



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

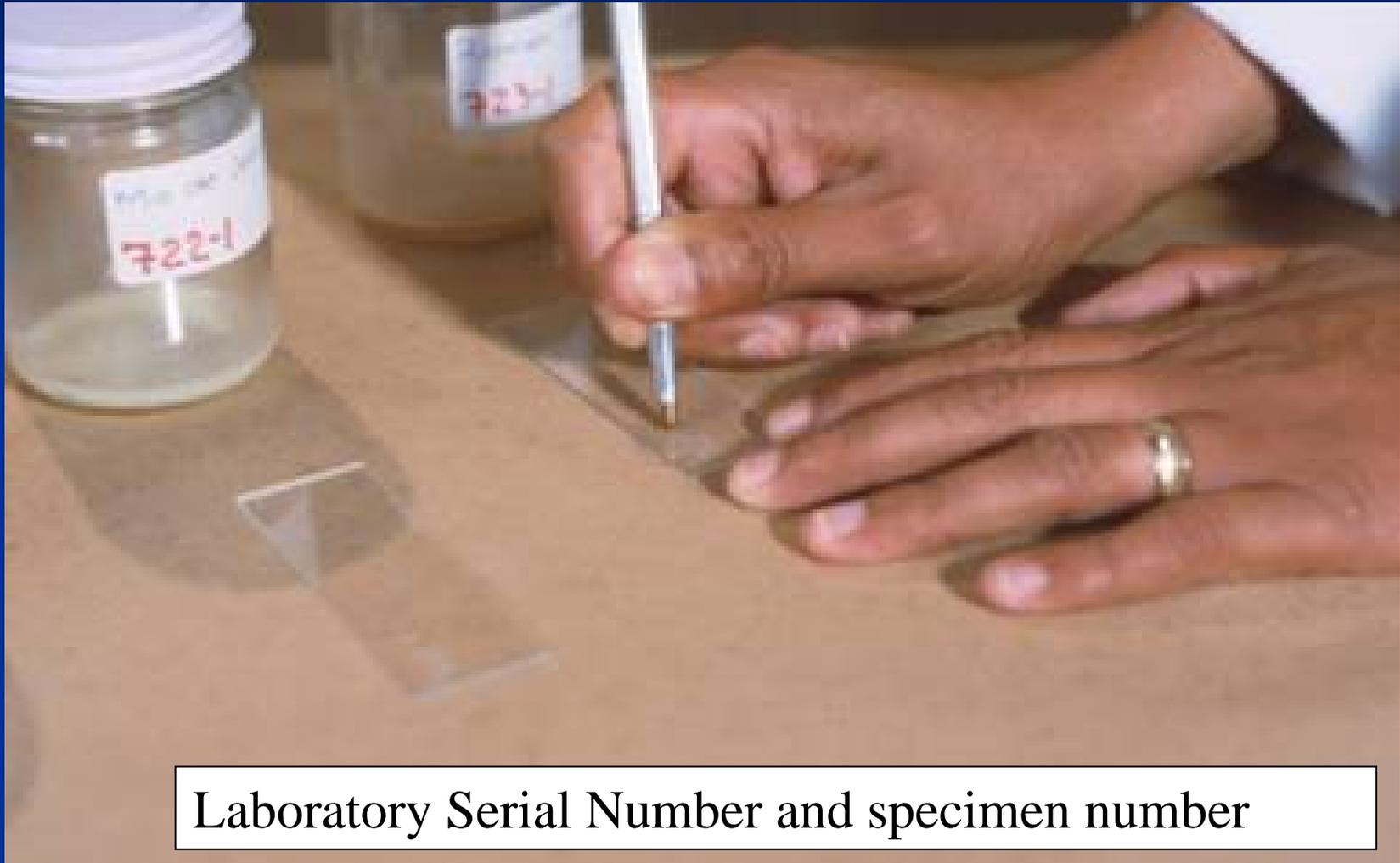
Labeling Frosted Slides with Pencil



Laboratory Serial Number and specimen number

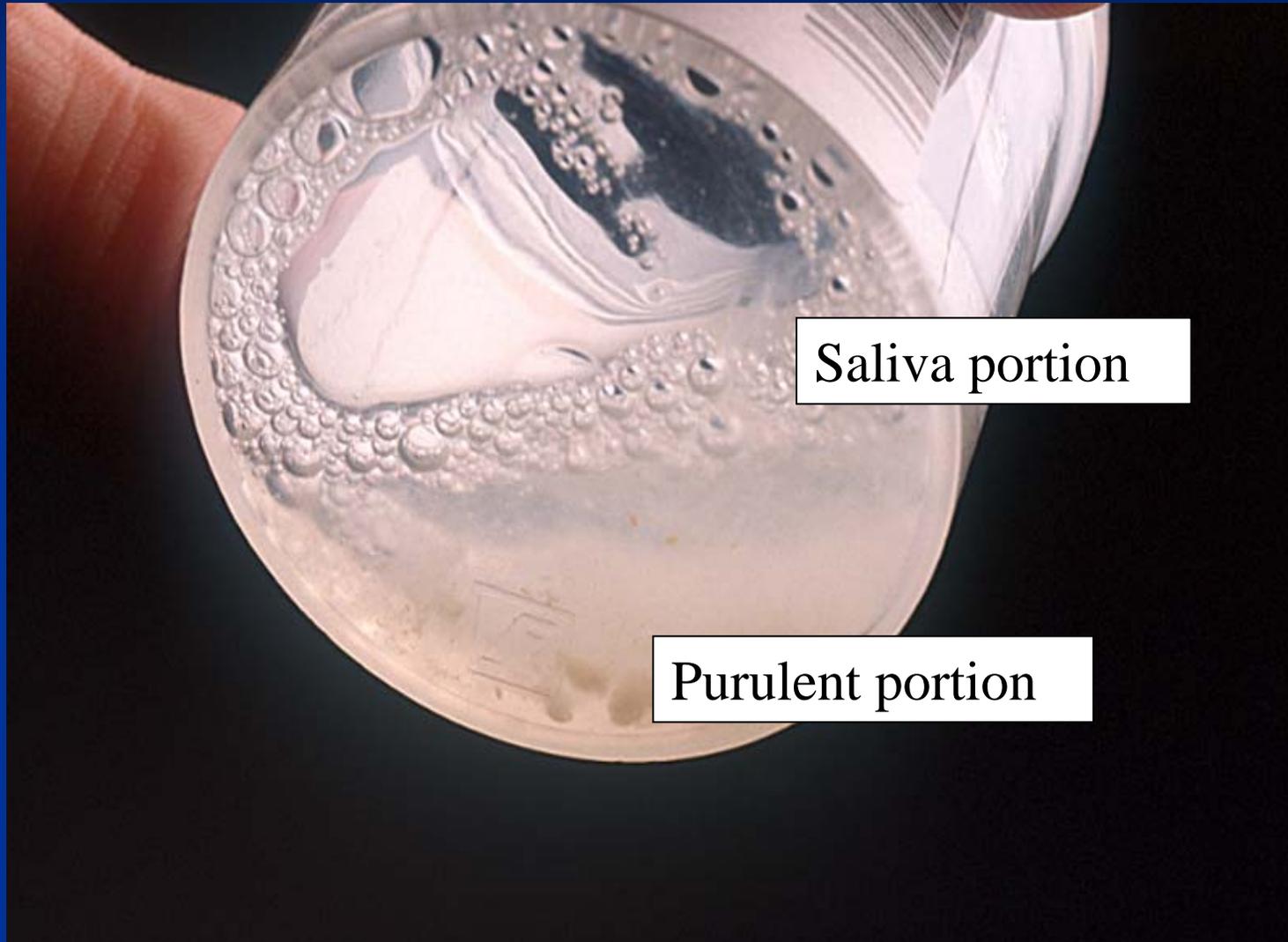


Labeling Non-frosted Slides with Diamond Pencil



Laboratory Serial Number and specimen number

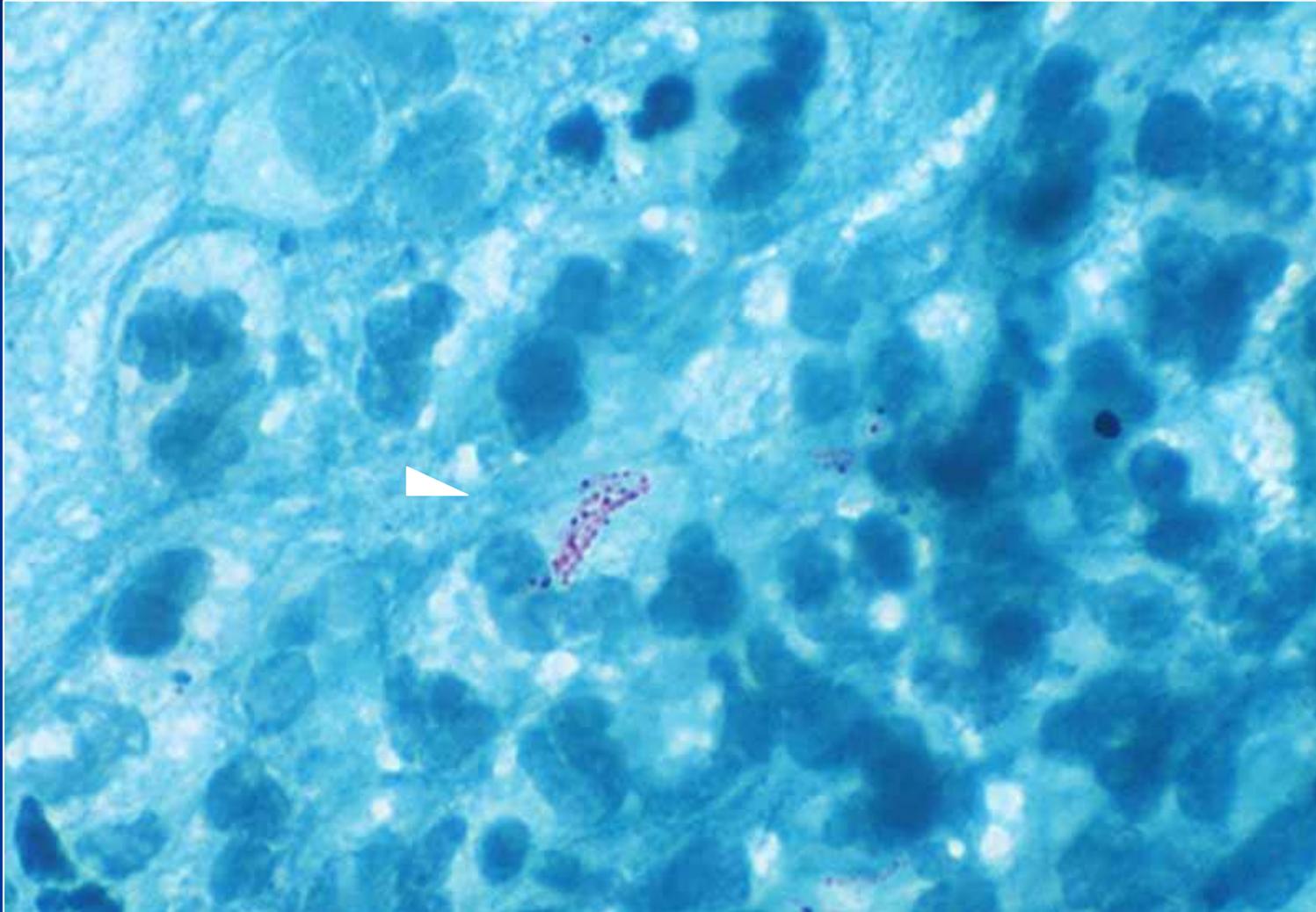
Specimen Appearance



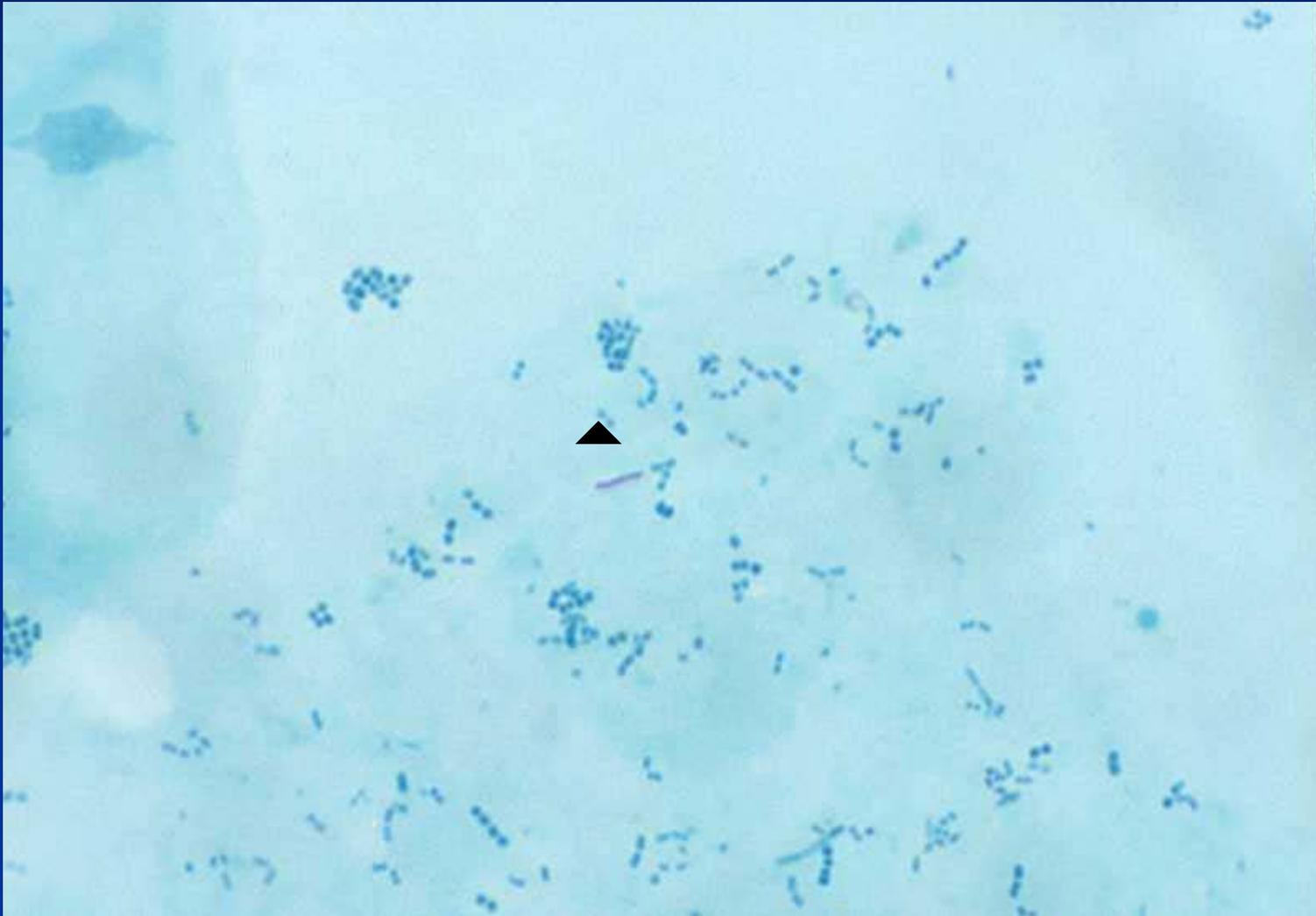
Saliva portion

Purulent portion

Purulent Portion



Saliva Portion



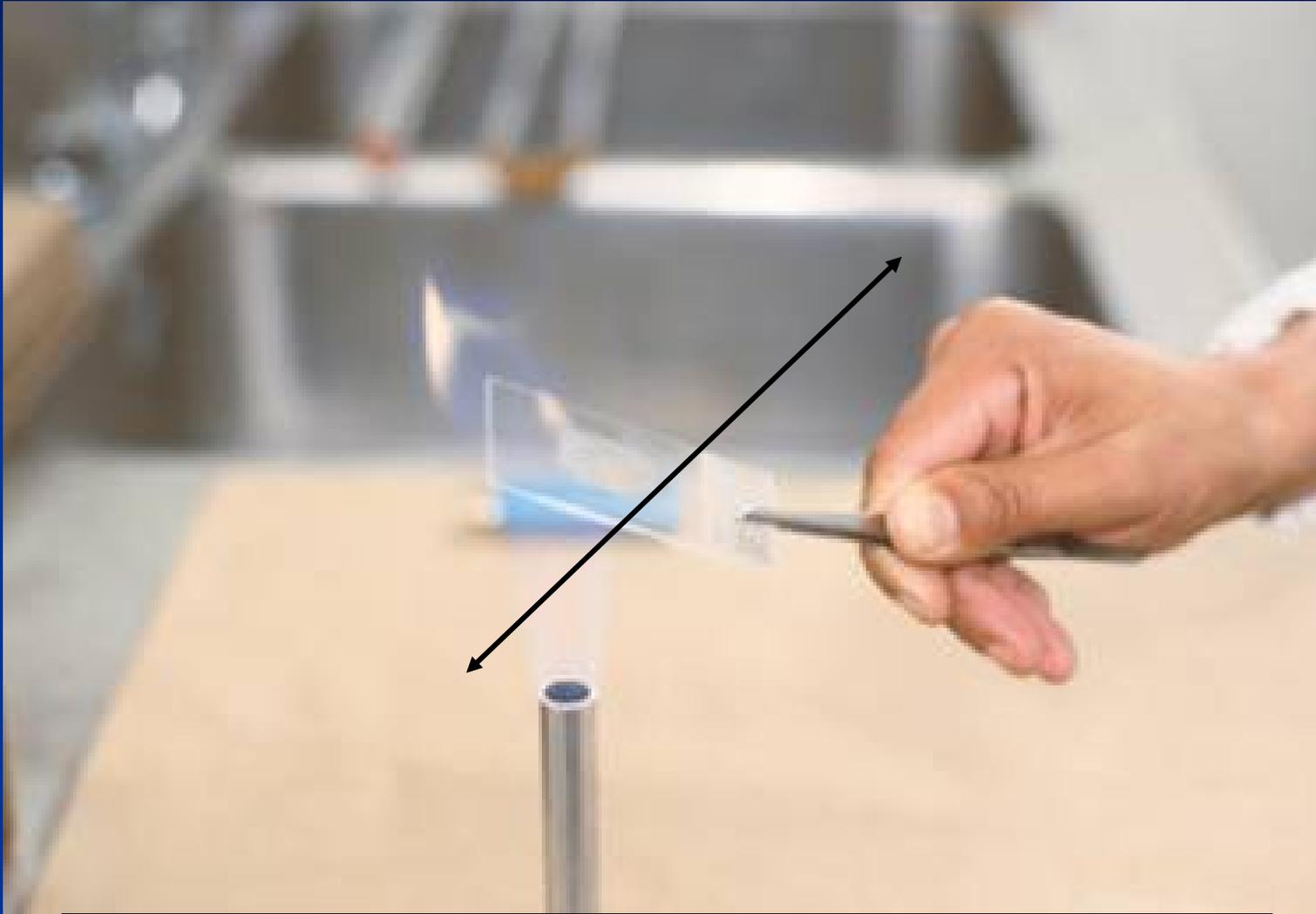
Consistent Quality of Smears



Making Smear with Loop



Heat Fixing a Smear



Pass the smear through the flame 2–3 times

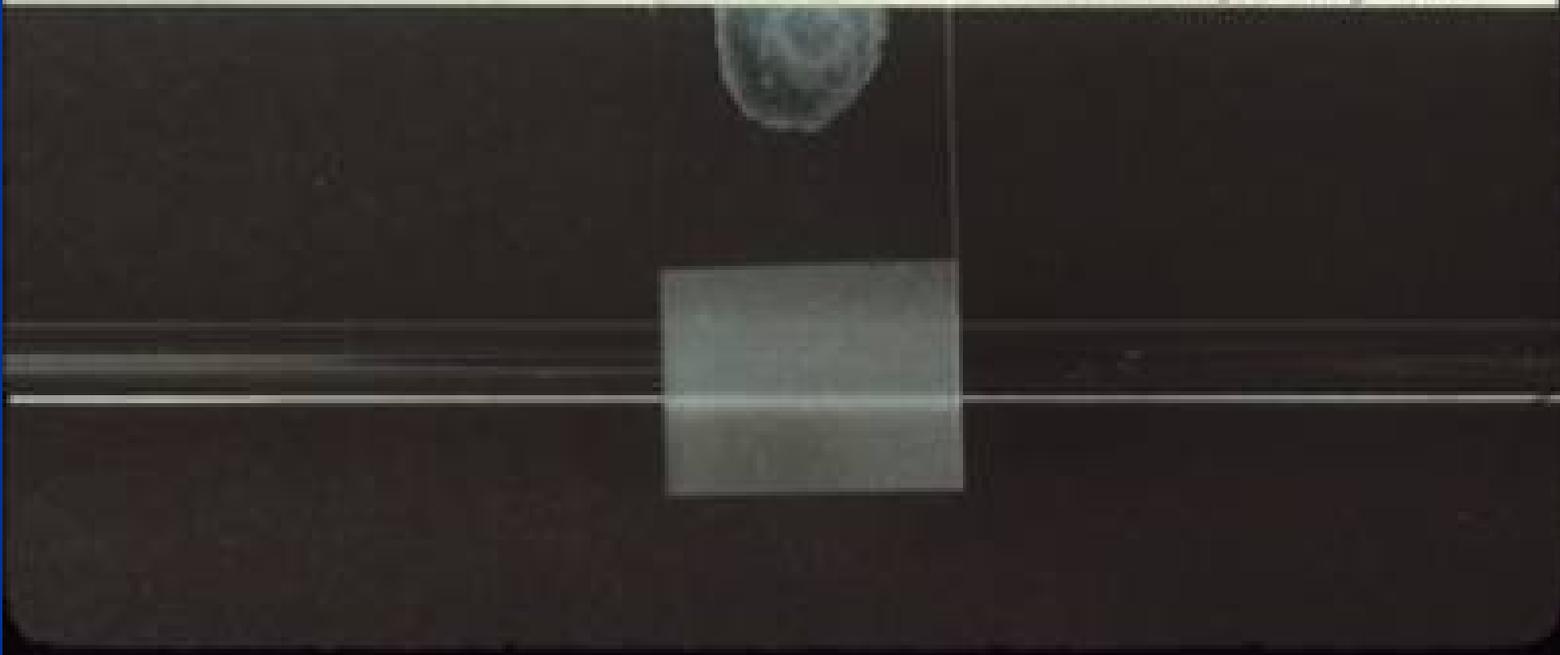
Proper Thickness of Smear

5. Centrifuge at about 2,000 x gravity for 15 min.
6. Pour off the supernatant and retain the sediment.
7. Resuspend the sediment in several drops of water and prepare the smear.



hypochlorite. This material and the crystals that form as the smear dries will wash off during staining, but the AFB will remain on the heat-fixed smear.

A direct or concentrated sputum smear should appear cloudy before staining. But it is too thick if you cannot read print in a newspaper through the smear when it is held 5 to 10 cm from the print. Smears that are too thick often wash off during staining or the



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

AFB

Non AFB



carbol fuchsin

+

HEAT



Principle of ZN Stain - 2

AFB



carbol fuchsin



decolouriser



Non AFB



Principle of ZN Stain - 3

AFB



carbol fuchsin



decolouriser



counterstain

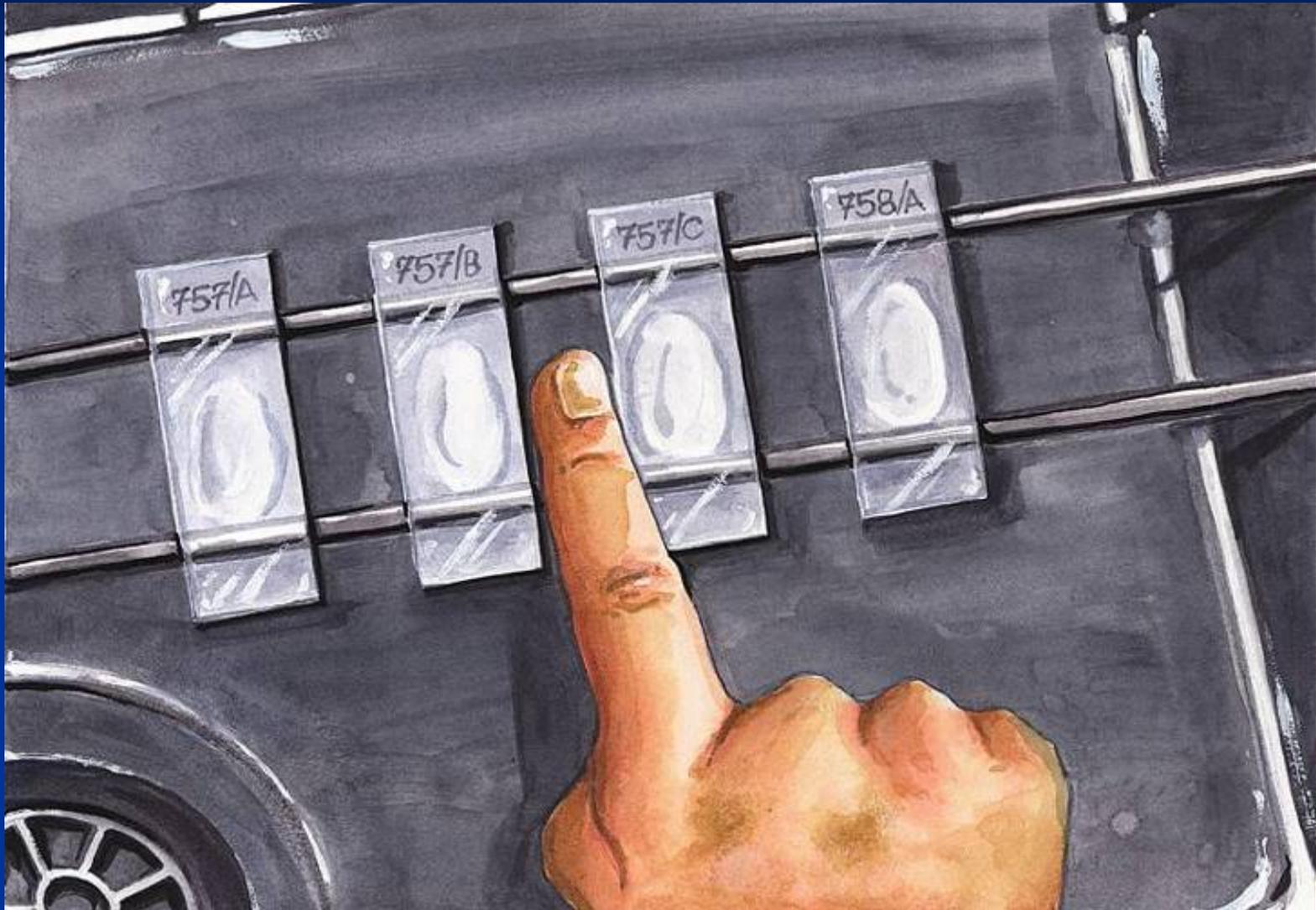
Non AFB



Overview of Staining Procedure

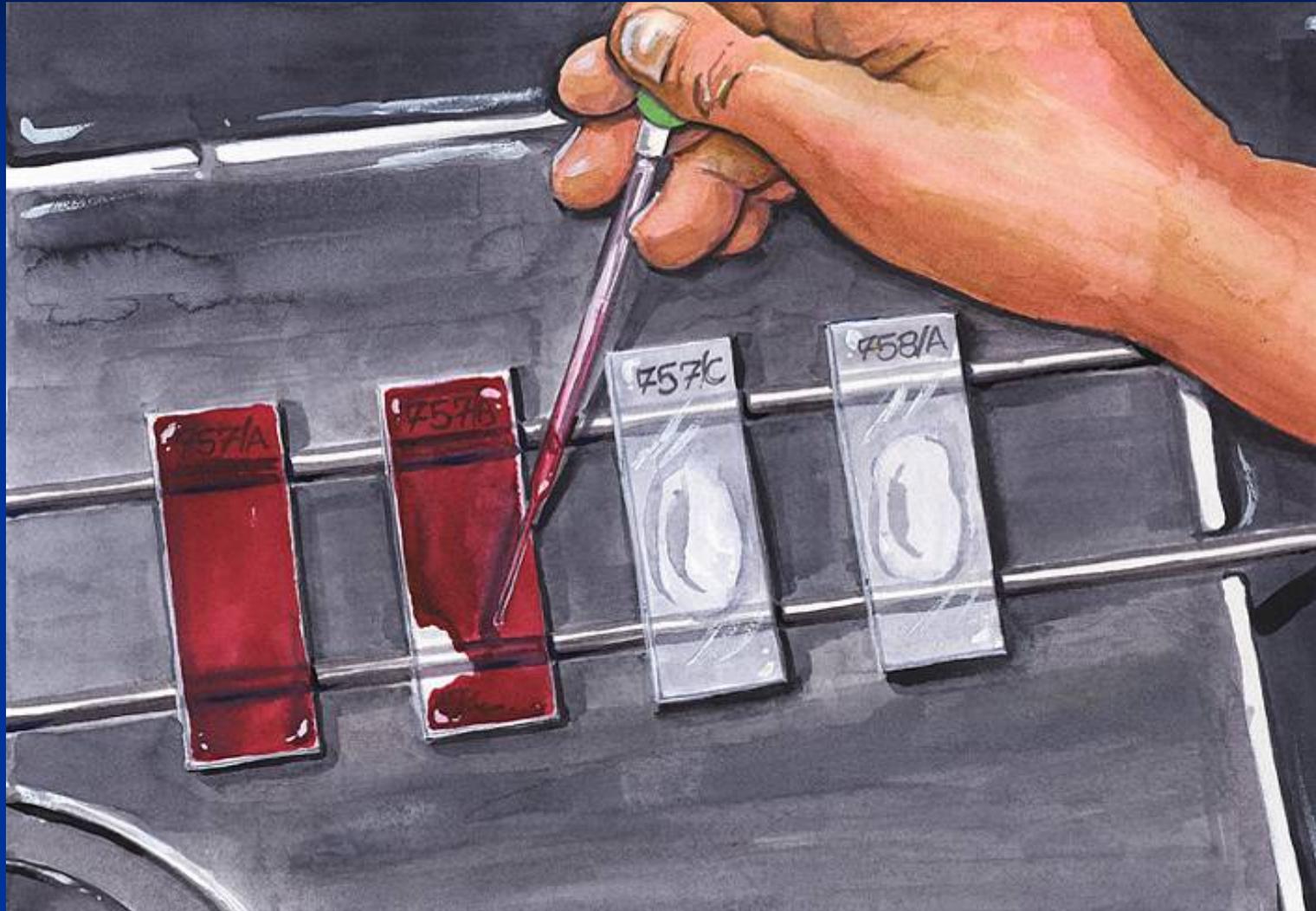
1. Arrange slides in serial order smear side up
2. Keep a finger-thickness between smears
3. Flood with carbol fuchsin
4. Heat to steaming once only
5. Leave for 10 minutes
6. Rinse with water, drain
7. Apply decolorizing solution 3 min.
8. Rinse, drain
9. Apply Methylene blue counterstain, 1 min.
10.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

Setting up Slides on Staining Rack

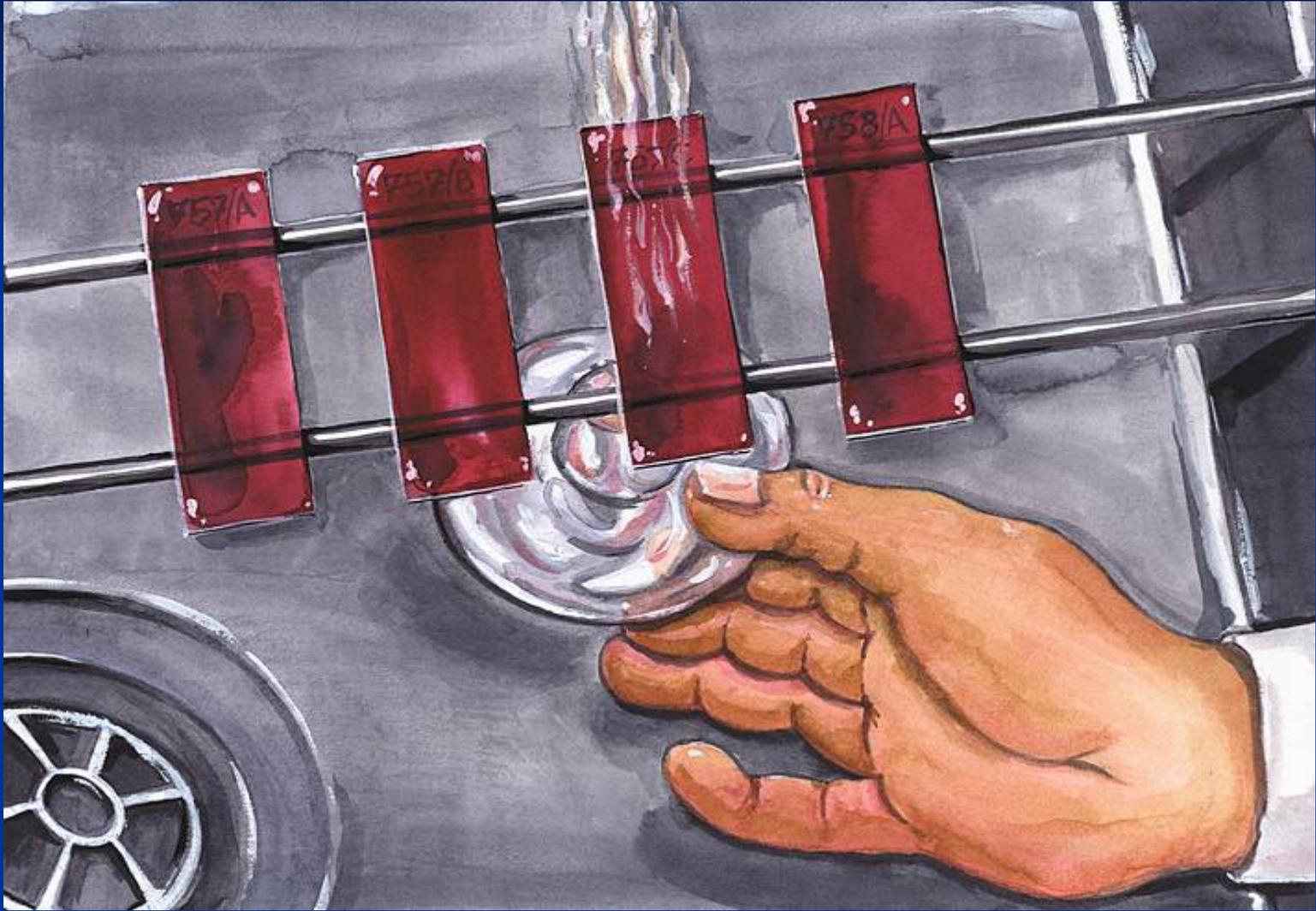




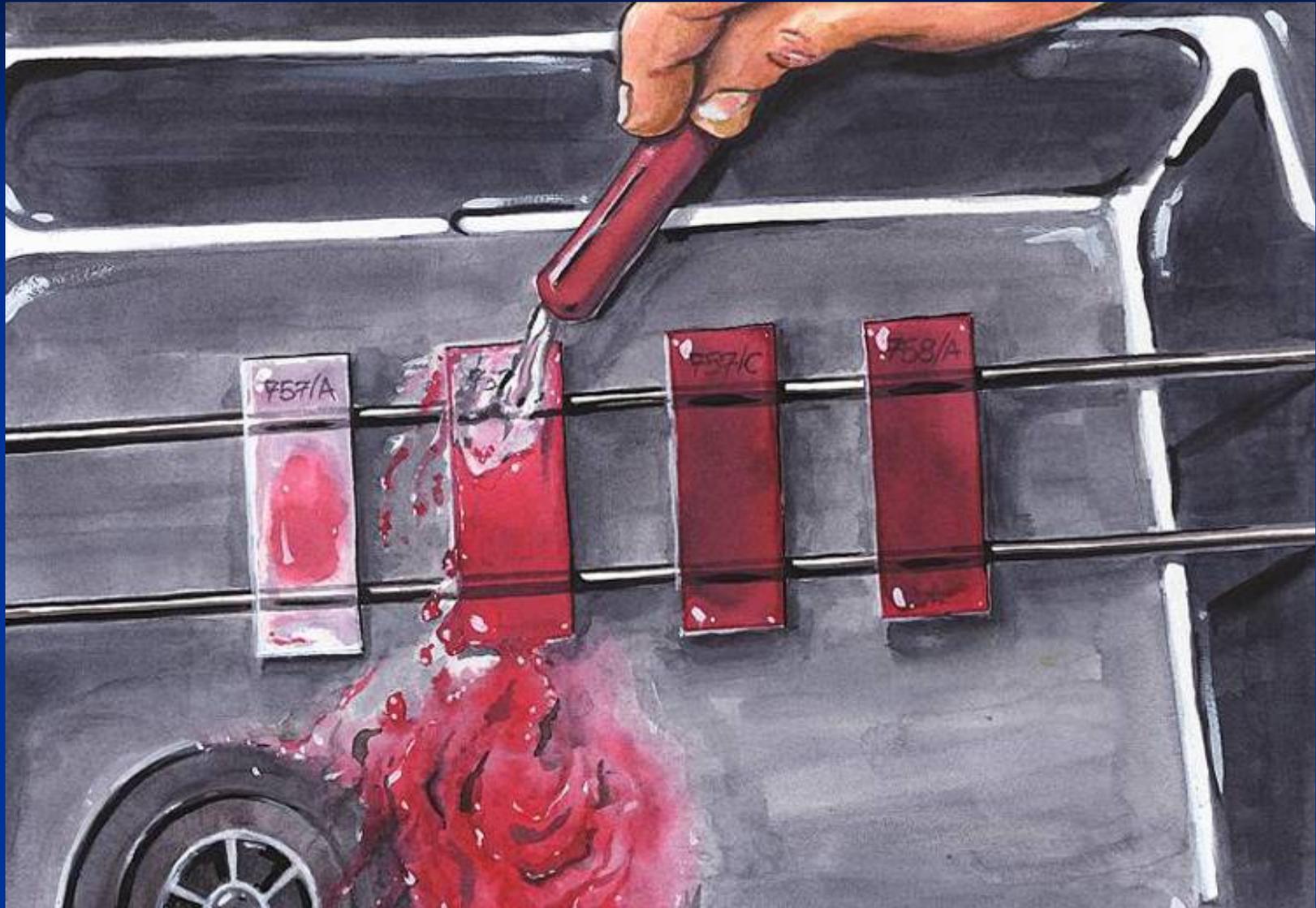
Carbol Fuchsin



Heating Carbol Fuchsin to Steaming



Rinsing Slides



Don't Splash Adjacent Slides





Decolorisation Step

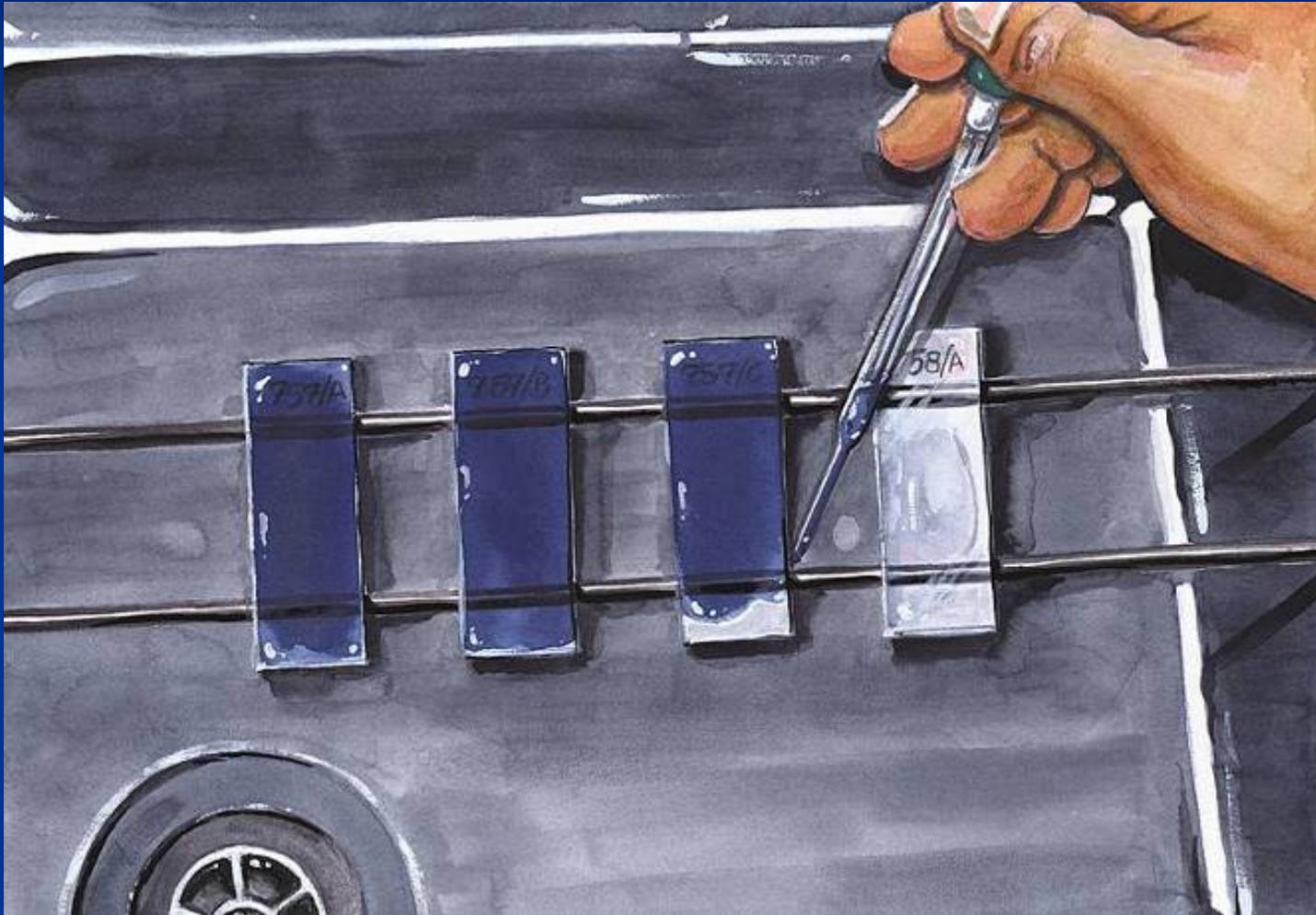


Rinsing Slides





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?**