Clinical Non-gyn
Procedure
nongyn030.01

Acceptance and processing of Non-gynecological specimens

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<tr>
<th>Final Approval:</th>
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List all stakeholder(s) and dates of approval:

- **Stakeholder Name(s):** Jeffery Hadley Date: July 29, 2010 Reviewed ☒ Revised ☐
- **Stakeholder Name(s):** Mark Magilner, MD Date: July 29, 2010 Reviewed ☒ Revised ☐
- **Stakeholder Name(s):** Jeffery Hadley Date: 8/17/11 Reviewed ☒ Revised ☐
- **Stakeholder Name(s):** Date: Reviewed ☐ Revised ☐
- **Stakeholder Name(s):** Date: Reviewed ☐ Revised ☐

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

New Procedure

Purpose/Policy Statement:

The general requirements of the laboratory for the collection, identification, labeling, and handling of the specimens submitted to Pacific Pathology laboratory. This procedure also includes preparation of fluid and tissue of Non-Gynecological specimens.

Definitions:

- Acellular-Containing few cells, not made of cells.
- CoPath- Anatomic pathology laboratory information system (LIS) software.
- Epic- Electronic medical record system used by Salem Health and Pacific Pathology Associates.
- Mysis- Salem Hospital lab information system (LIS) software.

POLICY CONTENT

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

SPECIMEN ACCEPTANCE:

The Order - The request slip or order in Mysis must be completed with the following information:

- Patient name, no abbreviations of first or last name
- Birth date
- Gender
- Specimen Source
- Clinician’s name

Preferred but not required-

- All pertinent history
- Patient billing address
- Patient insurance information
- Patient social security number

1. When slides are submitted, the patient’s name should be written on the frosted end of the slide with a #2 soft lead pencil or permanent lab marker (such as Statmark or Secureline). Do not use a ink/sharpie pen or sticky labels.
2. When non-gyn containers are submitted, the patient’s name and source of specimen should be written on the label on the container, not the lid.
3. All attempts will be made to obtain the minimal information before specimen is processed. The information may be obtained in one of the following ways:
   a. Call the respective location or clinician for missing or unclear information.
   b. Fax clinician with Pacific Pathology Associates cover page. Once correct fax is received place with the requisition.
4. Once the correct information is obtained, the deficiency, resolution, date, and to whom you spoke to is noted in Copath under specimen/req deficiencies.

Papanicolaou stain slides- Immediate and proper fixation is a prerequisite for cytologic evaluation. Slides must be fixed immediately after preparation. The preferred method of fixation is Cyto-spray fixative or slides fixed in 95% alcohol.

Diff Quick stain slides- Slides must be air-dried before staining. If slides received from outside location and thought to be fixed call clinician for verification. If it can’t be determined see Operations manager or Pathologist.

Note: All specimens are kept and refrigerated after processing for at least 1 week.

**CYTOPSPIN PREPARATION:**

a. Most specimens need to be centrifuged to concentrate cellular material before making cytospin preparations.

b. Specimens need to be in a 15 or 50mL conical tube before centrifugation. If necessary, aliquot up to 50mL of patient sample into conical tube from specimen container. 40-50mL of patient sample is preferred but some specimen types are difficult to collect or may have a limited volume. In those cases, centrifuge as much of the specimen as possible. See “Centrifugation of Non-Gynecological Specimens” policy for centrifuge instructions.

c. While specimens are in the centrifuge, label slides and place slide and cytofunnel into the metal clip.

d. Once specimen has been centrifuged decant in one fluid movement the supernatant (unless sample is acellular then you would use a pipet to clear the supernatant) leaving a few drops of fluid to mix with the cell button.

e. Vortex the tube to mix fluid and cell button.

f. Using a disposable pipet drop 1-4 drops of fluid (depending on cellularity) into the cytofunnel chamber/chambers. Then place cap on cytofunnel.

g. Ensure the cytofunnel/clip are balanced before placing lid on the base. Place in Cytospin.

h. Spin for pre-set time (1300RPM for 1 min.).

i. While spinning, place a few clean paper towels under the bio hood to catch the used funnel chambers. And enough paper towels to lay the slides out to dry.

j. When Cytospin alarm sounds that spin is complete. Remove and place under the biohood.

k. Remove the lid. First remove cytofunnels one at a time starting with spins to be fixed.

l. Open clip (with clip side facing the roof of the biohood). Allow cytofunnel to drop away.

m. Quickly and gently spray 1-4 pumps of fixative on the slide and set on paper towel allowing the slide to dry.

n. When all fixed spins are completed, take air-dried spins 1 at a time following step i, this time allow the slide to air dry.

o. Place clips into decontamination fluid for 15 minutes before rinsing with tap water.

**SPUTUM**

Acceptance-

1. First-morning deep cough specimens are preferred, although all sputum specimens collected at any time will be accepted.

2. For outside locations, it is preferred each sputum sample have equal amounts of liquid fixative to amount of sample added to it promptly after collection. Fresh refrigerated samples are also acceptable.

Processing- For optimum screening results make direct pick and smear and blended smear preparation as follows:

a. Select any discolored and or solid particles if present and place a small pea size drop of specimen on 1 slide near frosted end. If no discolored or solid particle present, go directly to step c.

b. Take 2nd glass slide and press the particle of sputum between the slides gently dispersing the sample evenly. Immediately spray fix both slides. After slides are dried soak slides in 95% alcohol for 15 minutes before staining.

c. Blend the rest of the specimen with a small amount of saline.

d. Centrifuge specimen for 8 minutes at 1800 RPM.

e. Decant supernatant, leaving a few drops of fluid to mix with the granular, sediment (cell button).

f. Vortex the tube to re-suspend the sediment
g. Take 2 glass slides and press the particle of sputum between the slides gently dispersing the sample evenly. Immediately spray fix both slides. After slides are dried soak slides in 95% alcohol for 15 minutes before staining.

h. Prepare a cellblock if appropriate.

BRONCHIAL WASHING:
Acceptance-
Refrigerated fresh samples are preferred with 0.5ml of fluid minimum. Less than 0.5ml of fluid acceptance will be determined by the operations manager. Fixed samples (such as Saccamanno or CytoRich Red) are also acceptable.

Processing-
Determine how thin or thick the sample is before preparation. If the sample is thin 2 double fixed cytospins are prepared. If the sample is mucoid and thick make 2 direct smears as follows:

a. Pour specimen into a blender container and place lid on. Blend at high speed for 3-5 seconds.

b. Pour blended specimens in a specimen tube. If flecks and fine threads are visible, return to blender for an additional 5 seconds. Note: The sample may be so small saline or liquid fixative may be used to transfer specimen out of the blender into the tube.

c. Centrifuge specimen for 8 minutes at 1800 RPM.

d. Decant supernatant in one fluid movement, leaving a few drops of fluid to mix with the cell button.

e. Vortex the tube to re-suspend the sediment.

f. Prepare smears by placing a few small drops of specimen near the frosted end of 1 slide. Using another slide gently press the specimen between the slides coating evenly.

g. Spray fix the slides. Allow to air-dry. Place in 95% alcohol before staining.

h. Prepare a cellblock if appropriate.

BRONCHIAL LAVAGE:
Acceptance-
Refrigerated fresh samples are preferred with 0.5ml of fluid minimum. Less than 0.5ml of fluid acceptance will be determined by the operations manager. Fixed samples (such as Saccamanno or CytoRich Red) are also acceptable.

Processing-
Mix sample gently in original container. Decant sample into centrifuge tube labeled with patient’s name.

a. Centrifuge for 8 minutes at 1800 rpm.

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

d. Make 3 double fixed cytospins. If the sample is acellular make only 1 double fixed cytospin.

e. Spray fix slides. Allow to air-dry then soak in 95% for 15 minutes before staining.

f. Prepare a cellblock if appropriate.

BRONCH BRUSHING:
Acceptance-
Bronch brush in fluid and/or bronch brush slides are acceptable specimens.

Processing-
If brush is received, dislodge particulate material from the brush using forceps and vortexing.

b. Prepare 1 double cytospin.

If slides only are received spray fixed, soak in 95% alcohol for 15 minutes before staining. If slides are already in alcohol stain them.

BRUSHINGS OTHER THAN BRONCHIAL:
Acceptance-
Esophageal, ureteral and brushings of other sites should be processed in similar manner to bronchial brushings. See protocol for bronchial brushings above.

BREAST SMEAR (NIPPLE SECRETIONS, NIPPLE DISCHARGE).
Acceptance-
Preferred specimen is a direct fixed smear. Also fluid received fresh refrigerated or fixed.

Processing-
a. Prepare up to 2 double fixed cytospins. If ovarian cyst use positive charged slides. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
b. Prepare a cellblock if appropriate

TZANCK SMEAR:
Acceptance-
Fixed direct smear is preferred. If smear is received air-dried, make a note in Copath under specimen/req deficiency.

Processing
Fixed smear-
a. Soak slide or slides in 95% alcohol for 15 minutes before staining.

Air-dried smear-
a. Stain slide using Diff-Quik method.

SYNOVIAL FLUID:
Acceptance-
Fluid received fixed or fresh refrigerated is acceptable.

Processing-
a. Make 2 direct smears
b. Spray fix slides. Allow to air-dry then soak in 95% for 15 minutes before staining.

Note: If specimen is too viscous blend with a small amount of saline for up to 5 seconds.

BODY FLUIDS (Pleural, Ascites, Pericardial):
Acceptance-
The minimal amount of fluid for evaluation is 0.5ml depending on cellularity. 50ml or greater is preferred. A fresh and immediately refrigerated sample is preferred if the laboratory can receive the specimen within 24 hours. If immediate refrigeration and prompt transport are not possible, add equal volume of liquid fixative to the specimen. Never use formalin for cytology specimens.

NOTE: If specimen is fresh and stored at room temperature for greater than 4 hours make a note in Copath under specimen/req deficiency.

Processing-
Fresh specimen
a. Prepare 2 double fixed cytospins. Spray fix slides. Allow to dry then soak in 95% for 15 minutes before staining.
b. Prepare 1 double air dried cytospin.
c. Prepare 1 cellblock or if no cellblock material is present, prepare 2-4 double fixed cytospins and one double air-dried cytospin. If cellblock is made place completed block in container with 10% formalin.

Fixed specimen:
a. Prepare 2 double fixed cytospins and 1 cellblock. If no cell block material present prepare 2-4 double fixed cytospins Spray fix slides. Allow to dry then soak in 95% for 15 minutes before staining.

OTHER WASHES: (STOMACH, ESOPHAGUS, PERITONEAL, CUL DE SAC)

Acceptance-
Gastric washes are particularly sensitive to cellular degeneration. Preferred sample is fixed within 4 hours. Refrigeration doesn’t slow cellular degeneration in these types of specimens. All other washes are acceptable refrigerated or fixed.

Processing-
a. Prepare 2 double fixed cytospins.
b. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
c. Prepare cellblock if appropriate.
CEREBROSPINAL FLUIDS:
Acceptance-
Preferably the CSF specimen should be received fresh within 1 hour of the sample collection. If delivery to the laboratory is to be delayed, the sample should be diluted with an equal volume of liquid fixative. Minimum volume of specimen is 0.1ml. Preferred volume is 1-4ml or greater.

Processing- Use positive charged slides
For fixed CSF’s:
   a. Volume up to 4ml in volume, prepare 1 single fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
   b. Volume 4ml or more, prepare 1 double fixed cytospin and spray fix, allowed to dry and then soak in 95% alcohol for 15 minutes before staining.

For fresh CSF’s:
   a. Volume up to 2ml in volume, prepare one single fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
   b. Volume 2ml or more, prepare 1 single fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
   c. Prepare 1 single air dried cytospin.

URINE: (BLADDER AND URETERAL WASHES)

Acceptance-
Preferred specimen is a second morning voided urine sample or any catheterized urine sample fresh or with an equal volume of liquid fixative. A minimum of 1ml of urine is preferred.

Processing-
   a. Prepare 1 double fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
   b. Prepare cell block if appropriate.

CYST FLUIDS:

Acceptance-
Fresh or fixed specimens are acceptable.
A minimum of 1ml is a preferred volume of cyst fluid.

Processing-
   a. Prepare up to 2 double fixed cytospins. If Ovarian cyst use positive charged slides. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
   b. Prepare a cell block if appropriate.

FINE NEEDLE ASPIRATIONS (FNA)

Acceptance-
Specimen received in FNA’s include needle rinse in fluids such as Cyto Rich Red, Saline or Saccomanno. FNA specimen can also be received fresh in a capped syringe. Slides include fixed and air dried.

Processing-
Note: Cell blocks are not prepared on Thyroid FNA’s unless requested by a pathologist.
   a. Prepare cell block with the particular material present within the needle rinse. Place completed block in 10% formalin.
   b. If no cell button prepare 1 double fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.

For fixed specimen with slides:
   a. Prepare cell block with the particular material present within the needle rinse. Place completed block in 10% formalin.
   b. If no cell button prepare 1 double fixed cytospin. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.

For fresh specimen received without slides:
a. Prepare 2 double cytopins. Spray fix, allow to dry and then soak in 95% alcohol for 15 minutes before staining.
b. Prepare 1 double air dried cytospin.
c. Prepare cell block if particular material is present within the needle rinse. Place completed block in 10% formalin.

For fixed specimen received without slides:

a. Prepare 2 double cytopins.
b. Prepare cell block if particular material is present within the needle rinse.
c. Place completed block in 10% formalin.

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**Equipment/Supplies** (If Applicable):
Centrifuge
Cytocentrifuge
Vortex
Blender

**Material, Reagents**
CytoRich Red, Saccomanno’s fixative, 50% Alcohol, 95% Alcohol, Acid alcohol, Diff quik stain, Pap stain, Frosted end slides, Positive charged slides, Cell block cassettes, Tissue paper, Histo Gel, Metal spatula, Disposable pipet, Disposable Cytofunnel, Metal cyto clip, Disposable centrifugation tube, No. 2 pencil, Permanent lab pen, Black.

**Form Name & Number or Attachment Name** (If Applicable):
nongyn030

**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):
Operating instructions for Cytospin 3. Centrifugation of Non-Gynecological specimens.

**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

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n/a

**Policy, Procedure or Protocol Cross Reference Information:**
n/a