Clinical Non-gyn Procedure
nongyn28.01

Staining Procedures- Papanicolaou and Diff-Quik

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List all stakeholder(s) and dates of approval:

- **Stakeholder Name(s):** Jeffery Hadley  Date: 7/29/10 Reviewed [X] Revised [ ]
- **Stakeholder Name(s):** Mark Magilner, MD  Date: 7/29/10 Reviewed [X] Revised [ ]
- **Stakeholder Name(s):** Jeffery Hadley Date: 8/22/11 Reviewed [X] Revised [ ]
- **Stakeholder Name(s):** Jeffery Hadley Date: 8/30/12 Reviewed [X] Revised [ ]
- **Stakeholder Name(s):** Date:  Reviewed [X] Revised [ ]

Describe briefly the most recent revision made to this policy, procedure or protocol & why:
New Procedure

Purpose/Policy Statement:
The modified Papanicolaou staining method is used to stain non-gynecologic alcohol or spray-fixed slide preparations.

Definitions:
- n/a

POLICY CONTENT

NOTE: APPROPRIATE PROTECTIVE DRESS FOR THE HANDLING OF CHEMICALS MUST BE WORN: GOGGLES OR SAFETY GLASSES, LAB COAT AND GLOVES.

PAPANICOLAOU STAINING PROCEDURE

The cytology laboratory uses a progressive polychrome stain after hydration in which the nucleus is stained with Gill's hematoxylin to the desired intensity and set with a "bluing agent". Following hydration, the cytoplasm is slightly tinted with two counterstains, EA-50 (which contains eosin Y, light green S.F. yellowish, and Bismarck brown Y) and OG-6. Further dehydration and clearing results in good cellular transparency after which the slide is cover-slipped.

1. Non-Gynecologic specimen preparations are stained on the auto-stainer. If there is a known or suspected malignant non-gynecologic specimen, the specimen will be stained last on the run or hand stained to prevent cross-contamination. At least one blank slide is included in each staining run to ensure cross-contamination is not present. Blank slides are cover slipped and checked by a Senior technologist for cells before the next rack is stained. Results of examination are recorded on the Non Gynecological Stain QC log. If cells are present, the stain setup will be filtered before another run and the Pathologist will determine if further action or reprocessing is necessary. See Documentation of Specimen Cross Contamination. Floater cell blank slides are disposed of after examination by the technologist.

2. All non-gynecologic specimens that have a high potential for cross contamination are stained separately or last. These include:
   a) All known highly suspicious specimens.
   b) All specimens that appear to be exceptionally thick or bloody or known to be malignant through previous detection (i.e. from assisted FNA procedure).

3. CSF’s are stained with new or filtered solvents and clearing agents due to the manner of collection, which makes this an irretrievable sample.

4. Prior to staining, all spray-fixed slide preparations require immersion into 95% ethanol for period of 15 minutes to remove all traces of the fixative.
5. Stains are routinely filtered:
   a) From the plastic storage bottles or from the new containers into staining dishes prior to staining.
   b) After staining known malignant specimens, floater cells are found on the blank slide, or at the end of each day
      if alcohol looks too diluted with stain.
   c) Into the storage bottles upon the completion of staining. The plastic bottles are stored in a flammable cabinet
      for safety and to prolong the effectiveness of the stains.

6. All the tap water rinses are changed after each staining rack.

7. Alcohols and Clearing agents are:
   a) Filtered prior to staining.
   b) Kept free of water (note: the dishes are to be absolutely dry before the addition of 100% alcohol or
      clearing agents).
   c) Kept covered when not in use.
   d) Change if dirty (with the next alcohol or clearing agent staining dish respectively after filtering) rotated
      into the position of the dirty one and the fresh solution placed at the end of the particular series. And all
      recycled at the end of each day.
   e) Never poured into a dirty staining dish (the Alcohol dishes are rinsed multiple times with tap water, and
      dried thoroughly prior to the return of the reagent. Clearing agent dishes are only wiped with clean paper
      towels, no H20)
   f) Monthly dump stains and replace with new filtered stains. (this is a good time to do the monthly stainer
      maintenance).

8. Dishes (other that clearing agent dishes and funnels) are washed thoroughly with 10% bleach solution. A weak
   bleach solution is used to remove precipitated stains from staining dishes. The dishes are then thoroughly rinsed
   in tap water to remove all traces of detergent or bleach. Dishes are then dried thoroughly prior to the addition
   of any solutions. Metal utensils are washed with cleaning solution and rinsed with tap water.

9. Quality control charts are maintained daily on all preparations stained. Any problems are documented.

Stain Set Up:

   Note: General staining times and reagent order are followed. Automated stain setups are programmed into the
   automatic stainers.

RAPID PAPANICOLAOU STAINING PROCEDURE (Ultrafast Papanicolaou Stain)

A rapid staining technique performed on air-dried slides used for rapid interpretation and specimen adequacy
determinations is done in the Gross Room. See Histology for written procedure.

DIFF-QUIK STAINING FOR AIR DRIED SLIDE PREPARATIONS

Purpose/Policy Statement:
The air-dried Romanowsky/modified Wright-Giemsa Diff-Quik preparation and staining technique is an ancillary procedure
used in conjunction with Papanicolaou staining methods for:

1. The rapid determination of specimen adequacy on fine needle aspiration biopsy materials.
2. Providing an alternative visual definition of nuclei, cytoplasm, and cellular products.
3. To facilitate the recognition of reticulo-endothelial / hematopoietic type cellular abnormalities in cerebrospinal fluid and
   other body fluids and lymph node specimens.

Definitions:
• n/a

PROCEDURE:

1. Direct smears and cyto centrifuge preparations are allowed to fully air dry. Filter the alcohol and stains after each part
   type. When new stain is used note on Non Gynecological Stain QC Log by initialing the New Stain column.
Note: Even if the multiple part types are the same patient they must be stained separately.

2. Immerse the air-dried slide into solution #1 (95% alcohol or methanol) for 15-20 seconds until slide appears shiny. Let excess run off slide onto a paper towel.

3. Dip the slide gently into solution #2 several times (15-20 dips) until the color development is achieved. Color should be orange-pink. Remove slide and blot off excess stain.

4. Dip the slide gently into solution #3, several times (15-20 dips) until appropriate color development is achieved. Color should be deep purple. Remove slide and rinse in cup with tap water. There should be no blue haze over the surface of the slide.

5. Allow the slide to dry completely. The slide maybe viewed in the wet state without a coverslip. For rapid interpretation and adequacy determinations.

6. Once the slide is completely air-dried dip slide into clearing agent and cover slip either by automated cover slipper or by hand.

**PROCEDURE NOTES:**
The results of the Romanowski type stains are similar to those of the May-Grunwald Giemsa stain: Nuclei stain blue; cytoplasm pink to rose; bacteria blue; fungi blue; and stroma magenta.

**PROCEDURE NOTES:**
Changes in any of the staining protocols are done only at the discretion of the operations manager and medical director.

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**Equipment/Supplies (If Applicable):**
**MATERIALS:**
The Cytology Laboratory currently uses the Richard-Allen brand of Gill #1 hematoxylin Papanicolaou, EA-50 and OG-6 stain solutions in the gynecologic slide Staining procedure. Expired stains are not used in the staining process and are discarded according to regulations

95% Alcohol or Methanol

**Form Name & Number or Attachment Name (If Applicable):**
nongyn28

H:\Lab\Anatomic Pathology\CYTOLOGY\2010 Cytology\Maintenance Logs\Non Gyn Logs\Non-Gyn Stain QC Log

**Author Position:**
Operations Manager

**Review/Revision Authority (Position Not Individual Name):**
Medical Director
Operations Manager

**Expert Consultant Position/s (Not Individual Name/s):**
n/a

**References (Required for Clinical Documents):**
n/a

**Is there a Regulatory Requirement? **

[ ] Yes [ ] No

If yes, insert requirement information here:

**Review History (No Changes):**
n/a

**Revision History (Note changes in area under header):**
n/a