INTENDED USE

The Sigma-Aldrich Papanicolaou Staining system is intended for staining exfoliative cells in cytologic specimens. Papanicolaou staining reagents are for “In Vitro Diagnostic Use.”

Papanicolaou staining techniques, reviewed in a concise report by Street,1 have changed little in the past 40 years. The stain is used for examining exfoliative cells of sputum as well as vaginal, cervical, and other body secretions. In general, cell are fixed to a slide, treated with a hematoxylin nuclear stain and counterstained with a mixture of orange G, eosin Y and fast green FCF (a replacement for light green SF yellowish). These treatments impart characteristic color to nuclei and cytoplasmic components.2

REAGENTS

PAPANICOLAOU STAIN OG 6, Catalog No. HT40116/HT40132/HT40180/HT401128
Certified orange G, 0.3% w/v, phosphotungstic acid, 0.015% w/v, in denatured alcohol. PAPANICOLAOU STAIN MODIFIED EA, Catalog No. HT40232
Certified eosin Y, 0.25% w/v, certified fast green FCF, 0.004% w/v, phosphotungstic acid, 0.4% w/v, in denatured alcohol with stabilizer.

PAPANICOLAOU STAIN EA 50, Catalog No. HT40316/HT40332/HT40380/HT403128
Certified eosin Y, 0.23% w/v, certified fast green FCF, 0.08% w/v, certified bismarck brown, 0.05%, phosphotungstic acid, 0.2% w/v, in denatured alcohol.

PAPANICOLAOU STAIN EA 66, Catalog No. HT40432
Certified eosin Y, 0.23% w/v, certified fast green FCF, 0.01% w/v, certified bismarck brown, 0.05%, phosphotungstic acid, 0.2% w/v, in denatured alcohol.

STORAGE AND STABILITY:
Store reagents at room temperature (18-26°C). Reagents are stable until expiration dates shown on labels.

PREPARATION:
Differentiation Solution is ready to use.
Scott’s Tap Water Substitute is prepared by mixing 1 part of Scott’s Tap Water Substitute Concentrate with 9 parts deionized water.
Filter Papanicolaou Staining System reagents before use.

PRECAUTIONS:
Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet and product labeling for any updated risk, hazard or safety information.

PROCEDURE

SPECIMEN COLLECTION:
It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No known test method can offer complete assurance that blood samples or tissue will not transmit infection. Therefore, all blood derivatives or tissue specimens should be considered potentially infectious.
Smears are prepared and fixed immediately with a spray fixative or placed in 95% ethanol for a minimum of 15 minutes.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED:
Differentiation Solution, Catalog Nos. A3179 or A3429
Reagent Alcohol, Catalog No. R8382, or Ethanol (absolute)
Hematoxylin Nuclear Stain:
Harris Hematoxylin Solution, Modified, Catalog No. HHS16/ HHS32/ HHS80/ HHS128
Hematoxylin Solution Gill No. 1, Catalog No. GHS116/ GHS132/ GHS128
Hematoxylin Solution Gill No. 2, Catalog No. GHS216/ GHS232/ GHS290/ GHS2128
Scott’s Tap Water Substitute Concentrate, Catalog No. S5134
Prepare Scott’s Tap Water Substitute Solution as indicated on bottle label.

Xylene or xylene substitute.

NOTES:
1. Separate staining solutions should be maintained for gynecologic smears and nongynecologic smears.
2. All solutions preceding the hematoxylin solutions should be filtered after each staining sequence and all solutions should be filtered daily.
3. Cellulose membrane filters should be dehydrated (Procedure 1, step 14 and 15) in isopropyl alcohol to prevent dissolution.
4. Staining times may be varied for individual color preferences.
5. Ethanol may be substituted for Reagent Alcohol.
6. For cytospin preparations, slides coated with poly-L-lysine may prevent cell loss during staining (See Catalog Nos. P0425 or P0820).
7. Appropriate control slides should be included in each run.
8. The data obtained from this procedure serves only as an aid to diagnosis and should be reviewed in conjunction with other clinical diagnostic tests or information.

PROCEDURE:

PROCEDURE 1 (Standard Method)
1. Reagent Alcohol, 95%.................................................................15 minutes
2. Tap water..................................................................................rinse
3. Hematoxylin solution...............................................................1-3 minutes
   NOTE: Time will vary with selection of hematoxylin solution.
4. Tap water..................................................................................rinse

PROCEDURE 2 (Rapid Papanicolaou Stain)
Reagent Preparation: Prepare Papanicolaou Stain OG 6–EA Solution by mixing equal volumes of Papanicolaou Stain OG 6 and one of the EA stains.
1. Wash smears in running water.............................................1 minute
2. Stain in Hematoxylin Solution Gill No. 2.............................1-3 minutes
3. Tap water..................................................................................rinse
4. Scott’s Tap Water Substitute....................................................20 seconds
5. Reagent Alcohol, 95%.................................................................20 seconds
6. Rinse in two changes of Reagent Alcohol, 95%, 10 seconds
7. Clear in two changes of xylene or xylene substitute.
8. Coverslip and examine microscopically.

PERFORMANCE CHARACTERISTICS

Nuclei are stained blue while cytoplasm displays varying shades of blue, orange, pink or red. If observed results vary from expected results, please contact Sigma-Aldrich Technical Service for assistance.

REFERENCES

1. Street CM: Papanicolaou Techniques in Exfoliative Cytology. IN Laboratory Technique in Biology and Medicine, 3rd ed. EV Cowdry Editor, Williams & Wilkins, Baltimore, 1952, p 253
5. Schiffgraben 41
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