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Papanicolaou Stain for Cytological Preparations - Technical Memo

SOLUTIONS:	**500 ml**		**1 Gallon**	
Hematoxylin Stain, Gill I	Part 1180A		Part 1180C	
Hematoxylin Stain, Gill II	Part 1180D		Part 1180F	
Papanicolaou Stain, OG-6	Part 1330A		Part 1330B	
Papanicolaou Stain, EA-50, Fast Green	Part 1310A		Part 1310B	
Papanicolaou Stain, EA-65, Fast Green	Part 1320A		Part 1320B	
Papanicolaou Stain, EA-50, Light Green	Part 1312A		Part 1312B	
Additionally Needed:				
Alcohol, Ethyl Denatured, 95%	Part 10842			
Lithium Carbonate, Saturated Aqueous	Part 12215	or	Scott Tap Water Substitute	Part 1380
Alcohol, Ethyl Denatured, 70%	Part 10844			
Alcohol, Ethyl Denatured, 100%	Part 10841			
Xylene, ACS	Part 1445			

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Papanicolaou (Pap) Stain provides classic staining solutions for cytology preparations, allowing for crisp, distinct nuclear detail and differentially stained cytoplasm. Gill Hematoxylin is the optimal nuclear stain and the two counterstains, Orange Gelb (OG) and Eosin Azure (EA) provide the subtle range of green, blue, and pink hues to the cellular cytoplasm.

Papanicolaou Stain, EA, is comprised of a combination of two dyes: Eosin Y and Fast Green SF or Light Green. EA-50 and EA-65 denote the varying proportions of dyes in each solution. The stains are suitable for both Gyn (EA-50) and Non-Gyn (EA-50, EA-65) specimens. Formula of choice will depend upon staining preference.

METHOD:

Technique/Fixation: Preparation technique and choice of fixative is dependent on specimen types.
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

STAINING PROCEDURE:

1. Fix in 95% Ethyl Alcohol for 5-10 minutes.
   a. *Fixation times may vary depending on specimen type.*
2. Rinse in running distilled water for 1 minute.
3. Stain in Hematoxylin Stain, Gill I or Gill II for 2 to 6 minutes, depending on preference of nuclear stain intensity.
4. Wash in distilled water until clear.
5. Blue slides in Lithium Carbonate, Saturated Aqueous (12215) or Scott Tap Water Substitute (1380) for 30 seconds.
6. Wash in running distilled water for 30 seconds.
7. Dehydrate in 70% Ethyl Alcohol: 10 dips.
8. Dehydrate in 95% Ethyl Alcohol: 10 dips.
9. Stain in Papanicolaou Stain, OG-6 for 1-2 minutes, depending on specimen type and preferred stain intensity.
10. Rinse in two changes of 95% Ethyl Alcohol; 10 dips each.
11. Stain in Papanicolaou Stain, EA-50 or EA-65 for 3-5 minutes, depending on specimen type and preferred stain intensity.
12. Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Chromatin	Blue
Keratin	Orange
Squamous cells	Shades of pink
RBCs, nucleoli, cilia	Shades of pink
Cytoplasm	Shades of blue-green

PROCEDURE NOTES:

1. Pap stains can be implemented for either manual or automated staining. Timings may vary depending on staining platform used.
2. Solutions should be filtered or replaced daily to prevent cross-contamination and maintain optimal staining.
3. If using a xylene substitute, closely follow the manufacturer's recommendations for the clearing step.

REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 127-128.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 326-327.
3. Koss, Leopold G. *Diagnostic Cytology and Its Histopathologic Bases*. 3d ed. Philadelphia: Lippincott, 1979. 1218.
4. Modifications developed by Newcomer Supply Laboratory.