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Steiner Stain Kit (For Spirochetes)

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The Steiner Stain Kit (For Spirochetes) is designed for demonstrating Fungi, Helicobacter Pylori, Legionella pneumophila, and Spirochete infected tissue. Kit may be used on formalin fixed, paraffin-embedded tissue as well as frozen sections.

Spirochetes: Black to Brown
Helicobactor Pylori: Black to Brown
Fungi: Black to Brown
Legionella pneumophila Black to Brown
Background: Yellow to Tan

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.

Recommended Positive Control

1. Helicobacter Pylori infected stomach

Reagents Provided

Kit Contents	Volume	Storage
Oxidizer Solution	125 mL	Room Temperature
Zinc Formalin Solution	125 mL	Room Temperature
Gum Mastic Solution	125 mL	2-8°C
Hydroquinone	1.5 Gram	Room Temperature
Silver Nitrate Solution (0.2%)	125 mL	2-8°C
Silver Nitrate Solution (1%)	9 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

1. Combine:
   i. 25mL 1% Hydroquinone (0.25 gram Hydroquinone in 25 mL Distilled Water)
   ii. 15 mL Gum Mastic Solution (2.5%).
   iii. Mix thoroughly and filter through medium filter paper.
2. Add 6 Drops (240 µL) Silver Nitrate Solution (1%) & Mix thoroughly.

Staining Procedure

1. Preheat Water Bath to 70° Centigrade.
2. Deparaffinize sections if necessary and hydrate to distilled water.
3. Incubate slide in Oxidizer Solution for 20 minutes.
4. Rinse thoroughly in distilled water.
   Note: Place 20 ml of Silver Nitrate Solution (0.2%) in water bath to preheat.
5. Incubate slide in Zinc Formalin Solution for 5 minutes.
6. Rinse thoroughly in distilled water.
7. Incubate slide in preheated Silver Nitrate Solution (0.2%) for 5 minutes at 70° Centigrade. (Note: Discard solution after this step)
8. Rinse slide thoroughly in distilled water.
9. Dehydrate slide in 2 changes of Absolute Alcohol.
   Note: Prepare Reducing Solution (above) and place in water bath to preheat.
10. Incubate slide in Gum Mastic Solution for 3 minutes.
11. Air dry slide for 1 minute or until gum mastic is completely dry.
12. Incubate slide in preheated Reducing Solution for 10-15 minutes or until section is tan to brown at 70° Centigrade. (Note: Discard solution after this step)
13. Rinse slide quickly in distilled water.
14. Dehydrate quickly in 3 changes of absolute alcohol.
15. Clear, and mount in synthetic resin.

Staining Procedure (Microwave)

1. Combine:
   i. 25ml 1% Hydroquinone (0.25gm Hydroquinone in 25ml Distilled Water)
   ii. 15ml Gum Mastic Solution (2.5%)
   iii. Mix thoroughly and filter through medium filter paper.
2. Add 6 Drops (240µl) Silver Nitrate Solution (1%). Mix thoroughly.
3. Deparaffinize sections if necessary and hydrate to distilled water.
4. Incubate slide in Oxidizer Solution for 20 minutes.
5. Rinse thoroughly in distilled water.
6. Incubate slide in Zinc Formalin Solution for 5 minutes.
7. Rinse thoroughly in distilled water.
   Note: In a loosely capped Slide Jar heat 20ml of Silver Nitrate Solution (0.2%) in a microwave oven for 10 seconds at full power. Repeat as needed until solution is hot, but do not allow solution to boil. Remove Slide Jar from microwave, tighten cap and agitate to equalize temperature.
8. Incubate slide in hot Silver Nitrate Solution (0.2%) for 2 minutes with occasional agitation. (Note: Discard solution after this step)
9. Rinse slide thoroughly in distilled water.
10. Dehydrate slide in 2 changes of Absolute Alcohol.
11. Incubate slide in Gum Mastic Solution for 3 minutes.
    Note: Prepare Reducing Solution (above).
12. Air dry slide for 1 minute or until gum mastic is completely dry.
13. In a loosely capped Slide Jar heat 20ml of Reducing Solution in a microwave oven for 10 seconds at full power.
14. Repeat as needed until solution is hot, but do not allow solution to boil. Remove Slide Jar from microwave, tighten cap and agitate to equalize temperature.
15. Place slide in loosely capped Slide Jar and return to microwave. As before heat Slide Jar containing slide for 10 seconds at full power. Repeat as needed until solution is hot, but do not allow solution to boil.

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16. Incubate slide in hot Reducing Solution for 3 minutes and then reheat again at full power until solution is hot.
17. Incubate slide for an additional 2-3 minutes or until section is tan to brown. (Note: Discard solution after this step)
18. Rinse slide quickly in distilled water.
19. Dehydrate quickly in 3 changes of absolute alcohol.
20. 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.

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 burns. 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.

References

I. Leung, K., Gibbon, K.J. A Rapid Staining Method for Helicobacter Pylori in Gastric Biopsies, Journal of Histochemistry, Volume 19, Pages 131-132. 1996

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