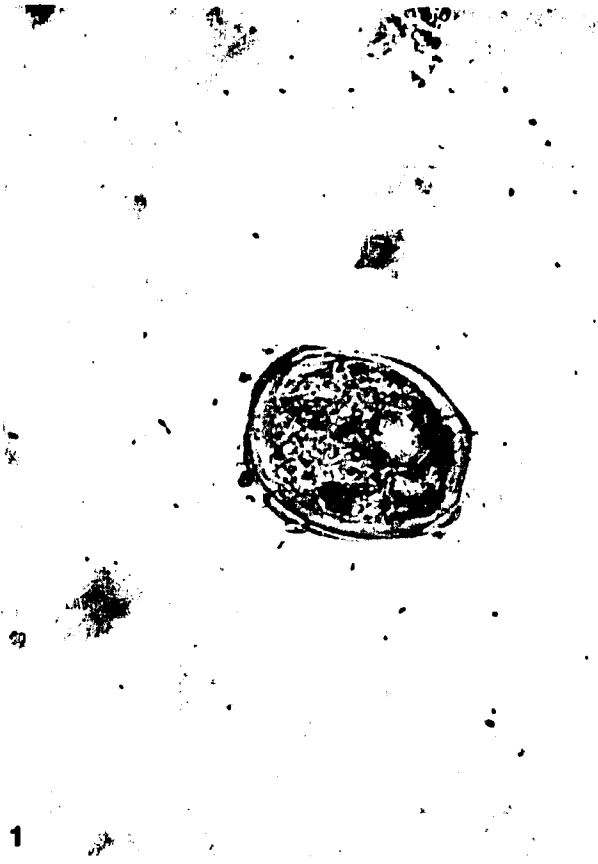
## PLATE 29 Fecal Debris

- Figure 1 Plant material that has a thick wall and might be confused with a helminth egg.
- Figure 2 This mite egg occasionally found in feces and may be confused with hookworm.
- Figure 3 This is the egg of a plant parasite that occurs on root vegetables such as radishes and turnips, can be confused with hookworm eggs.
- Figure 4 A stage of algae.

### ۲۹ تصویر

- (دکومتیازو) دغایطه موادوبقایاوی (پاته شونی)
- ۱- شکل :- نباتی مواد چه پنهدیوال (جدار) لری اوشایددچینجی له هگی سره غلط شی.
- ۲- شکل :- د هگی (د خورو یاغذاچینجیان) چه کله کله په دکو - متیازو (موادغایطه) کی موجود وی. شاید دچنگک ډوله چینجیانو سره غلط شی.
- ۳- شکل :- په دغه شکل کی دیوه نباتی پرازیت هگی تولید کیږی چه دریبته - کله ملیواو تپیرو (شلفمو) موندل کیږی دچنگکی چینجیانو دهگیو سره غلطیږی.
- ۴- شکل :- د بحری نبات یعنی د اوبو خزی ( ) یوه مرحله لیدل کیږی.





# URINE SEDIMENT

## PLATE 30

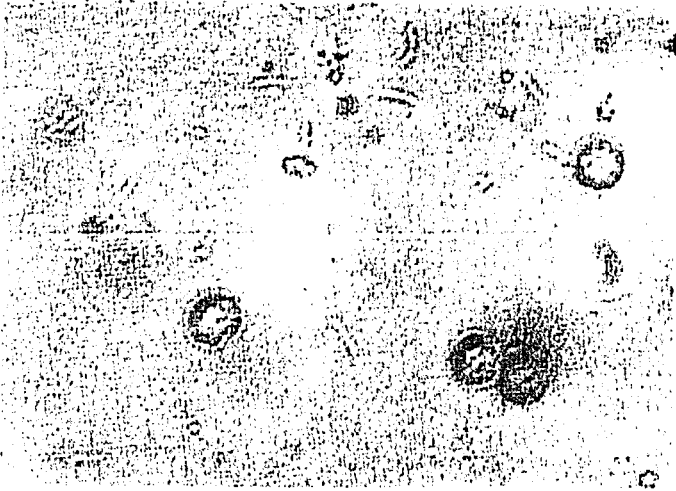
### Cells in Urine

- Figures 1-2 White Blood Cells (WBC's): Found singularly or in clumps.
- Figures 3-4 Red Blood Cells (RBC's): Fig. 3 shows fresh normal cells and fig. 4 shows RBC's starting to crenate (shrink).
- Figures 5-6 Epithelial Cells: figure 5, Squamous Cells that indicate contamination. Figure 6, Renal Cell: Take note of the cell and the large nucleus.

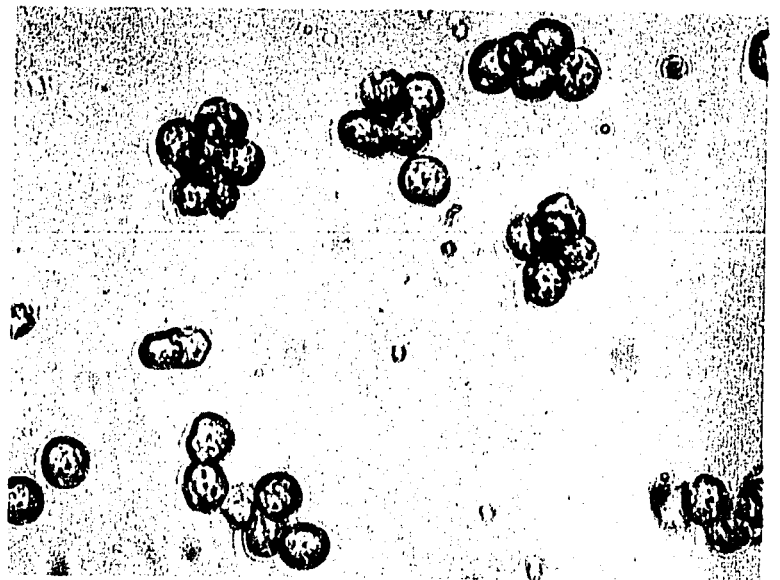
#### دیر شم تصویر

#### دحجرو موجودیت په تشو متیازوکی (ادرار)

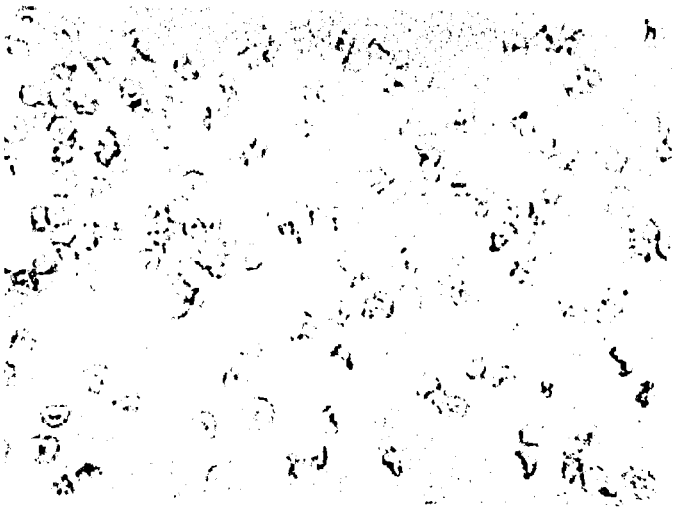
- ۲-۱ شکلونه :- دویښی سپینی حجرې : چه په یوازی اودله ایره توگه لیدل کیږی
- ۴-۳ شکلونه :- دویښی سری حجرې :- اول شکل: . نورمالی تازه حجرې ښی .  
او دوهم شکل گونجی یا چملکی شوی (متکمشی) او نا منظمی حجرې ښی .
- ۵-۶ شکلونه :- داپیتل حجرې :- ۵ شکل خشت فرشی حجرې چه د تشومتیازو (ادرار) -  
نا پاکي (ملوثیت) ښی . ۶ شکل:- دپښتورگی حجرې اولوئی هسني وگوری .



1



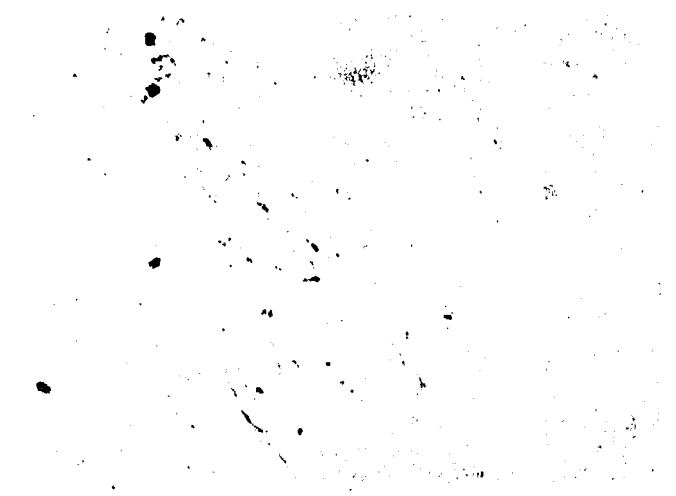
2



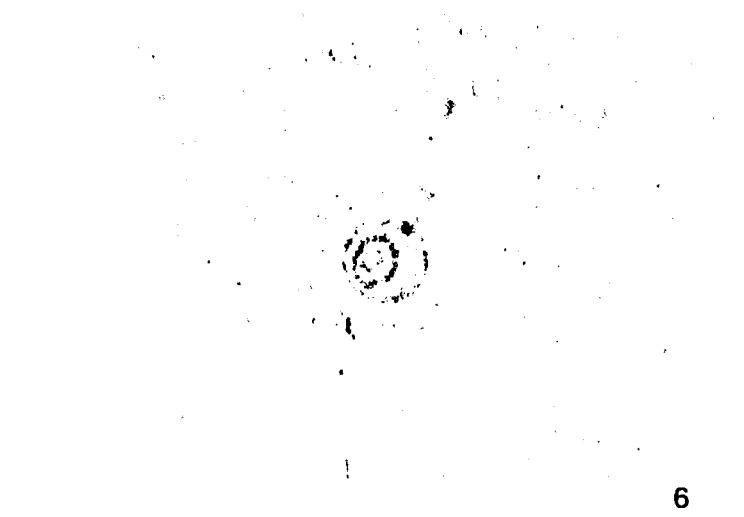
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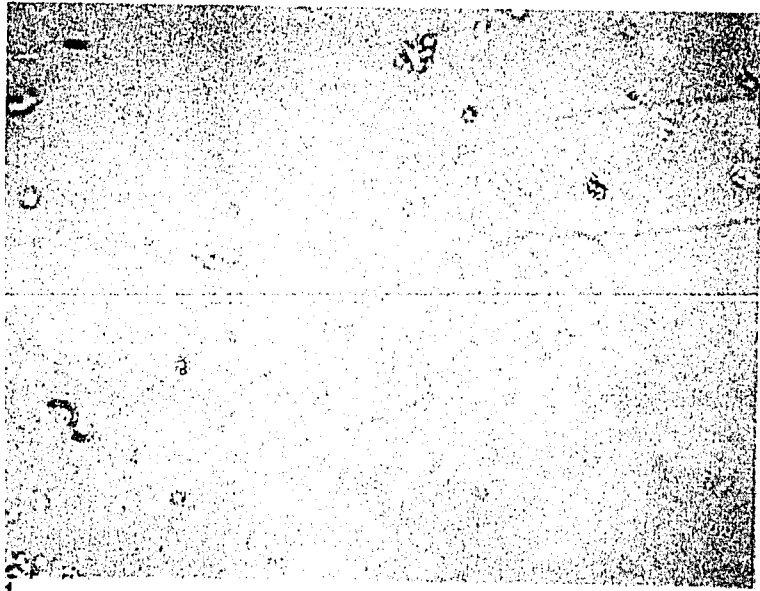
## PLATE 31

### Casts Found in Urine

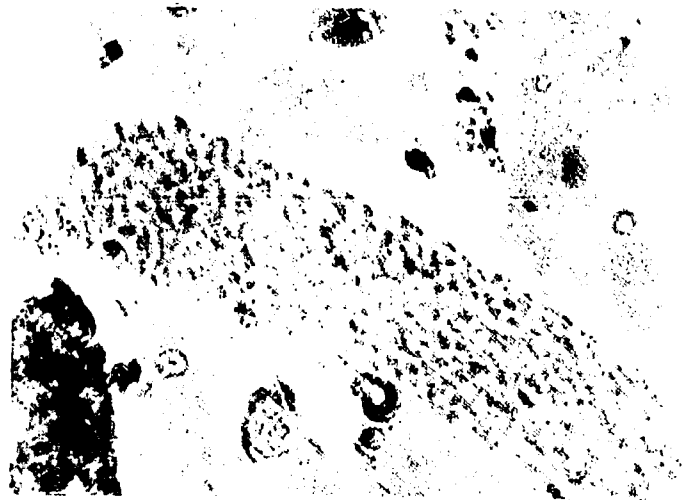
- Figure 1** Hyaline Casts: Usually colorless cylinders that are best seen with low intensity light.
- Figure 2** Granular Cast: Contain fine or coarse granules throughout the cast. Very refractile.
- Figure 3** White Blood Cell Cast: The cast should be more than 1/3 full of WBC's to be termed a WBC cast.
- Figure 4** Red Blood Cell Cast: These present in the urine indicate disease or injury in the kidney.
- Figure 5** Epithelial Cell Cast: The Cells in the cast are much larger than those found in WBC casts.
- Figure 6** Waxy Cast: A relatively broad cast with a brittle appearance. They can be yellow in color.
- Figure 7** Broad Casts: They are usually waxy, granular, or cellular and 2 to 6 times larger than ordinary casts. They are found associated with severe dehydration and severe renal disease.

#### یو دیرشم تصویر

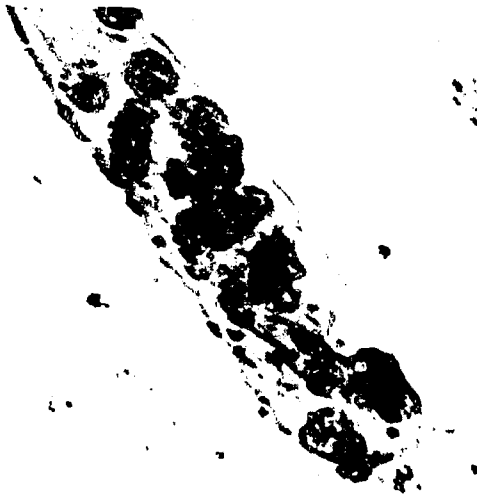
- هغه ( ) کستونه چه به تشو متيازو (ادرار) کی لیدل کیږی  
اول شکل :- دهیالین کستونه :- اکثره بی رنکه استوانوی شکل لری او په لیږه روښائی کی په لیدل کیږی .
- دوهم شکل :- کرنیولرکست (جیویکست) :- په ټولکست کی کوچنی کوچنی دانی (گرنیولونه) لیدل کیږی چه نورته انکسار ورکوی بریسی او حلیری .
- دریم شکل :- دوینی دسپینو حجرو کستونه :- هر کله چه دیوه کست ثلاث (۱/۳) برخه ئی سینو حجرو دکه کره نو هغه ته دسپینو حجرو کستونه وائی
- خلورم شکل :- د سرو حجرو کستونه :- کله چه په تشو متيازو (ادرار) کی ولیدل شی نو بیا به دپنټورکو (بدووی) ناروغی وی او یا دبدووی تپیا جزه .
- پنجم شکل :- داپیتیل د حجرو کستونه :- دنومووی کست حجری دسپینو حجراتو دکستو په پر تله ،یری لوئی دی .
- شپږم شکل :- شحمی کست :- لږ څه هوار او نازک ښکاريږی او ږیر رنګه لری .
- اوم شکل :- هوار او پراخه کستونه :- دا همیشه شحمی ،کرانیولر ، یا حجروی وی نوموړی کستونه د معمولی کستو نوپه پرتله ۲-۶ ځله لوی دی .
- په شدید دیهایدرپیشن او د بدووی په شدید ناروغی کی لیدل کیږی .



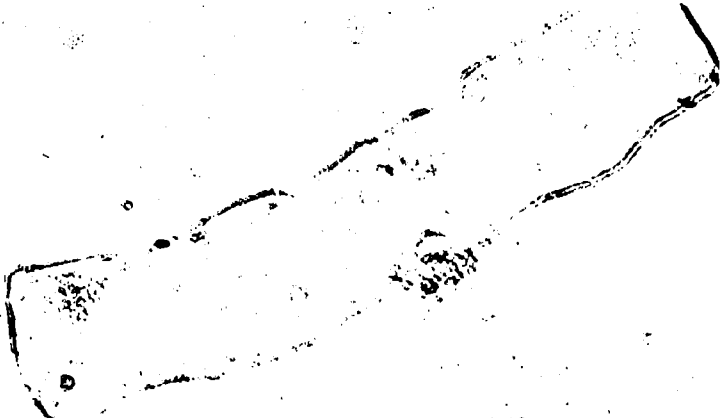
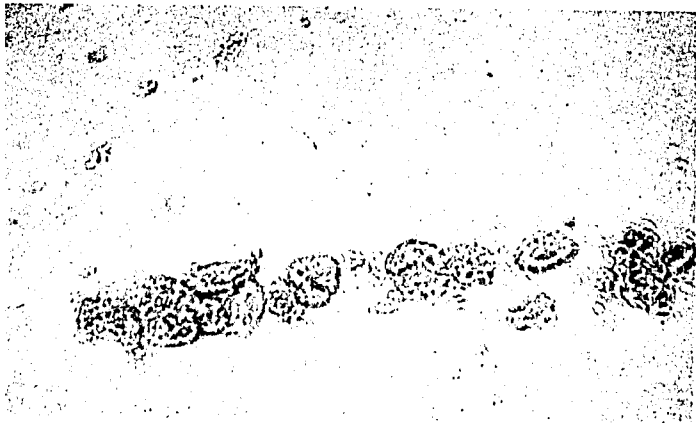
1



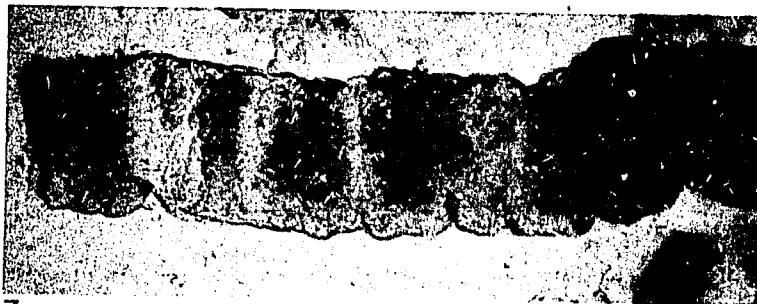
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## PLATE 32

### Crystals Found in Urine

- Figure 1      Uric acid: Found in acid urine and associated with gout.
- Figure 2      Cystine: Very rare, but can be found in acid urine of a patient with cystinuria.
- Figure 3      Tyrosine: An abnormal crystal found in acid urine of patients with liver problems.
- Figure 4      Leucine: An abnormal crystal found in acid urine of patients with liver problems. DO NOT confuse with Taenia.
- Figure 5      Calcium Oxalate: Crystals that can be found in any pH and are most often associated with kidney stones. Note the refractile "X".
- Figure 6      Triple Phosphate: Usually found in alkaline urine but can be found in acid urine.
- Figure 7      Ammonium Urates (Buirate): A yellow - brown crystal seen in urine when there is ammonia formation in the bladder.

- هغه ڪر ستالونه چه په تشومتيازو (ادرار) کي ليدل کيږي
- اول شکل :- يورنگ اسيد :- په اسيدی ادرار (تشومتيازو) کي دنقرص دنا روغي سره يو ځای ليدل کيږي.
- دوهم شکل :- سيستين :- ډير لږ ليدل کيږي . دهغو مريضانو په تشومتيازو کي چه سيستين توري لري او تشي متيازوئي اسيدی وي مشاهده کيږي.
- درېم شکل :- تايروزين :- يو غير نورمال ڪرستال دی چه د هغه مريضانو په تشومتيازو (ادرار) کي ليدل کيږي چه د ځگر ناروغي ولري .
- څلورم شکل :- ليوسين :- يو غير نور مال ڪرستال دی چه دهغه مريضانو په تشومتيازو کي چه ځگر ناروغي لري او تشي متيازو ئي اسيدی وي ليدل کيږي.
- پنځم شکل :- کلسيم او گزلات :- دا ڪرستالونه په هر pH کي ليدل کيږي خو (پښتورگي) بدودی د ډبري سره يوځای ليدل کيږي . او حلیدونکی (X) ته په ڪرستل کي پاملرنه وکړي.
- شپږم شکل :- تريپل فوسفات :- اکثراً په قلوې ادرار (تشومتيازو) کي ليدل کيږي . خو په اسيدی ادرار کي هم ښکاره کيدای شي.
- اوم شکل :- امونيم يورات (بيورت) :- يو ډير نساوي رنگه ڪرستال دی . کله چه په مثانه کي امونيا جوړه شي نو بيا دا ڪرستل ليدل کيږي.

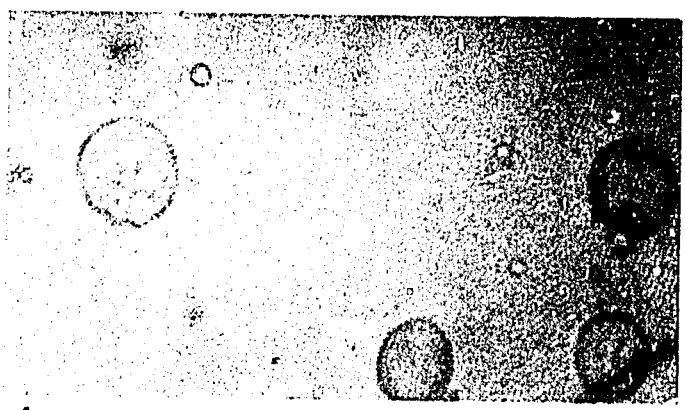


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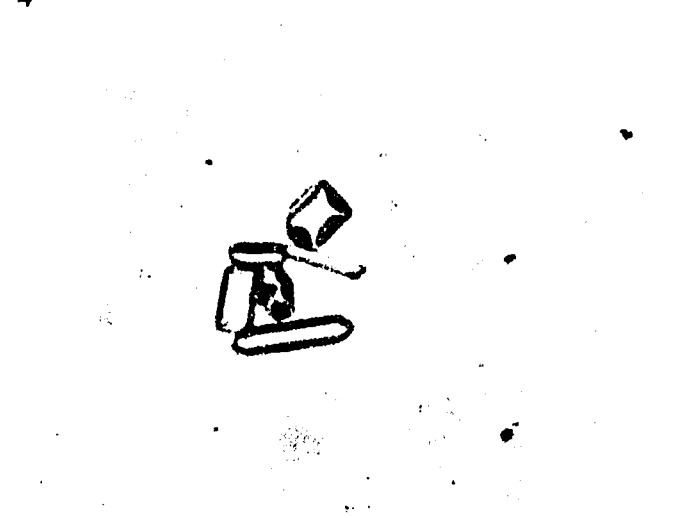
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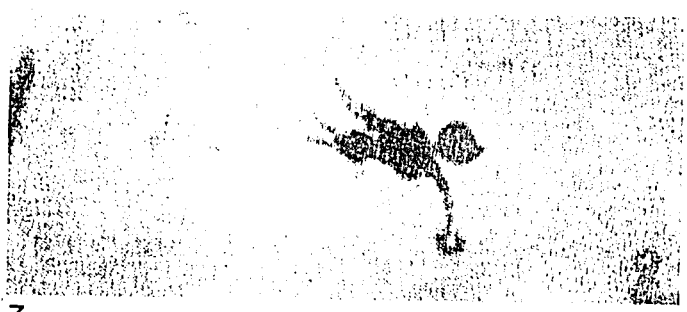
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## PLATE 33

### Miscellaneous Structures in Urine

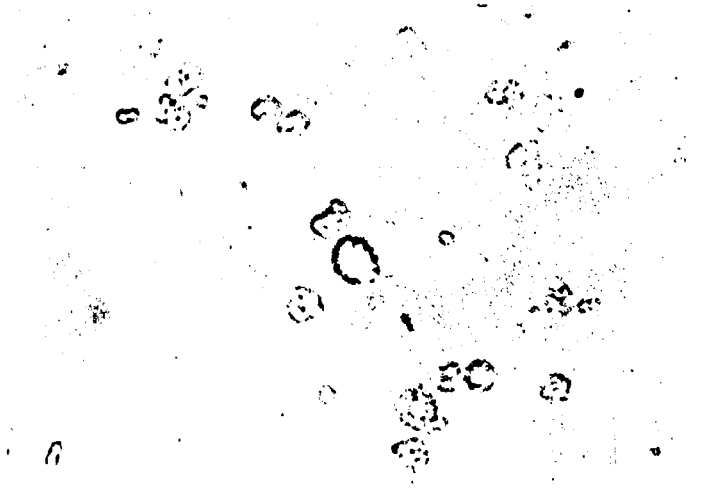
- Figure 1                      Bacteria: May be seen in urine sediment as a result of either urinary tract infection or contamination.
- Figure 2                      Trichomonas vaginalis: The most frequently seen parasite in urine. They are recognized by their swimming motion in the sediment.
- Figure 3 and 4                Yeast: Do not confuse with RBC's. They are ovoid in shape and may frequently show budding.
- Figure 5                      Spermatozoa: Oval bodies with long delicate tails. They may be mobil (moving) or stationary and can be found in both male and female urine.
- Figure 6                      Urine Debris: Starch granules
- Figure 7                      Urine Debris: Cotton fibers

#### ۳۳ تصویر

- دمتفرقه اجسامو موجودیت په تشو متنازو کی (ادرار کی) اول شکل :- باکتیریا :- دادرار به رسوب کی هغه وخت لیدل کیږی چه یاپد بولی طرفوکی انتان موجود وی او یا تشومتیازی ملوئی (ناپاکی) وی .
- دوهم شکل :- تریکومونالیس وجینالیس :- یوازینی پرازیت دی چه په تشومتیازو (ادرار) کی زیات لیدل کیږی . څرنگه چه د تشومتیازو (ادرار) په رسوب کی ځانگړی لامیوهونکی حرکتونه لری نو له د کبله ژر پیږندل کیږی .
- دریم او څلورم شکلونه :- خمیره : . باید دوینو سرو جراتو سره غلظنه شی . بیضوی ډوله شکل لری او معمولا (جوانه زدن) نیئی . پنځم شکل :- سیرماتوزوا :- تنه بیضوی او نری لکی لری . شاید خوځیدونکی ( متحرکه ) او شاید ثابت اوبی حرکتونه وی . د نر اوسنځی په تشومتیازو (ادرار) کی لیدل کیږی .
- شپږم شکل :- دادرار بقایا وی ( Leucocytes ) :- دنشایستی گرنیولون (دانی) . اووم شکل :- دادرار بقایا وی ( Starch granules ) :- د مالوچ تارونه .



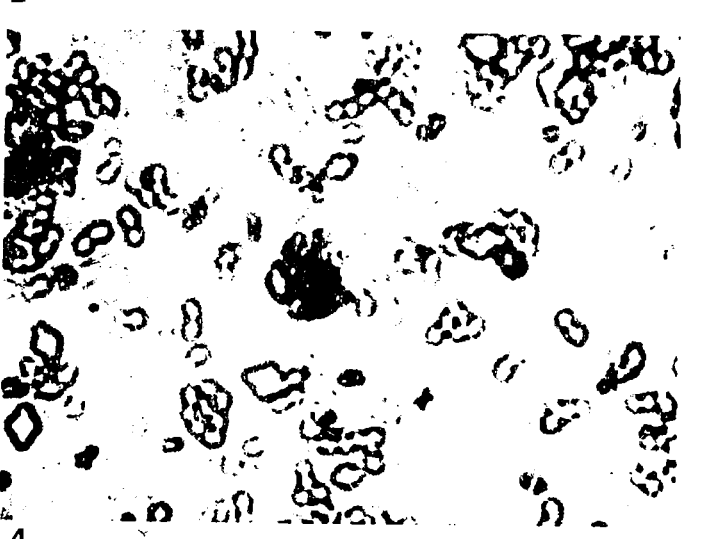
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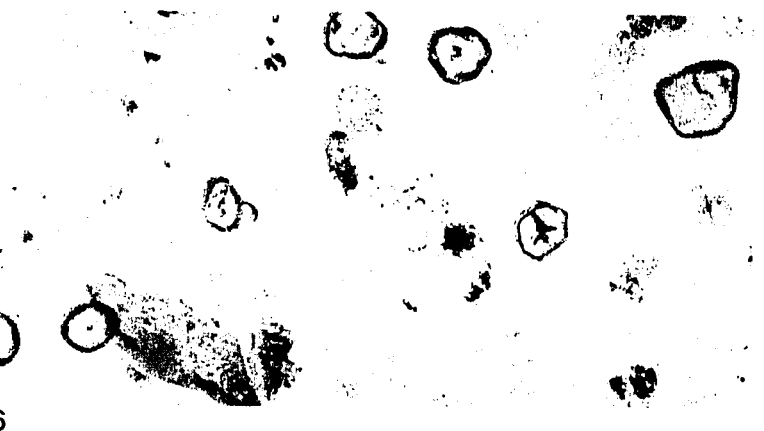
3



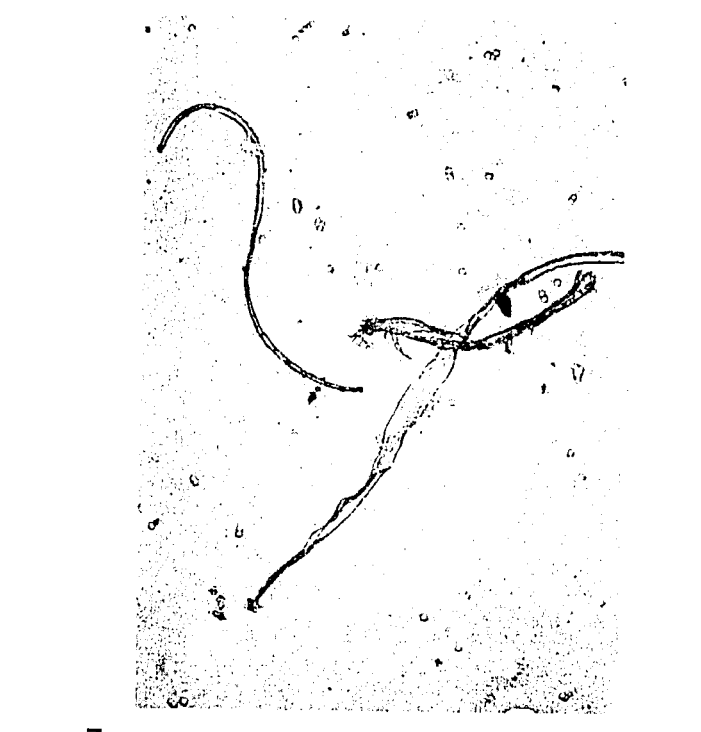
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# **BACTERIOLOGY**

## PLATE 34

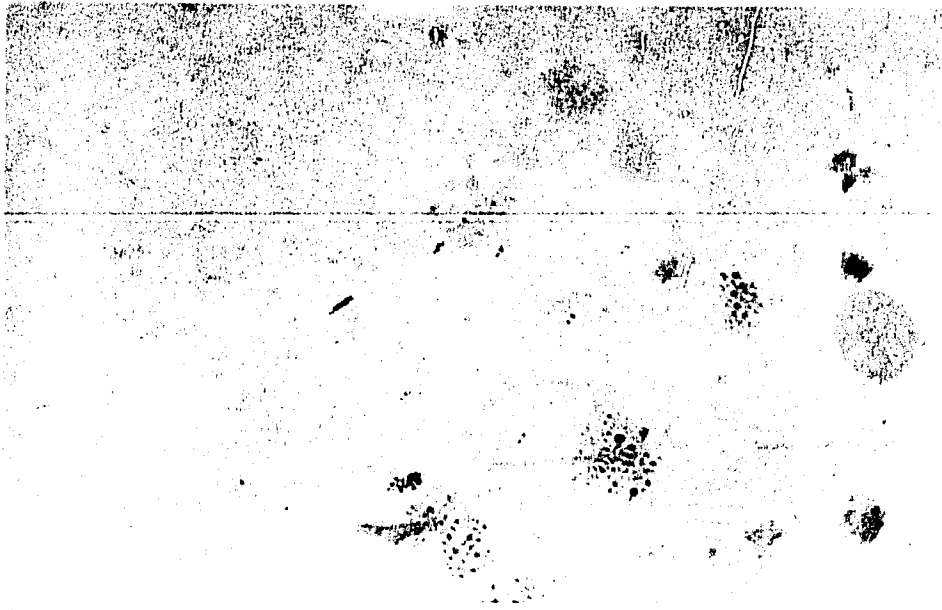
### Mycobacterium Tuberculosis

- Figure 1 One Acid-fast bacillus seen in a microscopic field (reported as + — or numbers seen).
- Figure 2 Singular bacillus and a few bacilli that are branching (considered as 1+).
- Figure 3 A heavier infection considered as 2+ if seen in 10 differ microscopic fields.

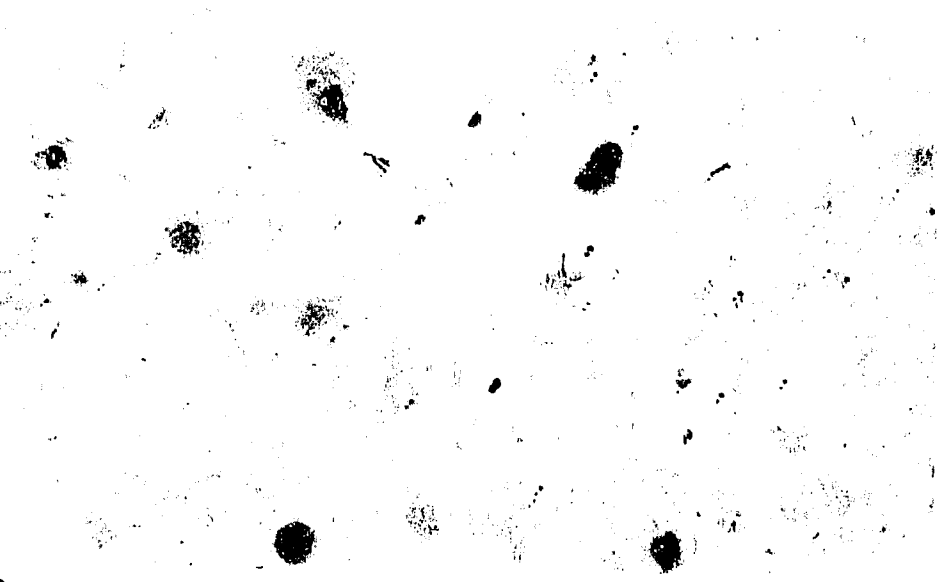
All are sputum samples stain with Ziehl-Neelsen.

ما يكو با كتر يوم تو بركلوز

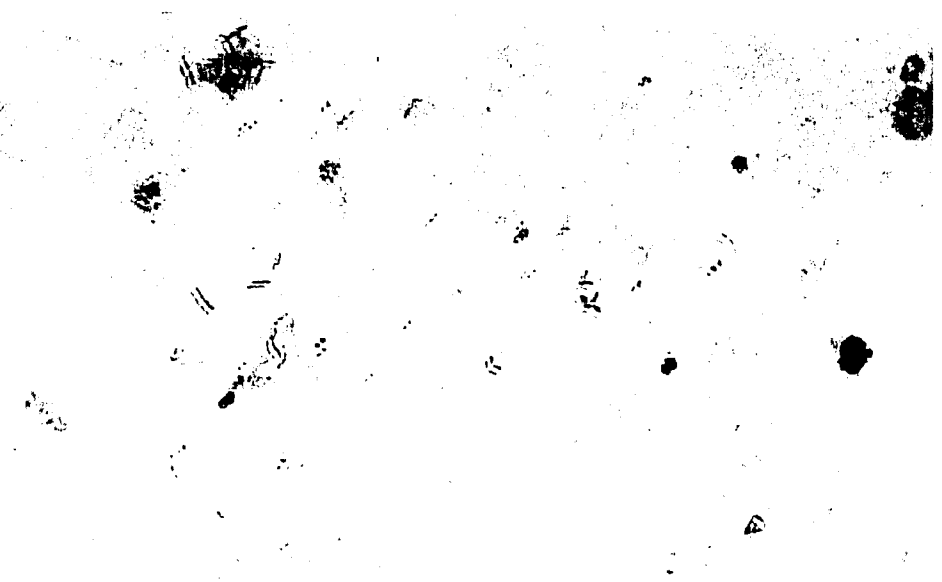
- لمری شکل :- يو داسيد په مقابل باسيل دمايكرسكوب په ساحه كی ليدل كيږي (چه په ۱ + ۱- او يا په زياتو تعداد سره راپور ورکړ شوی)
- دوهم شکل :- يوه واحده باسيل او خو نور باسيلونه چه سره منشعب شوی وی ( ۱+ په توگه مشاهده کيږي)
- درېم شکل :- که په لسو مختلفو مايکروسکوپيکي سا حوکي د ۲+ په توگه وليدل شي يو شديد انتان نښي .
- دا ټول د بلغم نموني دي چه دزييل نلسن په مينودتلوين شويدي .



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## PLATE 35

### Mycobacterium Tuberculosis

- Figure 1 and 3      Seen as a more infective case of T.B. depending on how many bacilli are seen per microscopic field.
- Figure 2            This is an acid-fast stain of a T.B. culture that demonstrates cord formation and is associated with the virulence potential of the T.B. organism.

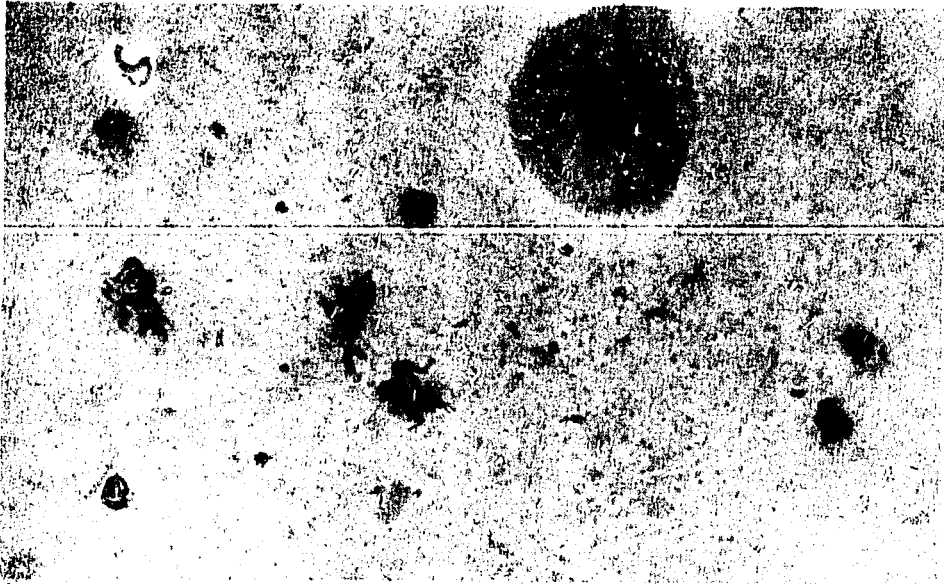
مايكوباكتريوم توبركلوز

لمرى اودريم شكل : له رويه ددى چه په شه تعداد باسيل په يوه ساحه كى : كى دميكروسكوپ .

ليدل كيرى دايو توبركلوز بيره منتنه واقعہ بنكاريرى

دوهم شكل : داديو اسيد په مقابل مقاوم توبركلوز ارگانيزم دويرونانس شدت سره

ارتباط نيسى.



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## PLATE 36

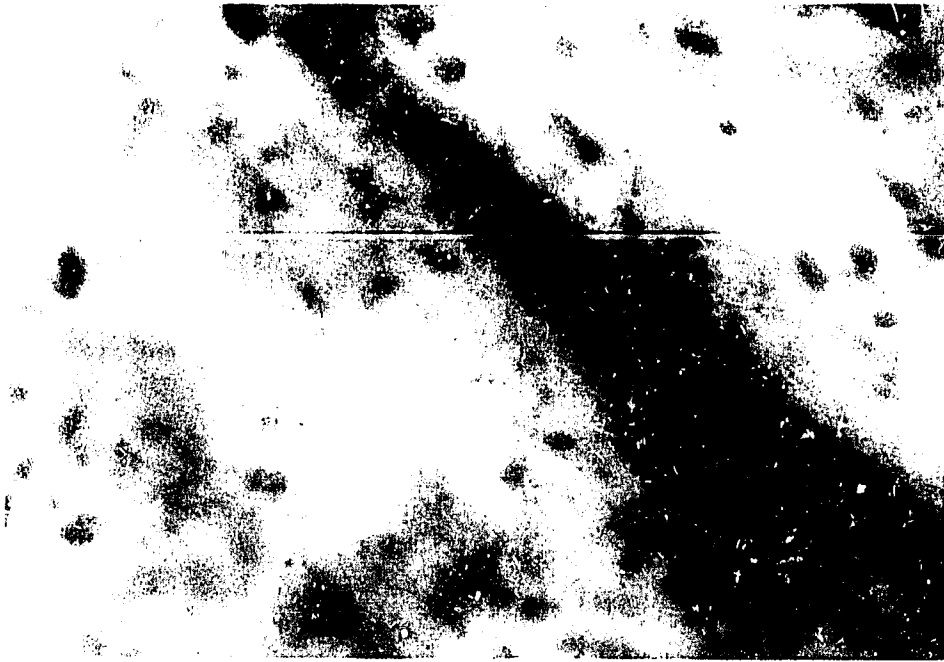
### Inappropriate Slides

- Figure 1 A sputum smear stained with Ziehl-Neelsen that has not been decolorized properly.
- Figure 2 A sputum smear that has been overheated (boiled).
- Figure 3 A sputum slide that was made too thick and is unreadable. A new slide will have to be made.

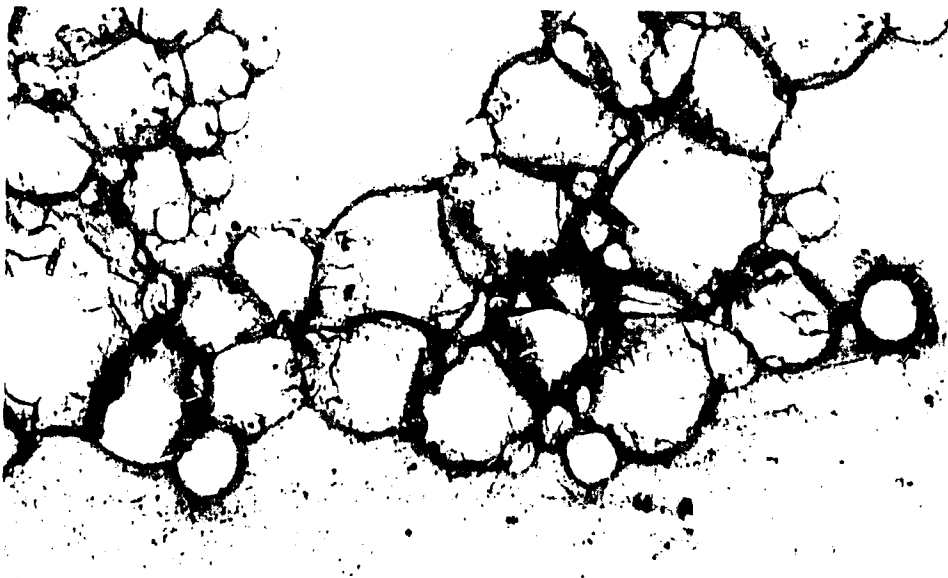
#### ناقص سلايدونه

- لمری شکل : د ذیل نلسن میتود سره یو د بلغم سمیر چه په منسبه توکه دیکالرایزشوی نه دی.
- دوهم شکل : یو د بلغم سمیر چه په زیاته اندازه حرارت ورکړل شوی دی .
- دریم شکل : یو د بلغم سلايد چه ډیر ضخیم جوړ شوی دی او دویلوور ندی. یونوی سلايد باید چه تهیه شی.

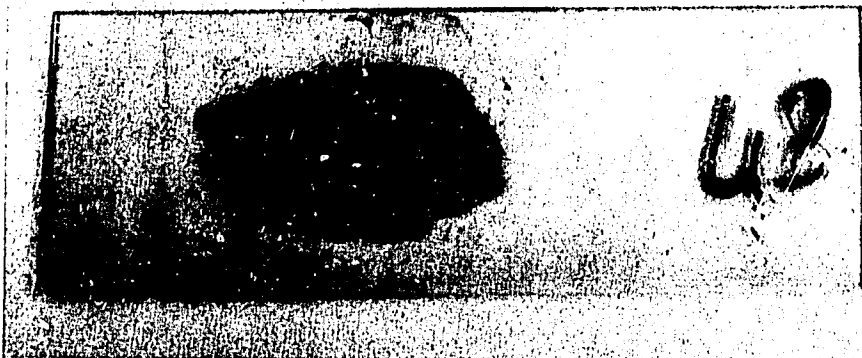




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2



2

## REFERENCES

1. L.W. Diggs, M.D., Dorothy Sturn, Ann Bell, M.S.,:  
*The Morphology of Human Blood Cells*: Abbott Laboratories, North Chicago, Illinois, 1978.
2. Lawrence R. Ash, Thomas C. Orihel: *Atlas of Human Parasitology*, 2nd ed. Chicago, Illinois, ASCP Press, 1984.
3. *Modern Urine Chemistry*: Ames Division, Miles Laboratories, Inc., Elkhart, Indiana, 1987.
4. Microphotographs courtesy of Dr. Menno Bouma, MSF Holland and Italian Cooperation for Development.

ماخذ

۱ :-

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ومن الله التوفیق

د اکتړغلام ربانی "وردک

دنریوال روغتیا ئی سازمان

دانگریزی ترجمان.