MODULE 6
Smear Preparation and Staining

Learning Objectives

At the end of the module, participants will be able to

- **Safely prepare sputum smears**
- **Prepare good quality sputum smears**
- **Identify problems with smear preparation**
- **Perform the Ziehl-Neelsen (ZN) method on sputum smears**
- **Trouble shoot problems with the ZN method**

Content Overview

- Labeling of slides
- Selecting the best portion of the sample for smear preparation.
- Techniques for preparing smears
- Principles of the Ziehl-Neelsen method
- The Ziehl-Neelsen staining procedure

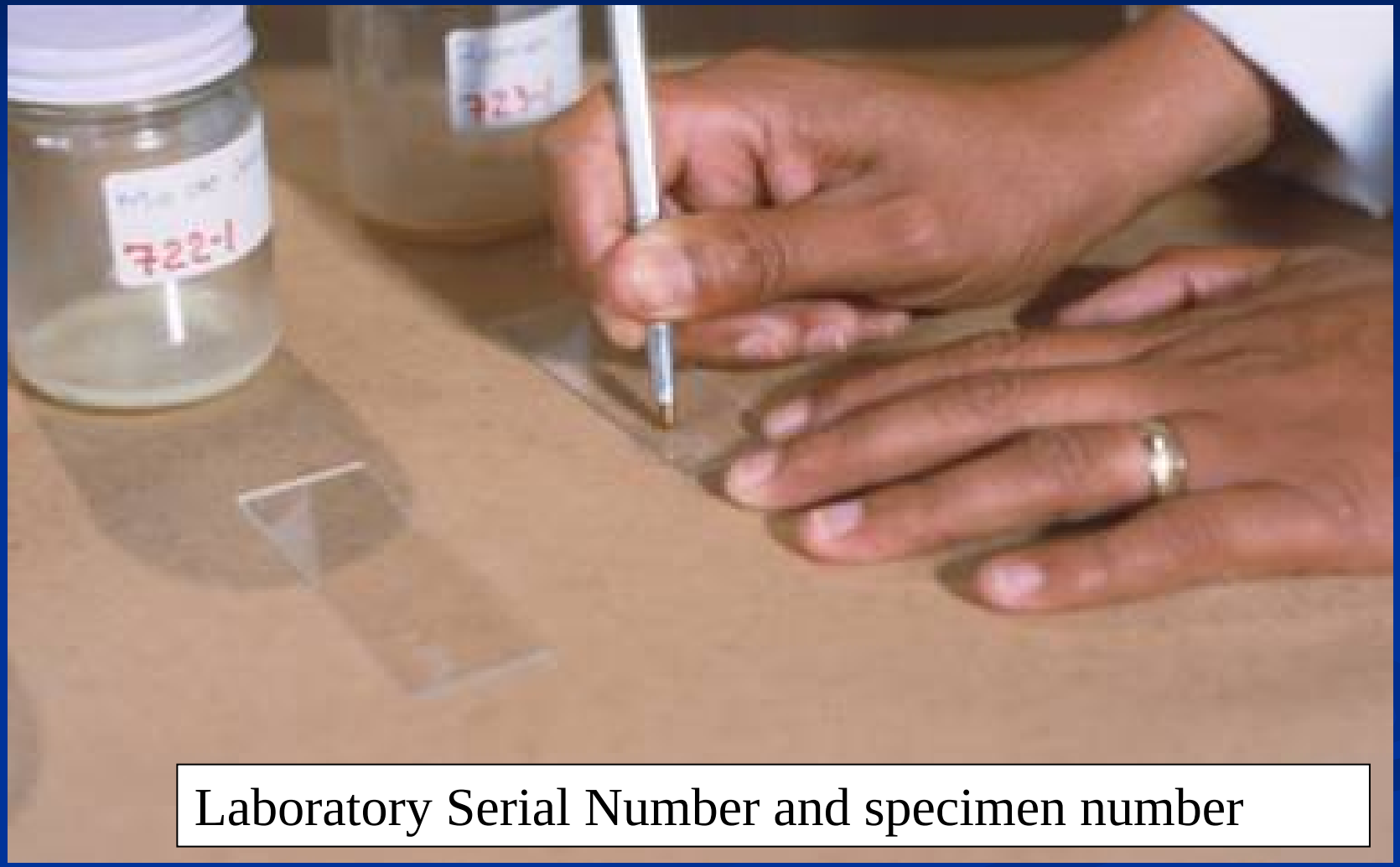
Overview of Smear Preparation

1. Label each slide
2. Make smear
3. Air dry
4. Heat fix smear

Labeling Frosted Slides with Pencil

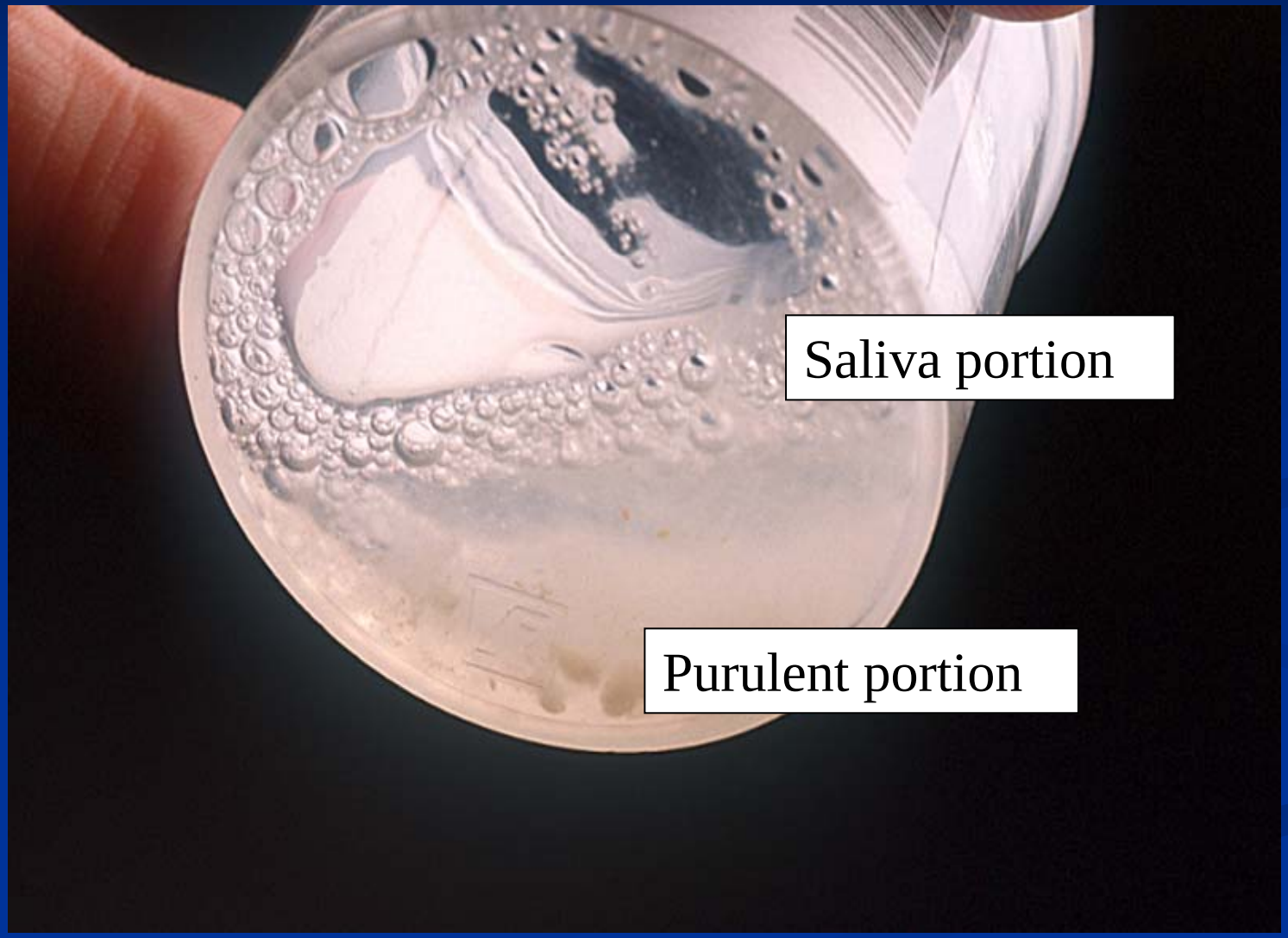
Laboratory Serial Number and specimen number

Labeling Non-frosted Slides with Diamond Pencil

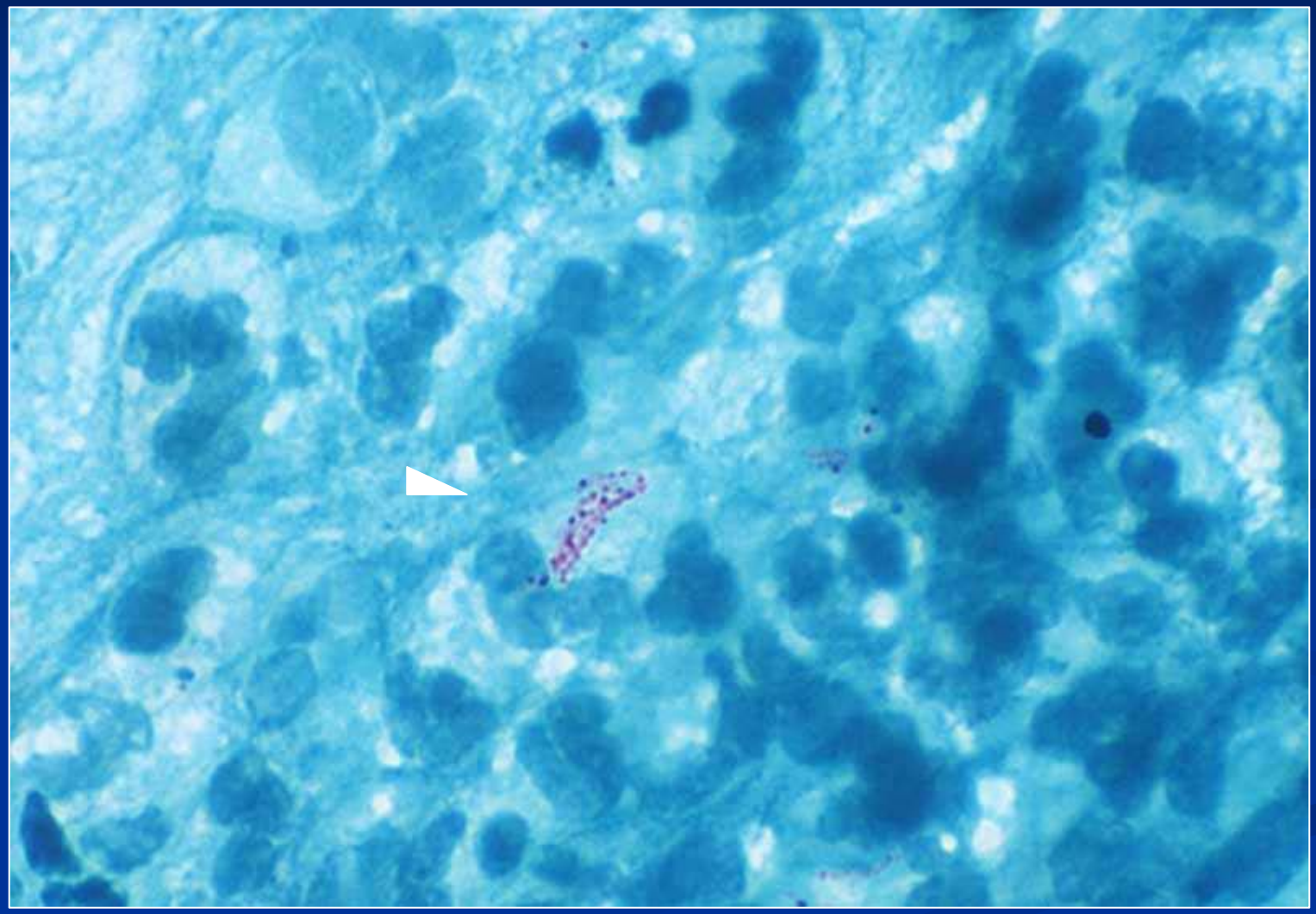


Laboratory Serial Number and specimen number

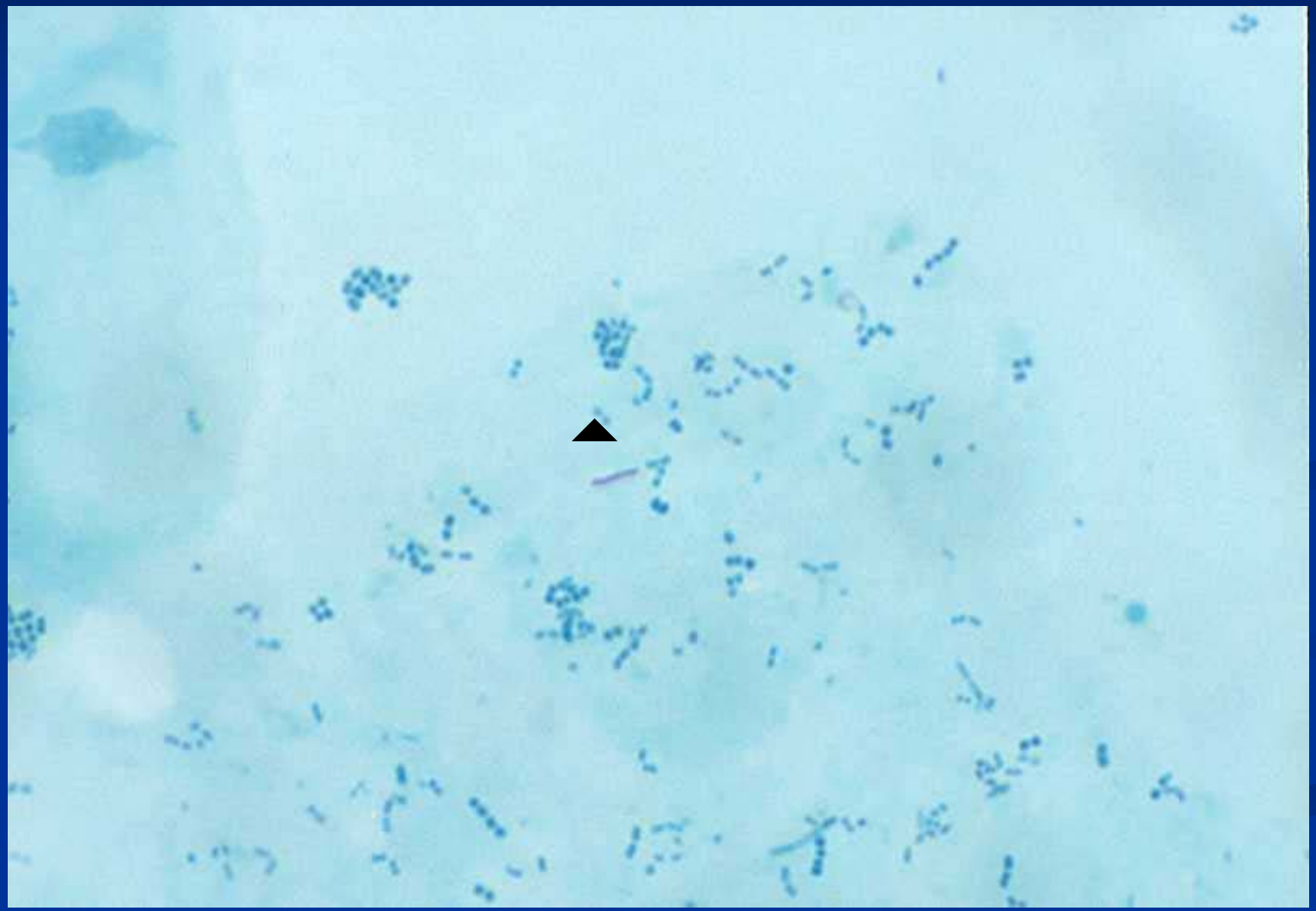
Specimen Appearance



Purulent Portion



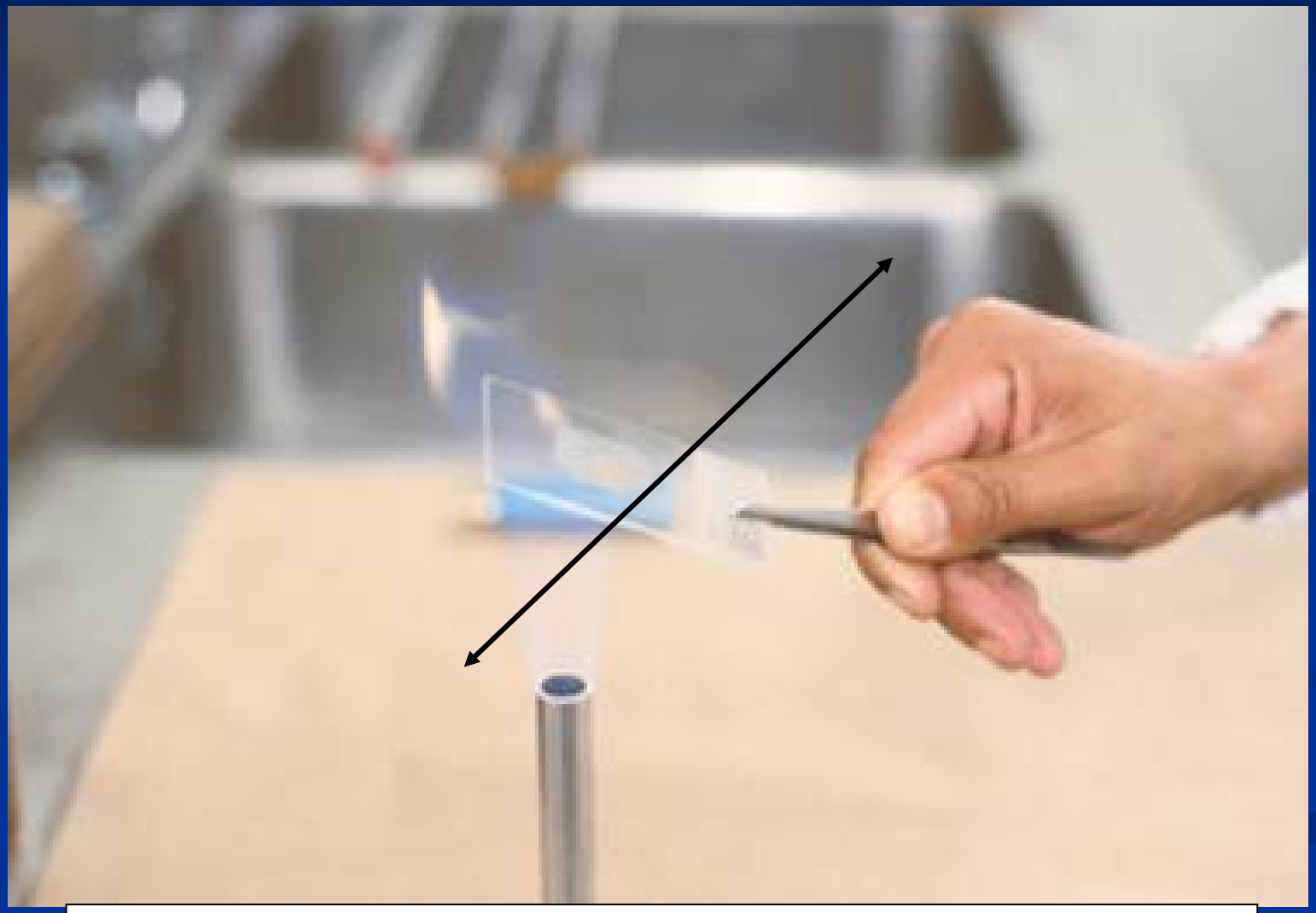
Saliva Portion



Consistent Quality of Smears

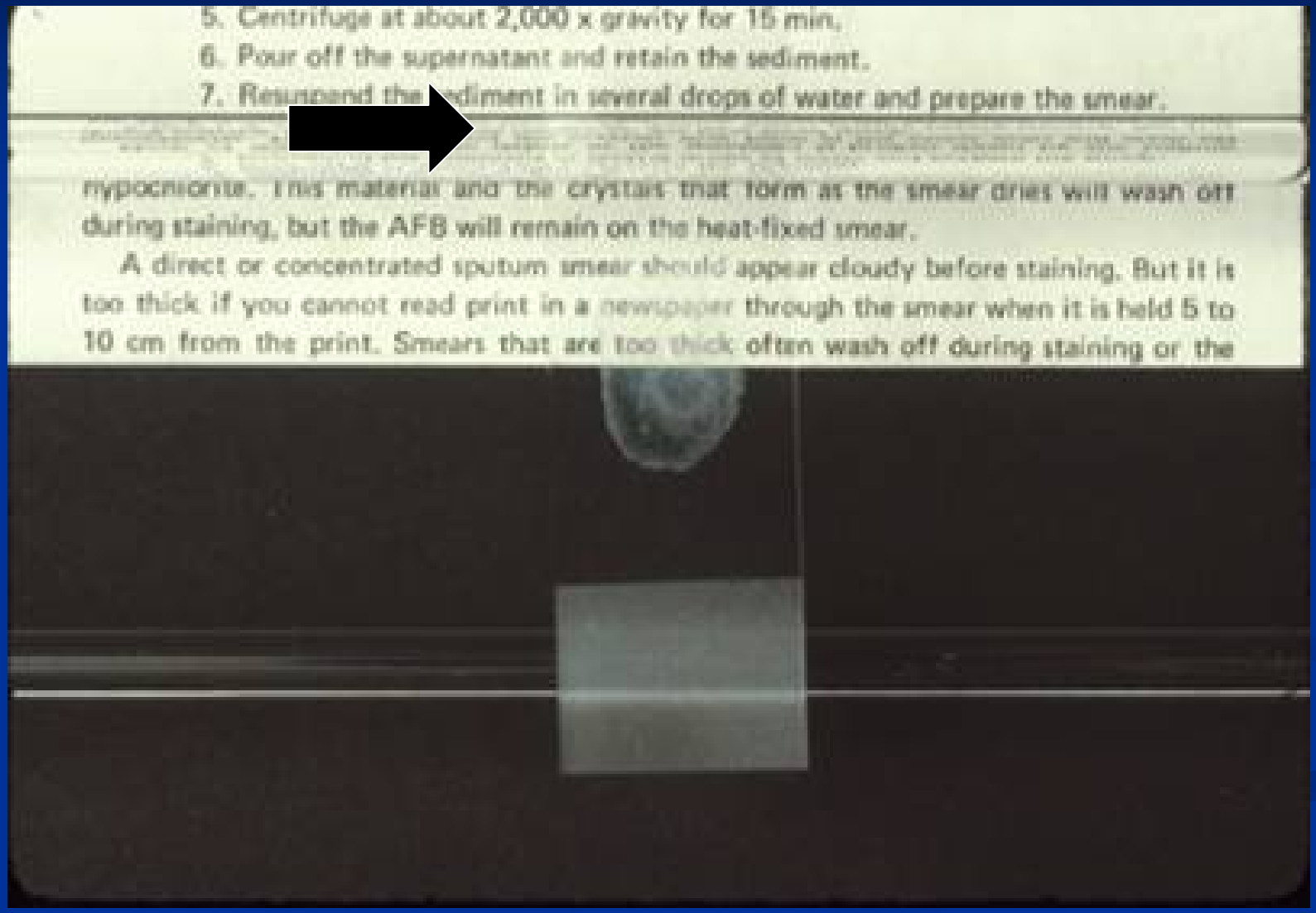
Making Smear with Loop

Heat Fixing a Smear



Pass the smear through the flame 2–3 times

Proper Thickness of Smear



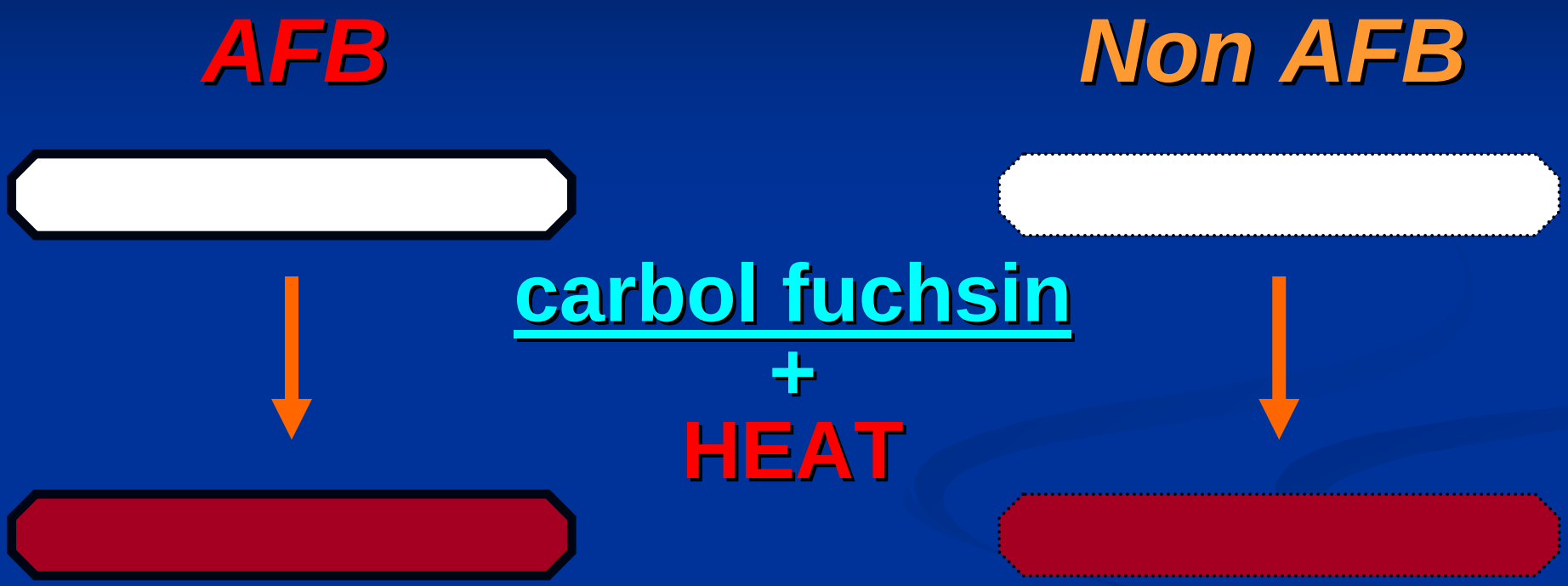
Ziehl-Neelsen Staining Procedure

1. Primary Stain (carbol fuchsin)
2. Decoloriser
3. Counterstain (methylene blue)

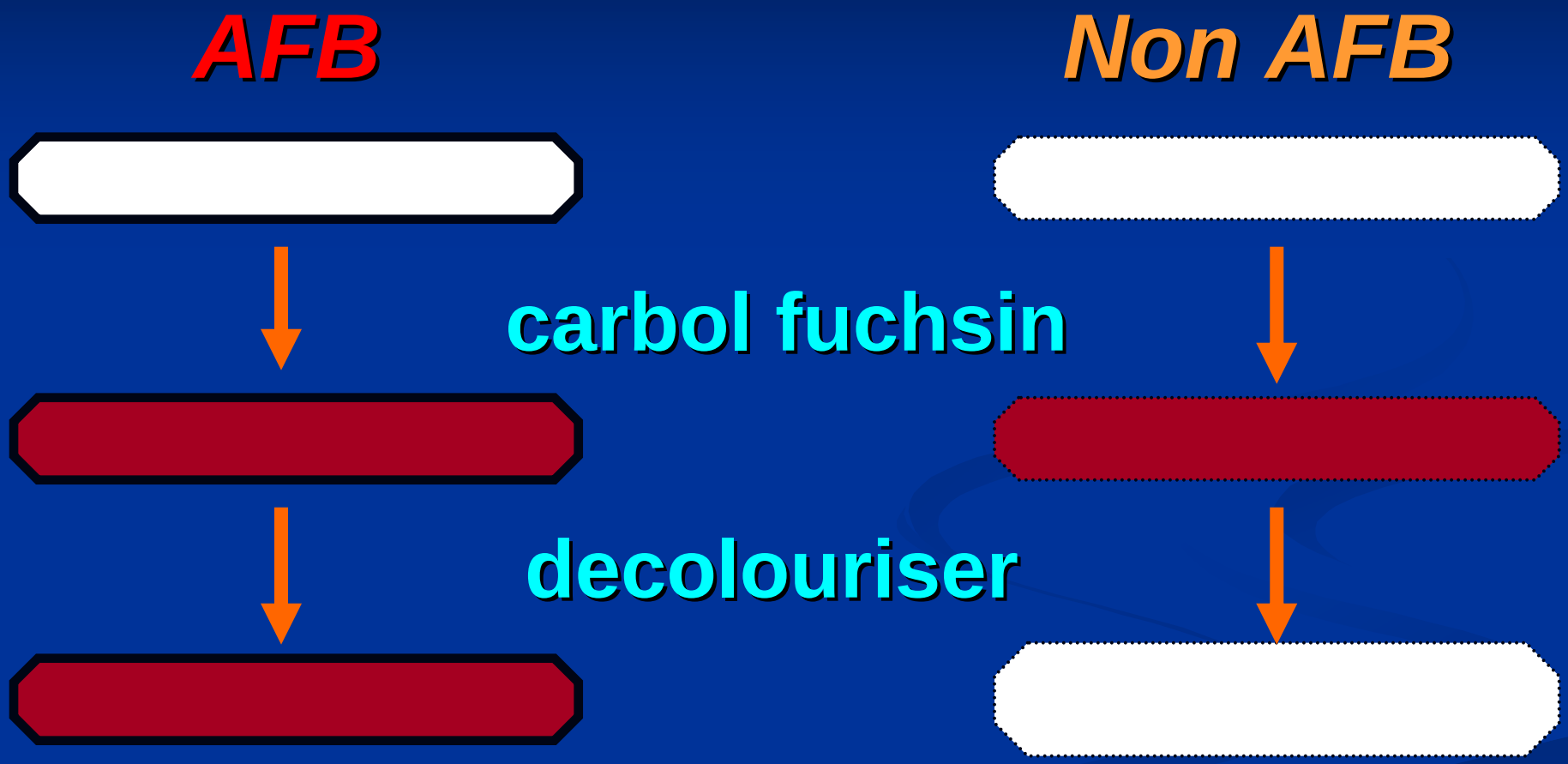
Acid-Fast Principles

- Primary stain penetrates cell wall
- Intense decolorization does not release primary stain from the cell wall of AFB
- Color of AFB-based on primary stain
- Counterstain provides contrasting background

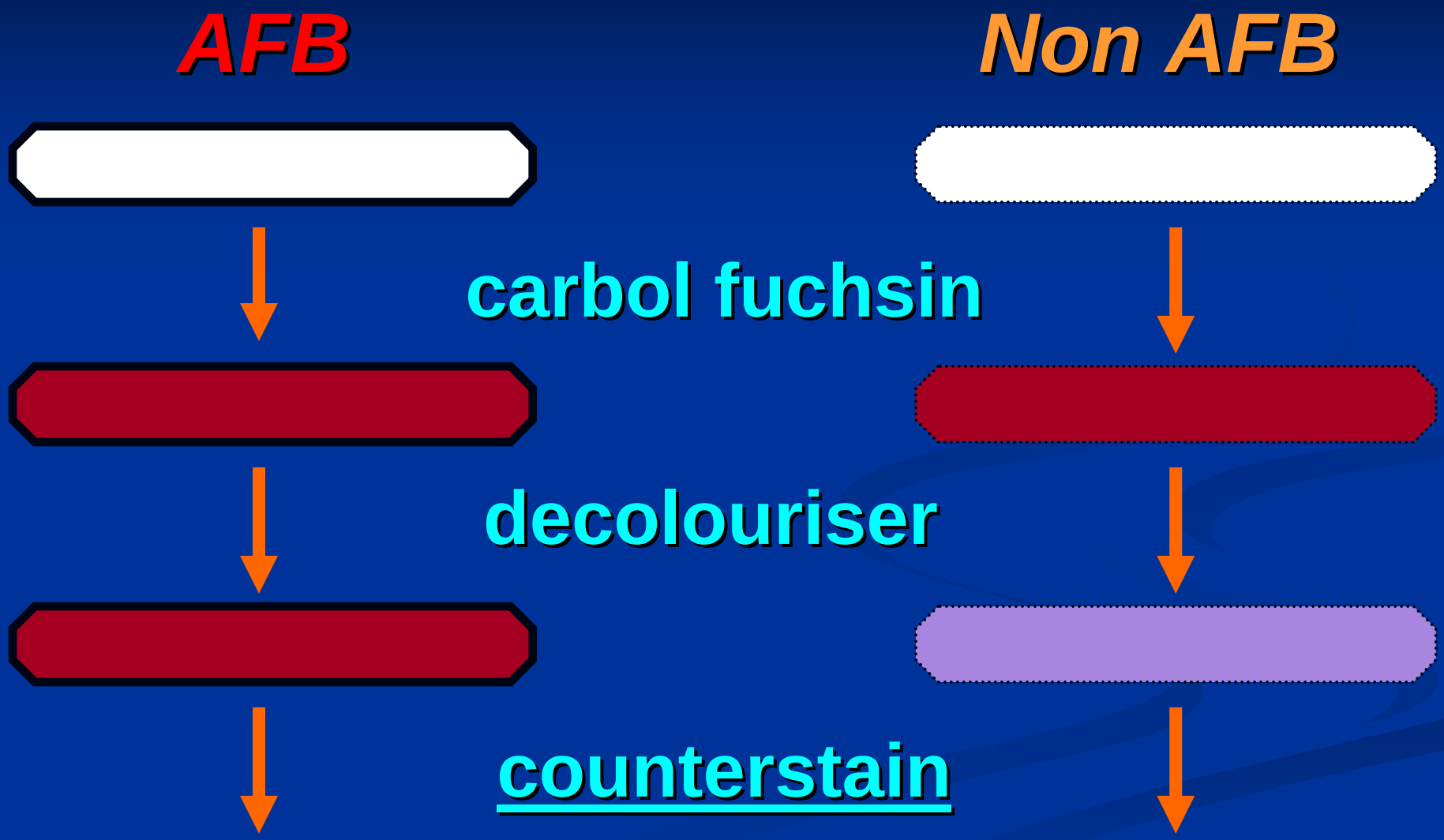
Principle of ZN Stain - 1



Principle of ZN Stain - 2



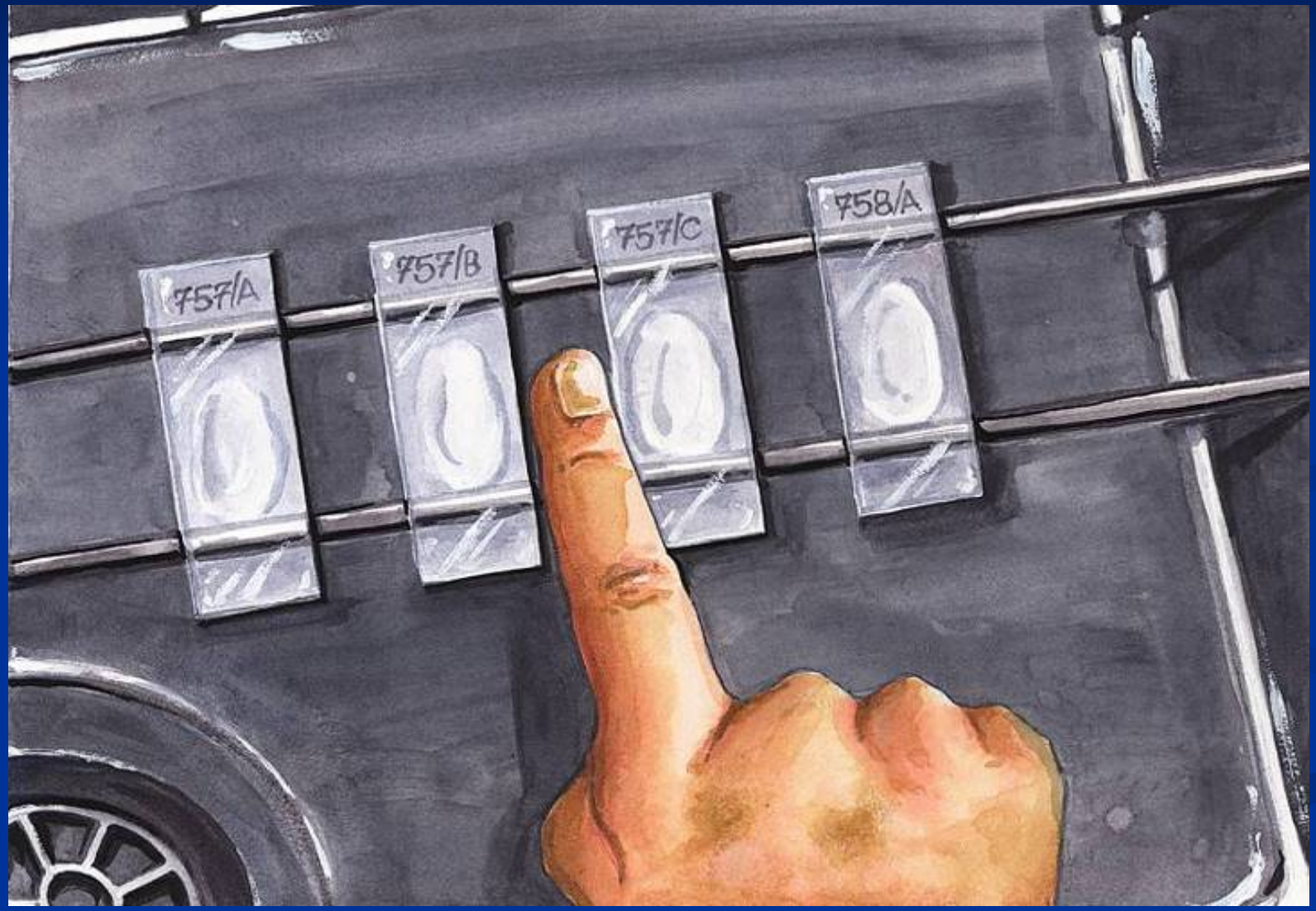
Principle of ZN Stain - 3



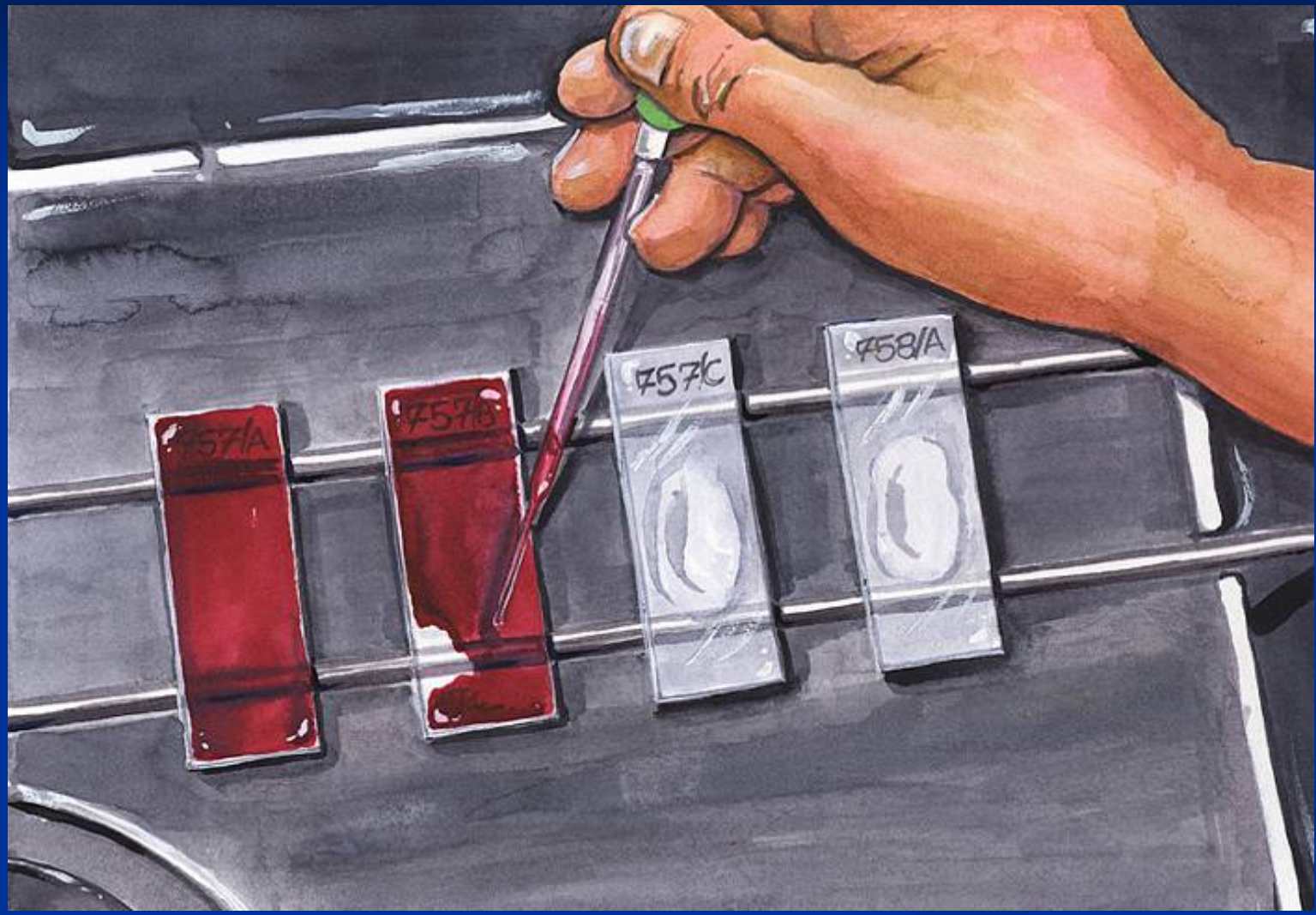
Overview of Staining Procedure

1. Arrange slides in serial order smear side up
2. Keep a finger-thickness between smears
3. Flood with carbol fuchsin
4. Heat to steaming once only
5. Leave for 10 minutes
6. Rinse with water, drain
7. Apply decolorizing solution 3 min.
8. Rinse, drain
9. Apply Methylene blue counterstain, l min.
10. Rinse, drain
11. Air dry

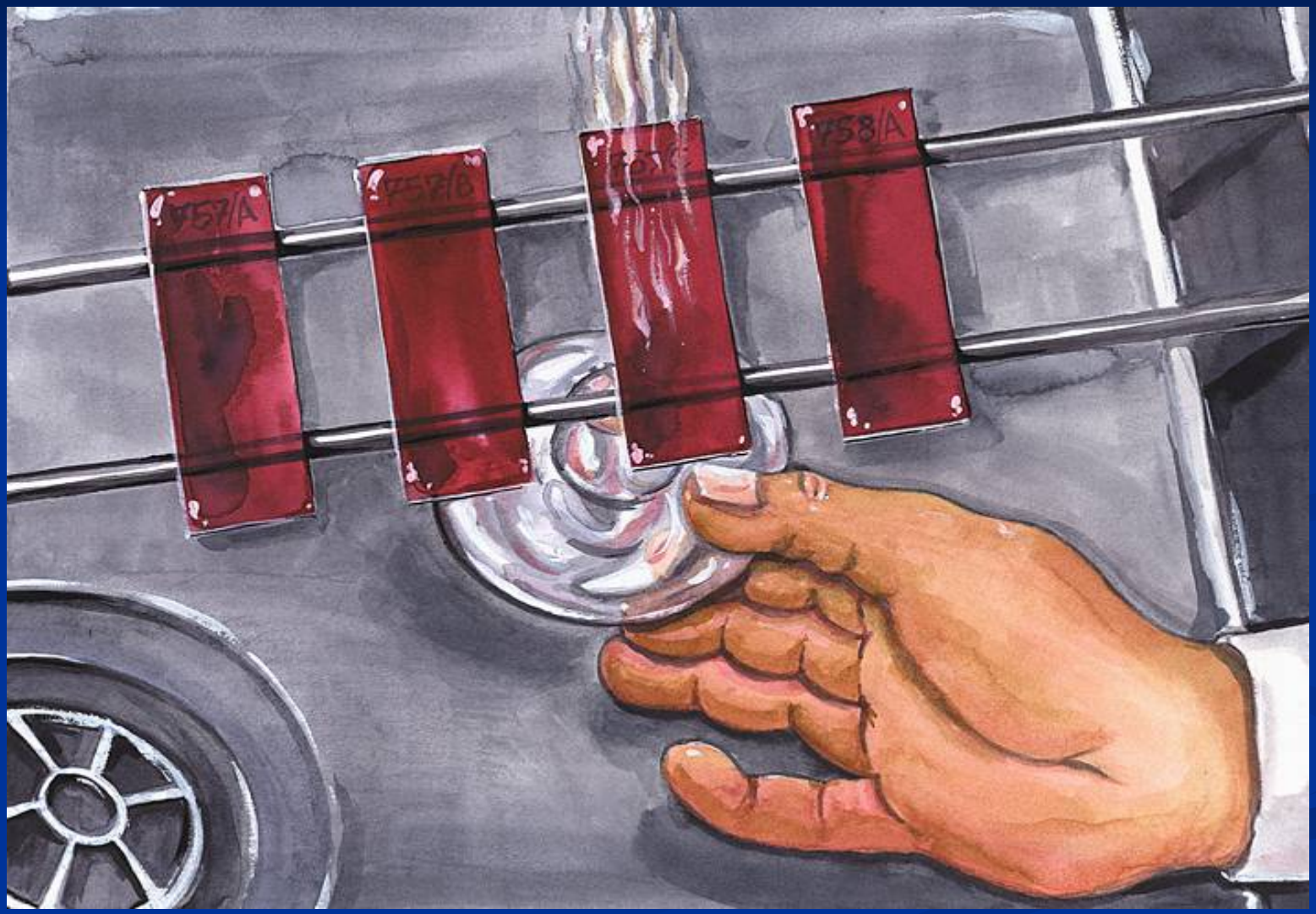
Setting up Slides on Staining Rack



Carbol Fuchsin



Heating Carbol Fuchsin to Steaming



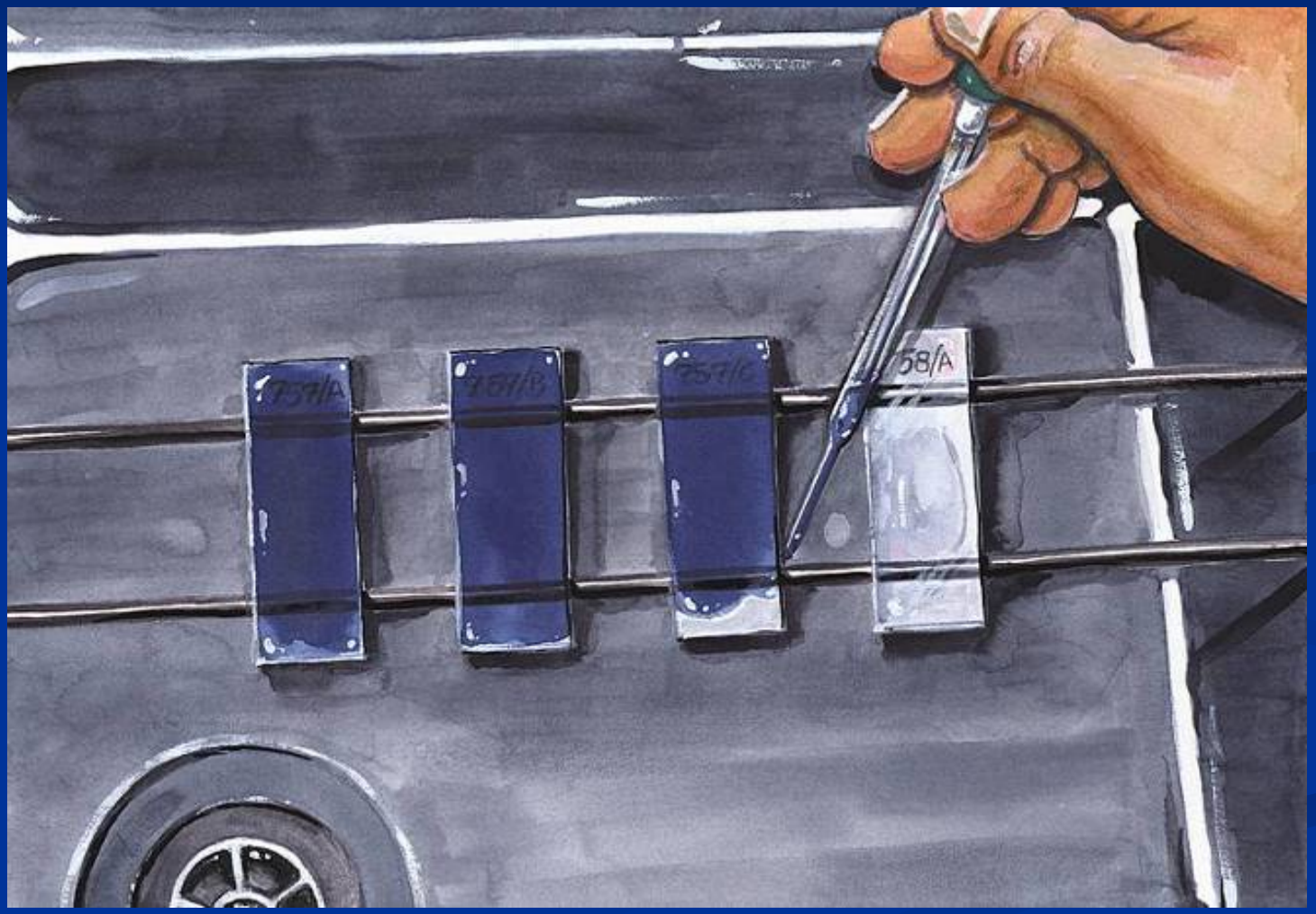
Rinsing Slides

Don’t Splash Adjacent Slides

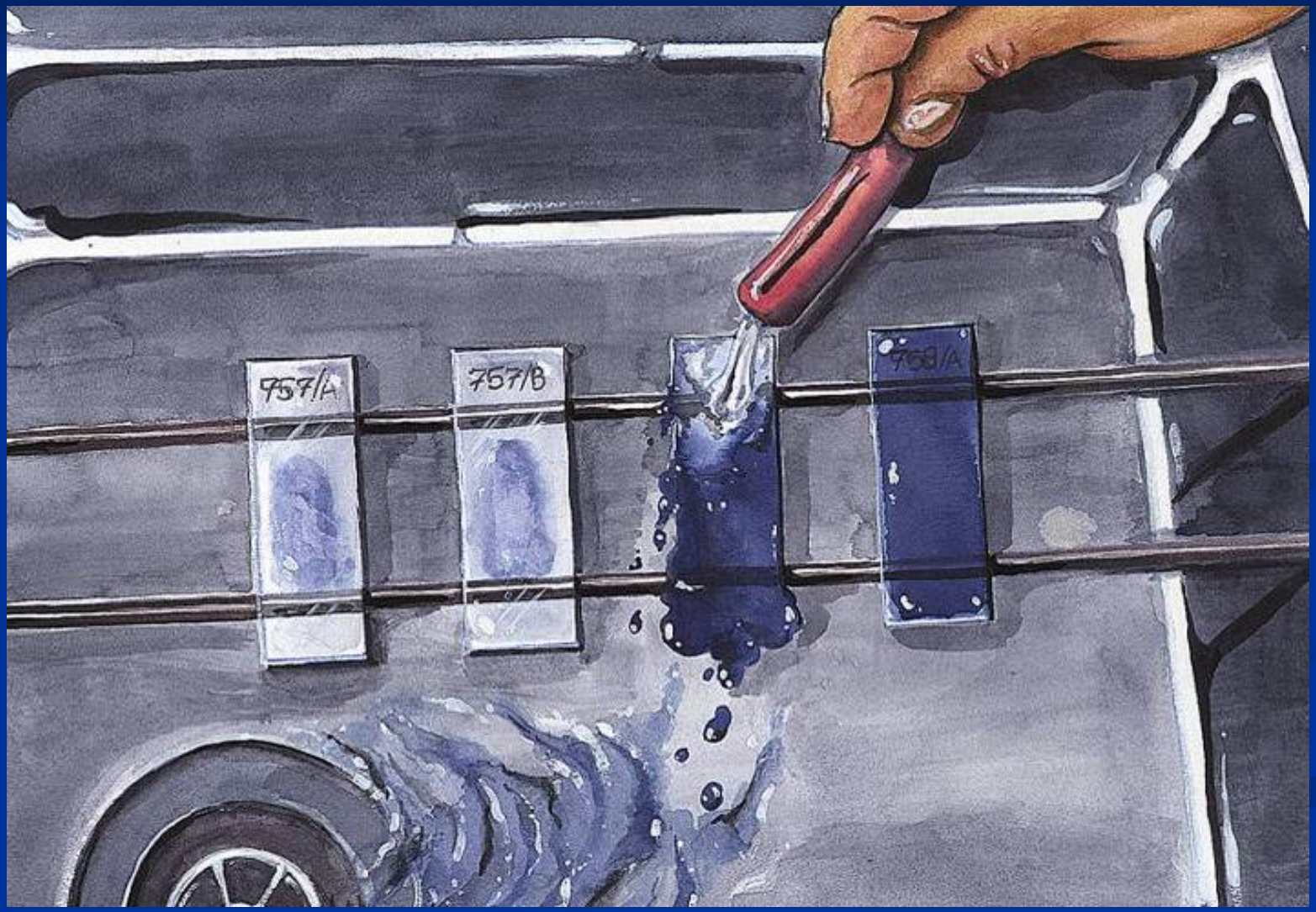
Decolorisation Step

Rinsing Slides

Counterstaining



Rinsing off Counterstaining



Drying the Stained Slides

Quality Control of Routine Staining

- All labs performing AFB-smear microscopy
- Ideally daily but at least weekly
- Positive control smear (1+)
  - in routine series for staining
  - check for the number and color of AFB
- Record results in the laboratory register

Summary

- What labelling information is needed on a slide?
- How do you select the best portion of the sample for smear preparation?
- How can you determine the correct size and thickness of a sputum smear?
- What are four critical steps in ZN staining?