


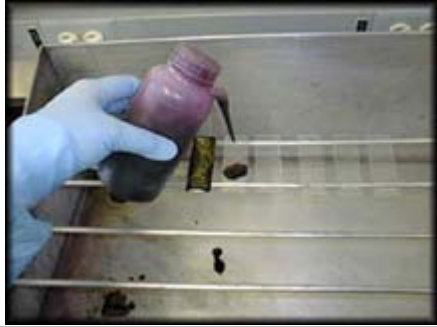





# Ziehl-Neelsen Staining Procedure

## Method Stepwise

When you see this icon  click on it to view what the slide would appear like if that step of the staining procedure was not performed correctly.

<b>STEP 1:</b>  Flame slides to heat fix	
<b>STEP 2:</b>  Flood the entire slide with <a href="#">Carbol Fuchsin</a>  Ensure enough stain is added to keep the slides covered throughout the entire staining step.  	 
<b>STEP 3:</b>  Using a Bunsen burner, heat the slides slowly until they are steaming. Maintain steaming for 5 minutes by using low or intermittent heat (i.e. by occasionally passing the flame from the Bunsen burner over the slides)  Caution: Using too much flame or heat can cause the slide to break.  	

**STEP 4:**

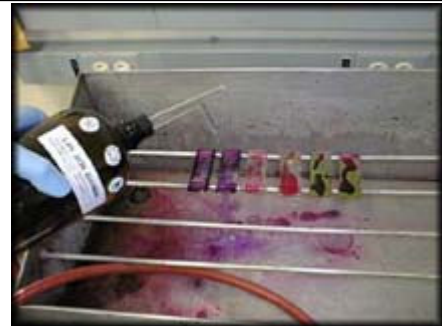
Rinse the slide with water.

**STEP 5:**

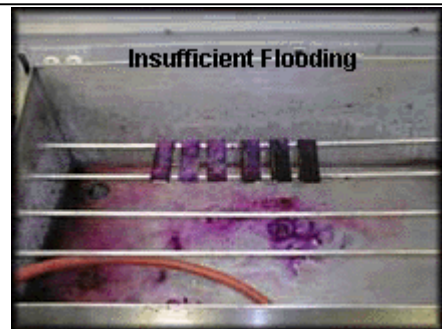
Flood the slide with [3% acid-alcohol](#) and allow to decolorize for 5 minutes.

Throughout the 5 minutes, continue to flood the slides with 3% acid-alcohol until the slides are clear of stain visible to the naked eye.

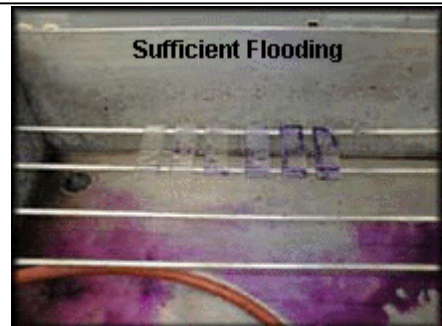
To the right are examples of slides insufficiently and sufficiently flooded with 3% acid-alcohol.



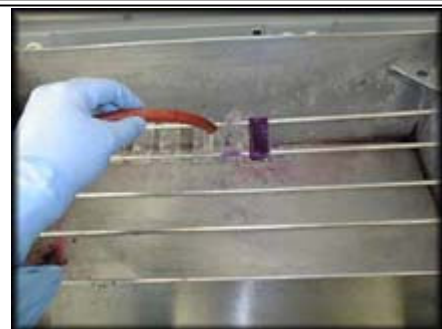
Insufficient Flooding



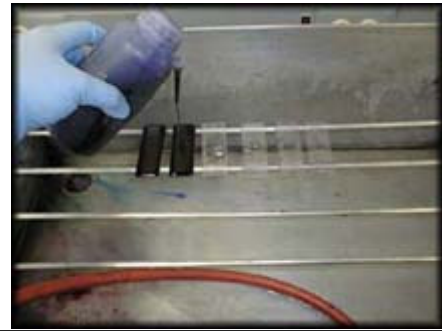
Sufficient Flooding

**STEP 6:**

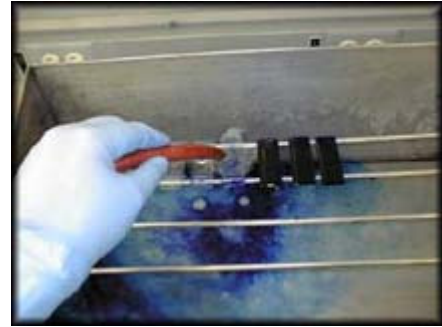
Rinse the slide thoroughly with water and then drain any excess from the slides.

**STEP 7:**

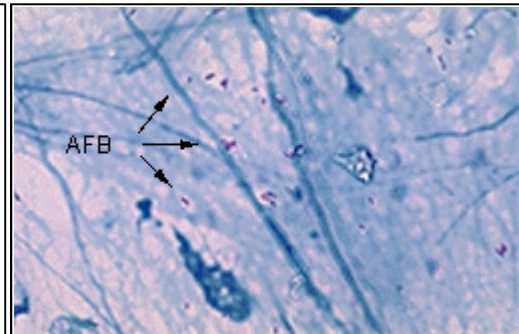
Flood the slide with the counterstain, [Methylene Blue](#). Keep the counterstain on the slides for 1 minute.

**STEP 8:**

Rinse the slide thoroughly with water.



If all steps are performed correctly you should have a slide that looks like this.



## Quality Control Parameters

A [positive and negative control slide](#) should be included with each run of stains. This will verify the correct performance of the procedure as well as the staining intensity of the acid-fast organisms.

Control slides should be reviewed before patient smears are read to confirm that the mycobacteria stain acid-fast. If the results of the QC slides are acceptable, go on to the patient smears. If, however, the control slide(s) are unacceptable, review procedures and reagent preparations. When the problem has been identified and corrected, remake and stain all of the patient's slides from the problem run along with a new set of controls.

Negative Control		Positive Control	
Acceptable Results	Unacceptable Results	Acceptable Results	Unacceptable Results
Only blue	Remains red after decolorization	Red bacilli against a	Bacilli are not stained red

background	Background is not properly decolorized	blue background	Background is not properly decolorized
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