Warthin-Starry Stain for Spirochetes in Tissue

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I had recently been involved in a computer group discussion about the Warthin-Starry stain. No one seems to like to do it, except me. Here is a procedure that my histology laboratory (The Toledo Hospital, Toledo, Ohio) received from Lee Luna, who was a consultant to our laboratory, just before his death. Any "tips-of-the-trade" I have added.

A. Principle

The Warthin-Starry technique is an argyrophilic stain. This means that certain tissue elements will absorb silver from a silver solution, but need a separate reducing agent solution to reduce the absorbed silver to a visible metallic state. The pH and heat applied affect the staining intensity. Pyrocatechol, not hydroquinone, is used in the developing solution, as pyrocatechol allows silver to reduce to a metallic state faster. Due to the small size of the organisms, it is recommended that an oil immersion lens be used to review the slides.

B. Clinical Significance

The Warthin-Starry method is used primarily to demonstrate spirochetes (spiral-shaped bacteria) in tissue. Several varieties are pathogenic to humans. These include *Treponema pallidum* (syphilis), *Treponema pertenue* (yaws), *Treponema careteum* (pinta), *Borrelia* and *Leptospira.*

Other organisms that may be detected by Warthin-Starry are Donovan bodies, legionnaires bacteria, campylobacter (*H. pylori*), nocardia, klebsiella & the bacteria that cause Lymes Disease & Cat Scratch Fever.

Fusoform bacteria are demonstrated exceptionally well by this technique. Fusoform bacterium (*Fusobacterium*) is an important cause of chorioamnionitis & prematurity.

C. Specimen

Paraffin-blocked tissue is cut at 5 µm and sections are put on glass microscope slides.

D. Reagents

1. Chemicals
   a. Deionized (minimum Type II) water
   b. Tap water
   c. Xylene
   d. 100% denatured ethanol (USP grade)
   e. Citric acid (granular; certified ACS; Fisher A104)
   f. Silver nitrate (Fisher S181)
   g. Gelatin (purified grade; Fisher G7)
   h. Catechol (Pyrocatechol; SigmaC-9510)
2. Working Solutions
   a. 95% denatured ethanol:
      100% denatured ethanol: 950 mL
      Deionized (minimum Type II) water: 50 mL
   b. 1% Citric acid solution:
      Citric acid: 1 g
      Deionized (minimum Type II) water : 100 mL
   c. Acidulated water:
      Deionized (minimum Type II) water : 500 mL
      Add 1% citric acid solution, drop by drop, to bring pH to 4.0.
      (The Acidulated water can be made up ahead of time as stock solution. At least 200 mL is needed for this procedure.)
   d. 1% & 2% Silver Nitrate solutions. *Make fresh each time and use acid-cleaned glassware!*
      Silver nitrate crystals: 0.8 gram
      Acidulated water: 40 mL
      Withdraw 10 mL of the silver nitrate solution.
      This is the 2% silver nitrate solution.
      To the 30 mL left, add 30 mL of acidulated water.
      This is the 1% silver nitrate solution.

e. 5% Gelatin solution. *Make fresh each time!*
Gelatin 1.0 gram
Acidulated water: 20 mL

f. 0.15% Pyrocatechol (catechol). *Make fresh each time!*
Catechol: 0.15 gram
Acidulated water: 100 mL

g. Developing Solution. *Wait to mix until directed to by the procedure and use acid cleaned glassware . Combine in this order:*
2% silver nitrate solution: 1.2 mL or 2.4 mL
5% gelatin solution: 3.75 mL or 7.5 mL
0.15% pyrocatechol : 2.0 mL or 4.0 mL

(Note: How much developing solution is needed depends upon how many slides there are. I usually use the 2nd set of amounts or double that if I have a lot of slides. No matter which you use, you have enough of all the needed reagents.)

E. Equipment

- Besides the obvious to make up the reagents,
- Coplin jars
- Water baths set at 43°C and 58°C, deep enough to cover the Coplin jars to the rim. For the water baths, I use old paraffin heaters from a tissue processor that we threw out. I had them reset to the proper temperatures.)
- pH meter
- incubator, 56-58°C (optional)

F. Quality control

A known spirochete (or fusoform bacteria) positive-staining control is stained at the same time as the case slides. Each case has its own control slide. Slides are reviewed by the histologist to ensure that the expected results on the positive control slide have occurred.

G. Procedure

1. Fill water baths with water to allow them to get to the proper temperatures
2. Deparaffinize & hydrate slides.(Xylene, 100% ethanol, 95% ethanol, water)
3. Make up silver nitrate solutions at this time. Also make up the gelatin & catechol solutions. These two solutions are to be prewarmed in the 58°C waterbath or in an incubator (56-58°C). (Either way works to prewarm these two solutions.)
   Leave the 2% silver nitrate solution at room temperature.
   Do not combine the final developing solution until the 30 minute incubation is completed.
4. Put slides in an acid-cleaned Coplin jar containing 1% silver nitrate solution. Place Coplin jar in preheated 43°C water bath for 30 minutes.
5. Prepare Developing Solution near the end of the 30 minute incubation.
6. Place 2 GLASS rods or pipettes over the 58°C water bath to form a staining rack. Use tape to secure them if necessary.
   The water level should be as high as possible. (I have found that out the hard way. It takes the reaction longer & you get more background if the water is too low.)
   This step can be tricky if you use this water bath for the reagents, but just wait until taking out the reagents before placing the pipettes.
7. Make up the developing solution.
   Place the slides on the 'rack' & flood with the developing solution.
   Allow slides to develop until they are a light brown or yellow, about 5 minutes.
8. Wash slides quickly & thoroughly in hot tap water, approximately 56°C.
9. Rinse in deionized (minimum Type II) water.
10. Dehydrate in 95% & 100% ethanols.
11. Clear in xylene.
12. Mount with permanent mounting media.

H. Results

Use oil immersion lens on microscope.
Spirochetes, Donovan bodies, borrelia, leptospira, legionnaires, campylobacter, nocardia, klebsiella, Lymes, cat scratch, and fusoform bacteria black/gray
Background: pale yellow or lightbrown

1. Personal communication from Lee Luna.
   His references:
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