Diagnostic BioSystems

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Warthin-Starry Stain Kit

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Intended Use

For In Vitro Diagnostic Use

Summary and Explanation

The Warthin-Starry Stain Kit is intended for use in the visualization of Spirochetes, Helicobacter pylori, Legionella pneumophila, and Cat Scratch Fever bacteria. This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Helicobacter pylori: Black
Legionella pneumophila: Black
Spirochetes: Black
Cat Scratch Fever Bacteria: Black
Klebsiella: Brown/Black
Nuclei: Brown
Background: Yellow

Control Tissue

Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Reagents Provided

Kit Contents	Volume	Storage
SpiroPrep	125 mL	15-30°C
Gelatin (4%), Acidulated	125 mL	2-8°C
Silver Nitrate Solution (0.5%) Acidulated	125 mL	2-8°C
Hydroquinone Solution (0.1%) Acidulated	2x30 mL	2-8°C
Silver Nitrate Solution (0.2%)	125 mL	2-8°C
Silver Nitrate Solution (2%), Acidulated	30 mL	2-8°C

Storage and Handling

Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Prepare the Following Solutions Before Use

Warm Gelatin (4%), Acidulated to liquefy prior to beginning.
Prepare Reducing Solution in an unused plastic Slide Jar.
Combine:

i. 12.5 mL Gelatin (4%) Acidulated.
ii. 20-30 Drops Silver Nitrate Solution (2%) Acidulated.
iii. 7.5 ml Hydroquinone Solution (0.1%) Acidulated.

Staining Procedure

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. For spirochete staining only, place slide in room temperature SpiroPrep for 5 minutes (Omit this step for other listed bacteria). **Note:** Use of SpiroPrep will not affect staining of Helicobacter pylori, Legionella pneumophila, or Cat Scratch Fever bacteria.
3. Pour 20ml of Silver Nitrate Solution (0.5%), Acidulated in an unused plastic staining jar and place in a water bath at 65-70°C for 5 minutes.
4. Place slide in warmed Silver Nitrate Solution (0.5%), Acidulated and incubate for 3-5 minutes with repeated agitation.
5. Transfer slide into previously prepared Reducing Solution and agitate. Place staining jar in a water bath at 65-70°C with frequent agitation until tissue section is brown (approximately 1-5 minutes).
6. Rinse slide carefully in hot tap water for 2 minutes.
7. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
8. Clear and mount in synthetic resin.

Rapid Procedure (Microwave Oven)

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. For spirochete staining only, place slide in room temperature SpiroPrep for 5 minutes (Omit this step for other listed bacteria). **Note:** Use of SpiroPrep will not affect staining of Helicobacter pylori, Legionella pneumophila, or Cat Scratch Fever bacteria.
3. Pour 20ml of Silver Nitrate Solution (0.5%), Acidulated in an unused plastic staining jar and microwave at full power for 30 seconds. Invert several times to equalize temperature.
4. Place slide in warmed Silver Nitrate Solution (0.5%), Acidulated and incubate for 5 minutes with repeated agitation.
5. Warm previously prepared Reducing Solution in microwave at full power for 20 seconds. Invert several times to equalize temperature.
6. Transfer slide into warmed Reducing Solution and with frequent agitation until tissue section is brown (approximately 2-5 minutes).
7. Rinse slide carefully in hot tap water for 2 minutes.
8. Dehydrate slide through 3 changes of fresh Absolute Alcohol.
9. Clear and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

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Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

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