Preparation Of Non-Gynecologic Specimens

Final Approval: October 2010 Effective: October 2010

List all stakeholder(s) and dates of approval:

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Purpose/Policy Statement:
An explanation of the processes used to prepare various routine non-gynecologic specimens for transfer to glass slides and cytologic screening.

Definitions:
- n/a

POLICY CONTENT

PROCEDURE:

NOTE: APPROPRIATE PROTECTIVE DRESS FOR THE HANDLING OF CHEMICALS MUST BE WORN: GOGGLES OR SAFETY GLASSES WITH SIDE FACE PROTECTION, LAB COAT WITH PLASTIC APRON OR FLUID-IMPERVIOUS GOWN, AND GLOVES.

1) SPUTUM:

a) PREFERRED SPECIMEN:
   i) First morning specimens are preferred. Deep cough sputum samples or saline washes of the bronchus should be collected into a container containing Saccomanno’s fixative or 50% ETOH in equal volumes to that of the sputum sample. The minimum amount of specimen is approximately 0.5 gm of a deep cough sample.

   Note: Specimens that are collected as fresh specimens must be immediately refrigerated and they are adequate for evaluation for less than 48 hours after collection. Prepare two smears (sm).

b) PICK AND SMEAR TECHNIQUE for making direct smear preparations:
   i) Select any bloody, discolored, or solid particles, if present, and place a small portion of each particle not larger than a small pea on each of two plain slides. With a clean glass slide press the particle of sputum onto each of the two slides using a rotary motion, then with overlapping and horizontal strokes spread the preparation evenly over the surface of the slide. For fresh specimens, place slides immediately in 95% ETOH. For fixed (Saccomanno), let slides air dry before placing in 95% ETOH.

   Note: If no particles or abnormal areas are identified in the specimen grossly, at least 4 different portions of the specimen must be selected and smeared.

   ii) Cellblock preparations may be made from the remaining specimen if indicated.
c) SACCOMANNO’S TECHNIQUE (THICK SPECIMENS)
   i) Pour specimen into a blender container and blend in a Warren blender at high speed for {3 - 5 seconds}. Do not remove the lid of the container during blending.

   ii) Pour blended specimens into a 50 ml test tube; if flecks and fine threads are still visible, return to blender for an additional five seconds.

   iii) Centrifuge specimen for 8 minutes at {1800 RPM}.

   iv) Decant supernatant, leaving a few drops of fluid to mix with the granular, pale sediment.

   v) Vortex the tube to re-suspend the sediment.

   vi) Prepare smears by placing a few drops of the re-suspended sediments in the center of the clean slides. Place a second clean slide over the material. Spread out evenly between the two slides and gently pull the slides apart with an easy sliding motion.

   vii) Allow the slides to air-dry (Saccomanno fixative fixes the slide) until ready for staining.

   viii) The slides should be soaked in 95% ETOH for 15 minutes before staining to remove the Saccomanno fixative completely.

2) BRONCHIAL WASHING:
   a) Bronchial washing should be sent to the lab fresh whenever possible. For specimens that are delayed more than 1 day, collect into containers containing Saccomanno fixative or into a container with 50% ETOH added in equal volume.

   b) If the specimen is mucoid, the Saccomanno technique should be used to prepare two direct smears as outlined above.

   c) If the specimen is watery, prepare two double cytospin preparations.

   d) Prepare a cellblock if appropriate.

3) BRONCHIAL BRUSHINGS:
   a) If the brush is received, dislodge the particulate material from the brush by agitation. Prepare one double cytospin preparations if direct smears are also provided or if no direct smears are provided, prepare two double cytospin preparations.

4) BRONCHOALVEOLAR LAVAGE:
   a) Preferred specimens are those above 10 mls of wash fluid sent to the lab fresh whenever possible. For specimens that are delayed more than 1 day, collect into containers containing Saccomanno’s fixative or into a container with 50% ETOH added in equal volume.

   b) Mix specimen well, break up small clots and divide the specimen, pouring it into 50 ml centrifuge tube(s) and cap.

   c) Centrifuge for 8 minutes at 1800 rpm.

   d) Decant supernatant leaving an equal amount of fluid as there is cell button with which to re-suspend the cell button.

   e) Cytocentrifuge at 1300 rpm for one minute using high acceleration.

   f) Remove cytofunnels from the cytocentrifuge. Remove the slides and fix immediately. Bronchial specimens that were received fresh should be dropped into 95% ETOH. Specimens that were received fixed in Saccomanno should be spray fixed immediately and left to dry thoroughly then place in 95% ETOH.
Note: If cell buttons on slides after cytocentrifugation appear too thick, smear lightly with the edge of a coverslip and then fix immediately.

g) Soak slides prepared from fresh specimens in 95% ETOH for 15 minutes. Stain all slides with Papanicolaou stain.

h) Cover slip.

Cytospin procedure notes:
If there is tissue in the specimen and sparsely cellular cytospin material, a cellblock should be made.

5) BODY FLUIDS (PLEURAL, ASCITES, PERICARDIAL):

a) Fluids should be collected into containers containing anti-coagulant such as heparin. Two mL of 1-% heparin per liter of fluid volume should be added to the specimen container.

Note: If transport is to be delayed, or the specimen cannot be refrigerated, an equal volume of Saccomanno’s fixative or 50% ETOH should be added to the fluid. For specimens of large volume a well-mixed aliquot of 100 mls is adequate, and this 100 mls should be mixed with an equal volume of Saccomanno’s fixative or 50% ETOH to produce a total of 200 mls to be submitted to the laboratory. A fresh refrigerated specimen is acceptable for 24 hours. A fresh specimen kept at room temperature is acceptable for 4 - 6 hours. A fixed specimen is acceptable for evaluation for an indefinite period of time.

The minimal amount of fluid for evaluation is 0.1 mls. 50 mls or greater is preferred.

b) Fresh specimen:
   i) Prepare two double cytospin preparations (fixed in 95% ETOH) to be stained with Pap stain and
   ii) One cytospin preparation (air-dried) to be stained with Quik-Dip stain and one cell block or
   iii) Prepare 2-4 double cytospin preparations (fixed in 95% ETOH) to be stained with Pap stain and one cytospin preparation (air-dried) to be stained with Quik-Dip stain.

c) Fixed specimen:
   i) Prepare two double cytospin preparations (Fixed in 95% ETOH) to be stained with Pap stain and one cell block or
   ii) Prepare 2-4 double cytospin preparations (Fixed in 95% ETOH) and one cytospin preparation (air-dried) to be stained with Quik-Dip stain.

d) Excessively bloody specimen:
   See manager for instructions.

e) Clotted specimens:
   Gently press the cloth around a wooden applicator stick and touch it against the side of the container to wring out fluid and trap cells. The fluid should be processed as described in #1 and #2 above, and the remaining clot should be processed as a cellblock.

f) Prepare a cellblock if appropriate.

6) CEREBROSPINAL FLUIDS:

a) Minimum volume of specimen: 0.1 mls.
   Preferred specimen: 1 - 4 ml or greater.

b) The CFS specimen should be received fresh in the cytology laboratory within one hour of the sample collection. If delivery to the laboratory is to be delayed, the sample should be diluted with an equal volume of Saccomanno’s fixative or 50% ETOH for cellular preservation. Use positively charged slides.

c) For fixed CSF’s:
   i) Up to 4 ml in volume, prepare one single circle cytospin preparation (fixed in 95% ETOH) for Pap stain.
ii) Volume 4 ml or more, prepare one double cytospin preparation (fixed in 95% ETOH) for Pap stain.

d) For fresh CSF’s:
   i) Up to 2 ml in volume, prepare one single circle cytospin preparation (fixed in 95% ETOH) for Pap stain.
   ii) Volume 2 ml or more:
       (1) Prepare one single cytospin preparation (fixed in 95% ETOH) for Pap stain, and
       (2) Prepare one single cytospin preparation (air-dried) for Quik-Dip stain.

   Note: If the requisition states that there is concern for a hematologic malignancy, leukemia, or lymphoma, one cytospin preparation should be stained with Papanicolaou stain and the second cytospin preparation should be air-dried and stained with the Quik-Dip stain. (The Quik-Dip specimen must be prepared from fresh fluid only.)

   e) Prepare a cellblock if appropriate.

7) URINES:

   a) Preferred specimen is a second morning voided urine sample or any catheterized urine sample with an equal volume of Saccomanno’s fixative or 50% ETOH added immediately. Bladder and ureteral washes should also be submitted in Saccomanno’s fixative or 50% ETOH added immediately upon collection. The requisition form should state whether the urine is a voided urine, catheterized urine, bladder or ureteral wash.

   b) At least 1 ml of urine is preferred for cytologic evaluation.

   c) Prepare one double cytospin preparations and stain with Papanicolaou stain.

   d) Prepare a cellblock if appropriate.

8) OTHER WASHES:

   a) Saline washes of other sites such as stomach and esophagus should be combined with an equal amount of Saccomanno fixative or 50% ETOH immediately upon collection of the sample.

   Note: Gastric washes are particularly sensitive to cellular degeneration if not placed in fixative. There will be significant cell degeneration if refrigerated without fixative in 24 hours and if left at room temperature, significant cell degeneration occurs in 4 hours.

   b) Prepare two double cytospin preparations, then stain with Papanicolaou stain.

       Note: If the specimen is extremely thick, after centrifugation prepare two direct smears and dilute the sample to prepare two cytospin preparations if possible.

   c) Prepare a cellblock if appropriate.

9) CYST FLUIDS:

   a) Fluid aspirated from cysts of any anatomic site (often breast or ovary).

   b) A minimum of 0.1 ml is a preferred volume of cyst fluid.

   c) Prepare up to two double cytospin preparations and stain with Papanicolaou stain on positive charged slides, if appropriate

       Note: If the specimen is extremely thick, saline may be used to flush the fluid from the syringe.

   d) Stain fixed direct smears with Papanicolaou stain.

   e) Prepare a cellblock if appropriate.
10) BRUSHINGS OTHER THAN BRONCHIAL:

Esophageal, ureteral and brushings of other sites should be processed in similar manner to bronchial brushings. See protocol for bronchial brushings above.

11) BREAST SMEAR (NIPPLE SECRETIONS, NIPPLE DISCHARGE) AND SKIN SMEAR:

a) Preferred specimen is a direct smear, made of the nipple secretion or skin lesion secretion, that is immediately fixed.

b) Direct smears are to be stained with Papanicolaou stain and cover slipped for microscopic evaluation.

12) FINE NEEDLE ASPIRATIONS (FNA):

a) Stain fixed direct smears with Papanicolaou stain.

b) Stain air-dried direct smears with Quick-Dip stain.

c) For fresh specimens with slides:
   i) Prepare one single cytospin preparation and stain with Papanicolaou stain and
   ii) Prepare one single cytospin preparation and stain with Quick-Dip stain or
   iii) Prepare a cell block.

d) For fixed specimens received with slides:
   i) Prepare a double cytospin preparation and stain with Pap stain or
      ii) Prepare a cellblock from the particular material present within the needle rinse specimen.

e) For specimens received without slides:
   i) Prepare two double cytospin preparations and stain with Pap stain and
      ii) Prepare one cell block, unless there isn’t sufficient specimen available.

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Equipment/Supplies (If Applicable):

**EQUIPMENT:**
- CENTRIFUGE
- CYTOCENTRIFUGE
- VORTEX
- BLENDER

**MATERIALS, REAGENTS:**
- SACCOMANNO’S FIXATIVE
- 50% ETHANOL
- 95% ETHANOL
- QUIK-DIP STAIN
- PAP STAIN
- FROSTED END SLIDES

Form Name & Number or Attachment Name (If Applicable):
nongyn21

Author Position:

Review/Revision Authority (Position Not Individual Name):
Expert Consultant Position/s (Not Individual Name/s):

References (Required for Clinical Documents):

Is there a Regulatory Requirement? Yes ☐ No ☐
If yes, insert requirement information here:

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