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Papanicolaou Staining (Progressive)

Reagents Needed:

Hematoxylin Stain. Use one of the following:

Hematoxylin Stain, Modified Harris	RICCA CHEMICAL COMPANY Cat. No. 3530
Hematoxylin Stain, Gill 1 Formulation	RICCA CHEMICAL COMPANY Cat. No. 3535
Hematoxylin Stain, Gill 2 Formulation	RICCA CHEMICAL COMPANY Cat. No. 3536
Hematoxylin Stain, Gill 3 Formulation	RICCA CHEMICAL COMPANY Cat. No. 3537

OG Counterstain. Use one of the following:

Papanicolaou Stain, OG-6	RICCA CHEMICAL COMPANY Cat. No. 5510
Papanicolaou Stain, Gill's Modified OG-6	RICCA CHEMICAL COMPANY Cat. No. 5512

EA Counterstain. Use one of the following:

Papanicolaou Stain, EA-36-50	RICCA CHEMICAL COMPANY Cat. No. 5500
Papanicolaou Stain, EA-65	RICCA CHEMICAL COMPANY Cat. No. 5505
Papanicolaou Stain, Gill's Modified EA	RICCA CHEMICAL COMPANY Cat. No. 5511

Bluing Reagent

Scott's Bluing Reagent	RICCA CHEMICAL COMPANY Cat. No. 6697

Recommended Method:

1. Use fresh, unfixed specimens which can be spread out and flattened on a slide or collected on a membrane filter. Immediately wet-fix in 95% Ethanol (or 95% Reagent Alcohol, 95% Denatured Alcohol, or 80% Isopropyl Alcohol) for 15 minutes. Air drying before or after fixation is not recommended.
2. Dip in suitable tap water or purified water 2 times for 10 dips each.
3. Stain with Hematoxylin Stain (see below). Time required depends on Hematoxylin stain used and personal preferences for stain intensity and may vary slightly from lot to lot. Exact times may be determined by running pilot slides with new lots of stain and/or checking each batch of slides visually during processing at appropriate slide processing steps.
   Harris Hematoxylin Stain is the classical formulation and is suitable for general staining. For this staining procedure, add 4 mL of Glacial Acetic Acid to 100 mL of filtered Harris Hematoxylin Stain just before use. Staining time is approximately 45 seconds. Harris Hematoxylin is subject to precipitation as it ages. Filter before use.
   Gill 1 Hematoxylin Stain is recommended for lower intensity staining for delicate nuclei. Use full strength in this procedure. Staining time is approximately 2 minutes.
   Gill 2 Hematoxylin Stain is recommended for more intense staining of nuclei. Use full strength in this procedure. Staining time is approximately 2 minutes.
4. Dip in suitable tap water or purified water 2 times for 10 dips each.
5. Dip is Scott's Bluing Reagent for 1 minute.
6. Dip in suitable tap water or purified water 2 times for 10 dips each.
7. Dip in 95% Ethanol 2 times for 10 dips each.
8. Counterstain with Papanicolaou Stain, OG-6 or Gill's Modified OG-6, for 1 ½ - 2 minutes.
   Note: Addition of 10 mL of Glacial Acetic Acid per 990 mL of Gill's Modified OG-6 just before use reduces staining time to 15 seconds.
9. Dip 3 times for 10 dips each in 95% Ethanol.
10. Counterstain with Papanicolaou Stain, EA-36-50, EA-65, or Gill's Modified EA, for 8 - 10 minutes.
11. Dip 4 times for 20 to 30 dips each in 95% Ethanol.
12. Dip 3 times for 10 dips each in absolute Ethanol.
13. Dip 3 times for 10 dips each in Xylene.

Satisfactory Staining Results:

Chromatin is blue to blue-black.
Nucleoli are stained faintly so their redness can be seen when subsequently stained by Eosin Y.
Barr bodies are stained conspicuously.
Cytoplasmic staining is so slight that differentiation in acid is unnecessary.

Unsatisfactory Staining Results:

Overoxidized Hematoxylin stains chromatin an undesirable brown color. Gill Hematoxylins are slower to overoxidize than Harris Hematoxylin.

This is a typical staining procedure. These reagents may be suitable for other staining procedures. Consult staining reference books or standard operating procedures for other suitable uses of these products.