Mycobacterial infection diagnosis

Practical Microbiology

Semester 2

Instructor information

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- Practical: Mycobacterial infection diagnosis
- Module (Respiratory and Renal Module RAR)

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Learning outcomes

By the end of this lecture student will be able to:

- Explain the proper sample to recover mycobacteria.
- Describe the principles and procedures for the stains used to demonstrate mycobacteria.
- Outline the different culture media used for the isolation of mycobacteria.
- Compare the new techniques used in detecting mycobacteria.
- State the principle and value of the tuberculin skin test.

Lecture outline

- General characteristics of *Mycobacteria*
- Laboratory diagnosis of *Mycobacteria*
- Specimen collection
- Microscopic examination
- Isolation and identification

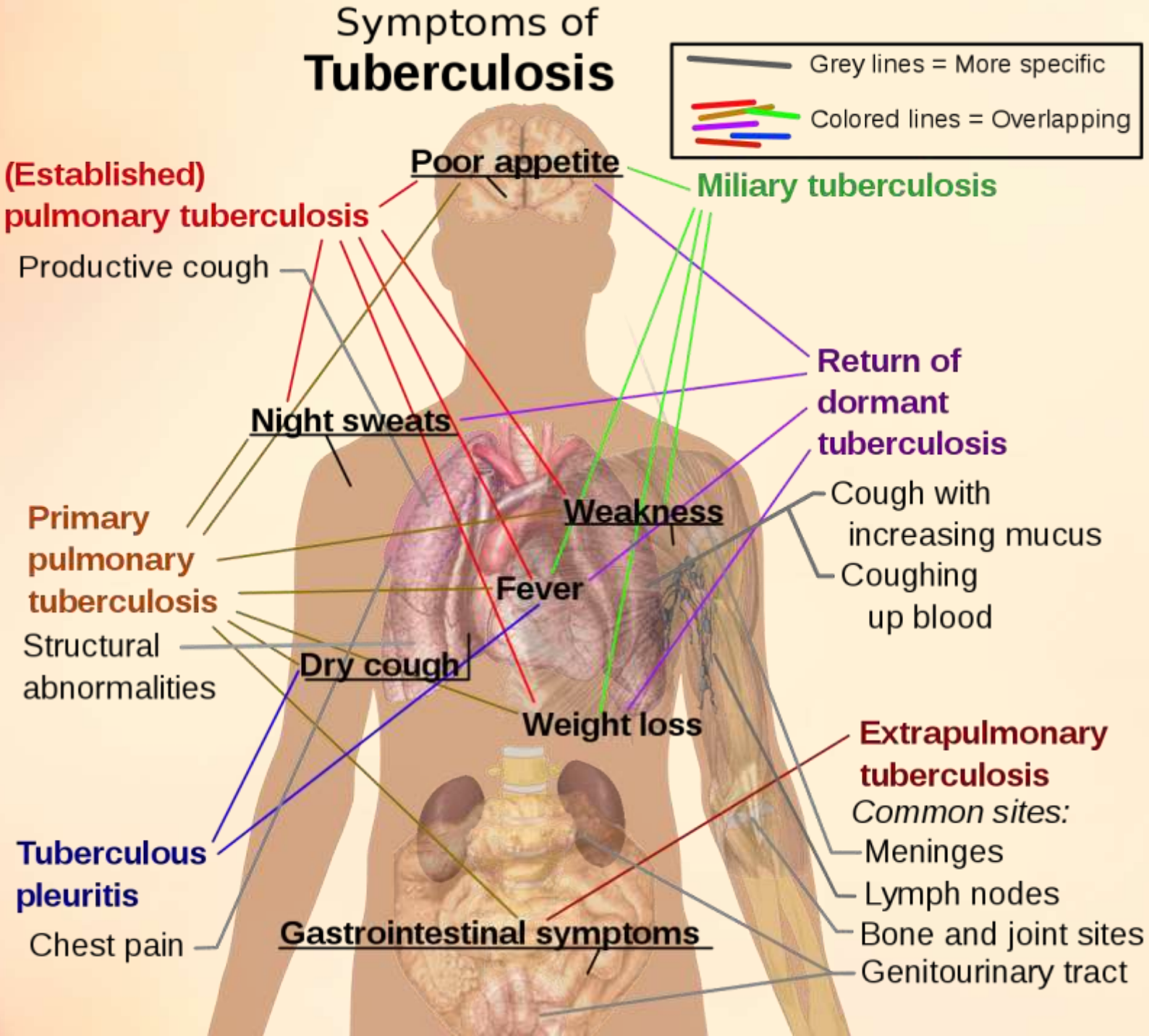
mycobacteria

1- M. tuberculosis complex (M. tuberculosis, M. bovis)

2- M. leprae

3- Non tuberculous "Atypical"

4- Saprophytic



M. Tuberculosis diseases:

1- Pulmonary

Gastrointestinal

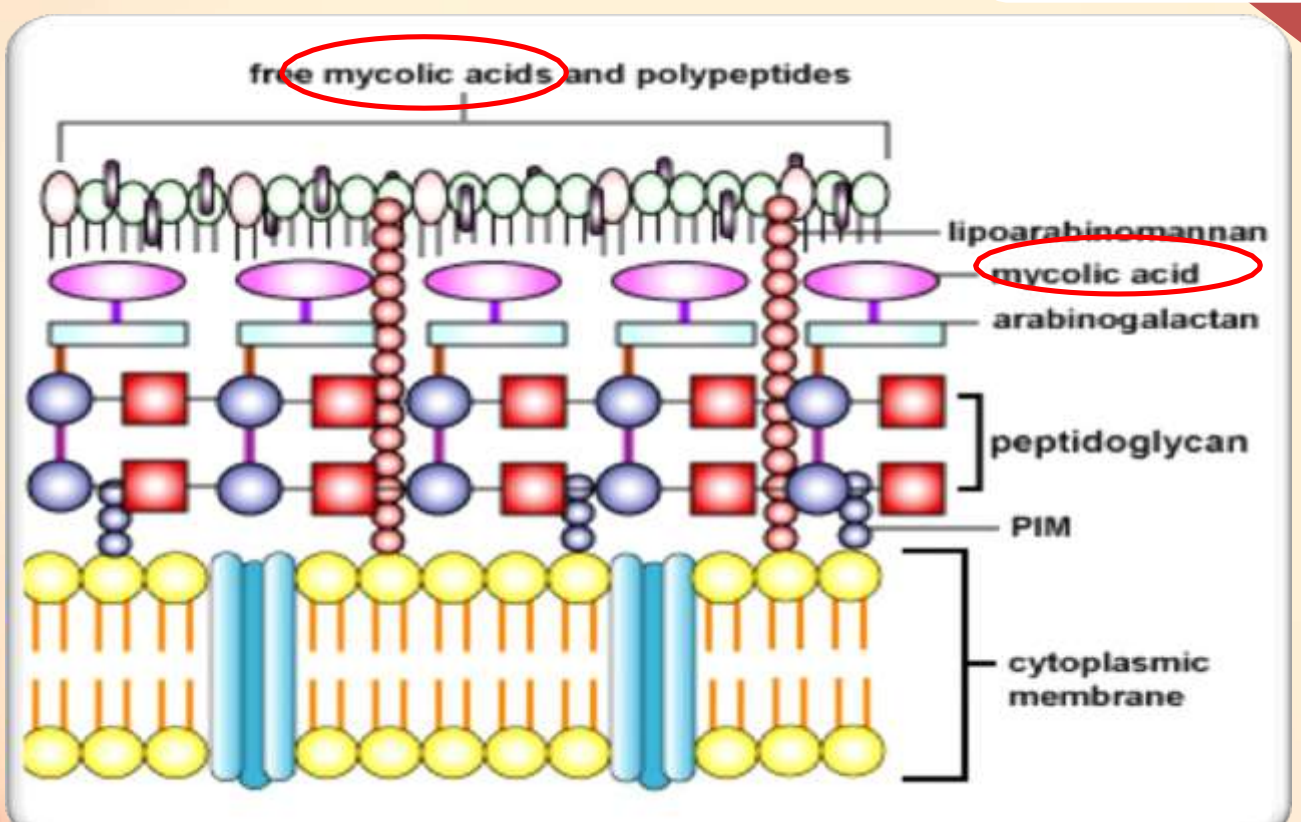
2 Renal

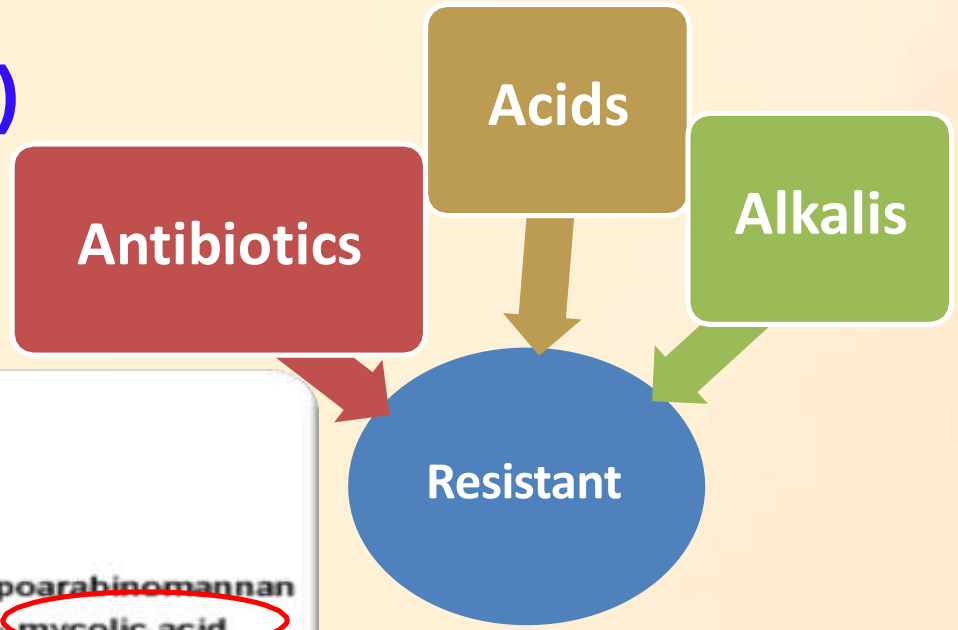
3 Meningitis

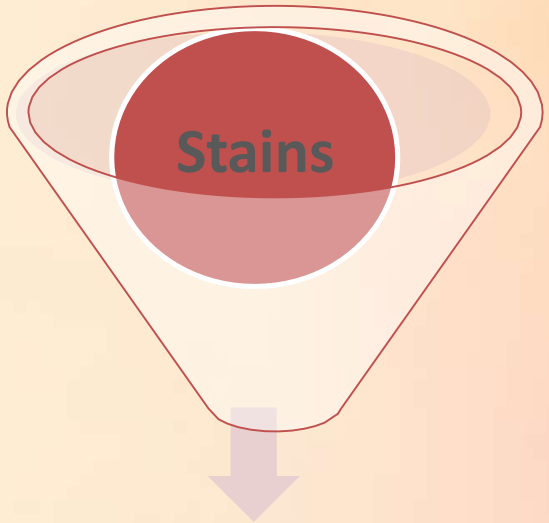
4 Spread (pleural, bone,..).

Mycobacterial cell wall

High lipid content (40-60%)







Impermeable

Gram stain


MYCOBACTERIUM
TUBERCULOSIS

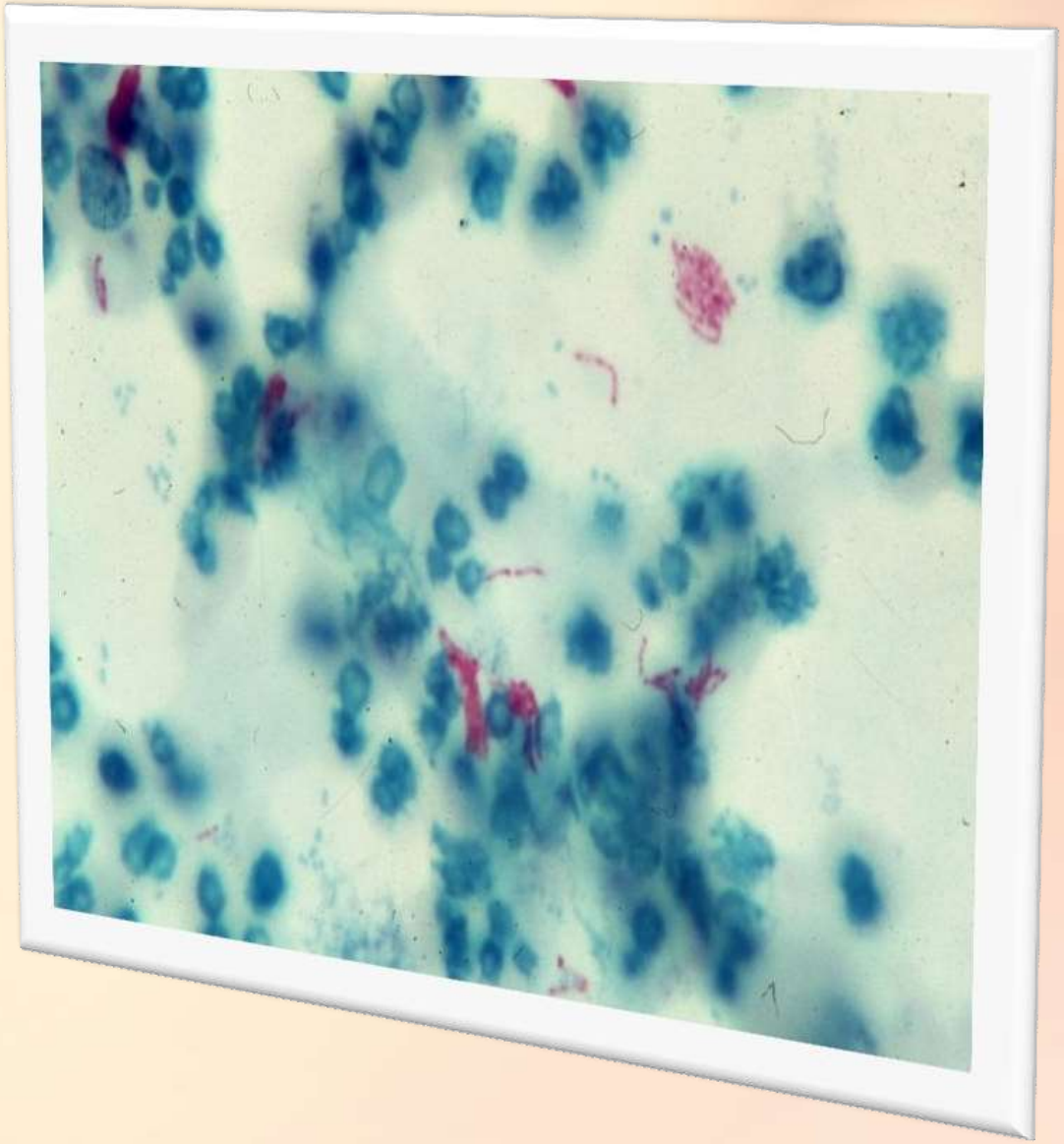
- One of the '**Mycobacterium tuberculosis complex**'

- **Human** is the only reservoir.

- Etiologic agent of tuberculosis "TB".

- F. **intracellular** 'Macrophages'. Small Straight or slightly curved Non motile Non spore forming Occur in singles, pairs or masses.

- **Acid and alcohol fast**
  i.e: resist decolourization by them



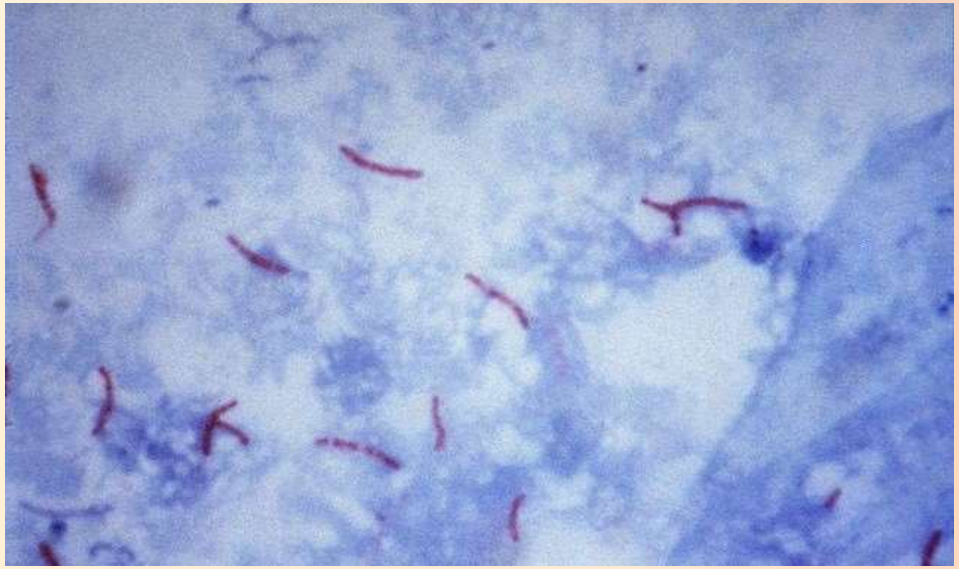
Lab diagnosis of pulmonary tuberculosis

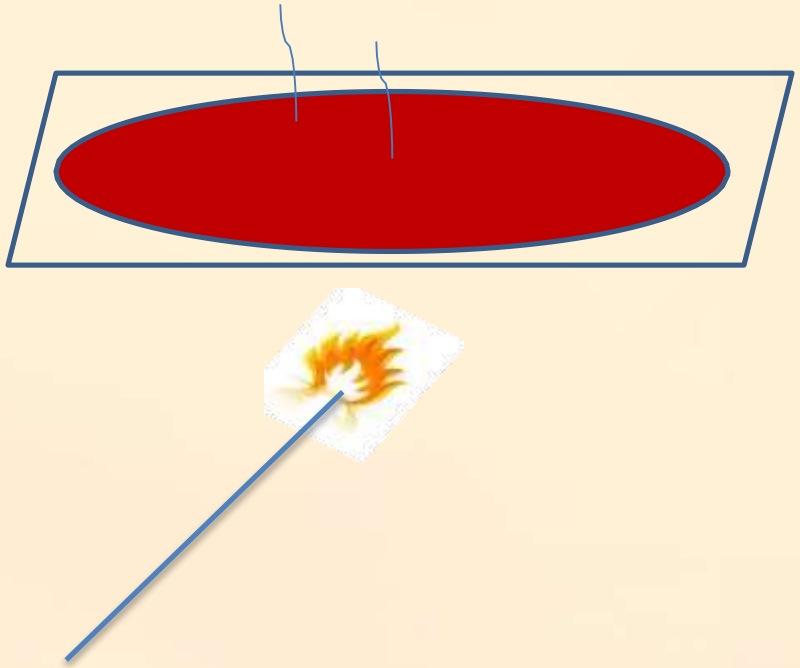
- **1- Sample: ?**
- Sputum, bronchial or gastric washings.
- At least **3 morning** samples of sputum are required for diagnosis.

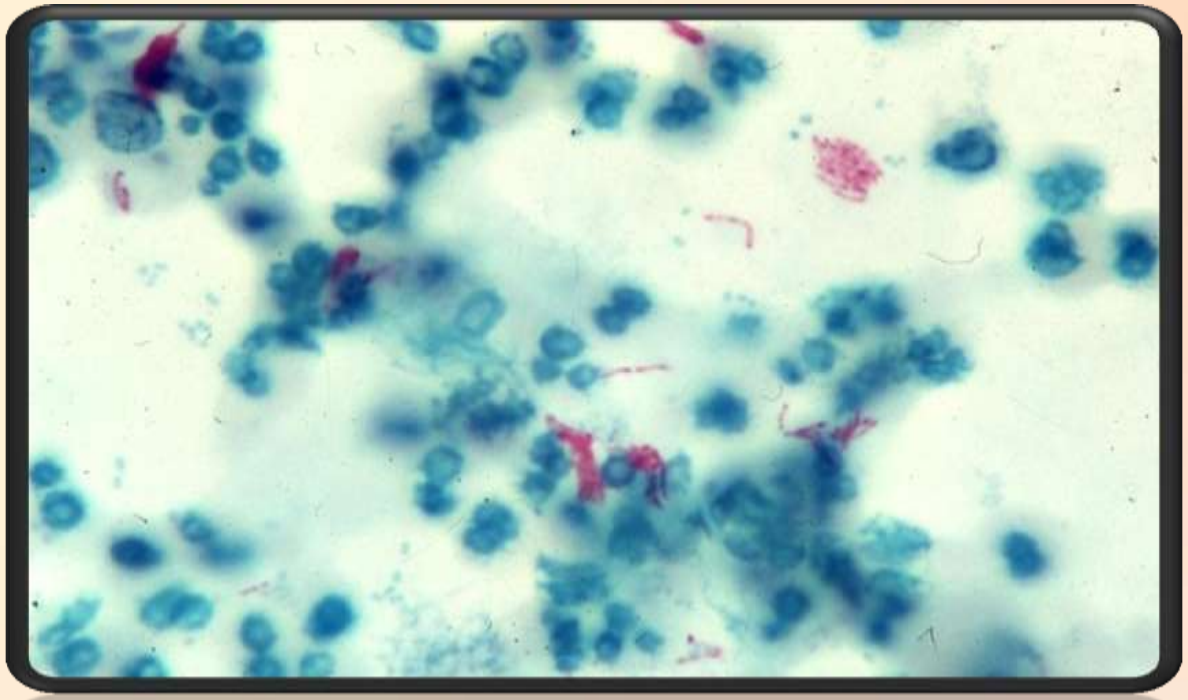
2- Direct film ‘smear’:

Z.N. stain

(after liquefaction→ by N-acetyl L-cystein)



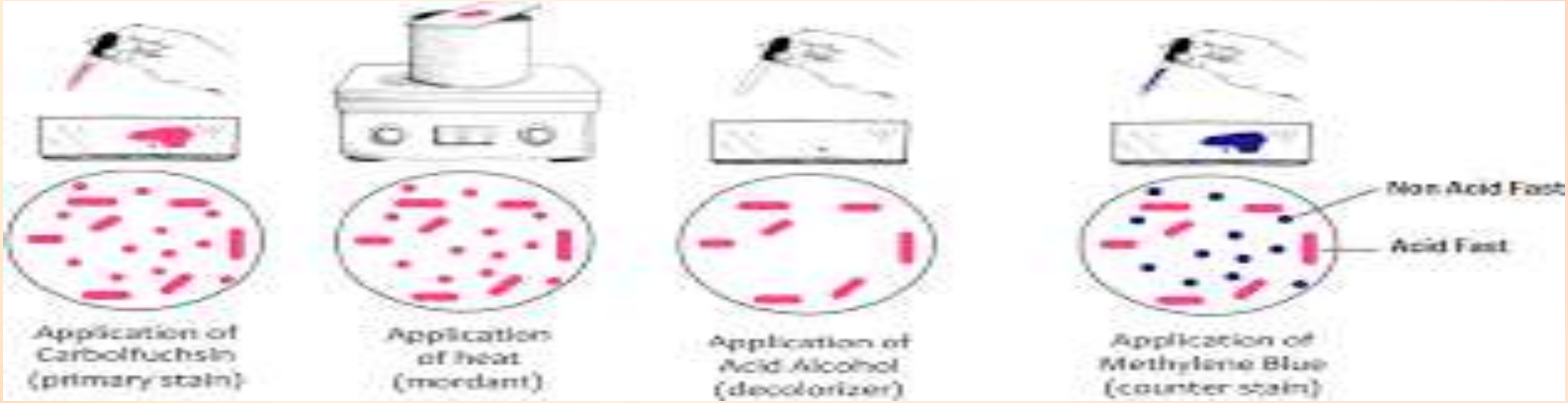




Ziehl- Neelsen Stain

Principle

- Some organisms contain many lipids in their cell wall that prevent staining with Gram stain.
- These organisms can be stained by concentrated dyes as concentrated carbol fuchsin in presence of heat.
- These organisms resist decolourization by acid (acid fast).



Requirements

- Concentrated carbol fuchsin.
- Sulphuric acid 20%.
- Alcohol 95 %.
- Methylene blue.

The Ziehl-Neelsen staining procedure requires:

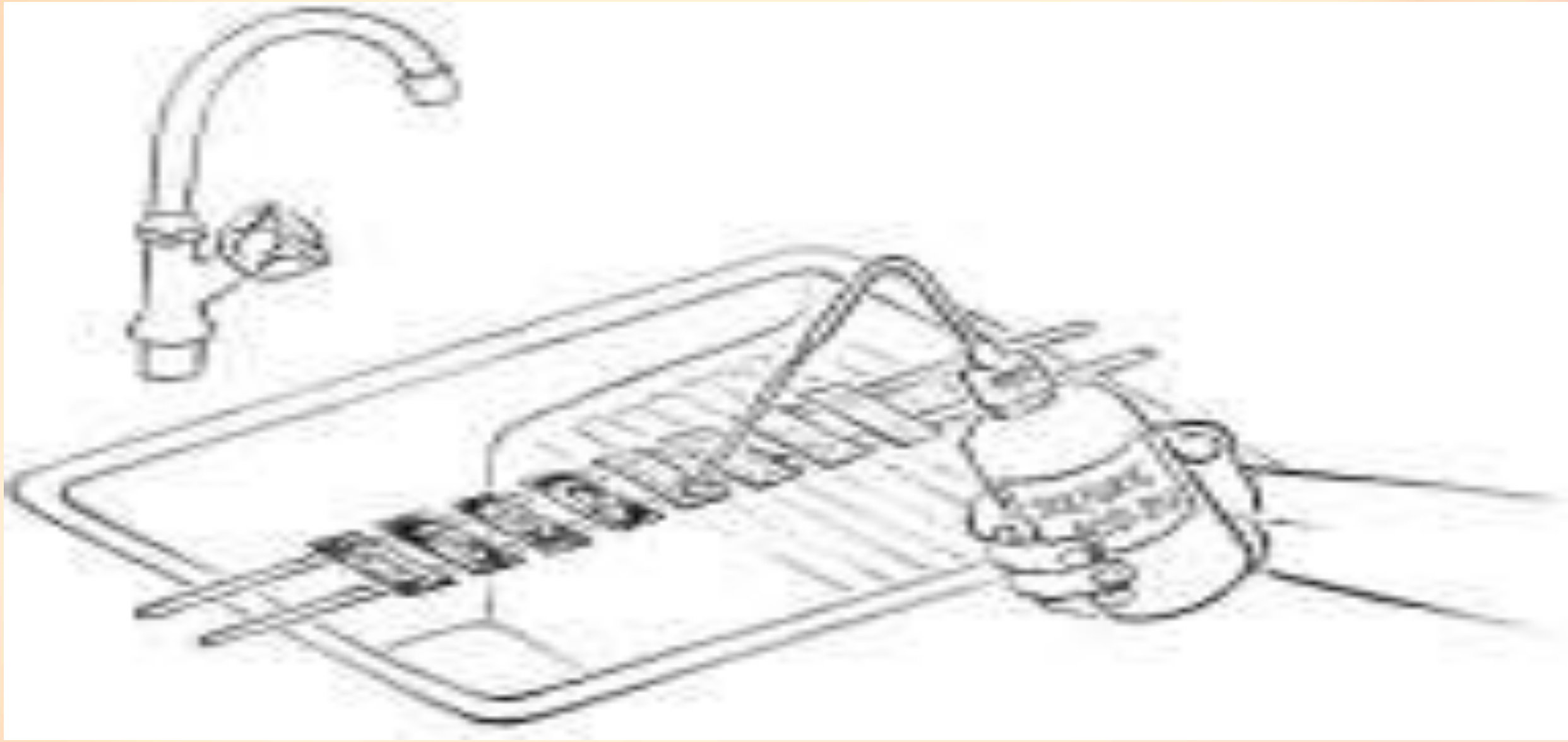
- Staining for 5 minutes
- Decolourising for 3 minutes
- Counterstaining for 1 minute

Cons. Carbol Fuchsin stain

Steam then cool

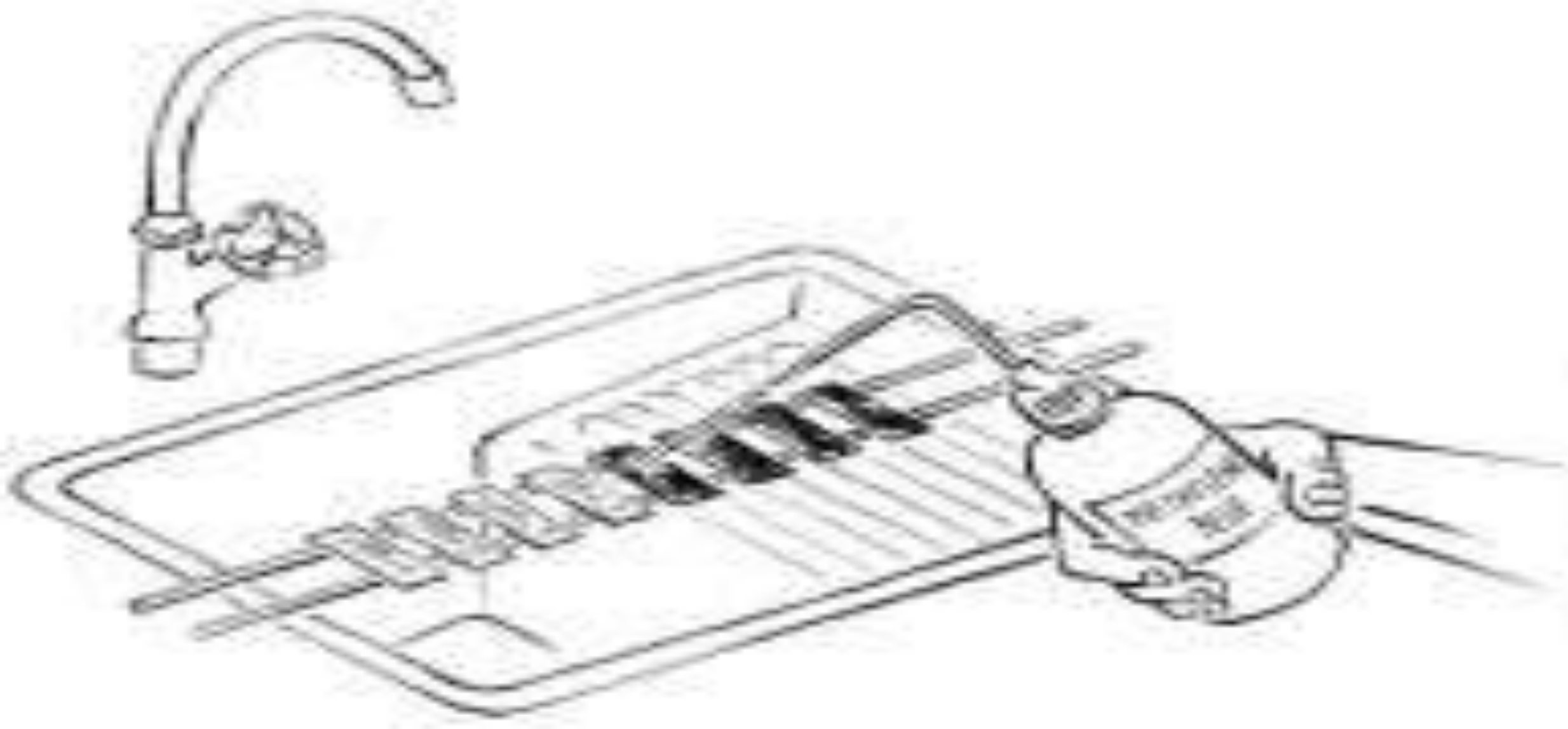
Rinse with water

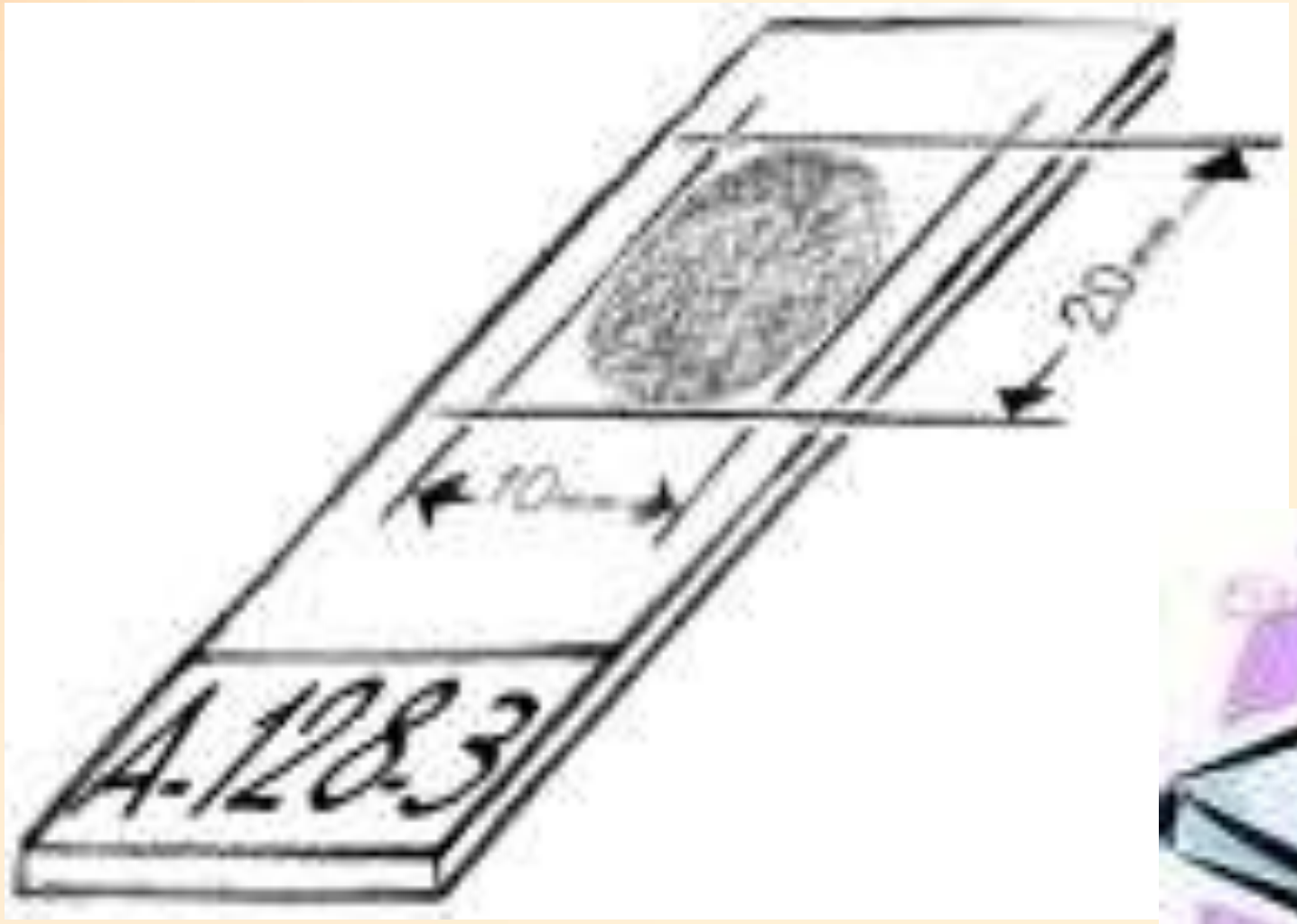
acid alcohol



Rinse with water

Methylene Blue

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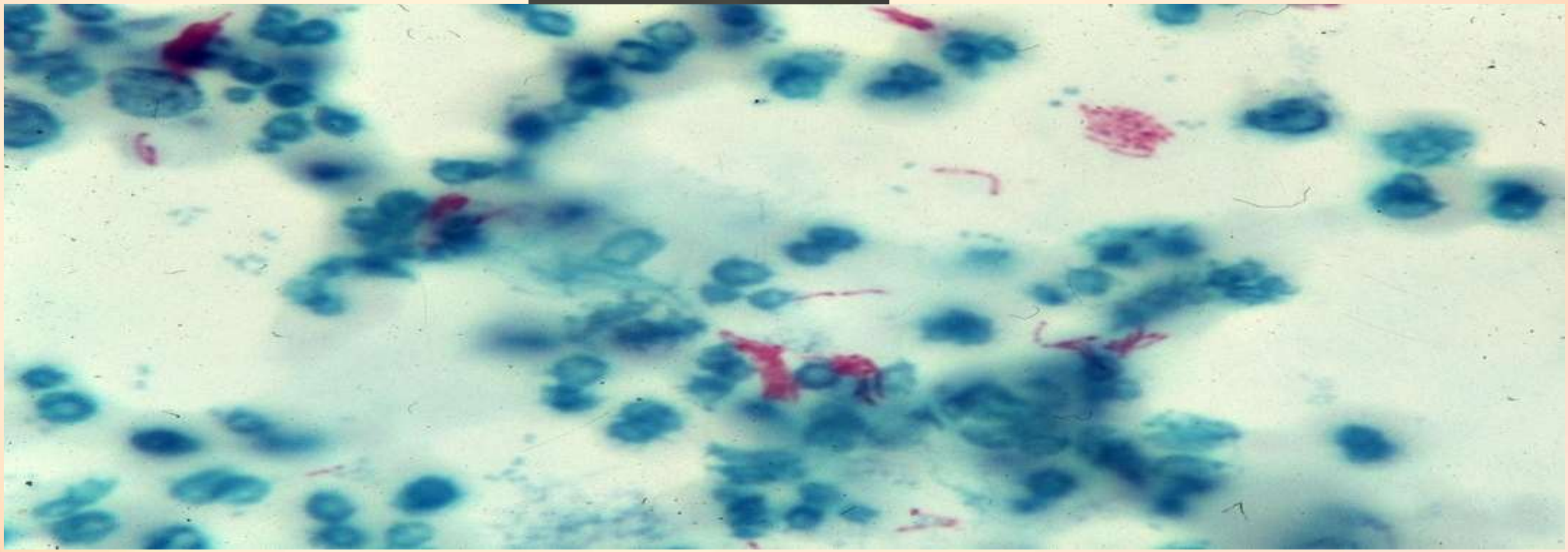
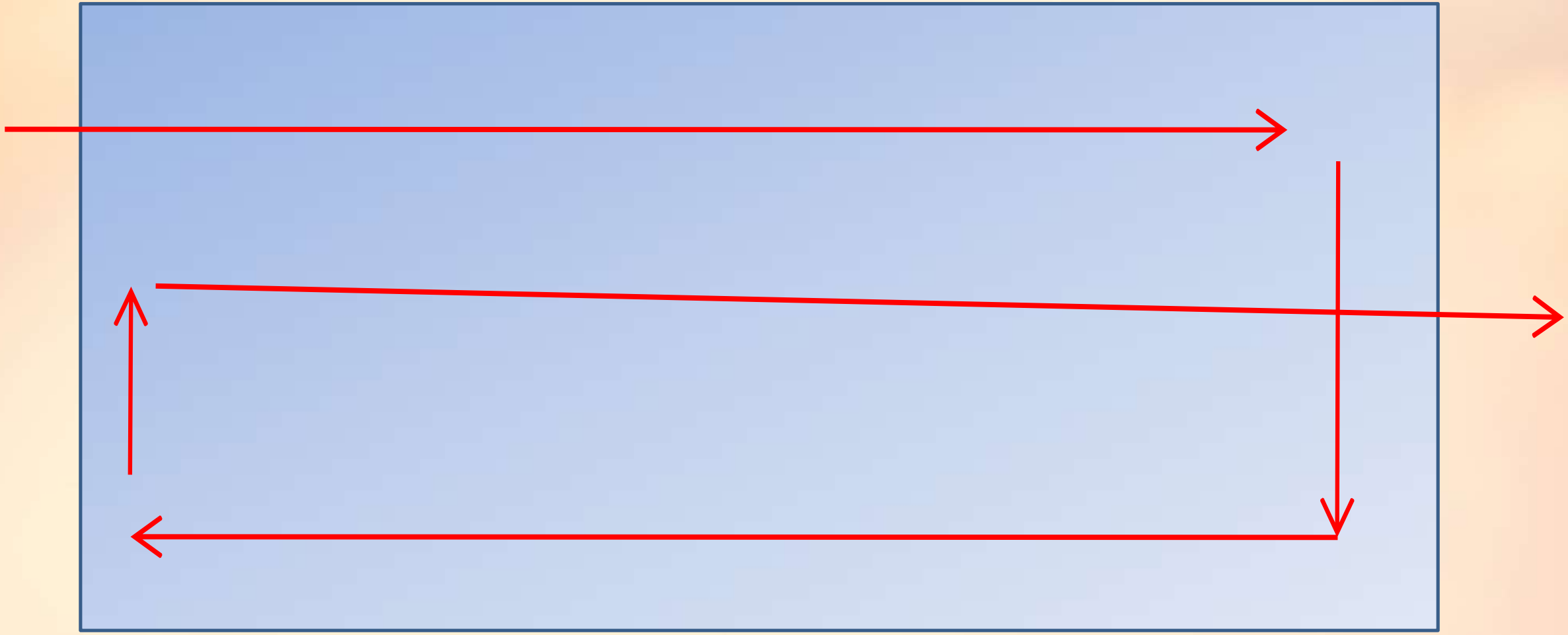
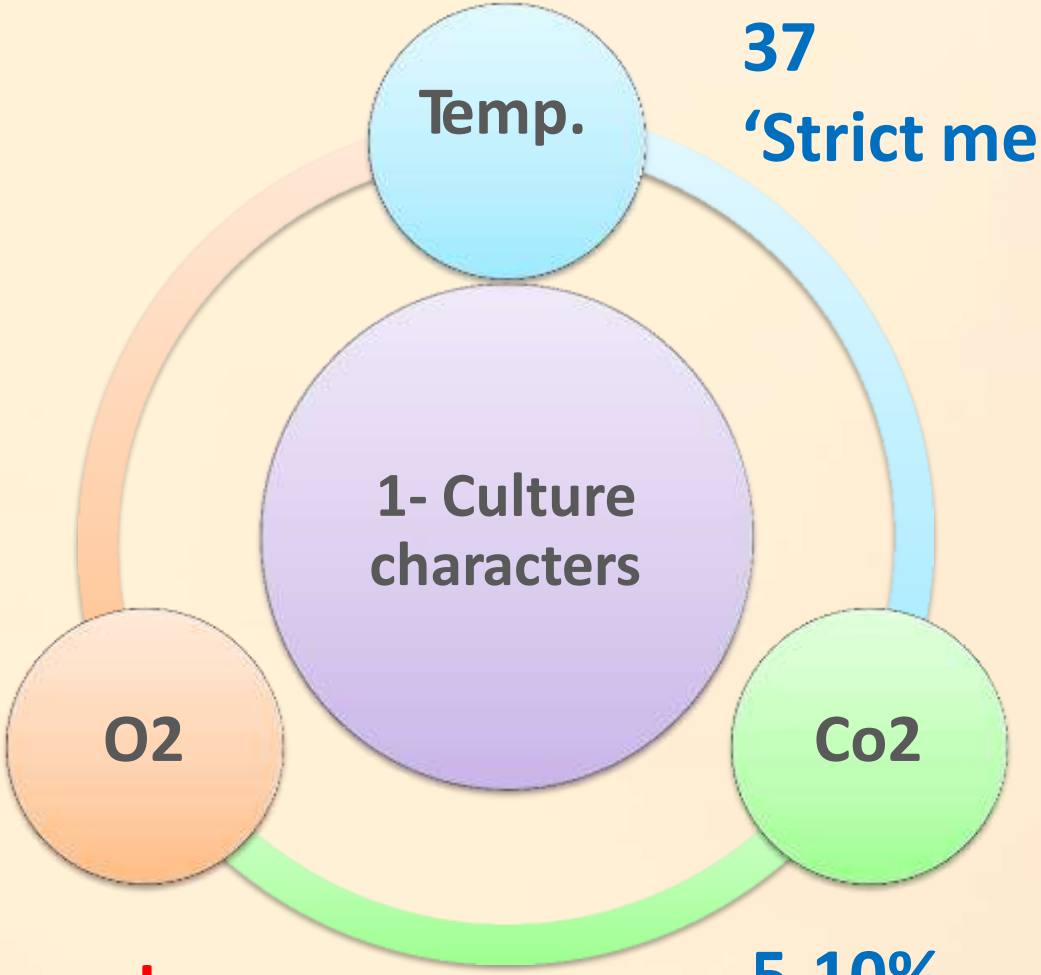




Before saying “Negative” examine well.
& Ask for another sample (3).

fluorochrome stain

3- Culture: (after decontamination; NaOH)



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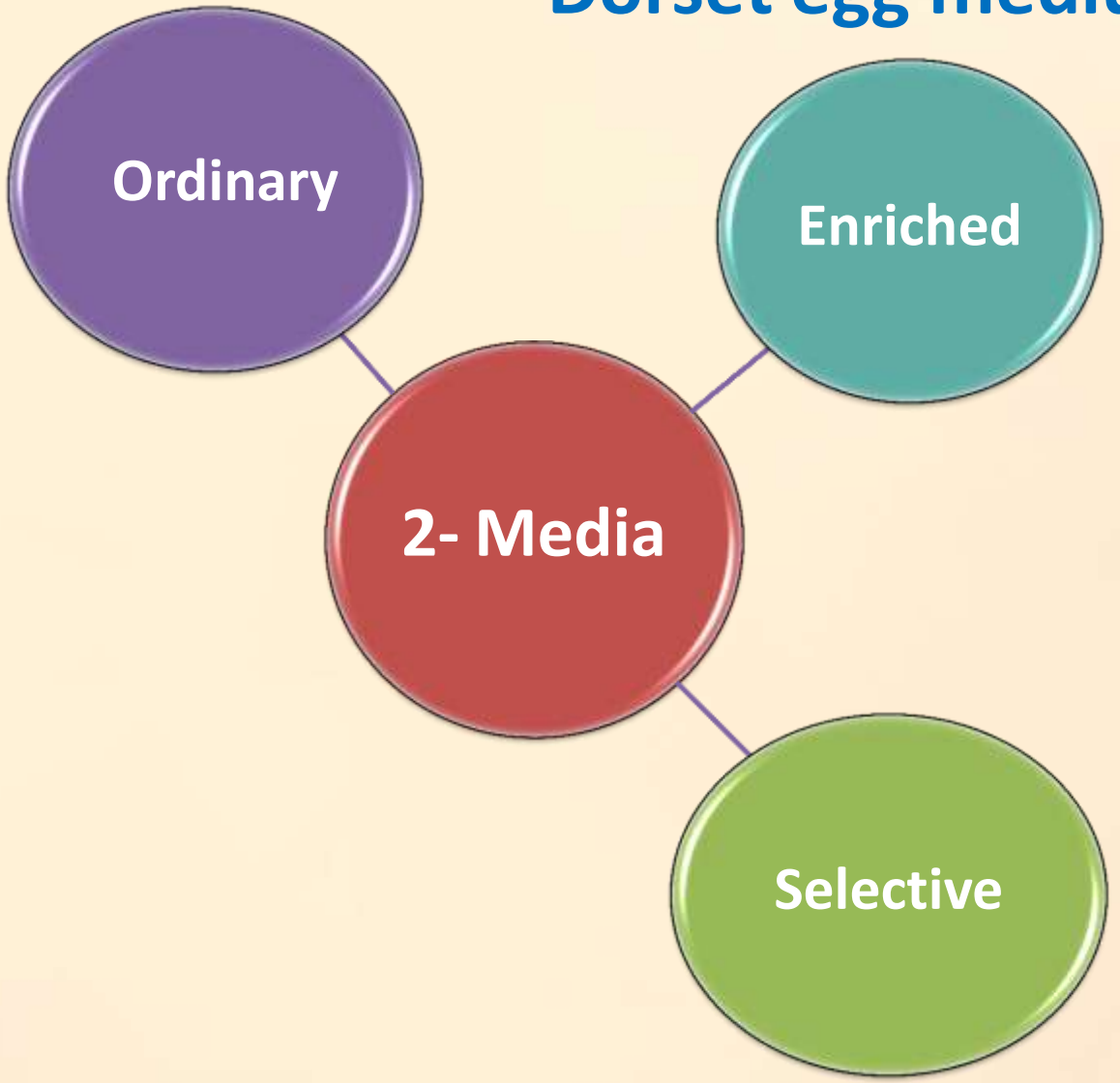
'Strict mesophile'

- Obligate aerobe

- 5-10% enhance growth

Dorset egg medium

No growth



* Very slow grower (4-8 weeks)

- Lowenstein-Jensen medium
- Middlebrook medium ‘agar-based’

Colony Identification

ZN Stained film

Characters

Dry, raised, rough, grayish, confluent “eugenic growth”

4- Animal pathogenicity: Guinea pig

5- Serology:

6- Recent methods for diagnosis:

- DNA probes
- PCR
- Gas-liquid chromatography.
- Quantiferon TB → latent TB diagnosis.

Rapid culture method (BACTEC System):

- More rapid.
- Broth media containing radio-labeled ‘palmitic acid’ as the sole carbon source, antibiotics.
- M.TB. multiplies, → radio-labeled CO_2 is released.
- Detected in 9-16 days .

Tuberculin test:

hypersensitivity skin test

-I.D. injection of (0.1 ml) containing (5 tuberculin units) of **PPD**.

- Read after 48-72 hours.

previously exposed to M.TB → induration > 10 mm

Tuberculin test:

Negative reaction → Never exposed to M.TB

False negative →

- **Impaired CMI response immune**
- **HIV, immunosuppressive drugs, malignancy, malnutrition, late stages of tuberculosis, acute viral infections, Improperly stored PPD.**

False positive →

- **other mycobacteria**
- **Vaccination „BCG".**
- **Operator error**
- **reading errors**

References

Koneman, W., Allen, SD., Janda,WM., Schreckenberger, R. and Winn, W. (1997): Color Atlas and Text Book of DiagnosticMicrobiology, 5th ed. , PP, Philadelphia. .Lippincott – Raven.


THANK YOU