

Beaumont

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Histology Special Stain - Diff-Quik - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide a procedure for the demonstration of *Helicobacter pylori*, which is present in many gastric biopsy specimens with active gastritis. It can also be used to non-differentially stain bacteria and for the demonstration of some parasites

II. PRINCIPLE:

Diff-Quik is a modified Giemsa/ Romanowsky stain. Diff-Quik solution I contains eosin, which gives a pink color to cytoplasm. Diff-Quik solution II contains Azure A and Methylene Blue. The methylene blue is an impure dye, and will oxidize into azure A, azure B, and methylene violet, thereby giving a wider range of colors. Both solutions contain buffers, which establish the best pH for staining.

III. SPECIMEN COLLECTION AND HANDLING:

- A. Fixation
 - 1. Formalin fixed tissues preferred.
- B. Processing
 - 1. Standard, overnight processing.
- C. Section Thickness
 - 1. Routine specimens-5μ.
- D. Slide Drying
 - 1. 60 minutes at 60°C.

- E. Type of Slide
 - 1. Plain slides

IV. REAGENTS:

- A. **Diff-Quik Fixative**
Purchased Pre-Made
- B. **Diff-Quik Solution 1**
Purchased Pre-Made
- C. **Diff-Quik Solution 2**
Purchased Pre-Made

- D. **Acid-Alcohol Solution**

95% ALCOHOL	368.0 mL
Distilled water	132.0 mL
Acetic acid (HCOOH)	1.25 mL

Mix together alcohol and water. Slowly add, drop by drop, acetic acid to solution. Store at room temperature in a plastic or glass container; good for several months.

V. EQUIPMENT:

- A. Balance

VI. SUPPLIES:

- A. Erlenmeyer flasks
- B. Graduated cylinders
- C. Coplin jars
- D. Forceps

VII. QUALITY CONTROL:

- A. Gastric tissue with *H. pylori* preferred.
- B. Any tissue with small, gram-negative bacteria may also be used, such as *E. coli*

VIII. SPECIAL SAFETY PRECAUTIONS:

- A. Diff-Quik Fixative
 - 1. Contains methanol which is poisonous.
 - 2. May be fatal or cause blindness if swallowed.
 - 3. Cannot be made nonpoisonous.
 - 4. Liquid and vapor are flammable.
 - 5. Harmful if inhaled or absorbed through skin.

6. May cause skin and eye irritation.
- B. Diff- Quik Solution 1
1. This solution is not considered a hazard.
- C. Diff-Quik Solution 2
1. This solution is not considered a hazard.
- D. Acetic Acid
1. Is an acid.
 2. Add drop by drop to solution.
 3. May cause skin/eye burns.

IX. PROCEDURE:

Step	Action	Time	Notes
1	Deparaffinize and hydrate slides through graded alcohol to distilled water.		
2	Place slides into Diff-Quik Fixative Solution.	1 minute	If specimen is a frozen section, follow directions under procedural notes.
3	Place slides directly into Diff-Quik Solution 1.	2 minutes	
4	Place slides directly into Diff-Quik Solution 2.	4 minutes	
5	Rinse quickly in 2-3 changes of distilled water.	1-2 seconds each	
6	Dip quickly in the acid-alcohol solution.	1-2 seconds	Steps 6 and seven can be eliminated with FS and touch preps.
7	Rinse in distilled water, 2-4 changes.	5 seconds each	Use a microscope to determine the quality of staining.
8	Allow slides to air dry completely.		
9	Coverslip.		

X. LIMITATIONS:

- A. The following may influence the validity of test results:
1. Tissues fixed in Hollandes may stain false negative, due to the Hollandes solution creating holes in the walls of the bacteria. Do not use this stain with Hollandes fixed tissues. If tissues have been fixed in Hollandes, use the Steiner and Steiner procedure.

2. If specimen is a frozen section: Use the following procedure:
 - a. Do not deparaffinize and dehydrate (Step #1)
 - b. Place in Diff-Quik Fixative solution (blue), 1-5 minutes
 - c. Skip step 6 and 7.

XI. RESULTS:

- A. Bacteria, including Helicobacter pylori - **deep-blue**
- B. Fungus - **deep-blue**
- C. Nuclei - **blue**
- D. Cytoplasm, collagen, muscle - **varying shades blue and pink**

XII. REFERENCES:

- A. Manufacturer's notes that come with Diff-Quik Solutions
- B. Ray Skipper and Don B. KeStephano: A Rapid Stain for Campylobacter pylori in gastrointestinal sections using Diff-Quik. J. Histotechnol 12:303-304, Dec. 1989

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak