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MINISTRY OF HEALTH

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# Training on LJ culture method

## Module 10: Identification And Confirmation Methods

**DATE:**

**VENUE: SRL, Uganda**

**FACILITATOR:**

# Content outline

- Definitive M. tuberculosis identification

- Cultural tests:

absence of growth on LJ containing PNB

- Biochemical tests:

niacin

nitrate reduction

Catalase

- Immunochromatographic method

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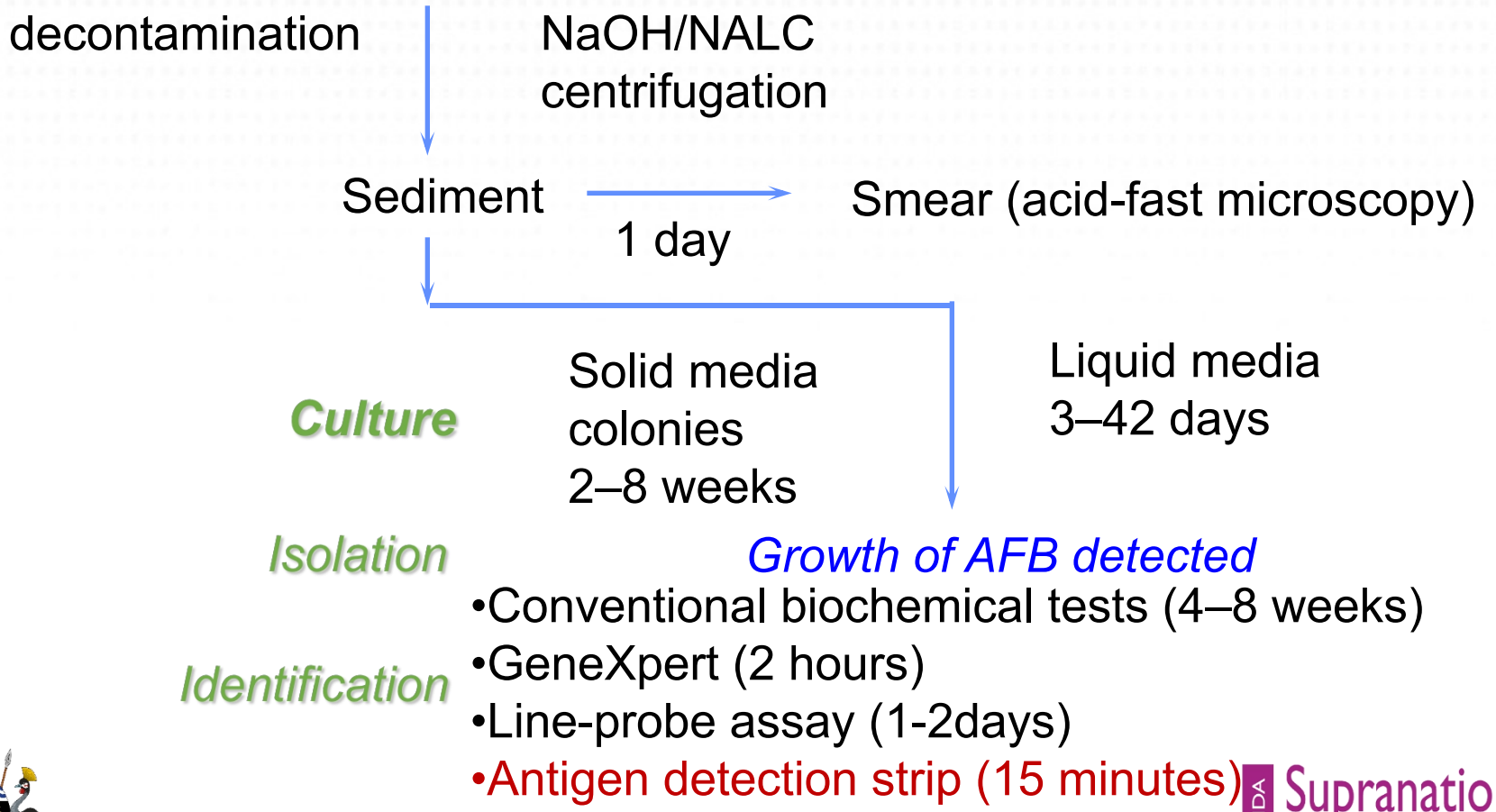
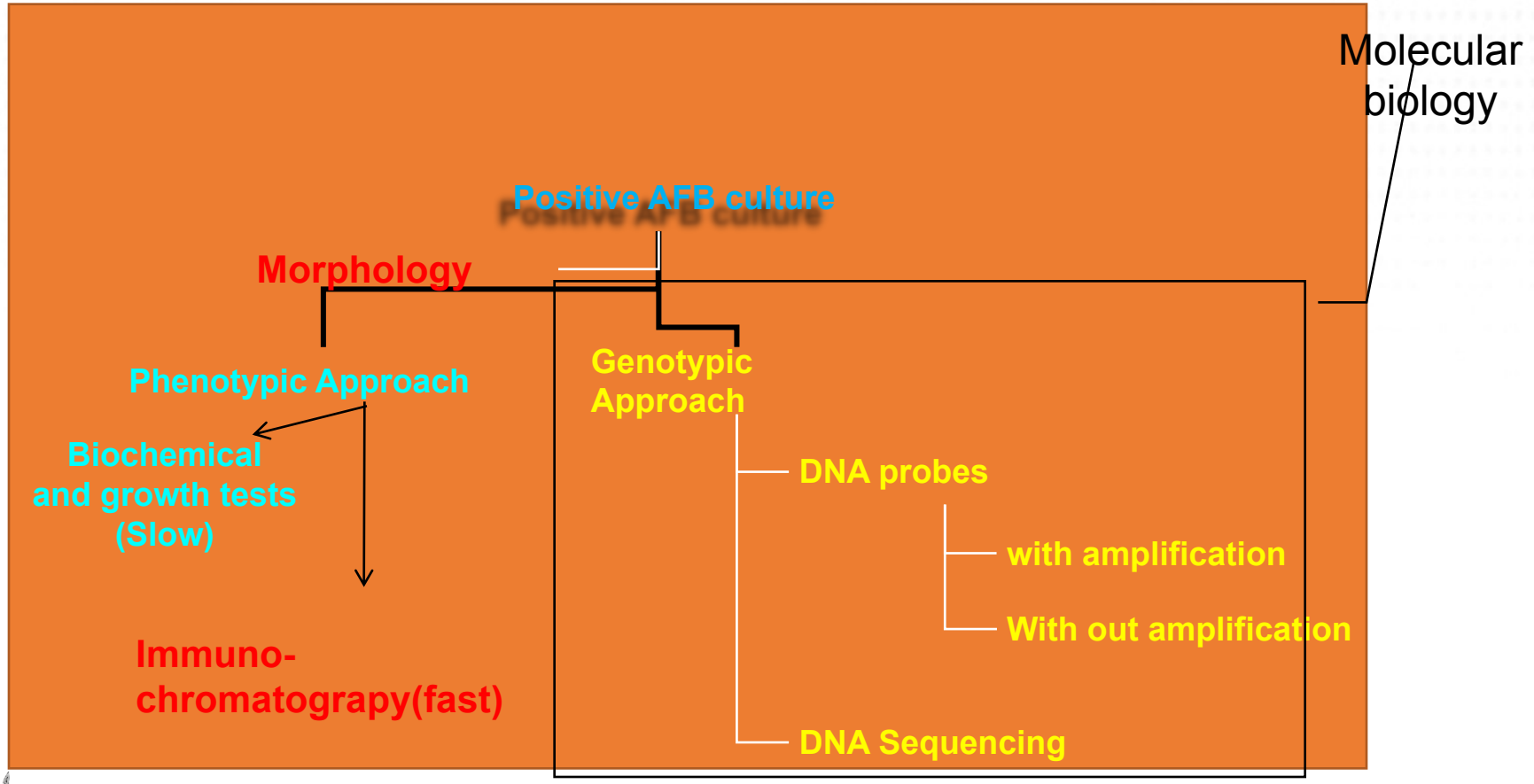
# Isolation and identification of mycobacteria

*Sample testing flow chat*



# *M. tuberculosis* identification

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# Phenotypic approach: biochemical tests

- Advantages:
- Inexpensive
- Will identify members of MTB complex
- Gives reliable results for some MOTT.

- Disadvantages:
- Labour-intensive
- Long turn-around times
- Technical expertise required
- Limited to solid cultures

# Genotypic approach

- **Advantages:**

1. Fast
2. Able to distinguish between members of MTB complex
3. Identifies NTM
4. Fewer biosafety concerns

- **Disadvantages:**

1. Expensive
2. Requires dedicated equipment
3. Requires technical expertise
4. Traditional methods still required for culture and DST

# Phenotypic identification

- Has to consider all the characteristics, including:
- Morphology (colonies/bacilli)
- Culture based
- Biochemical tests

# Culture based Technique:
# Growth on medium containing p-nitrobenzoic acid (PNB)

This is based on Selective inhibition for growth of MTB allowing only NTMs to grow

**Procedure**

1 LJ slant with PNB at 37 °C

1 LJ slant without PNB at 37 °C

Examine at 28 days

## Interpretation

- Abundant growth on both slopes: mycobacterial strain other than tubercle bacilli.
- Abundant growth on control tube and little or no growth on PNB medium: MTB complex strain.
- No growth on either slope: non-interpretable test, to be repeated.

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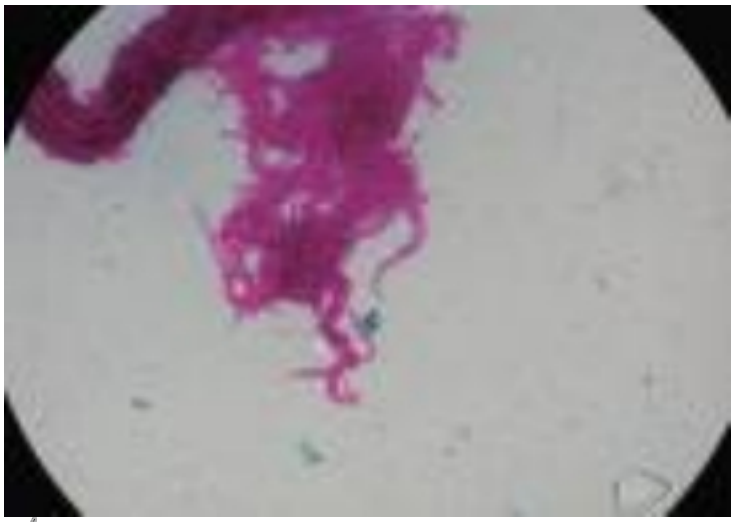
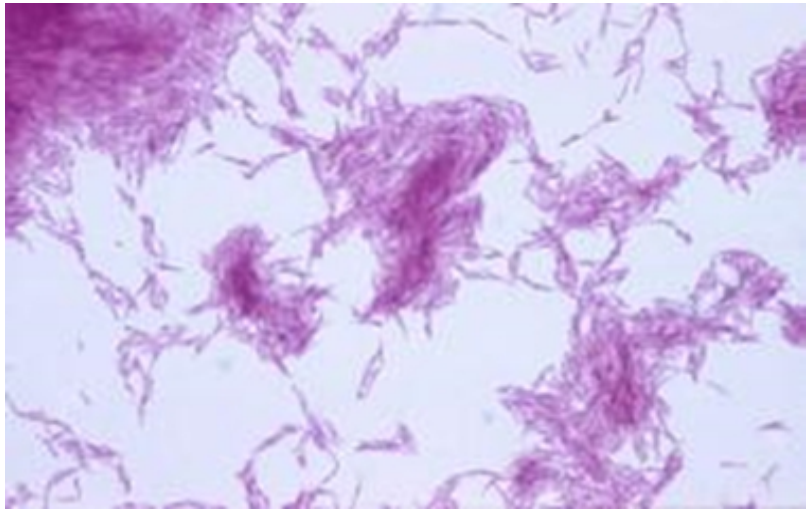
# Microscopy Identification

**This involves staining with ZN and Examining the morphology of the Bacilli**

**Cord-like Morphology for MTB**



**Striped" Morphology for NTM**



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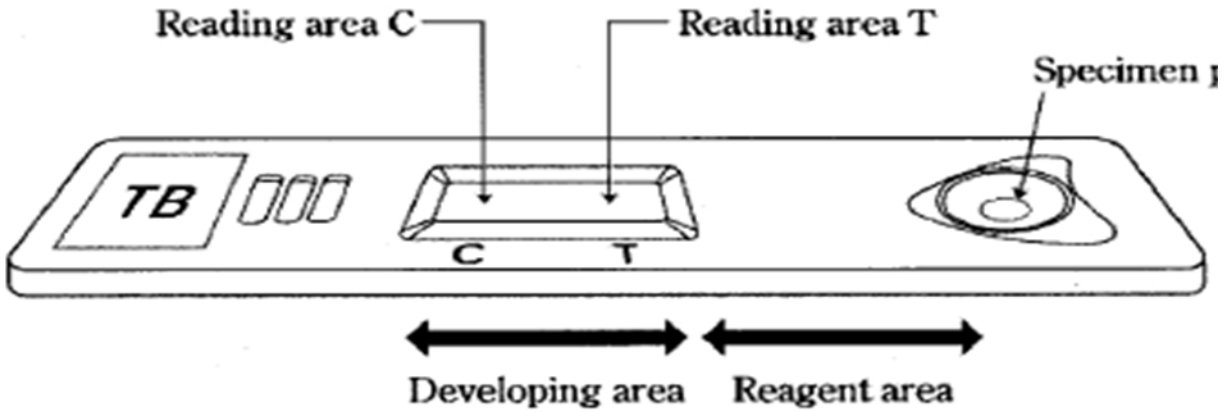
# Immuno-chromatographic assay: Antigen Test

- Immunochromatographic assay: Simple lateral flow speciation test, Monoclonal antibody technology
- Detects MPB64 antigen– predominant protein Ag, secreted specifically by members of the MTBC
- First monoclonal antibody labelled by colloidal gold particles reacts with MPB64 antigen in sample to form antigen-antibody complex
- Complex is then captured by a second monoclonal antibody fixed in the middle of the test zone

# Antigen detection using Immunochromatographic Assay

**Procedure:**

- A 100 µl suspension of growth from liquid or solid media is added to the specimen well.
- The test is read after 15 minutes
- All procedures above should be done in a certified BSC

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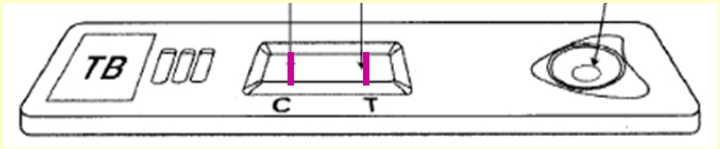
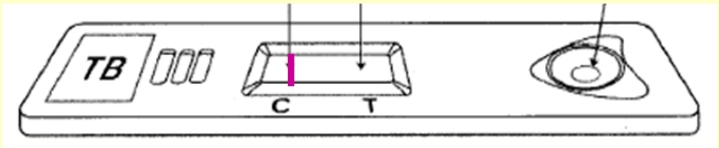
# Antigen detection using Capilia TB

## Interpretation:

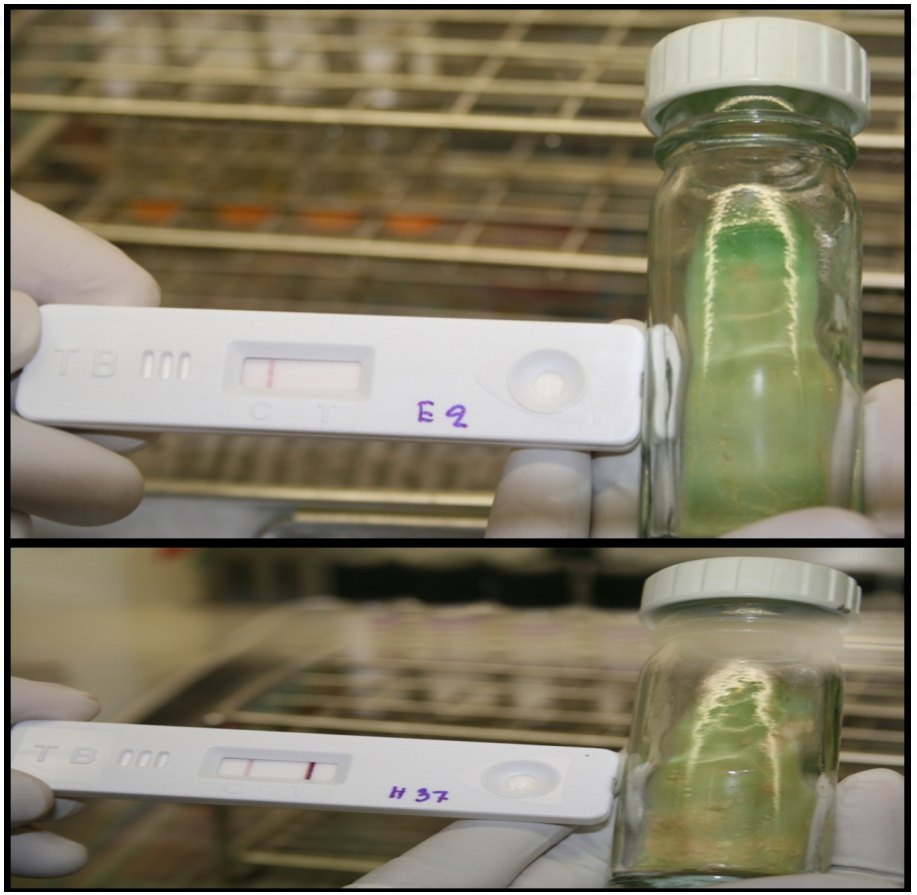
MTB complex – two purple-reddish bands at test and control sites




NTM or other bacterial species – one purple-reddish band only at control site




Note: The test cannot be interpreted if there is no band at the control site

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# Advantages of Immunochromatographic Assay

- Performed directly from the AFB positive liquid and solid culture
- Detects MTBC in pure, mixed and contaminated liquid cultures
- Does not require additional equipment or reagents
- Results immediately available

# Limitations of Immunochromatographic Assay

- Does not differentiate between members of the MTBC

- Requires culture (no direct inoculation from clinical specimen)
- Some substrains of M. bovis BCG are interpreted as negative (organism lacks MPB64)

- Strains of microbes, such as S. aureus, that produce protein A may produce a false positive result

- False Negative if the MPB64 concentration is below the detectable limit or if mutations have occurred in the MPB64 gene of MTBC

# Exercise (3 minutes)

True" or "false"

1. M. tuberculosis can be identified only by the morphology of the colonies.

2. Expired reagents affect the results of identification tests.

3. The Immunochromatographic test allows fast identification of TB complex from positive cultures.

# Biochemical tests

These methods are currently less applied, they include

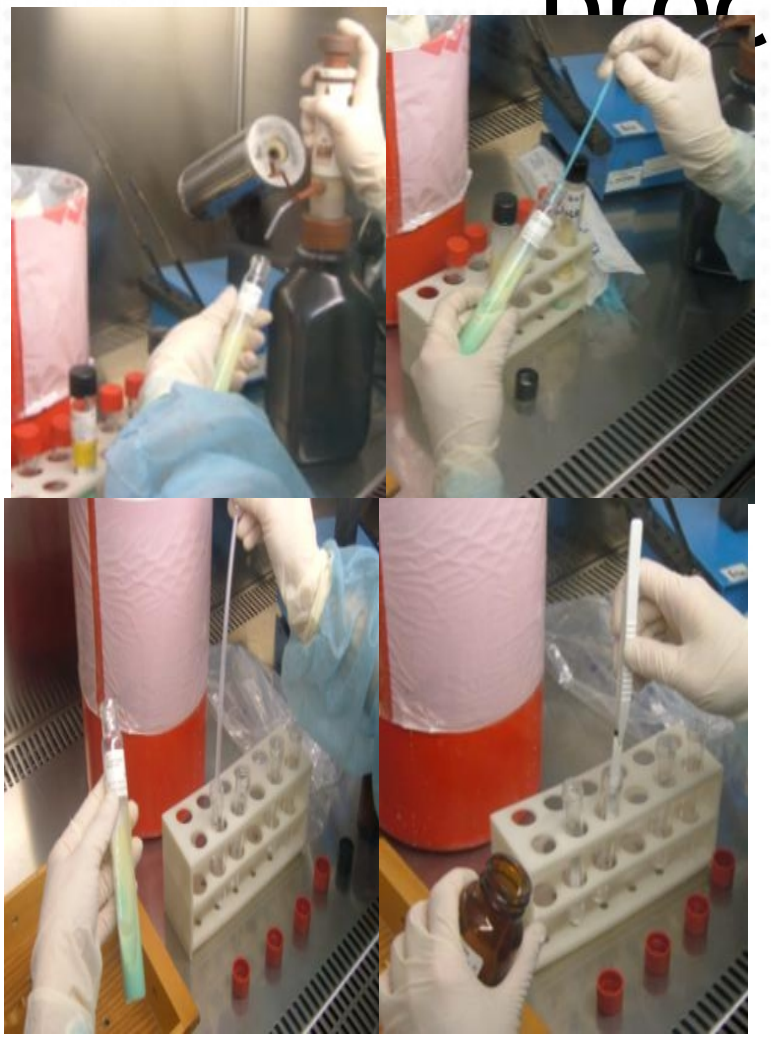
- Niacin production
- Nitrate reduction
- Catalase negative at 68 °C

# Niacin test-Principal

This is a Metabolite accumulating in medium during M.tb growth:

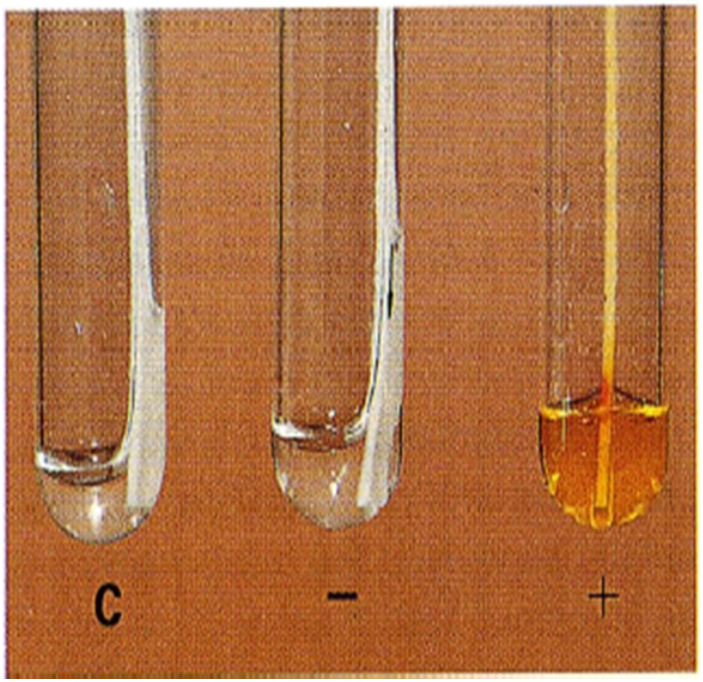
- Differentiates M. tuberculosis from other species.
- Rarely positive in mycobacteria other than M.tb.
- The test must be carried out on pure cultures otherwise it will yield false results
- Pure cultures of acid-fast bacilli grown on solid medium, age 21–28 days, with more than 50 colonies

# Niacin test – procedure



Commercially available test-strips used

**Interpretation**



# Nitrate reductase test

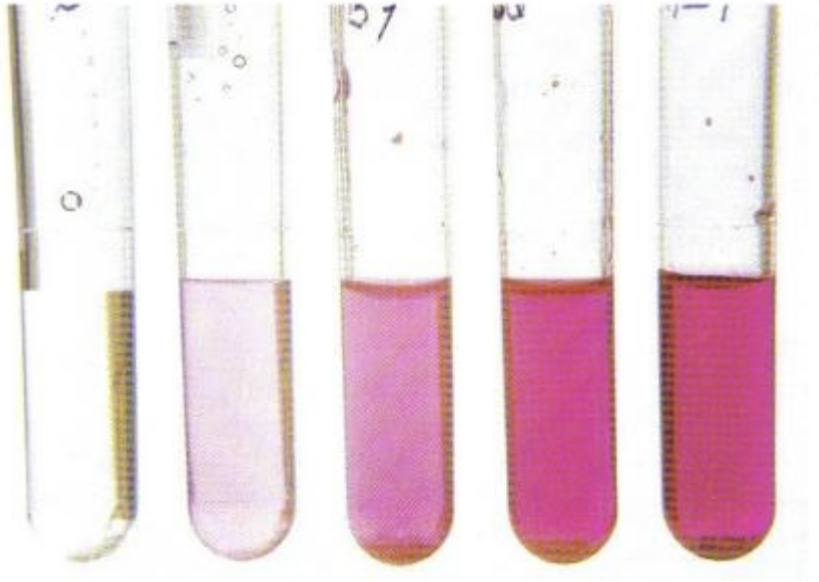
- *M. tuberculosis* reduces nitrates to nitrites.
- Test takes about 4 hours to report results.
- Cultures tested:
  - 4 weeks old
  - abundant growth.

# Nitrate reductase test – results and interpretation

- *Negative*: no colour.
- *Positive*: red colour, varying from pink to very deep crimson:

  *faint pink* = +/-(inconclusi
  clear pink = 1+
  deep pink = 2+
  red = 3+
  deep red = 4+
  purplish red = 5+

  POS



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# Catalase test: principle

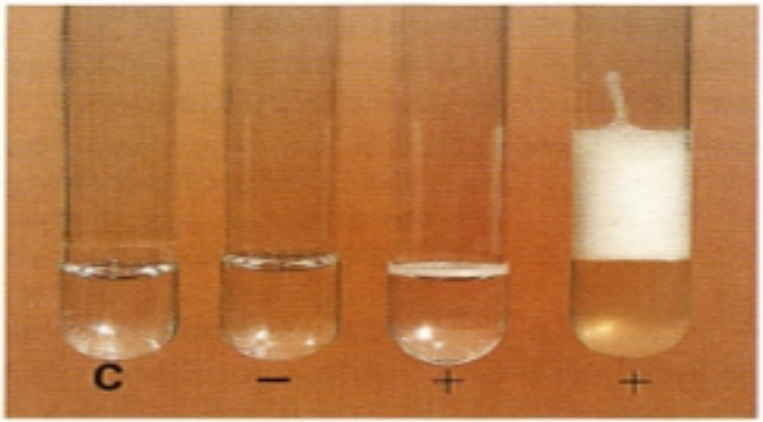
- Catalase converts $H_2O_2$ to water and $O_2$ bubbles .

- All mycobacteria produce catalase except for rare isoniazid-resistant mutants of M. tuberculosis and M. *bovis*.
- catalase enzyme present in most of the mycobacterial species is heat-stable at pH 7.0 except in tubercle bacilli

# Catalase test: Procedure and interpretation

- 1 tube: incubation at 68 °C for 20 minutes- No bubbles hence no MTBC

- 1 tube: incubation at room temperature for 20 minutes- bubbles hence Positive for MTBC




COLOR PLATE 8. Heat stable catalase test.

# Assessment

1. State the principal of the Immunochromatographic assay used in MTB identification.
2. List three advantages and two limitations of using Immuno-chromatographic assay test method

# Summary

## Characteristics of M. tuberculosis AFB

- Slow growth rate
- Growth temperature 35–37 °C only
- Typical morphology
- No pigmentation

## Additional

No growth on LJ medium containing p-nitrobenzoic acid

Niacin test: positive

Catalase test: negative at 68 °C

Nitrate reductase test: positive

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