

MODULE 6

Troubleshooting Smear Preparation and Staining

Interactive Exercise

Smear Preparation

First part of exercise will
cover troubleshooting
smear preparation

Uniform Smear Size



Size of Smear

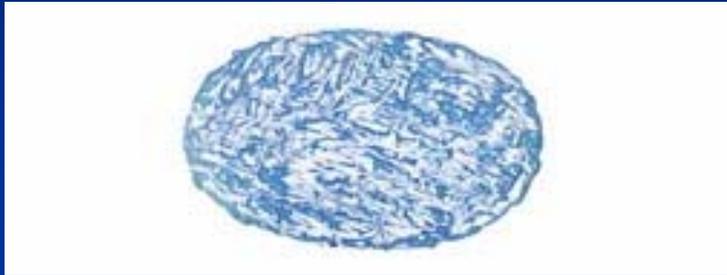


2 x 3 cm



1 x 2 cm

Evenness of Smear



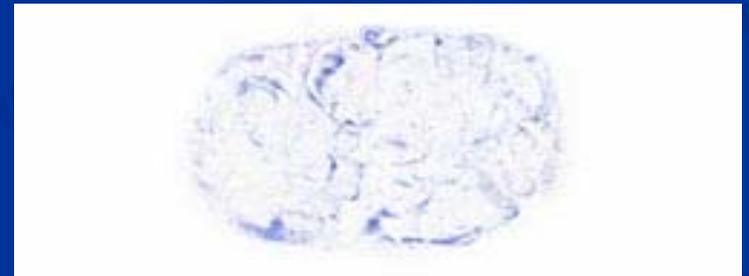
Good



Good

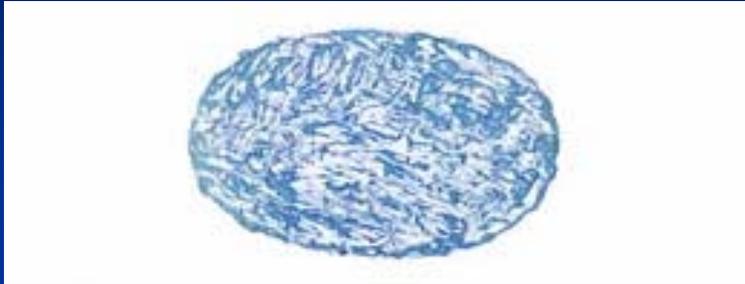


Sloughed off

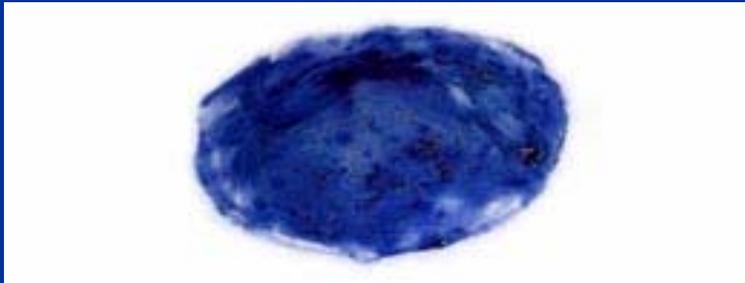


Uneven

Smear Thickness



Good

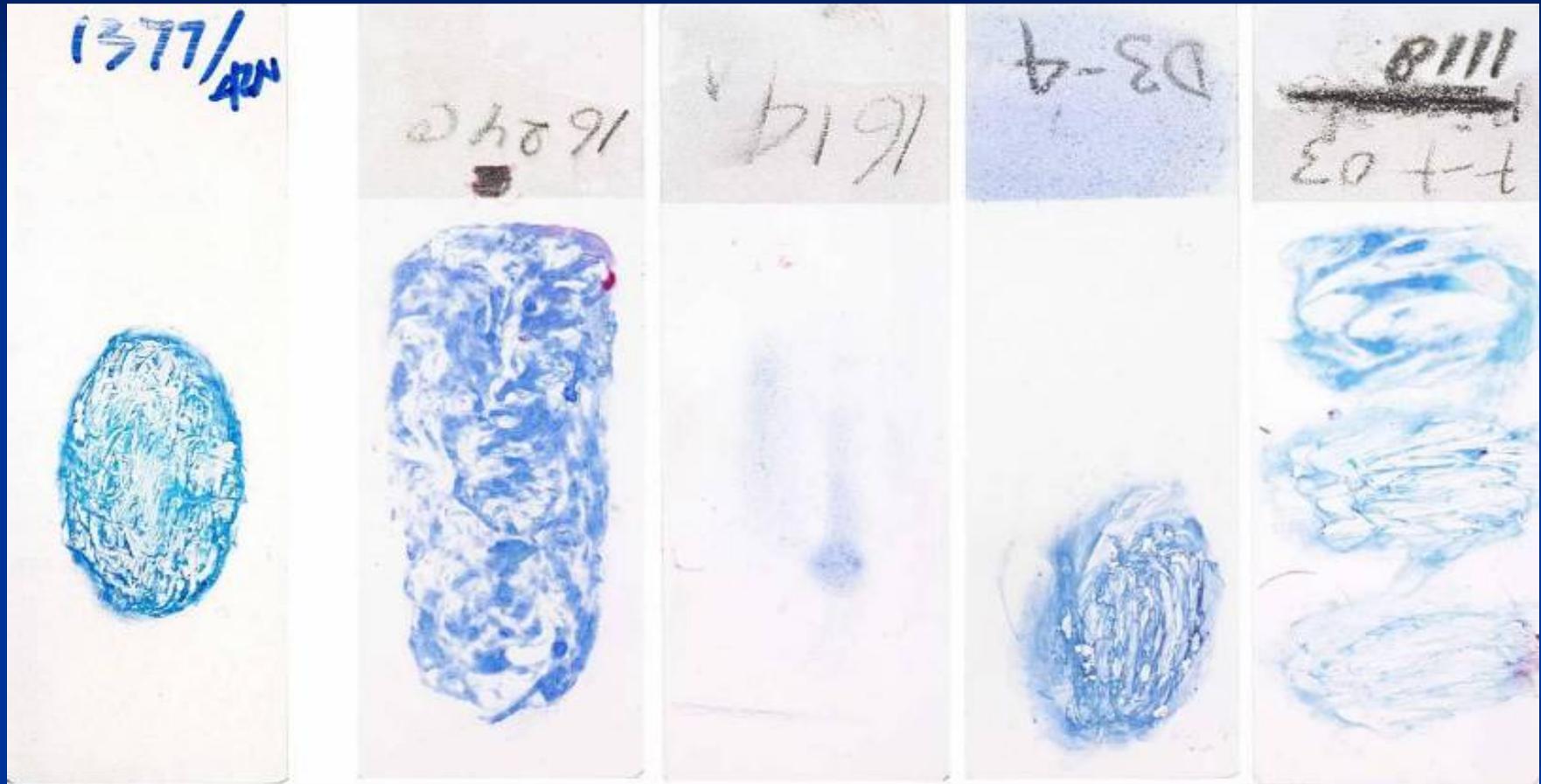


Too Thick



Too Thin

Question: Identify the Problem?



1

2

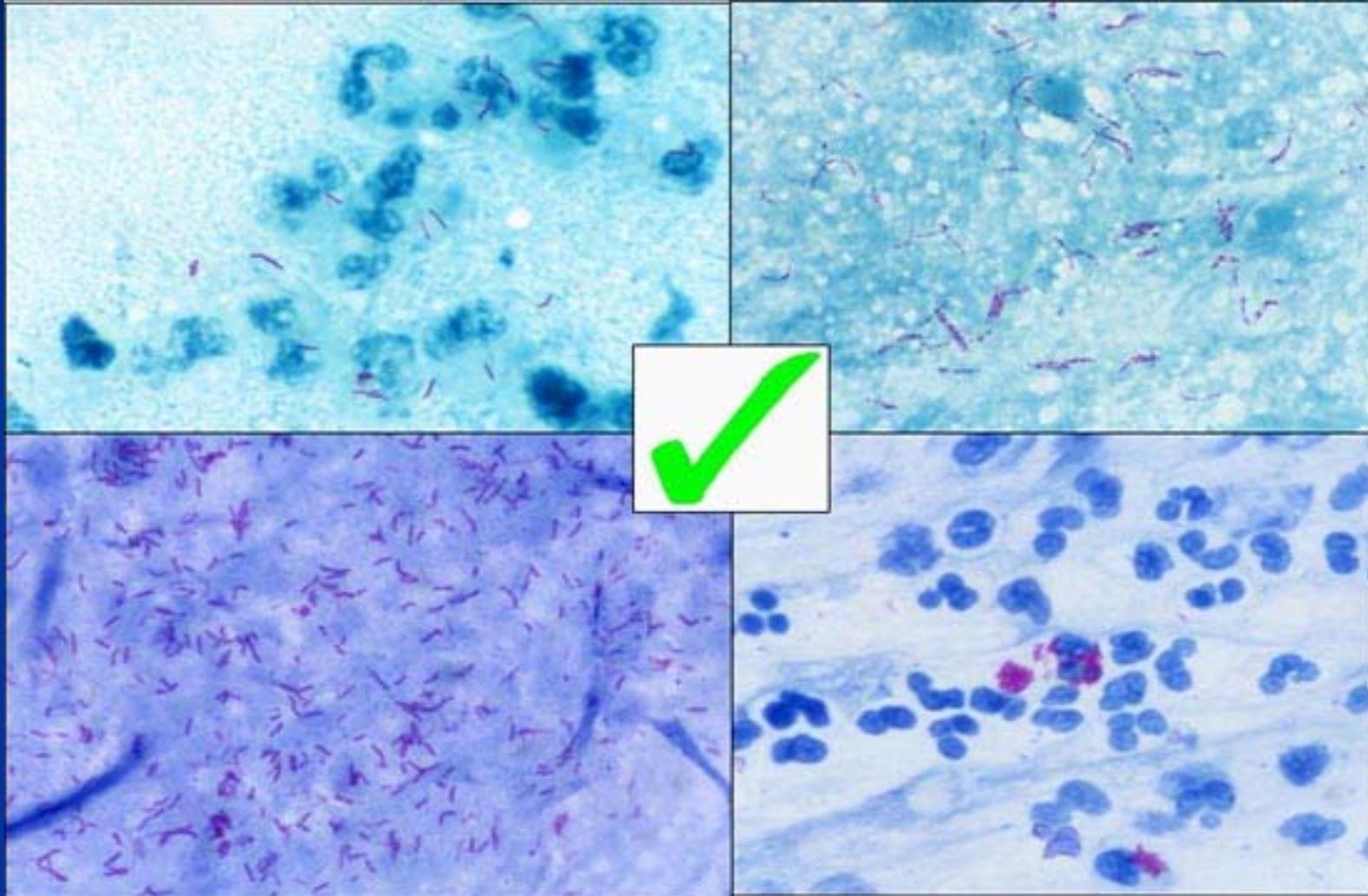
3

4

5

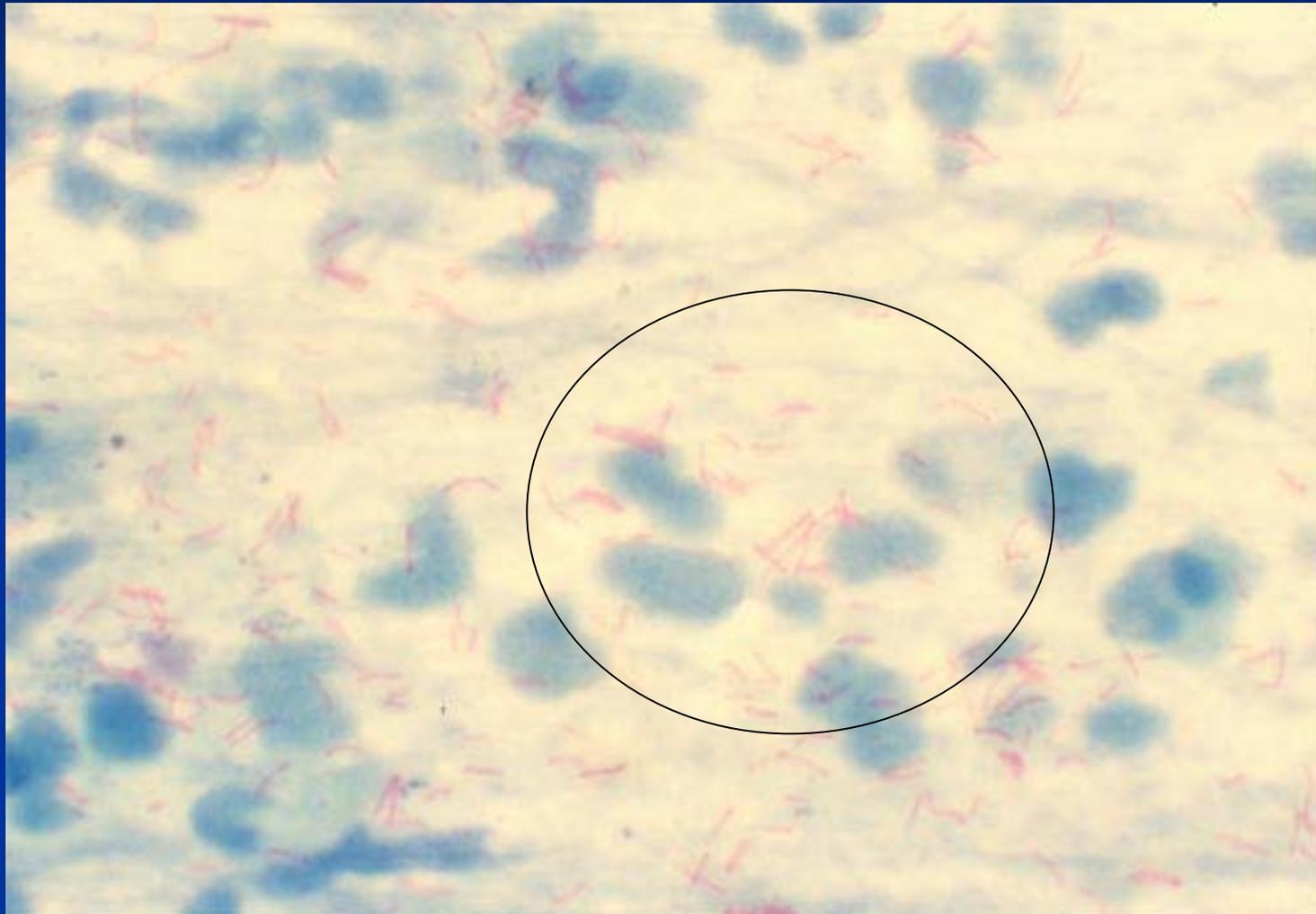
Staining

Good Quality Staining of AFB



What Are the Causes of Pale Staining AFB?

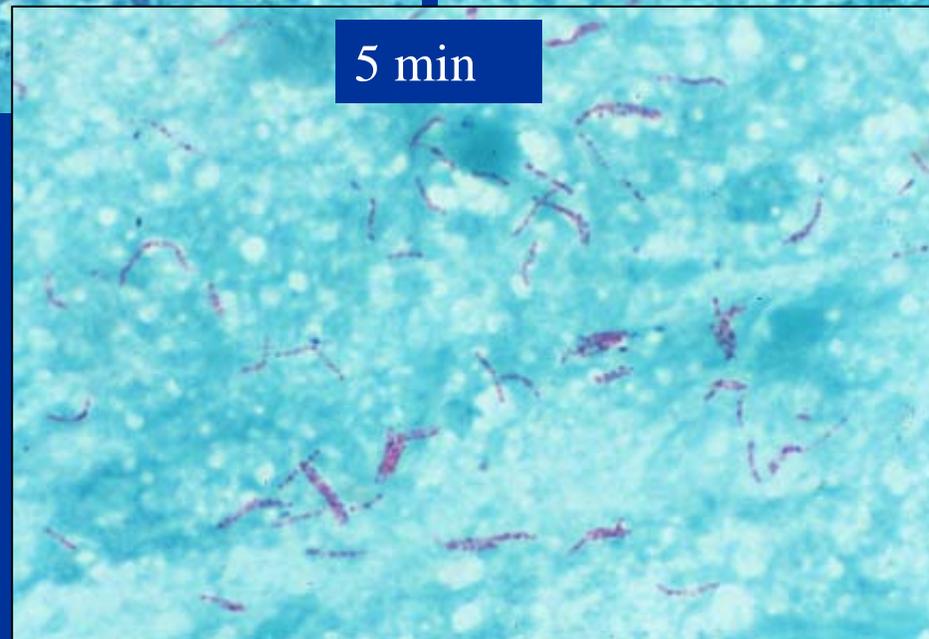
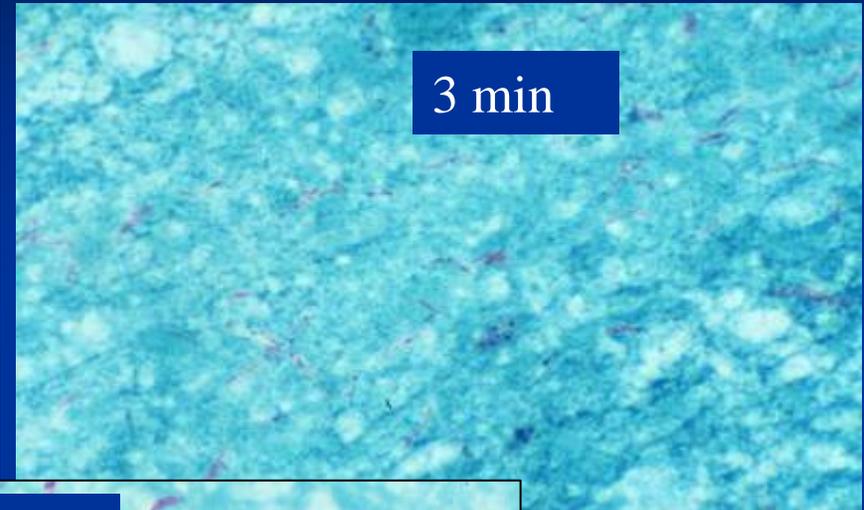
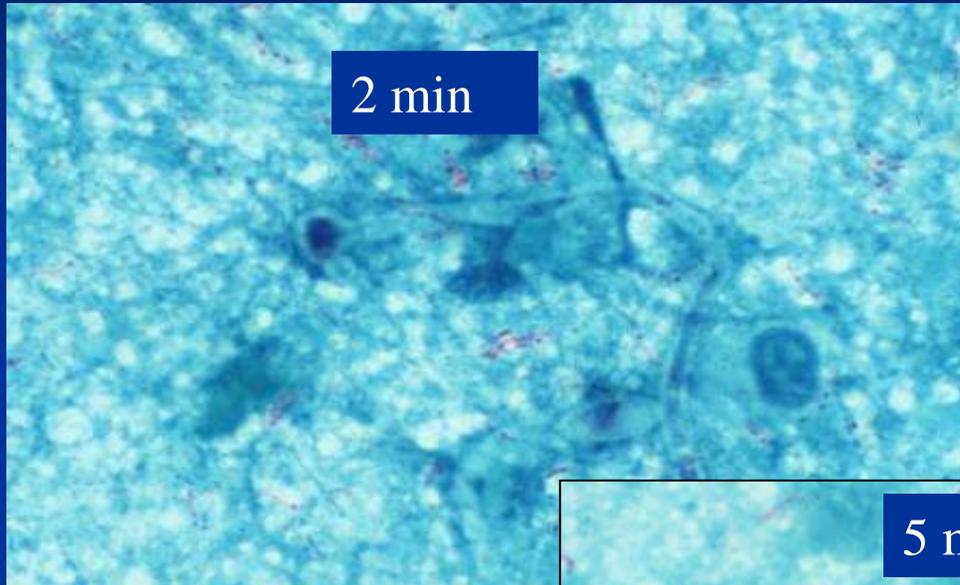
Pale Staining AFB



Causes of Pale Staining AFB

- Concentration of CF <0.3%:
- Staining time less than 5 minutes
- Inadequate heating of carbol fuchsin on the slide
- Excessive exposure time with an acid alcohol decoloriser may remove the carbol fuchsin from the AFB

Time Effect of Carbol Fuchsin on Intensity of AFB



Effect of Not Heating the Carbol Fuchsin to Steaming

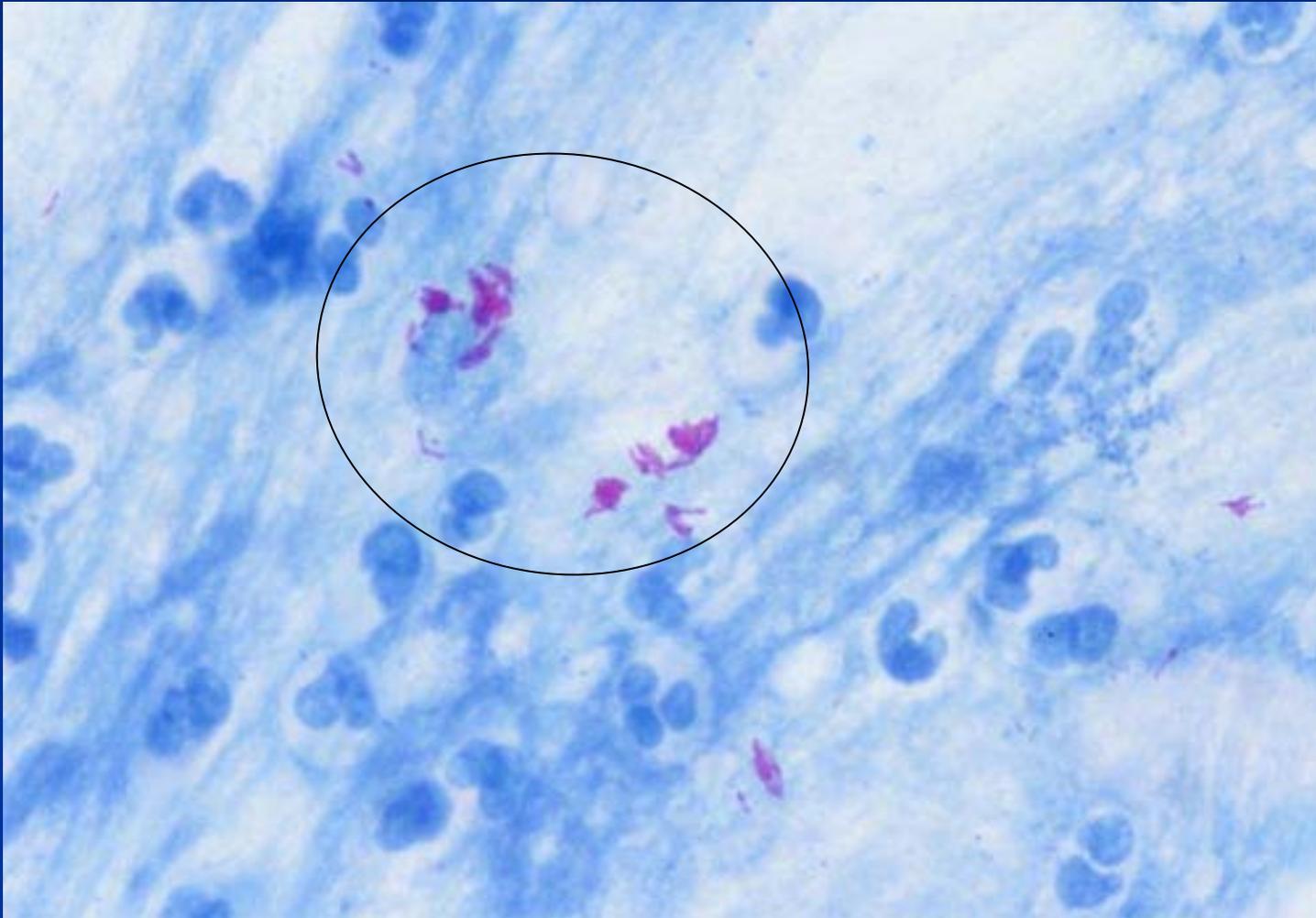


What Are the Causes of Excessive Counterstaining ?

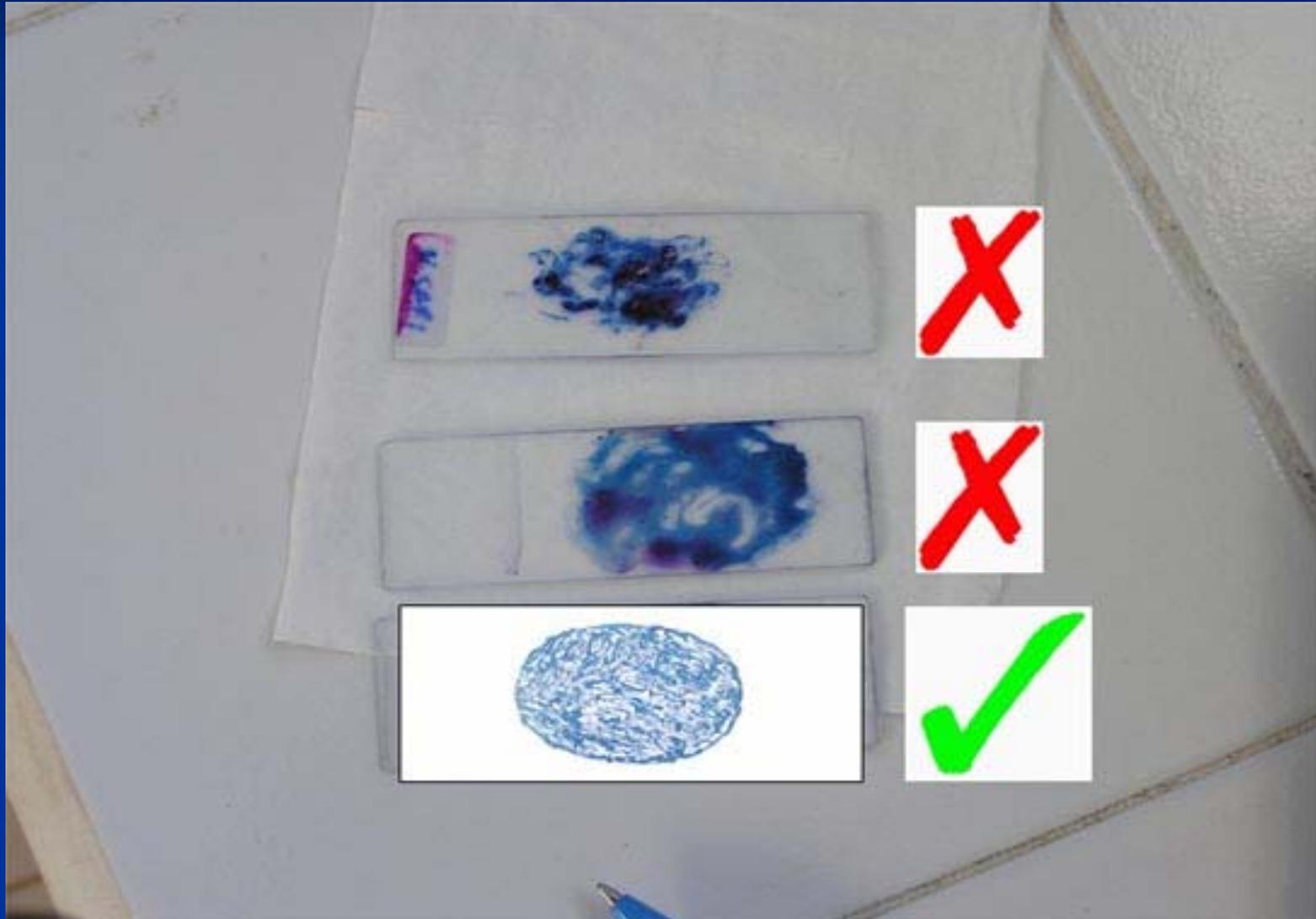
What Are the Causes of Excessive Counterstaining ?

- Leaving counterstain on slide for longer than one minute
- Methylene blue concentration $> 0.3\%$

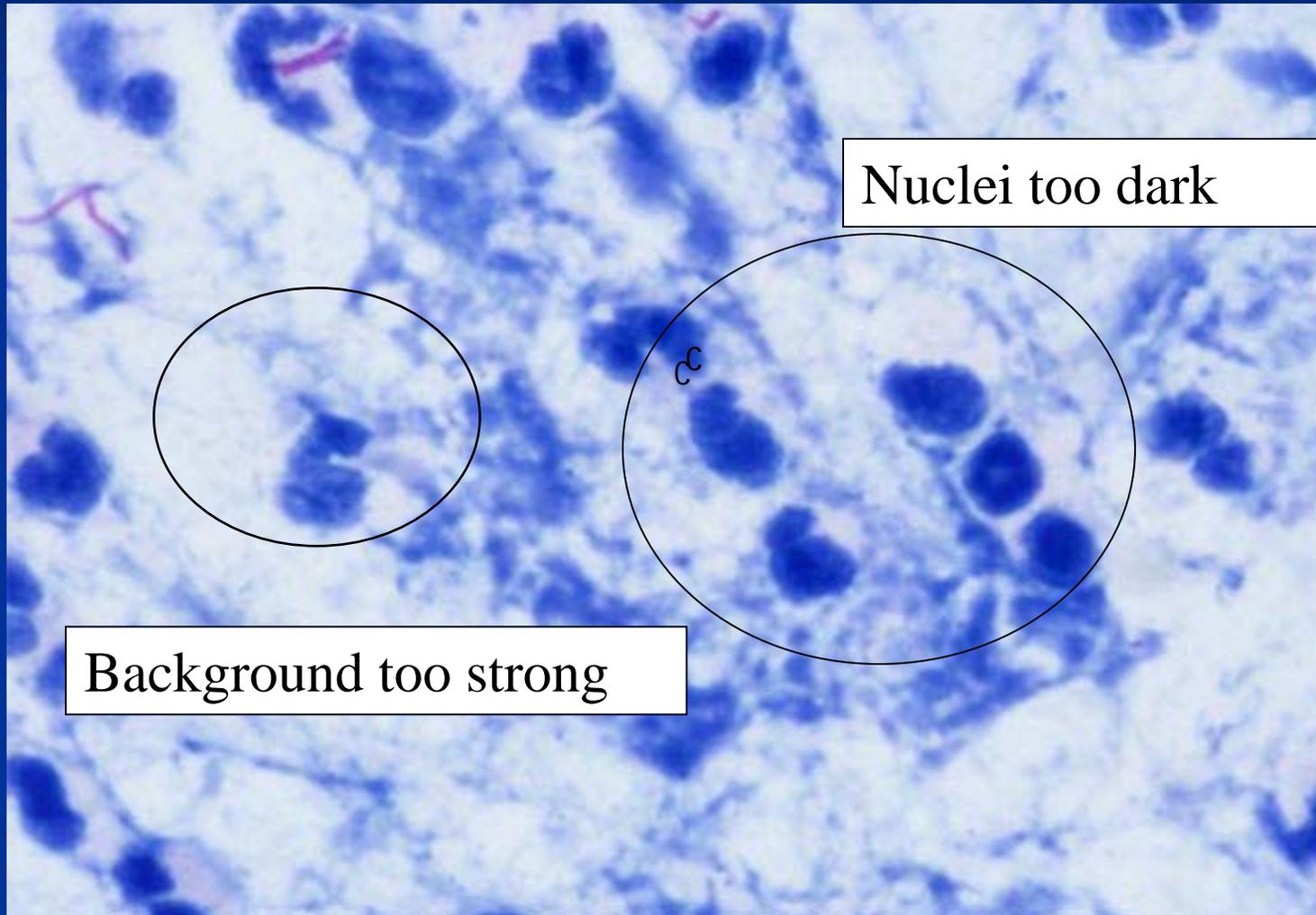
Good Quality Counterstain



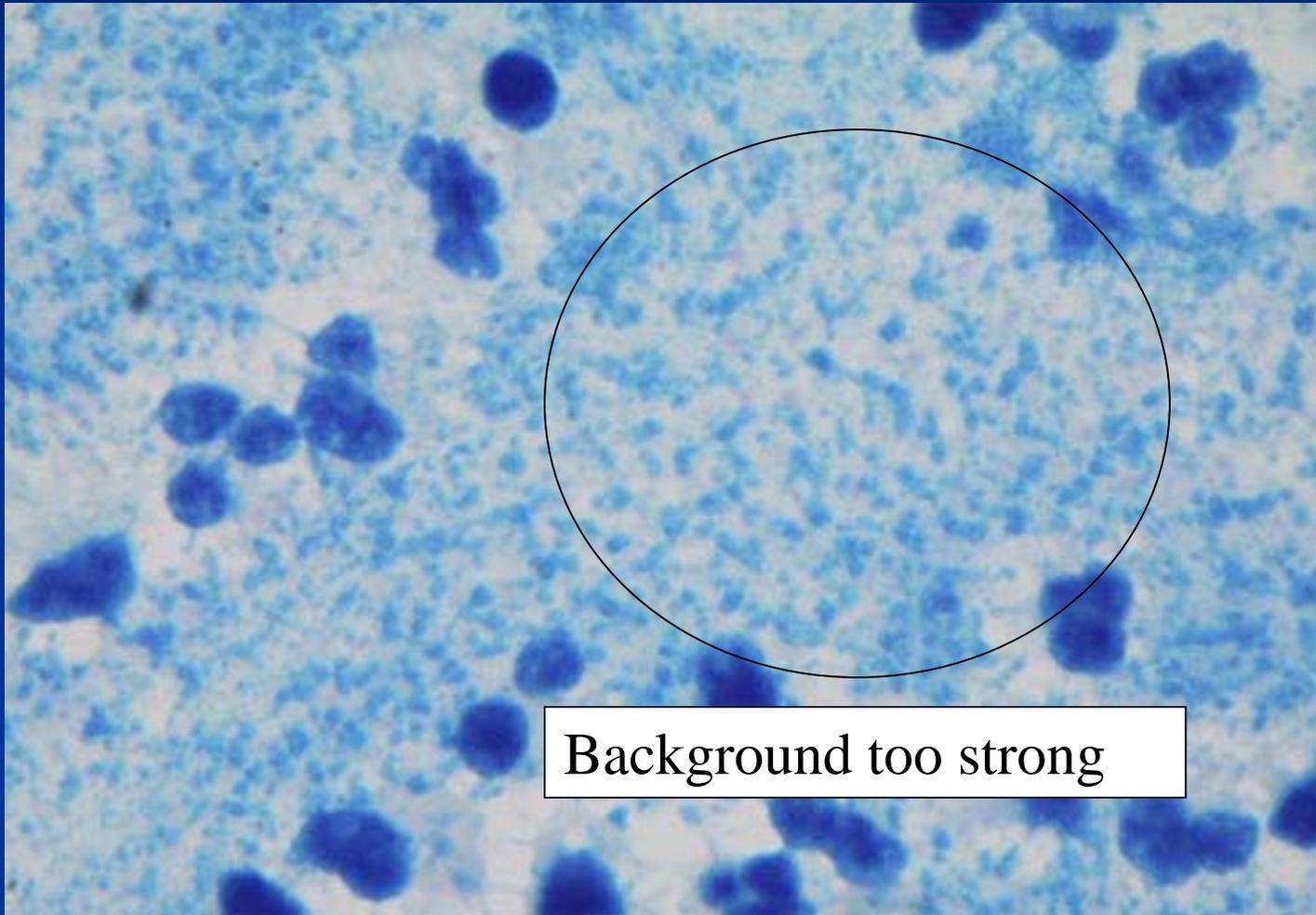
Excessive Counterstain



Effect of Excessive Counterstain-1

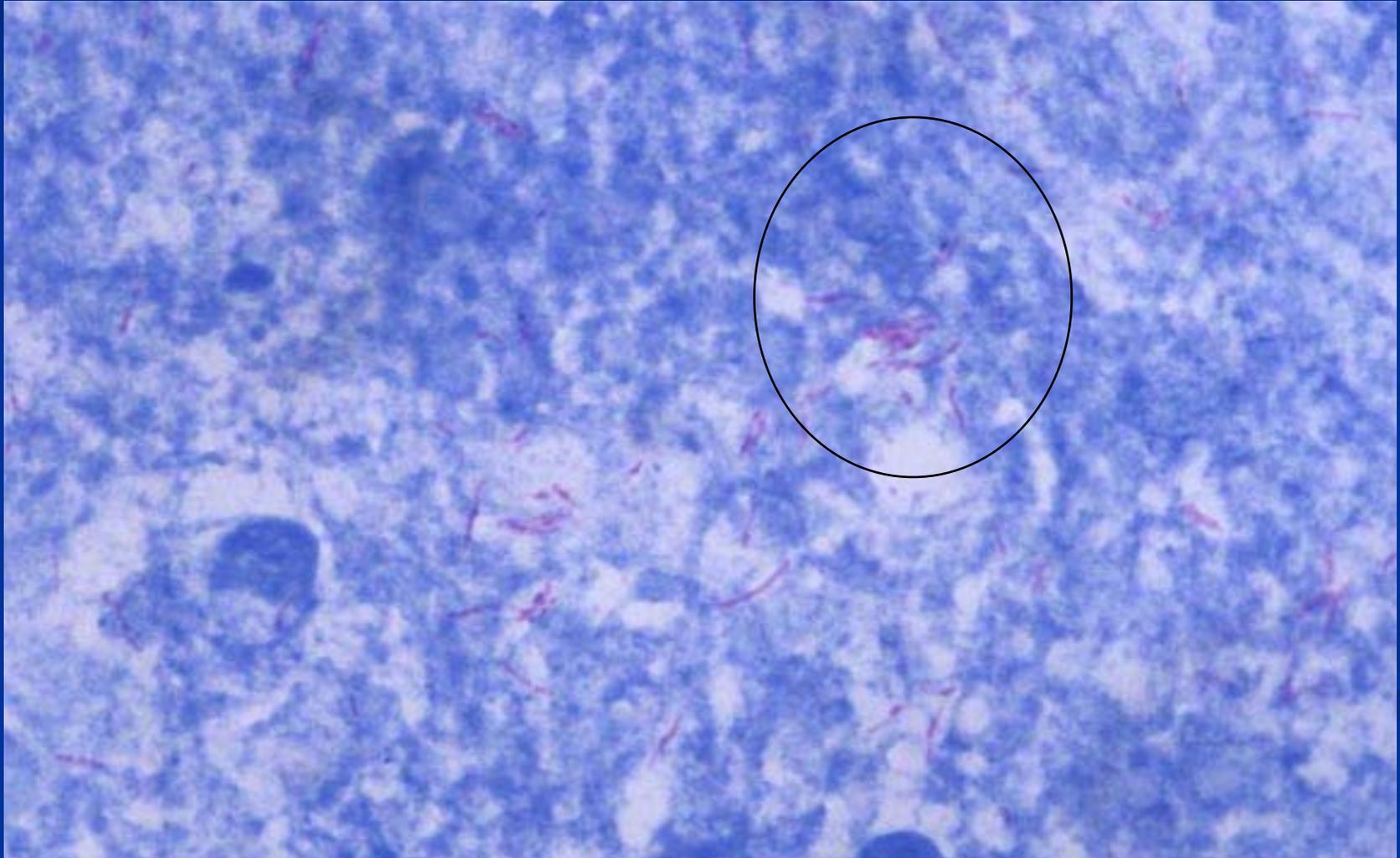


Effect of Excessive Counterstain-2

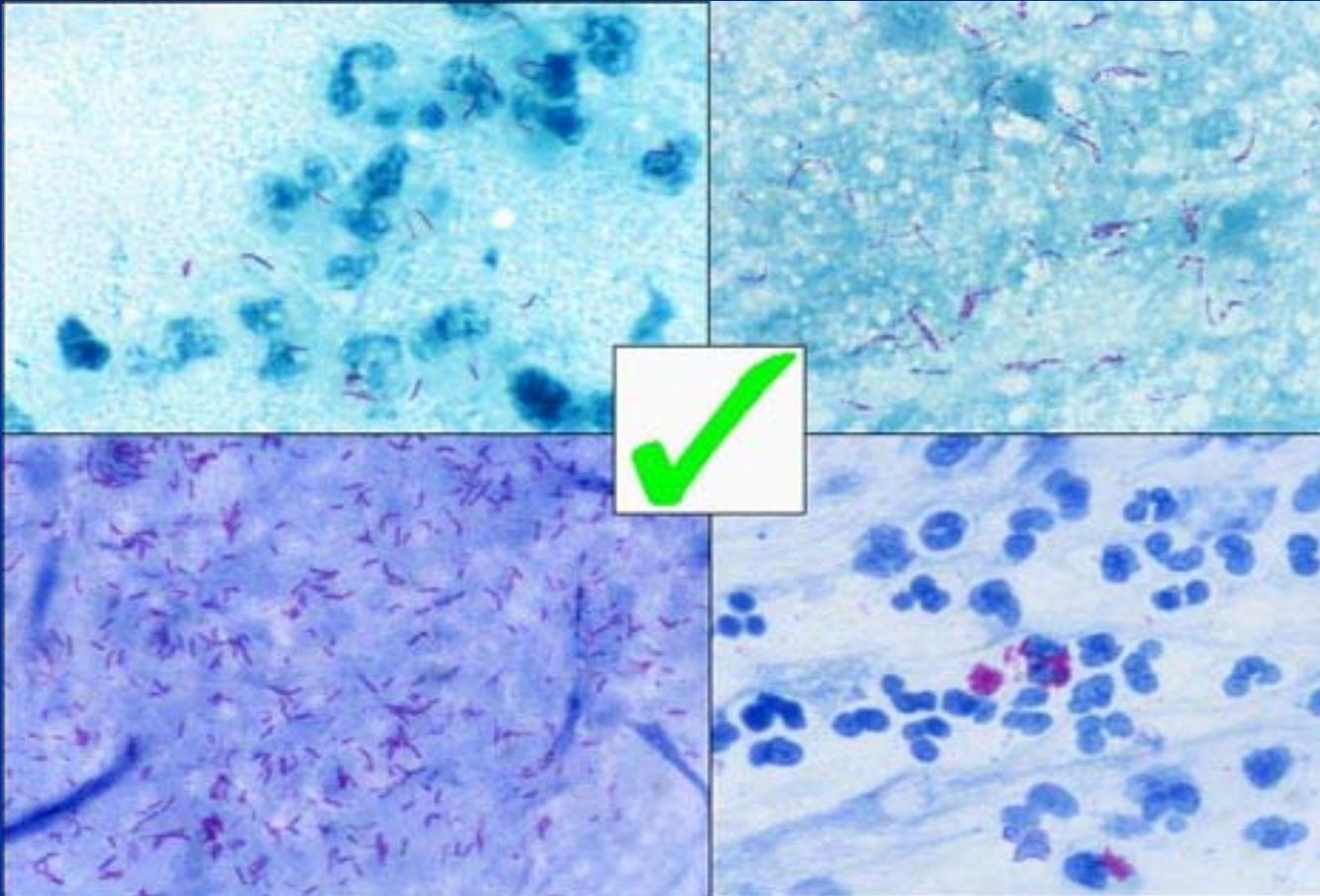


Background too strong

Combination of Excessive Counterstain & Poorly Stained AFB

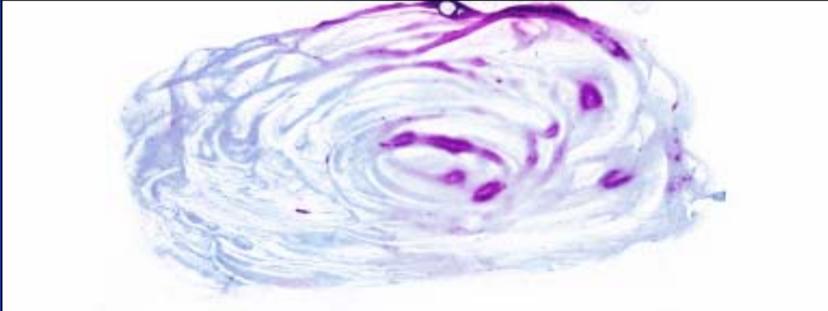


Good Quality Staining of AFB

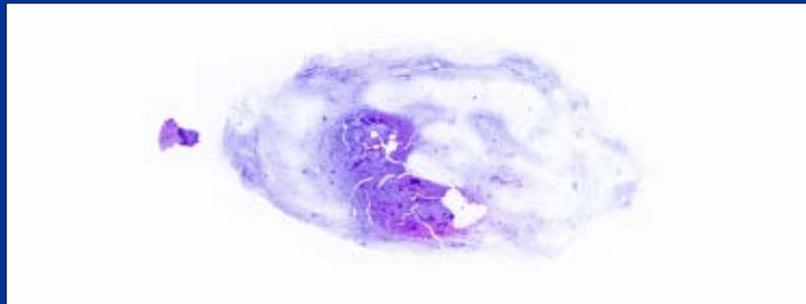


What Are the Causes of Insufficient Decolorisation?

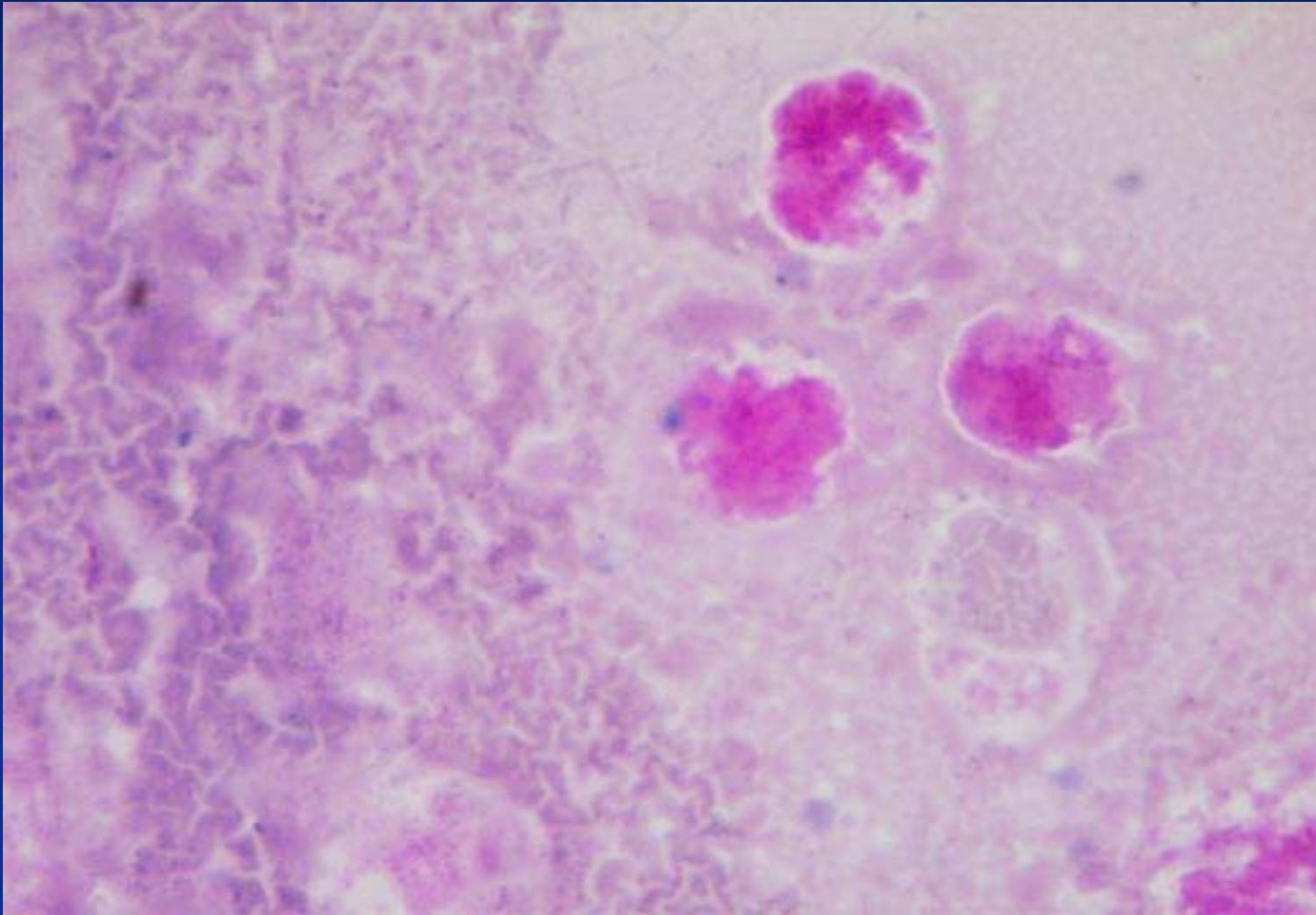
Assessment of Smear Quality



**Uneven smear preparation
and insufficient decolorisation**

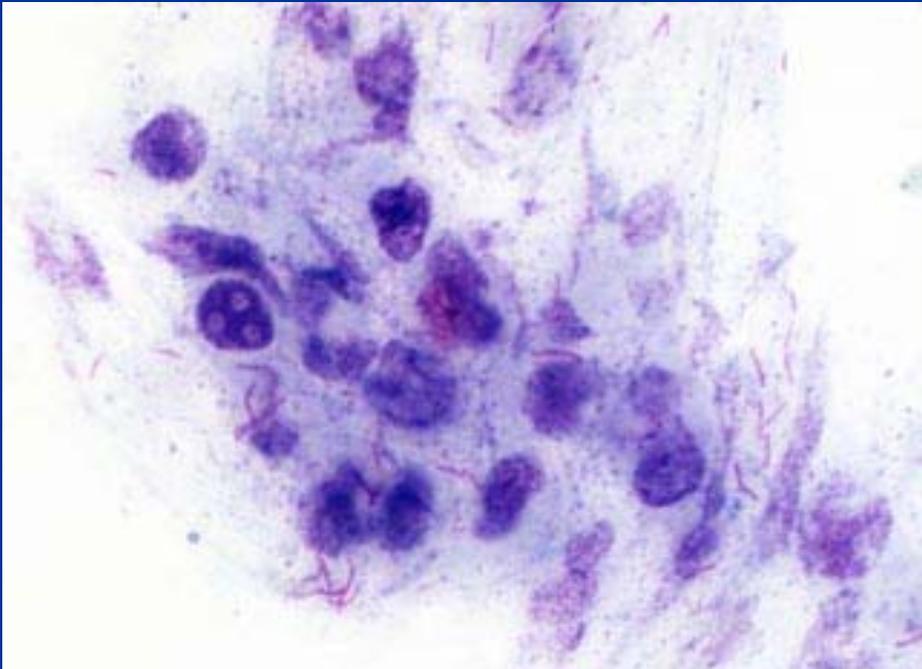


Insufficient Decolourisation

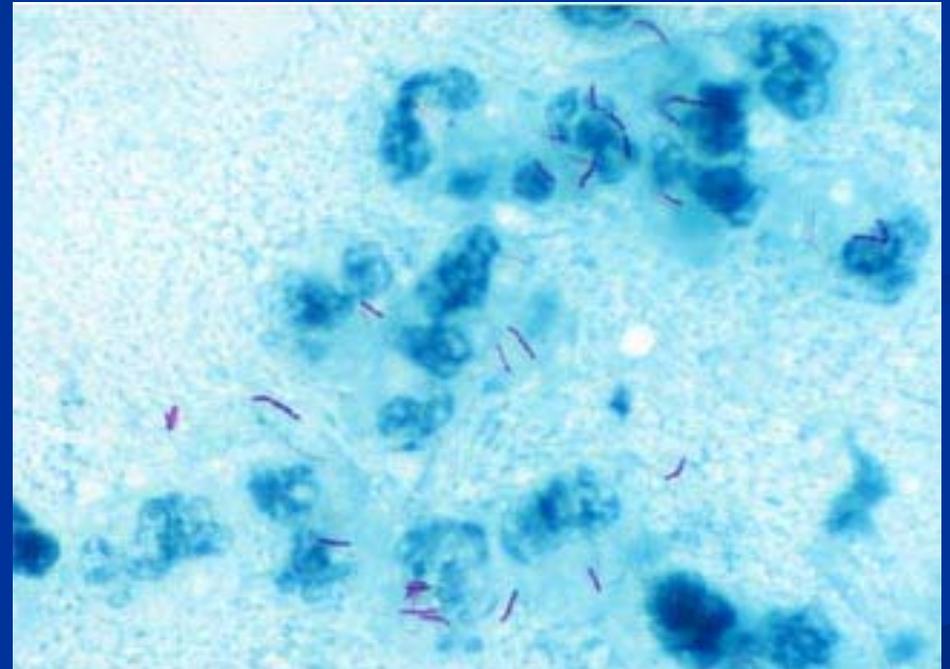


Importance of Correct Decolourisation

Insufficient



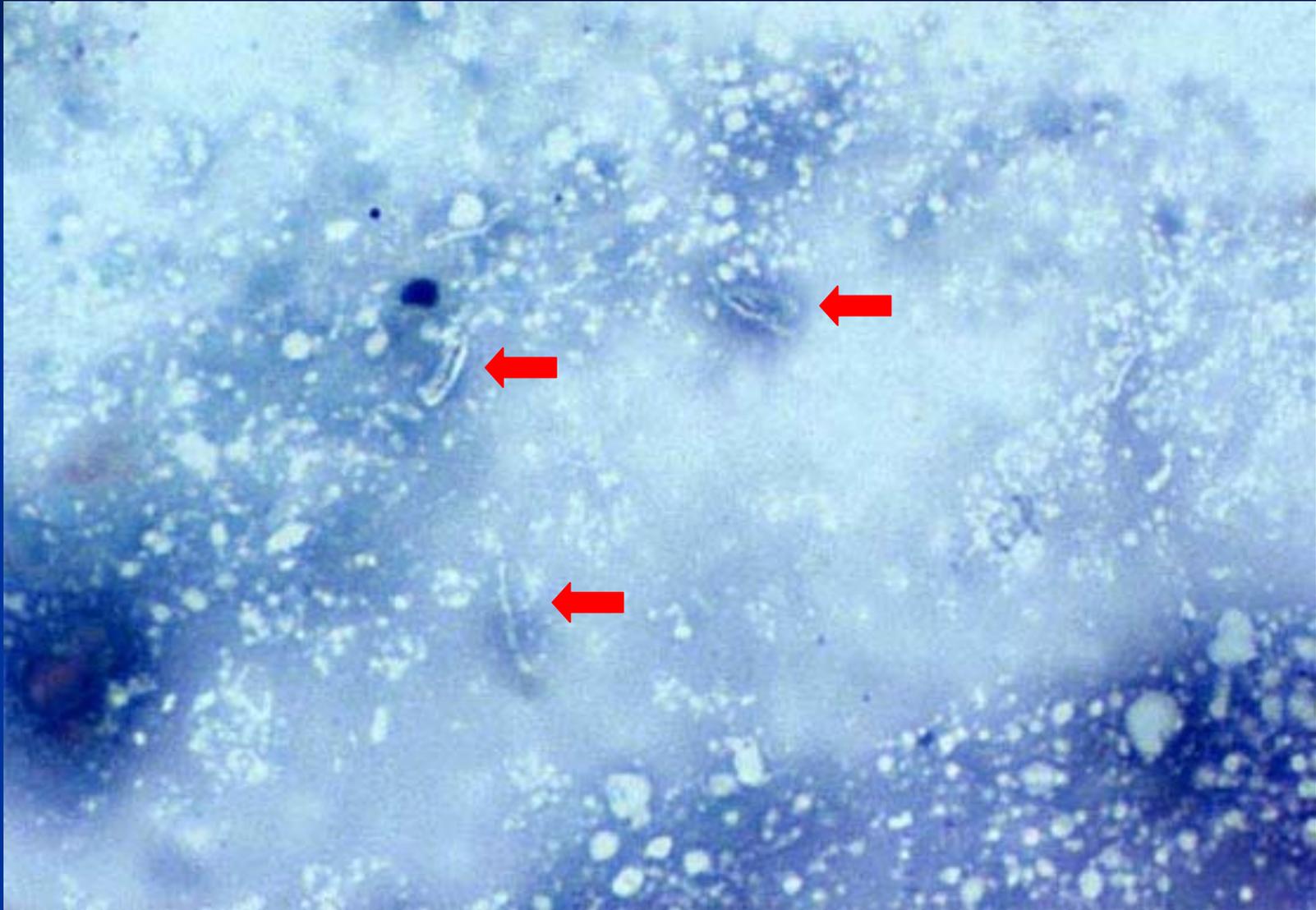
Correct



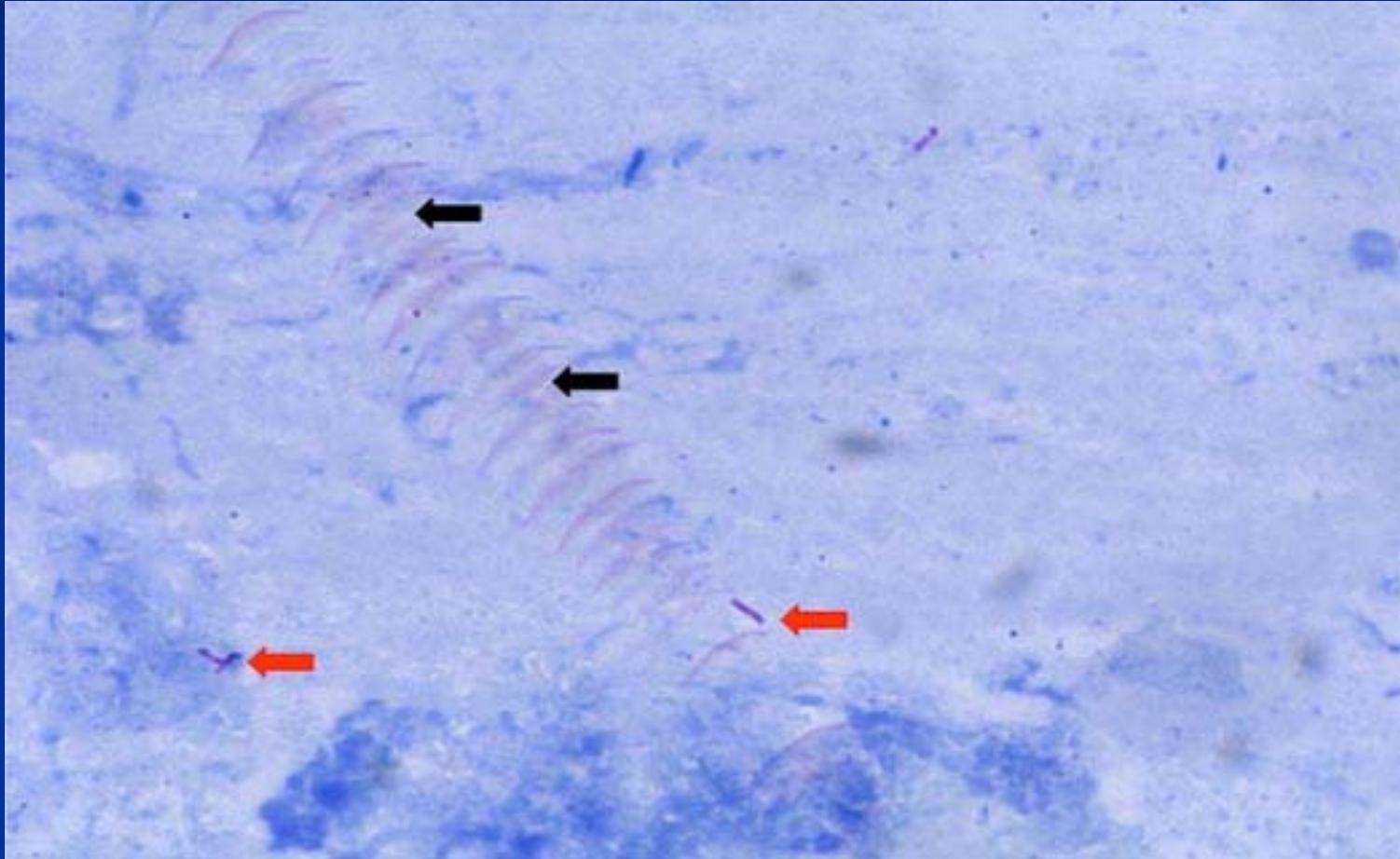
Troubleshooting and Miscellaneous

- Excessive heat-fixing of smear
- Glass scratches and AFB
- Soot on underside of smear

Excessive Heat Fixing of Smear



Glass Slide Scratch and AFB



Soot Deposit on Underside of Smear



Summary

- **Errors in smear preparation and staining contribute to false-negative and false-positive results**
- **Every step of the protocol needs to be performed to your best possible level**