



# *Wisconsin Veterinary Diagnostic Laboratory UNCONTROLLED Document*

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<i>Number</i>	PZNACIDFASTST
<i>Title</i>	Acid-fast bacteria on prepared formalin-fixed tissue slide by Ziehl-Neelsen staining method
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# Wisconsin Veterinary Diagnostic Laboratory

## Standard Operating Procedure

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### 1 Introduction

The Ziehl-Neelsen stain is used to determine the presence of acid-fast bacteria including mycobacteria in tissue.

### 2 Specimen submission

#### 2.1 Type

Paraffin embedded tissue block

#### 2.2 Special requirements for collection - NA

#### 2.3 Handling conditions - NA

#### 2.4 Criteria for rejection of sample - NA

### 3 Materials

#### 3.1 Equipment & Instrumentation

1. Automatic stainer
2. Automatic coverslipper
3. Stir plate
4. Balance
5. Fume hood
6. Shallow plastic tub
7. Slide racks and slide rack holders

#### 3.2 Reagents & Media

1. Ziehl-Neelsen carbol-fuchsin solution
2. 1% Acid alcohol solution
3. Methylene blue stock solution
4. Methylene blue working solution
5. 95% and 100% ethyl alcohol
6. Xylene
7. RO/DI H<sub>2</sub>O

#### 3.3 Supplies

1. Standard microscope slides - Blue
2. Charged microscope slides - Yellow
3. Disposable Pasteur pipettes
4. Pipette bulbs
5. Filter paper
6. 5 ml disposable serological pipettes

### 4 Safety Management

#### 4.1 Required Safety Training:

- Chemical fume hood operation
- SDS/Hazard communication

#### 4.2 Required personal protective equipment (PPE):

- Minimum:  Lab coat, Safety glasses and closed toe shoes– upon entry of lab  
Gloves:  Nitrile  Chemical resistant when immersing fingers in xylene  
Eyewear:  Splash Goggles or face shield-when pouring liquid

#### 4.3 Hazard Communication

##### A. Chemical: See MSDS

- Ethanol 100% & 95%** (MSDS-260 and 261)
  - Flammable liquid and vapor
- Xylene** (MSDS-574)
  - Flammable liquid and vapor. Possible cancer hazard. May cause cancer based on animal data. Harmful if absorbed through skin or if inhaled. Causes eye, skin, and respiratory tract irritation. Inhalation may cause central nervous system effects. Aspiration hazard if swallowed - can enter lungs and cause damage.
- Acid alcohol 1%**
  - **Hydrochloric acid 35 to 38%** (MSDS-826)
  - Causes burns by all exposure routes. May be harmful if inhaled.

##### B. Biological: Biosafety Level 2

#### 4.4 Disposal of waste

- Xylene is discarded in a designated waste xylene carboy and recycled using the in-house xylene recycler.
- Ethanol, ethanol contaminated with xylene, histological stains, heavy metal stains, acids and the xylene / peanut oil mixture must be dumped into a designated waste carboy. Document the type and amount of each solution on the Chemical Inventory Analysis For Waste Solvents In Carboys. Once the carboy is full, transport the carboy with the completed documentation to room 1101 for safe storage until it can be picked up by UW-Safety.
- All broken glassware and/or slides are disposed in the broken glass box located in Room 1124.

### 5 Preparation for procedure

#### 5.1 Equipment and instrumentation preparation

Turn on automatic stainer and coverslipper.

#### 5.2 Reagents and media preparation – See Section 12.1

#### 5.3 Standards/controls preparation

1. Use a validated AFB control slide cut at 5  $\mu\text{m}$  (PSECTIONING).

#### 5.4 Specimen preparation

1. Cut the diagnostic paraffin sections at 5  $\mu\text{m}$  (PSECTIONING).

### 6 Performance of procedure

*The following steps are applied to both the control and diagnostic slides.*

1. Deparaffinize and hydrate the slides using the H<sub>2</sub>O program on the automatic stainer.

2. Flood slides with freshly filtered carbol-fuchsin in the fume hood for 30 minutes.
3. Rinse the slides well in running tap water for 2-5 minutes.
4. After rinsing, decolorize the tissues by dipping one slide at a time in a glass container filled with 1% acid alcohol.
  1. Continue to dip until the carbol fuchsin no longer runs off the slide.
5. After each slide has been decolorized in the acid alcohol, stand it in the tub of running water until all slides are finished.
6. Apply the counterstain
  - a. Dip one slide at a time in a Coplin jar filled with the methylene blue working solution for 30 seconds.
  - b. Remove the slide from the methylene blue and quickly dip it 5-10 times in the running tap water tub.
  - c. Place the slide in a slide rack.
  - d. Repeat steps a-c until all slides have been counterstained.
9. Dehydrate and clear the slides using the "specials kwik" program on the automatic stainer or manually dehydrate:
  - a. Station 12 (95% alcohol)
  - b. Stations 13, 19-18 (100% alcohol)
  - c. Stations 17-14 (xylene)
10. Remove the slides from xylene and coverslip (PGLASCOVERSLIP).
11. Dump the unused, filtered, carbol-fuchsin back in the original container .
12. Clean all glassware. Glassware that has been used with the carbol- fuchsin must first be rinsed with 1% acid alcohol before rinsing in water.

## 7 Interpretation of results

1. Use the microscope to check the control slide, (See Section 12.1 for interpretation guidelines).
2. If the control slide stained properly, the stain can be considered valid and the pathologist can determine the results of the specimen slide.
3. If the control slide does not stain properly, a RAW-non-conformance must be created in QPulse.

## 8 Report of results

1. Record the completion of the stain in the [Histology Database](#).
2. Place slide in a flat and place in the outbox of the appropriate Pathologist.

## 9 Procedure notes

### 9.1 Details and helpful hints

1. Take care not to rinse out too much of the counterstain (methylene blue working solution) in tap water, or the background will be understained.

### 9.2 Limitations of procedure - NA

## 10 References

1. Carson FL. *Histotechnology: a self-instructional text*. 2nd ed. ASCP Press: Chicago 1997;180-187.
2. Prophet EB, et al. *Laboratory methods in histotechnology*. American Registry of Pathology, Armed Forces Institute of Pathology: Washington, DC 1992;219.

## 11 Summary of Current Revisions

1. General formatting changes.
2. Updated Safety section.
3. Section 5.2: Moved reagent preparation to Section 12.1.

## 12 Supplemental Information

### 12.1 Quick Procedure Reference – Acid Fast ZN

#### ❖ Reagent Preparation

##### Methylene blue stock solution

- Methylene blue.....1.4 gm
- 95% ethyl alcohol.....100 ml

*Stability:* Store in a sealed container at room temperature for 1 year.

##### Methylene blue working solution

- Methylene blue stock solution.....5.0 ml
- Distilled water.....45.0 ml

*Stability:* Store in sealed Coplin jar at room temperature for 6 months.

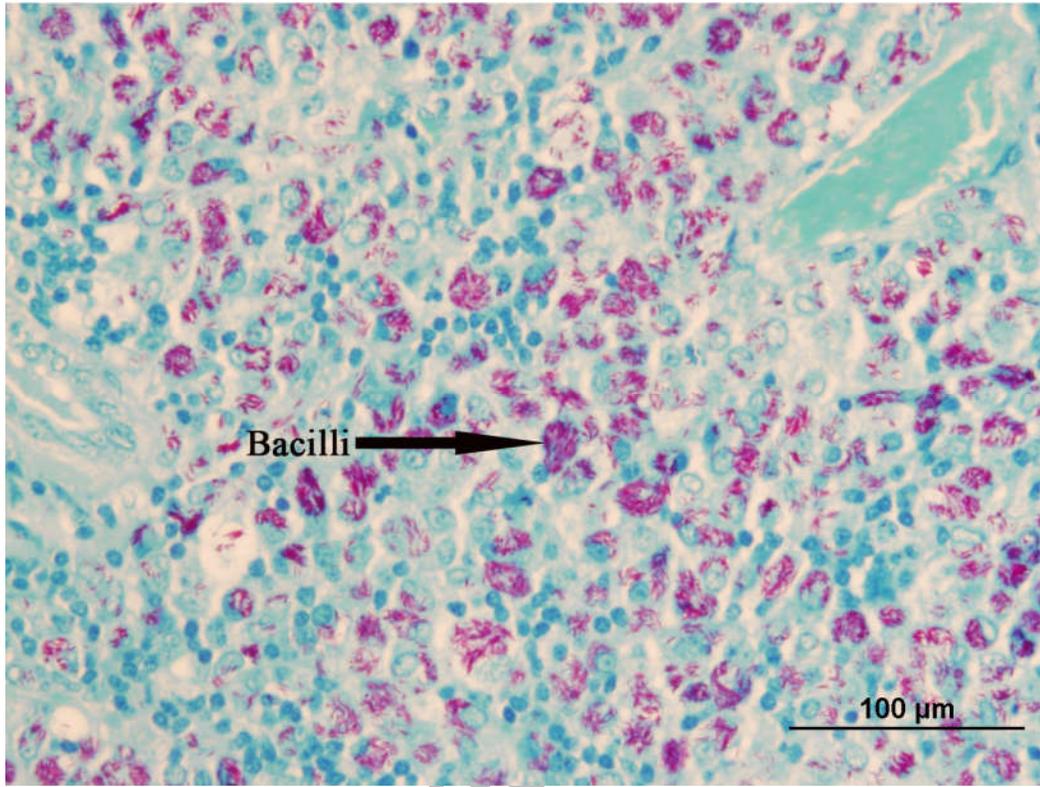
#### ❖ Ziehl-Neelsen Acid Fast Staining Method

1. Deparaffinize and hydrate the slides using the H2O program on the automatic stainer.
2. {In a Fume Hood} Flood slides with freshly filtered **carbol-fuchsin** for 30 minutes.
3. Rinse in running tap water for 2 – 5 minutes.
4. Decolorize with **1% acid alcohol** until the carbol fuchsin no longer runs off the slide.
5. Rinse in running tap water for 2 – 5 minutes.
6. Place slides in a coplin jar filled with **methylene blue working solution** for 30 seconds.
7. Dip 8-10 times in running tap water.
8. Dehydrate and clear using the “specials kwik” program on the automatic stainer
9. Coverslip (PGLASCOVERSLIP).
10. Clean all glassware.

##### Interpretation of Results

- Use the microscope to check the control slide.
- When stained properly, the control tissue will look as follows:

Acid-fast bacilli.....Bright red  
Erythrocytes.....Yellow orange  
Other tissue elements.....Blue



12.2 Flow Diagram - NA

12.3 Manufacturer's Information - NA