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Gill's Modified OG-6 and Gill's Modified EA

for Papanicolaou Staining

Description:
Gill's Modified OG-6 and Gill's Modified EA are the two cytoplasmic counterstain solutions that are used sequentially in the Papanicolaou staining method for clinical cytology.[1-3] Although not numbered, Gill's Modified EA is similar to EA-36 and the commercially numbered EA-50, but offers improved staining performance.

Summary of Method:
Cytomorphology reflects cellular health and disease. The cytoplasm indicates the functional differentiation of a cell and its nucleus signals normal or abnormal growth activity.[4] With the light microscope, interpreting the meaning of cytomorphologic patterns is done most conveniently with stained cells, for unstained cells are practically invisible. Biologic behavior of cells can be assessed qualitatively and quantitatively both in terms of growth activity and functional differentiation.

If properly stained with hematoxylin, the nuclei of cells are visible, though their cytoplasm would be poorly discernible and the likelihood of detecting and identifying abnormal cells during screening would be decreased. This is avoided by staining the cytoplasm of cells a contrasting color (i.e., counterstaining).

The use of a single counterstain would stain different cell types similarly and they would appear monotonous. When several counterstains are applied, different cell types then appear in different contrasting colors. Such a situation is referred to as differential counterstaining.[5] After well stained chromatin, differential counterstaining is a prime objective of the Papanicolaou stain and is achieved by the use of Gill's Modified OG-6 and Gill's Modified EA.

Limitations of Papanicolaou's Original OG-6 and EA Formulations:
Papanicolaou published his OG-6 and EA formulas three times in 18 years.[13] The formula for OG-6 remained the same throughout this period. The simplicity of the OG-6 stain allows good reproducibility, without applicable limitations.

EA is a complex stain with a complex history. In each of the three published descriptions of EA: (1) the quantities of the ingredients were changed, and in some cases not made clear, (2) lithium carbonate was included without demonstrable effect, and (3) two chemically incompatible ingredients (i.e., Bismarck brown Y and phosphotungstic acid) were used.[6] As a result, the proper formulation of EA is sufficiently obscure so that wide variations in performance are readily observed with unsatisfactory staining results being commonplace. The serious limitations in the formulation and performance of OG-6 and EA have gone uncorrected despite attempts by others to introduce modifications.[7-9]

Merits of Gill's Modified OG-6 and Gill's Modified EA:
Gill's Modified OG-6 and EA are based on pertinent chemical considerations. These counterstains are stable in solution and give predictable high quality staining results that heretofore have not been generally possible.

Chemical Principles of Gill's Modified OG-6 and Gill's Modified EA Formulations:
Gill's Modified OG-6 is an alcoholic solution of phosphotungstic acid and orange G. Phosphotungstic acid is a nonvolatile acid which acidifies the solution and increases the uptake of orange G, which is primarily a function of physical factors. Immediately following immersion in OG-6, the cytoplasm of all cells is stained by orange G. However, only physically dense materials retain the dye. Eosin and Fast Green FCF subsequently displace orange G from less dense cytoplasm, but they cannot dislodge it from inside the small submicroscopic spaces of material such as keratin.

Gill's Modified EA is an alcoholic solution of phosphotungstic acid, glacial acetic acid, Fast Green FCF and eosin Y. Phosphotungstic acid selectively excludes eosin from the cytoplasm of certain cell types and permits it to be stained by Fast Green FCF. Those sites remaining unstained by hematoxylin, orange G and Fast Green FCF are then stained by eosin. Acidification by acetic acid permits more intense eosin and Fast Green FCF staining.

Usage:
Gill's Modified OG-6 and Gill's Modified EA are used full strength. No mixing, dilution, or additions are required. If individuals prefer a weaker EA, they may dilute it with methyl or ethyl alcohol until the desired intensity of staining results is attained.

Filtration is not needed before use the first time. Filtration after every subsequent use is recommended to control cross-contamination.[10]

Specimen Preparation:
Well flattened cells are preferred for optimal staining and display. Fresh, unfixed cells are spread ideally as a monolayer on a slide, or collected on a membrane filter, and immediately wet-fixed in 95% ethyl alcohol.[11]

Non-flattened cells may take up sufficiently more stain and hinder microscopic examination. Non-flattening in cells may be attributable to the following: (1) collection in preservative (i.e., an equal volume of 50 to 70% alcohol), (2) spreading on albuminized slides, (3) thick spreads, (4) part of a tissue fragment, (5) suspension in a mucus stream, and (6) spray fixation. The foregoing circumstances are linked in that the cells are virtually in suspension at the time of fixation, and thus become hardened spheres that cannot flatten.

If ethyl alcohol is unavailable, one of the following alternatives is recommended: (1) absolute methyl alcohol (not for Millipore filters[11]), (2) 95% reagent grade alcohol,[11,12] or denatured alcohol, (3) 80% isopropyl alcohol[12], and (4) 90% acetone[13] (not for Millipore filters). Commercial fixatives or Saccomanno's preservative may also be used.[14]

Cell spreads that have been air-dried following fixation by commercial spray fixatives or collection in Saccomanno's preservative are coated with a water soluble wax. Such air-dried cells become more closely textured submicroscopically. Unless the wax is removed prior to staining, and the staining times in hematoxylin and Gill's Modified EA are lengthened, poor nuclear staining and differential counterstaining are likely to result.[15] (See Polysciences' Gold Standard Series Routine Histology and Cytology stains and Reagent Protocol, #70013.)

Unless cells are preserved or fixed and will be protected by a wax coating, cells should always be kept wet. Cells unprotected by wax should be air dried before or after fixation.[16, 17]

Notes:
(a) No distinction is made between gynecologic and non-gynecologic specimens with regard to staining times. If staining, rinsing, and cleaning are performed properly, differences in the average thicknesses of various preparations rarely create problems in visualizing microscopic details.
(b) Air-dried, fixed, Carbowax-coated cytologic material will display poor nuclear staining unless immersed in 95% ethanol for at least 10 minutes before being stained.
(c) Tap water in many localities is satisfactory. If doubtful about the chemical quality of tap water, use distilled water. Tap water baths should be changed after every other rack of material passes through them.
(d) Most laboratories can establish a regular schedule for replacing their OG-6 and EA solutions based on the volume of slides stained per week and storage conditions. Daily microscopic checks are recommended, as well as keeping a well-stained slide available for reference.
(e) The third dish of a three-dish 95% alcohol series should be stain-free. Alcohol rinses that become stain-laden begin to behave more as a stain and less as a rinse.

Results:
Chromatin is stained the classical rich blue to blue-black color that is best for contrast, resolution, and visibility (see Polysciences Data Sheet #192 for additional important information). Orange G stains cells that contain keratin (e.g., mature squamous cells in keratotic lesions and keratinizing squamous cell carcinoma), and may stain erythrocytes and the granules of eosinophils.

Fast Green FCF stains the cytoplasm of cells that had been metabolic shortly before the time of specimen collection. Typical examples are leukocytes, histiocytes, columnar cells, parabasal cells, mesothelial cells, transitional cells, and cells of large cell undifferentiated cancer, small cell undifferentiated cancer (i.e., oat cell carcinoma) and adenocarcinoma.

Eosin stains pink to red superficial squamous cells, cilia, nucleoli, and frequently erythrocytes. The cytoplasm of most well-preserved, properly fixed cells is stained in vivid hues. Cells retain their transparency.

Safety and Handling Precautions:
Warning: HARMFUL IF SWALLOWED. DO NOT DRINK. Contains methanol and cannot be made non-poisonous.[18]

Danger:
Extremely flammable! Keep away from heat, sparks and open flames. Keep container closed. Use with adequate ventilation.

Disposal and Storage:
Disposal of exhausted stain must comply with federal, state, and local regulations. Follow good laboratory practice for storage of dye solutions. Keep stock solutions in the dark at room temperature; avoid sparks and sources of ignition. Store in tightly capped containers. Shelf-life is two years. Determining whether OG-6 or EA is still useful is best done microscopically. Stain a test slide (e.g., a touch preparation of keratinizing squamous cell carcinoma for OG-6 and a buccal smear for EA) and compare the results with slides previously selected as references.

Ordering Information:

Cat. #	Description	Size
24245	Harris Hematoxylin, Mercury Free, Acidified This stains vaginal, cervical and endometrial smears in addition to its excellent nuclear staining properties of histology sections.	500ml 1000ml
09782	Gill's Modified OG-6 Cytoplasmic counterstain solution used sequentially with EA in the Papanicolaou staining method for clinical cytology. Gill's Modified OG-6 obviate the difference in commercially supplied OG-6 formulations	500ml 1000ml 3.75L
09783	Gill's Modified EA Cytoplasmic counterstain solution used sequentially with OG-6 in the Papanicolaou staining method for clinical cytology. Gill's Modified EA obviate the difference in commercially supplied OG-6 and EA formulations.	500ml 1000ml 3.75L
09859	Eosin Y, Alcoholic, 0.5% (contains acetic acid) Used as a counterstain with hematoxylin.	500ml 1000ml 3.75L
17269	Eosin Y, Alcoholic, 1% (contains no acetic acid) Used as a counterstain with hematoxylin.	500ml 1000ml 3.75L

Gill's Hematoxylin
Request Technical Data Sheet #192

Cat. #	Description	Size
24242	Gill's Hematoxylin - No. 1 for Cytology	500ml 1000ml 3.8L
24243	Gill's Hematoxylin - No. 2 for Histology and Cytology	500ml 1000ml
04571	Gill's Hematoxylin - No. 3 for Histology	500ml 1000ml
09860	Ethyl Alcohol, (95%) Histology Grade	1gal

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