# SIDE LAB MANUAL

## Developed by:

## THE UNIVERSITY OF ZIMBABWE
## DEPARTMENT OF MEDICINE

&

## THE NECTAR PROGRAM

(PI) Prof. J. Hakim
(Co PI) Prof. J. Matenga
(Dept Chairman) Dr M. Borok
(Partner) Dr. J. Gray
(Partner) Prof. N. Madinger
(Lab Scientist) B. Ndemera
(Lab Scientist) A. Taziwa

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# PREFACE

The laboratory has become more essential in the clinical examination of patients. However there has been a tendency for the clinician to relegate even simple tests to specialist laboratory workers. This has its disadvantages. First, the clinician has less insight into the meaning and potential errors of the test. Second, depending on the main lab the test results may become available later than if the tests were done in a ward side-lab. Finally, the side-lab tests may be the only results available to the clinician during an emergency at night.

- Buxton Ndemera

# SIDE LAB SAFETY REGULATIONS

1. Keep hands away from face, especially mouth, nose, and eyes

2. **NO** smoking, eating, drinking, or cosmetic application

3. Long hair must be pulled back from face and dressed in such a way that it cannot touch either patient specimens, lab materials, or open flames

4. Clothing and especially long sleeves should be tight enough to minimize contact with specimens, lab materials/reagents or flames

5. **NEVER** pipette by mouth

6. All sharp materials (glass slides, needles, etc.) **MUST** be discarded in the appropriate SHARPS container

7. Patient specimens **MUST** be discarded in the plastic dish marked "DISINFECTANT"

8. Wear gloves if there are open lesions on your hands

9. **CLEAN** surface contamination or spills by wiping up with an absorbant material. Patient specimen spills should then be cleaned with 10% bleach (sodium hypochlorite) solution. Tissue paper plus A4 sheet can be used to scoop up broken slides.

10. **REPORT** any accident to laboratory staff in room 52 (Dept of Medicine)

11. **WASH** hands after working with patient specimens AND before leaving the laboratory

12. Do **NOT** wear contaminated clothing into patient care or public areas

13. **IMMEDIATELY** turn the bunsen burner **OFF** after use.

14. **CLEAN** the area after use:
    a. Place specimens in the correct bin or discard container
    b. Wipe surfaces clean of spills

15. **CHECK** that the flame has been turned **OFF** before leaving the laboratory

# THIN FILM – FIELD'S A & B METHOD

1. PREPARE A THIN SMEAR AND LEAVE TO AIR-DRY.

2. PLACE SLIDE ON A STAINING RACK AND FIX WITH METHANOL FOR 5 MINUTES.

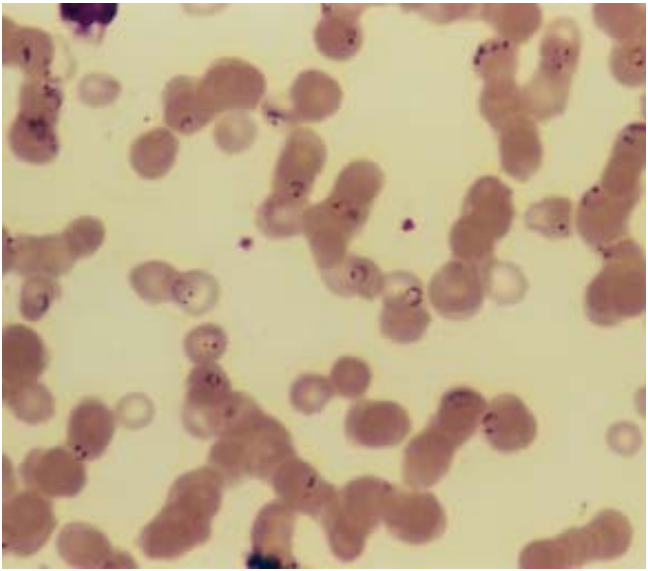
3. GENTLY WASH OFF FIXATIVE WITH WATER AND FLOOD WITH FIELD'S A.

4. MIX FIELD'S B AND LEAVE TO STAIN FOR 1 MINUTE.

5. WASH OFF STAINS WITH CLEAN WATER.

6. CAREFULLY WIPE THE BACK OF THE SLIDE CLEAN AND LEAVE SLIDE TO AIR-DRY.

7. SCAN THE SLIDE WITH 40X OBJECTIVE THEN DO A THOROUGH EXAMINATION USING 100X UNDER OIL IMMERSION.



*Thin film showing intracellular ring forms*

# THICK FILM – FIELD'S A & B METHOD

1. PLACE A DROP OF BLOOD ON A SLIDE AND PREPARE A THICK SMEAR.

2. STAND SLIDE TO AIR-DRY FOR 3 – 5 MIN.

3. DIP THE SLIDE IN **FIELD'S A** JAR FOR 5 SECONDS.

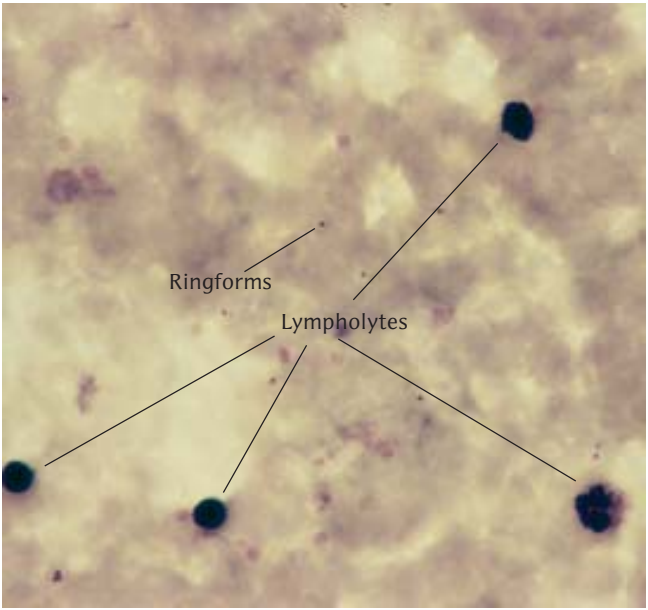
4. WASH GENTLY FOR 5 SECONDS IN CLEAN WATER. DRAIN OFF EXCESS WATER.

5. DIP THE SLIDE IN **FIELD'S B** JAR FOR 3 SECONDS. DRAIN OFF EXCESS STAIN.

6. WASH GENTLY IN CLEAN WATER.

7. CAREFULLY WIPE THE BACK OF THE SLIDE CLEAN AND LEAVE SLIDE TO AIR-DRY.

8. SCAN THE SLIDE WITH 40X OBJECTIVE THEN DO A THOROUGH EXAMINATION USING 100X UNDER OIL IMMERSION.




*Tropical view of a positive thick film*

# THIN FILM – LEISHMAN'S STAIN
## *ALTERNATIVE STAINING METHOD*

1. MAKE A THIN BLOOD FILM AND AIR-DRY.

2. PLACE SLIDE ON A STAINING RACK, COVER WITH **LEISHMAN'S STAIN** AND LEAVE FOR 1 – 2 MIN.

3. ADD **pH 6.8 BUFFER** FOR A 1:1 MIXTURE OF STAIN:BUFFER. BLOW GENTLY TO MIX AND LEAVE FOR 6 MIN.

4. POUR OFF STAIN MIXTURE AND WASH WITH **pH 6.8 BUFFER** FOR 1 MIN.

5. POUR OFF BUFFER, WIPE BACK OF SLIDE AND STAND UPRIGHT TO AIR-DRY.

# URINE MICROSCOPY

1. PUT ~ 12 mL OF FRESH URINE INTO A CENTRIFUGE TUBE.

2. BALANCE THE CENTRIFUGE WITH A SECOND TUBE WITH EQUAL VOLUME OF WATER.

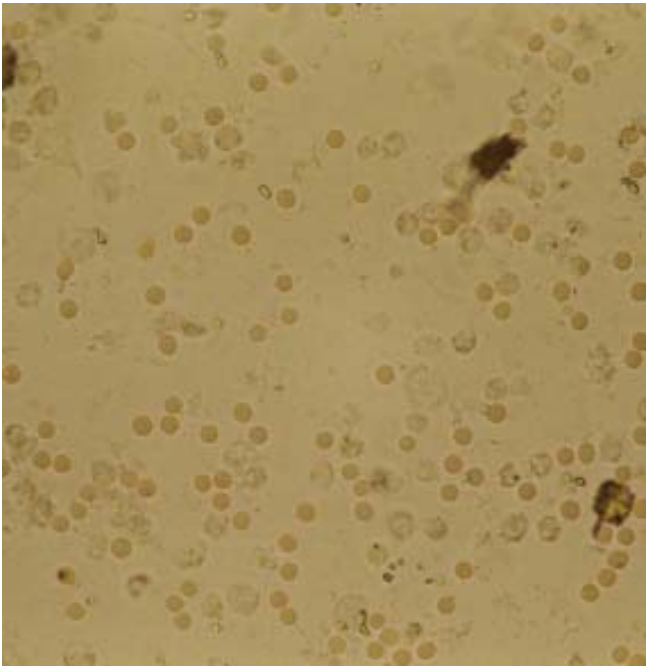
3. SPIN AT 1500 RPM FOR 2 – 3 MIN. AND DECANT THE SUPERNATANT.

4. RESUSPEND THE SEDIMENT IN THE REMAINING ~ 0.5 mL OF URINE BY FLICKING THE BOTTOM OF THE TUBE.

5. TRANSFER 1 DROP ON TO A CLEAN SLIDE AND PLACE A COVERSLIP ON TOP.

6. EXAMINE AT 10X WITH THE CONDENSER HOUSING LOWERED AND LIGHT DIMMED.

7. EXAMINE AT 40X WITH THE CONDENSER RAISED AND LIGHT INCREASED.



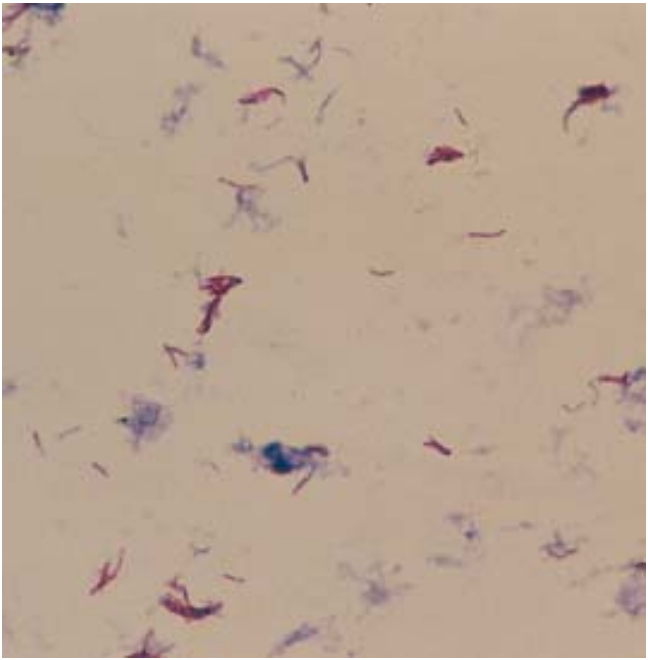
*Urine sediment showing lympholytes and red blood cells*

# GRAM'S STAIN

1. PREPARE SMEAR AND LEAVE SLIDE TO AIR-DRY.
2. FIX SLIDE BY PASSING THROUGH A FLAME 2 – 3 TIMES.
3. FLOOD WITH **METHYL VIOLET** 10 – 30 SEC.
4. WASH OFF WITH WATER AND COVER WITH **GRAM'S IODINE** 10 – 30 SEC.
5. DECOLOURIZE WITH **ACETONE** 2 – 3 SEC.
6. COUNTERSTAIN WITH **SAFRANIN** 5 – 10 SEC.
7. WASH WITH WATER AND BLOT DRY.
8. EXAMINE USING 40X THEN 100X OBJECTIVE UNDER OIL-IMMERSION.

# ZIEHL NEELSEN (AFB) STAIN
## BLEACH METHOD (PREFERRED)

1. ADD EQUAL VOLUME OF **5% SODIUM HYPOCHLORITE** TO SAMPLE AND STAND FOR 5 MIN.
2. MIX GENTLY WITH A TRANSFER PIPETTE.
3. TRANSFER 10 – 15 mL OF SOLUTION INTO A CENTRIFUGE TUBE.
4. CENTRIFUGE AT 2500 RPM FOR 3 – 5 MIN.
5. DISCARD SUPERNATANT COMPLETELY.
6. VORTEX DEPOSIT.
7. PLACE A SMALL DROP OF DEPOSIT ON A SLIDE TO MAKE A THIN SMEAR AND AIR-DRY.
8. PROCEED WITH TRADITIONAL METHOD, STEP 2.



*A sputum smear showing AFB*

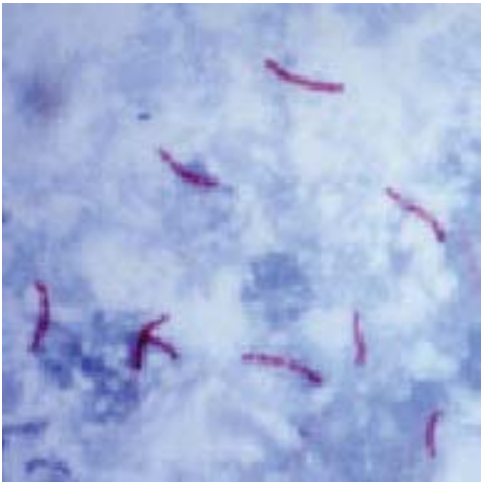
# ZIEHL NEELSEN (AFB) STAIN
## TRADITIONAL METHOD

1. PREPARE THIN SMEAR AND LEAVE SLIDE TO AIR-DRY THEN HEAT FIX.
2. COVER THE HEAT-FIXED FILM **WITH 2% CARBOL FUCHSIN**.
3. HEAT WITH A FLAME UNTIL IS STEAMS BUT NOT BOILS.
4. WASH WITH WATER.
5. FLOOD WITH **ACID ALCOHOL** (3% HCL IN 95% ETHANOL) AND LEAVE FOR 5 – 10 SEC.
6. WASH WITH WATER.
7. COUNTERSTAIN WITH **METHYLENE BLUE** FOR 15 – 30 SEC.
8. WASH WITH WATER AND BLOT DRY.

# GRADING AFB SMEARS

## ACID-FAST BACILLI APPEAR AS RED RODS

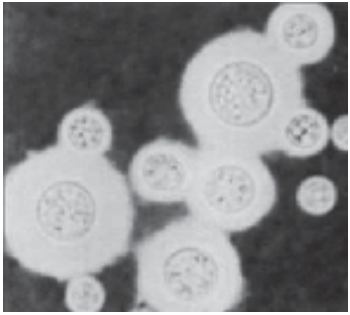
**+ = 1 – 10 IN 100 FIELDS**
**++ = 1 – 10 IN 10 FIELDS**
**+++ = 1 – 10 IN 1 FIELD**
**++++ = > 10 IN 1 FIELD**



# CSF INDIA INK STAIN

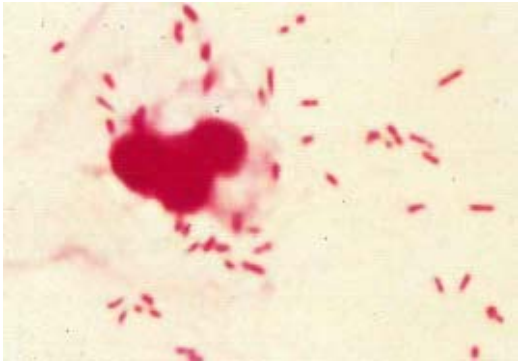
1. SPIN CSF IN CENTRIFUGE AT 1500 RPM FOR 3 MIN.
2. PLACE 1 DROP OF CSF SEDIMENT TO A CLEAN SLIDE.
3. PLACE 1 DROP OF INDIA INK TO SLIDE.
4. EXAMINE USING 40X OBJECTIVE

CRYPTOCOCCUS APPEAR AS ROUND STAINED CELLS SURROUNDED BY AN UNSTAINED CAPSULE

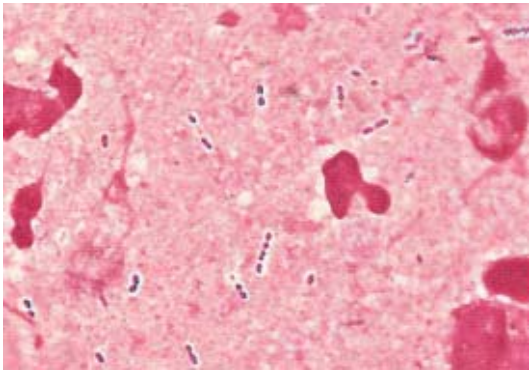


# CSF GRAM'S STAIN

1. SPREAD PURULENT CSF OR SEDIMENT OF CENTRIFUGED SAMPLE ONTO CLEAN SLIDE.
2. AIR-DRY AND FIX WITH **METHANOL** FOR 2 MIN.
3. FLOOD WITH **CRYSTAL VIOLET** FOR 30 – 60 SEC.
4. WASH OFF WITH WATER AND FLOOD WITH **GRAM'S IODINE** FOR 30 – 60 SEC.
5. DECOLORIZE WITH **ACID ALCOHOL** FOR 3 SEC THEN WASH OFF WITH WATER.
6. FLOOD WITH **SAFRANIN** FOR 2 MIN.
7. WASH WITH WATER AND BLOT DRY.
8. EXAMINE USING 40X THEN 100X OBJECTIVE UNDER OIL-IMMERSION.



*A Gram negative CSF smear*



*A Gram positive CSF smear*