Rice Memorial Hospital

CYTOLOGY COLLECTION - NON-GYN SPECIMEN COLLECTION PROCEDURE

I. **PRINCIPLE:** This procedure provides basic instructions on how to collect non-gyn cytology specimens. Rice Memorial Hospital (RMH) Cytology Lab accepts cytology specimens from virtually any body site. It is imperative that standardized collection procedures are followed to ensure the best possible specimen is collected, preserved, and available to process.

II. **RESPONSIBILITY:** All licensed providers and other clinical personnel responsible for the collection and submission of non-gyn cytology specimens and cytology personnel who routinely receive and process these specimens should have a working knowledge of these procedures.

III. **SPECIMEN REQUIREMENTS**: The following are submission requirements of all cytology specimens regardless of source:

A. Specimens must be collected and received only from licensed, authorized sources, such as physicians, nurse practitioners, and physician's assistants.

B. Specimen Labeling Requirements:

1. Specimens must be labeled according to RMH Laboratory procedure, *Zero Tolerance Specimen Labeling Procedure*. This procedure requires 2 unique identifiers. These identifiers may include 2 of the following:
   - Patient Name (*Last Name, First Name, MI).*
   - Date of Birth
   - Medical Record Number

2. Provider's name must be clearly annotated.

3. Date and time of collection.

4. Specimen Source: All submissions need exact anatomical site specified to include left or right designations.

C. Clinical Information and History: Specimens must be submitted with any pertinent clinical information and history.

D. Unacceptable specimens:
   - Unlabeled/Mislabeled specimens.
   - Specimens without a completed cytology request.
   - Specimens without a complete annotated source.
   - Specimens not collected in an approved manner.

NOTE: Certain cytology non-gyn specimens are considered "recollectable" and therefore may be rejected. These specimens will be defined in this procedure under section V. An attempt will be made to rectify any submission problems before any specimen is rejected. Cytology processing will contact the provider to correct any submission problems. The Cytology Technical Supervisor is the final authority for specimen rejection determinations.

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IV. MATERIALS:

A. Slide transport containers (for air-dried smears).
B. Liquid rated slide transport containers (for alcohol fixed smears).
C. 95% ETOH.
D. Cytology spray fixative.
E. Urine cups (for urine and sputum collection).
F. 50 ml centrifuge tubes (for the transport of fluid type specimens).
G. Containers of 10% Buffered Formalin (for FNA needle rinses)
H. Cytopreservative fluid (50% ETOH based).

NOTE: The above materials are available at no charge from RMH Cytology Laboratory.

V. PROCEDURES:

A. General Collection and Submission Guidelines: The following collection guidelines apply to all cytology specimen collections:

1. Most fluid specimens should be submitted fresh (unfixed) provided they remain refrigerated from the time of collection.
   a. If fixative is used, indicate the sample was fixed and what type of fixative was used.

   b. If bacteriology (cultures) or other clinical tests are to be performed on the same specimen, the specimen should **not** be fixed but rather handled according to RMH clinical laboratory procedures. (e.g., sterile containers for culture, RPMI for flow cytometry, etc).

2. When sending fresh (unfixed) specimens, **please keep refrigerated.**
   a. Some specimens tend to degenerate more rapidly than others if not fixed (e.g., Urine and CSF fluids) however even specimens that are generally more stable (pleural fluids and ascites) should also be kept refrigerated.

   b. We also recommend refrigerating even if a specimen is believed to be fixed (just in case – to avoid cell degeneration).

3. Never discard a portion of a specimen (e.g. even if there is 5 liters of ascites, please send all of the fluid).

4. Do not freeze any cytology specimens.

5. When preparing fixed smears or brushed slides for cytology:
   a. Label the slides according to our labeling policy but also indicate if the slides were fixed or air dried. (May mark "F" for fixed and "AD" for air-dried).

   b. Please note that **immediate** (within one second) fixation is crucial to cell preservation. Drop slides directly into 95% alcohol filled containers (preferred), or spray fix immediately. Note: if spray fixative is used then allow it to dry completely on the slide preparations before placing them in slide containers or they may become glued in place and difficult to remove.

6. When submitting FNA specimens that have a needle rinse collected in 10% buffered formalin, do not ship any air-dried smears and/or spray fixed alcohol smears with the formalin container. Formalin vapors coming from the formalin container may cause cytologic damage to the smears. Place the appropriately labeled formalin container in its own biohazard bag and send with the rest of the specimen.

   NOTE: If sending FNA smears in 95%ETOH filled slide shipping containers, it is then acceptable to send these containers and the formalin container in the same bag.

7. All specimens should be treated as biohazardous and handled using "Standard Precautions". Specimens must be placed in biohazard bags or containers for shipment/transport to RMH Laboratory.

8. If any questions arise about proper handling, please call the Cytology lab at 1-800-922-RICE.

B. Specimen Collection Instructions:

1. **Body Cavity Fluids (Pleural, Peritoneal, Pericardial, Ascites, Pelvic Wash, Synovial):**
   a. After obtaining the fluid, place in a sterile, properly labeled container. Any original collection containers are acceptable.

   b. Please indicate Right or Left side (if indicated).

   c. **Do not add fixative (recommended). PLEASE SEND ALL AVAILABLE FLUID.** (The sensitivity of cytology is improved by a larger sample size.).

   d. Keep refrigerated – but submit the specimen even if unable to keep chilled.

   e. If clinical lab tests are to be ordered on a given specimen (i.e. protein, cell count, cultures, etc.) the collection procedures for those tests should be consulted as well.

2. **Breast Specimens:**
   a. **Breast Cyst Fluid:**
      1) Higher volume specimens (> 0.5cc): Submitted fresh and refrigerated (preferred method). The cyst fluid can be transferred to an appropriate specimen container or remain in the collection syringe with capped needle.

      2) Low volume specimens (<0.5cc): Alternatively, slides can be prepared as follows:
         a) Drop a small amount of fluid onto a slide.

         b) Place another slide on top of the slide with the sample and let the sample spread out between the slides.

         c) Pull the slides apart.

d) Immediately place slides in 95% alcohol slide containers (preferred) or spray fix with cytology spray fixative.

b. **Nipple Discharges:**
1) Touch the slide directly to the drop of secretion on the nipple.

2) If the secretion is abundant or thick, it should be smeared by placing another slide on top and pulling the two slides apart.

3) It is recommended that one slide be immediately alcohol fixed (for Pap-staining) and one slide air dried (unfixed for Oil Red O stain for fat).
Note: The preferred method to fix the slide is to place it in a 95% alcohol transport container but spray fixing is also acceptable.

c. **Solid Masses**: Collected using fine needle aspiration (FNA) technique. Instruction in and experience with FNA technique is recommended for optimal results. Please follow the protocol for Fine Needle Aspiration of solid masses below in section 4.

3. **Cerebrospinal Fluids (CSF): NOTE:** This procedure is for cytology only. If clinical lab tests (i.e. cell counts, glucose, cultures, etc.) are to be ordered, the collection procedures for those tests should be consulted.
a. Optimally cytology should receive the middle tube of a collection set (i.e. tube #2 or #3 of a 4 tube set).

b. All CSF specimens are routinely received fresh and sent to the laboratory as soon as possible.

c. If there will be a processing delay of more than 12 hours, an equal volume of a 50% ethanol based cytopreservative should be added. All specimens should be immediately refrigerated after collection and remain so until processing.

Note: CSF can degenerate rapidly so preservation may be the best option if transport or processing could be delayed for any reason. **Only add preservative fluid to the tube designated for cytology.**

4. **Fine Needle Aspiration (FNA):** FNA procedures may be performed on palpable masses utilizing direct visual guidance and deep lying masses using radiologic guidance. The FNA procedure will yield either cellular material and/or cyst fluid, both of which may be submitted for cytology.

The Cytology section of RMH Laboratory is available to assist with the collection and perform immediate adequacy assessments of all FNA procedures performed at RMH. These services are performed during the normal business hours of 8:00am-4:30pm, M-F or by special arraignment. For FNA assistance or to schedule, call Cytology at 231-4826.

a. **Solid Mass:** Instruction in and experience with FNA technique is recommended for optimal results. Below is a basic guideline for collecting a FNA specimen:
   1) Standard Recommended Materials
      - 22-25 gauge needles.
      - 5, 10, or 20 cc syringes.
      - Alcohol swabs or betadine swabs.
      - Gloves.
      - Glass slides (frosted tip).
      - 95% alcohol transport containers (preferred) or spray fixative.
      - Small container of 10% buffered formalin (for needle rinses).
      - FNA syringe holder ("gun")

   2) Basic Procedure
      a) Confirm patient ID and collection site using active identification process.

      b) Label 2 slides for each pass with patient's name, date of birth, specimen source and pass number.

      c) Explain the procedure to the patient.

      d) Sterilize skin over area to be punctured (alcohol swabs usually suffice).

      e) Inject local anesthesia (into skin only), if desired.

      f) Position the nodule between second and third digits.

      g) Insert needle into lesion.

      h) Apply full suction.
         - If syringe fills with cyst fluid, keep applying suction until cyst has emptied. Remove syringe and submit fluid as cyst fluid. It is advisable to re-aspirate nodule after draining cyst fluid.
         - If no fluid is aspirated proceed to step i).

      i) Quickly make 5-10, 2-5 mm. in and out excursions into the lesion (do not extend time beyond 5-10 seconds).

      j) RELEASE SUCTION.

      k) Remove needle from the patient.

      l) Remove the needle from the syringe and draw in 5-10 cc air into syringe.

      m) Reaffix needle to the syringe.

      n) Squirt semi-liquid aspirate onto slide.

o) Immediately smear material on properly labeled slides by placing another slide onto first slide and pull both apart.

p) Immediately fix one slide (95% ETOH or spray fixative) within 1-2 seconds, let the other air dry.

q) Rinse any remaining material into a properly labeled container with 10% buffered formalin and submit for cell block.

r) Repeat entire process, performing 2-5 separate passes per lesion (depending on site and material obtained) for a total of 6-10 smears. Separate needles and syringes should be used.

s) If lymphoma is suspected, place at least 2 passes into RPMI transport media.

NOTE: For thyroid, lymph node, and parotid gland aspirations, air dry half of the smears and alcohol fix the other half. The air-dried smears will be stained with a Romanowsky type stain.

3) Submission: Place all slides in slide transport containers and send to RMH Laboratory.

b. **Cyst Fluid:** Many times during the FNA procedure, the only material collected is cyst fluid. Common sites that routinely yield cyst fluid are breast, thyroid, and some neck nodules.
1) Follow the same collection procedure as a FNA of a solid mass up to step h).

2) Remove needle and cap syringe.

3) It is advisable to re-aspirate any remaining nodule after draining the cyst fluid.

4) Properly label the syringe and submit cyst fluid fresh and refrigerate. If there will be a delay to process of more than 24 hours, place the fluid in properly labeled specimen container (50ml centrifuge tube or urine cup) and add an equal amount of 50% ETOH to preserve the sample. Annotate on the container that XXmls of preservative was added.

NOTE: Direct smears may be made when the fluid consists of only a few drops. Fix smears immediately by placing the slides in 95% alcohol transport containers (preferred) or with spray fixative.

5. **Gastrointestinal (GI) Tract Specimens (Gastric Brushes/Washes):** Gastrointestinal tract specimens include brushings and washings from various GI sites to include, bile duct, esophagus, duodenum, stomach, etc. Brushing specimens should be received fixed using 95% ETOH or cytology spray fixative. Washings may be received fresh in normal saline or preserved in an equal volume of a 50% ETOH cytopreservative fluid.

Due to the acidic nature and degenerative potential of gastric material, it is advisable to transport the specimens to RMH Cytology as soon as possible for processing. If there will be a delay of more than 12 hours to process, any washings should have equal amounts of 50% ETOH added to help preserve the sample. Specimens should remain refrigerated until processing.

a. **Gastric Brushings**:
   1) Label in pencil or solvent resistant marker at least two (2) slides per site with the patient's full name, date of birth, and the site of specimen. Note: There may not be enough room on the slides for full words but when using abbreviations please ensure that they are understandable and that they agree with what is on the requisition or order.

   2) Roll brush across slide 2-3 times using light pressure and **immediately** (without delay) place slides directly in 95% ETOH containers (preferred) or use cytology spray fixative. For spray fixed slides, place in plastic slide holders for transport.

   3) If possible, include the brush with the submission. The brush may be placed in a small, labeled container of normal saline or 50% ETOH cytology fixative.

   4) Biopsies may be obtained at the same time. These must be placed in properly labeled formalin containers and have the required test requisitions submitted.

b. **Gastric Washings**:
   1) Collect specimen in a sterile, labeled container, preferably a Luken's Trap type container.

   2) Submit the specimen to the laboratory fresh and unpreserved.
   3) Keep refrigerated during transport to the laboratory.

   4) If there will be a delay to process of more than 12 hours, add an equal amount of 50% ETOH to the container.

6. **Respiratory Specimens (Bronchial Brush/Wash, Bronchioalveolar Lavage (BAL), Sputum):** Respiratory tract specimens that may be collected for cytology include bronchial brushings, washings, bronchioalveolar lavage (BAL), and sputum. These specimens may be received fresh or preserved in an equal volume of a 50% ETOH based cytopreservative fluid.

   The Cytology section of RMH Laboratory is available to assist with the collection and all bronchoscopy procedures performed at RMH. These services are performed during the normal business hours of 8:00am-4:30pm, M-F or by special arraignment. For collection assistance or to schedule, call Cytology at 231-4826.

   a. **Bronchial Brushings:**
      1) Label in pencil or solvent resistant marker at least two (2) slides per site with the patient's full name, date of birth, and the site of specimen to include lobe site designation (i.e. Bronch Brush RUL, RML, etc). Note: there may not be enough

room on the slides for full words but when using abbreviations please ensure that they are understandable and that they agree with what is on the requisition or order.

2) Roll brush across slide 2-3 times using light pressure and **immediately** (without delay) place slides directly in 95% ETOH containers (preferred) or use cytology spray fixative. For spray fixed slides, place in plastic slide holders for transport.

3) If possible, include the brush with the submission. The brush may be placed in a small, labeled container of normal saline or cytology fixative.

4) Biopsies may be obtained at the same time. These must be placed in properly labeled formalin containers.

b. **Bronchial Washings and Bronchioalveolar Lavage (BAL):**
   1) Collect specimen in a sterile, properly labeled container, preferably a Luken's Trap type container.

   2) Submit the specimen to the laboratory fresh and unpreserved.

   3) Keep refrigerated during transport to the laboratory.

   4) If cultures are requested, consider splitting the sample into two containers or collect a second sample for cultures. **Do not add alcohol to the container if cultures are requested.**
      - A separate clinical lab requisition must be completed if cultures are requested.
      - If the sample is split, annotate on the container which specimen is for culture and keep them separate.

   5) If there will be a delay to process of more than 24 hours, add an equal amount of 50% ETOH based cytopreservative to the container.

c. **Sputum:**
   1) Collect three (3) early morning specimens on three consecutive days - one specimen per container.

   2) Before collection, the patient must clean the mouth of all foreign matter (i.e. food, tobacco, toothpaste, etc.).

   3) Have the patient cough deeply and expectorate into a properly labeled, wide mouthed specimen container (urine cup works well).

   4) Submit unfixed and refrigerated (preferred).

   5) If the specimens are likely to sit for an extended period (> 72 hours) then equal amounts of 50% ETOH cytopreservative can be used to preserve the sample.

- The patient may cough directly into the container with the preservative present.
- Refrigerate all collected samples until they are brought to the laboratory.

NOTE: Patient education regarding the nature of the preservative is required. The container must also be labeled annotating the preservative is added.

7. **Tzanck and Other Direct Smears:** Tzanck smears are probably the most commonly submitted direct smear but direct scrapings from any site are acceptable. Specimens may be received wet fixed in 95% ETOH, spray fixed with cytopreservative, or may be received air-dried depending on the specimen type.

   a. **Tzanck Smears:**
      1) Label in pencil or solvent resistant marker at least two (2) slides with the patient's full name, date of birth, and the site of specimen to include left or right designations if indicated (i.e. Left Hand, Right Thigh, etc). Note: There may not be enough room on the slides for full words but when using abbreviations please ensure that they are understandable and that they agree with what is on the requisition or order.

      2) Scrape lesion from the outer edge of the lesion to minimize necrotic debris and inflammatory response.

      3) Smear material directly on the slide in a large circular motion and immediately place the slides directly into 95% ETOH alcohol transport containers (preferred) or spray fix the slides immediately with cytology spray fixative.

      NOTE: Additionally, we strongly recommend ordering a Herpes PCR (See clinical test # 3388 "Herpes Simplex Virus / Varicella zoster Virus DNA detection by PCR, Dermal) which is both more sensitive and more specific.

   b. **Other Miscellaneous Direct Smears**: Follow the same procedures for Tzanck smears except if no alcohol fixation is available, send slides air-dried but collect at least 4 smears if possible so an attempt can be made to re-hydrate some of the smears in the cytology laboratory.

8. **Urinary Tract Specimens:** It is preferred all urinary tract specimens be submitted fresh and refrigerated when possible. Urinary tract specimens may come from the following sites:
   - Voided Urine
   - Catheterized Urine
   - Illeal Loop Urine
   - Urethral Brush/Wash
   - Ureter Brush/Wash (Specify Right or Left)
   - Renal Pelvis Brush/Wash (Specify Right or Left)

a. **Voided/Catheterized/Illeal Loop Urine:**
   1) A maximum of 100 mls and a minimum of 5 mls is the preferred volume; however, an attempt will be made to process all samples.

   2) Specimens may be submitted by the patient in a properly labeled urine cup or the submitting lab may transfer the urine into a properly labeled 50ml centrifuge tube for shipment.

   3) Indicate the source of the sample on the specimen container and requisition: Voided, Catheterized, or Illeal Loop.

   4) Voided urine samples should be collected using a clean catch technique to avoid an overabundance of squamous cells and other contaminants.

   5) First morning samples are not desirable due to the potential for degenerative changes of the epithelial cells from sitting in the urine overnight.

   6) Submit the specimen fresh and refrigerated. If there will be a delay to process of more than 24 hours, add equal parts of a 50% ETOH cytopreservative to preserve the sample.

   7) If clinical laboratory tests are ordered on the urine, consult the collection procedures for those tests. **Do not add 50% ETOH preservative to these samples.**

   8) If FISH studies are required, please call the lab at 1-800-922-RICE for a Mayo Labs UroVysion collection kit.

   9) Voided urines are considered a "recollectable" specimen and may be rejected.

b. **Urinary Brushes (Renal, Ureter, Urethral):**
   1) Label in pencil or solvent resistant marker at least two (2) slides per site with the patient's full name, date of birth, and the site of specimen to left or right designation (i.e. Left Renal Pelvis, Right Ureter, etc). Note: there may not be enough room on the slides for full words but when using abbreviations please ensure that they are understandable and that they agree with what is on the requisition or order.

   2) Roll brush across slide 2-3 times using light pressure and **immediately** (without delay) place slides directly in 95% ETOH containers (preferred) or use cytology spray fixative. For spray fixed slides, place in plastic slide holders for transport.

   3) If possible, include the brush with the submission. The brush may be placed in a small, labeled container of normal saline or 50% ETOH cytopreservative.

   4) Biopsies may be obtained at the same time. These must be placed in properly labeled formalin containers.

c. **Urinary Washes (Renal, Ureter, Urethral):**
   1) Collect specimen in a properly labeled container indicating the site of the specimen to include left or right designation.

   2) Submit the specimen to the laboratory fresh and unpreserved.

   3) Keep refrigerated during transport to the laboratory.

   4) If there will be a delay to process of more than 12 hours, add an equal amount of a 50% ETOH cytopreservative to the container.

VI. Procedural Notes:

a. **95% Alcohol Transport Containers** are intended to fix the specimen onto the slide and keep the sample slide in solution continuously from collection to transport to processing and staining in the lab. Please do not dip slides in the alcohol and then remove them to air dry. This does not adequately fix the specimen and will compromise the integrity of the cells. The slides must remain in the alcohol solution.
b. **Cytology Spray fixative** contains an additive (polyethylene glycol) that allows the specimen to be fixed on the slide yet the slide can be allowed to air dry after immediate fixation without compromising the integrity of the cells. These are transported in a 'dry' plastic slide transport container.

VII. RELATED PROCEDURES:

A. RMH Laboratory Procedure, *Zero Tolerance Specimen Labeling Procedure.*

VIII. REFERENCES:

A. ASCP Press, *Manual of Cytotechnology, 7th Ed., 1997,* Chapter 26.

B. Koss, L. G., *Diagnostic Cytology and Its Histologic Basis, 4*th Edition, J. B. Lippincott, USA, 1992, Chapter 32.