Cytology Specimen Collection Manual

The cytopathology laboratory is dedicated to a high standard of patient care. In order to achieve that, the cytology specimen collection manual is dedicated in providing information on how specimens should be collected and submitted. Following these instructions will help increase the features and number of diagnostic cells. All cytology specimens must be submitted with a cytology requisition.

Please see the *Cytology Specimen Requisition Procedure* for additional information.

There are specific guidelines that are recommended by the American Society of Cytopathology, American Society of Clinical Pathology, College of American Pathology and American Society of Cytotechnology in collecting cytology specimens. It is imperative to follow the guidelines set forth for the following specimens:

1. Respiratory
   - Sputum
   - Bronchoalveolar Lavage
   - Bronchial Brushings/Washings
2. Fluids
   - Serous (Pleural, Peritoneal, Pericardial)
   - Body Cavity Washings
   - Nipple Discharge
3. Urine
4. Gastrointestinal Tract
   - Oral/Anal
   - Esophagus, Stomach, Small Bowel, Colon, Common Bile Duct
5. Skin
6. Cerebral Spinal Fluid
7. Fine Needle Aspiration Biopsy
   - Lymph Node, Thyroid, Breast, Soft Tissue, Bone, Kidney, etc…
   - Palpable (superficial sites) and deep localized organs (CT & Ultrasound guided)
8. Gynecological (Pap Smear)
   - Liquid-Based Pap
   - Conventional Pap

Cytotechnologists, pathologists, and cytopreparatory technicians are available to answer any questions during business hours.

Cytology Reports are guaranteed in two business days, unless special testing is required (i.e. immunoassays, special stains, flow cytometry studies, etc.); in that case, final reports

may be delayed until ancillary studies are complete. Clinicians may contact the cytopathology department during this time, and request a preliminary interpretation from a pathologist for pending patient care purposes.

****Failure to follow Evangelical Community Hospital Cytopathology Laboratory Policy will result in rejection of the specimen. The specimen will be sent back to the clinician's office, which may cause delays in patient care. The office will be notified by phone before any specimens are sent back.****

Cytology Specimen Requisition Procedure

PURPOSE

In order to ensure an accurate interpretation on the correct patient, it is important to maintain reliable and consistent procedures in submitting cytology specimens (gynecological specimens and non-gynecological specimens) to the laboratory. The following policy defines the required requisition elements to be indicated for each specimen sent to the laboratory. See the following sample copy of the *Cytology Requisition.*

POLICY

1. A Cytology Requisition must accompany all cytology specimens. Without a requisition, the specimen will be rejected and sent back from wherever it originated. Please use separate requisitions if more than one specimen source is sent to the lab.

2. The specimen can only be received from licensed physicians and/or other persons authorized by law, and their name should appear on the requisition. Any copy of reports to other physicians should include the clinician's full name/location in the space provided.

3. The Cytology Requisitions must have the following information ***written clearly***:
   - A. patient's full name
   - B. date of birth
   - C. identification number (SSN and/or visit number)
   - D. ordering clinician
   - E. collection date or date of service
   - F. specimen source (either write in or check the appropriate box)
   - G. ICD-9 code and relevant clinical history

   For PAP smears:
   - H. type of Pap test (check appropriate box)
   - I. HPV DNA testing (when applicable)
   - J. last menstrual period (LMP) information, and any other relevant Pap history (check appropriate box)

   Without this information, the office will be contacted, and must complete the *Request for Correction/Additional Information* form (and return via fax).

4. The specimen must be in a sealed container with the patient's full name, date of service and specimen type written on the container or slide. Specimen type for Paps is not needed on the slide or container.

5. The specimen container must be in a biohazard bag, except for effusion fluid evacuated containers; the bottle must have a biohazard symbol or state *biohazardous material*. Lack of proper identification on the container will result in a phone call to the physician's office, nursing floor, or where ever it originated to identify the specimen. Incomplete information results that the specimen will be rejected and returned to it's place of origin.

****Failure to follow Evangelical Community Hospital Cytopathology Laboratory Policy will result in rejection of the specimen. The specimen will be sent back to the clinician's office, which may cause delays in patient care. The office will be notified by phone before any specimens are sent back.****

Name:

Address: A

Birth date: B Sex: ☐Male ☐ Female

SSN: C

Please attach copy of Insurance information

Ordering Physician:_____D_____ Collection Date:_____E____

(First and last name)

Nurse Practioner/Physician's Assistant:_____D_____

Report Copy to:_____ Report Copy to:_____

Specimen Source:_____F_____

Previous Clinical History/ICD-9 Diagnosis

G

GYN (PAP TEST)

☐ Pre-natal Liquid V22.0 or V22.1
☐ Routine Liquid V76.2
☐ Conventional Routine V76.2
☐ Conventional High Risk V15.89

☐ High Risk Liquid V15.89_____H_____ ☐ Pap DNA Routine (Pap smear & HPV testing) V76.2 I

☐ Pap DNA High Risk (Pap smear & HPV testing) V15.89_____

☐ Diagnostic Liquid include ICD-9 code: _____

vical Vaginal Endocervical Combination CE

LMP ____/____/____

CHECK ALL THAT APPLY

☐ PREGNANT J
☐ POST-PARTUM
☐ BREAST FEEDING
☐ HYSTERECTOMY
☐ SUPRA-CERVICAL HYSTERECTOMY
☐ POST MENOPAUSAL
☐ PERIMENOPAUSAL
☐ BIRTH CONTROL PILL
☐ BC - PATCH/NUVARING
☐ BC - DEPO PROVERA/IMPLANON
☐ IUD
☐ HORMONE Rx
☐ RADIATION Rx
☐ COLPOSCOPY/BIOPSY
☐ LEEP – CONIZATION
☐ CHEMOTHERAPY
☐ PREVIOUS ABNORMAL PAPS
Dx: _____
Date: _____

Non-GYN (MARK SPECIMEN TYPE BELOW)

URINARY
☐ Urine - Voided
☐ Urine – Cath Wash F
☐ Bladder Wash

BODY FLUID
☐ Abdominal/Paracentesis
☐ Pleural/Thoracentesis
☐ Peritoneal Washing
☐ Spinal Fluid

ESOPHAGEAL
☐ Esophageal Brush

GASTRIC
☐ Gastric Brush
☐ Gastric Wash

BRONCHIAL/PULMONARY
☐ Bronch Brush R L
☐ Bronch Wash R L
☐ Bronchoalveolar R L
☐ Sputum

MISC CYTOLOGY
☐ Endocervical Rotage
☐ Nipple Discharge R L
☐ Other:_____

NEEDLE ASPIRATION
☐ FNA (specify type)

☐ Cyst Fluid (specify site)

☐ Other:

FOR LAB USE ONLY

Assisted FNA Tech: _____ Quality Assurance
Specimen Source: _____
'equate Inadequate
son:_____
_____Passes
_____ Fix Slides
_____ Diff Quik Slides
_____ Container Fix/Unfix

NON-GYN DESCRIPTION
Amount:
Color/Appearance:

☐ UNSATISFACTORY
☐ CYTOSPIN ☐ 59 Mod
☐ DIRECT SMEAR ☐ 59 Mod
☐ FNA ADEQUACY ☐ 59 Mod
☐ FNA INTERPRETATION ☐ 59 Mod
☐ SPECIAL STAIN II ☐ 59 Mod
☐ CELL BLOCK ☐ 59 Mod
☐ HPV ☐ 59 Mod

CT Initials

Respiratory

SPUTUM

To obtain cells from an upper respiratory tract lesion, collection of a sputum specimen is recommended. Sputum is also useful in the diagnosis of fungal, viral and parasitic infections. Unsatisfactory sputum will be reported out if no *alveolar macrophages* are present or no diagnostic cells are present.

Directions:

1. To increase adequacy, sputum specimens should be obtained by an early morning, deep cough for 3-5 consecutive days.
2. Place sputum specimen directly into a clean container (or use a sputum collection device) within a biohazard bag, along with a properly completed requisition.
3. Bring specimen to laboratory immediately; if unable to bring to the laboratory, refrigerate specimen until it can be delivered.

BRONCHOALVEOLAR LAVAGE (BAL)

To obtain cells from lower respiratory tract lesions, BAL is recommended. BAL specimens are obtained by wedging a sub-segmental bronchus with a bronchoscope and lavaging the area with saline or balance salt solution.

This technique is most useful in diagnosing opportunistic infections in immunocompromised patients. This can also be useful in diagnosing interstitial lung disease, granulomatous disease (including sarcoidosis), hypersensitivity pneumonia, drug induced pulmonary toxicity, asbestosis, pulmonary hemorrhage, and neoplasm (benign and malignant). BAL can also evaluate transplant rejection.

Directions:

1. Place specimen in a clean container within a biohazard bag; include a properly completed requisition.
2. Bring specimen to laboratory immediately; if unable to bring to the laboratory, refrigerate specimen until it can be delivered.

BRONCHIAL BRUSHINGS/WASHINGS

This is most warranted when abnormal sputum has recently been reported, or a lesion is suspicious.

Directions for bronchial brushing specimens:

1. Spread the specimen onto glass slides that are labeled with patient's name and date of birth.
2. Immediately spray fix slides.

3. If no fixative is available, submerge slides in slide containing 95% alcohol immediately. DO NOT allow the slides to air dry.
4. Obtaining numerous slides is recommended.
5. Any viable tissue should be placed in 10% formalin.
6. Deliver to laboratory with properly completed requisition.

Directions for bronchial washing specimens:

1. Follow same protocol listed above for BAL specimens.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Body Fluids for Cytology

SEROUS FLUIDS (Pleural, Peritoneal/Ascites, Pericardial)

Fluid specimens are recommended for cytology when metastasis is suspected or when there is an increased fluid accumulation of unknown origin. Direct drainage provides the most favorable specimen. Heparin should be used as an anticoagulant in all fluids, especially bloody fluids.

If clinically suspicious for lymphoproliferative disorder (leukemia/lymphoma), it is recommended that 2-5cc of fluid be placed in RPMI for flow cytometry testing. Contact laboratory for vial of RPMI and any additional information.

Directions:

1. Collect fluid in heparinized evacuated container or other clean collection device. Alternatively, heparin (3-5iu per milliliter of fluid) can be added to the collection container. This step is especially important for bloody fluids.
2. Indicate on the specimen container that heparin has been added, along with the amount added.
3. Please label the specimen with: patient name, DOB, source of the specimen, and date collected.
4. Bring specimen to laboratory immediately; if unable to bring to the laboratory, refrigerate specimen until it can be delivered.

BODY CAVITY WASHINGS

When washing out a body cavity, saline or a balanced salt solution is recommended. Place specimen in a clean container, and sent to laboratory immediately; refrigerate specimen if there is a delay in transport.

NIPPLE DISCHARGE

Please note on the requisition if the discharge is from the left or right breast, or if it is bilateral; also note consistency and color of the discharge (for example: bloody, serous, mucoidal, etc.).

Nipple Discharge

Please note on the requisition if the discharge is from left or right breast, or if it is bilateral or unilateral, the consistency and color of the discharge (bloody, serous, thick, etc…).

Make slides using the *pull- apart technique* as follows:

Directions:

1. Place a small amount of specimen in the center of one slide, with the labeled side up. Make sure the slide is labeled in pencil with patient's name, DOB and specimen type. Also, indicate which slide is to be fixed, and which slide is to be air-dried.
2. Invert a second slide over the specimen (ensure that the proper side of each glass slide will be making contact with the specimen).
3. Pull/slide the two slides apart, gently spreading the specimen; this technique produces a *pull smear* with an evenly distributed amount of specimen over the face of the glass slide.
4. Allow one slide to air-dry, and apply spray fixative to the other (available from the laboratory).
5. If no spray fixative is available, place the slide(s) to be fixed in a slide container containing 95% alcohol immediately.
6. Bring to laboratory immediately for processing.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Urine

Urine cytology is useful in the diagnosis of diseases involving the mucosal surface. Knowing the type of exfoliated urinary tract specimen is essential for an accurate cytologic diagnosis; therefore, clinical information should include whether the specimen is voided, collected from catheterization, or the product of a bladder washing. The *clean catch* voided urine is recommended for screening purposes, but if a bladder tumor is suspected, a bladder washing is preferred. Sending fresh specimens to the laboratory is preferred, but receipt of urinary specimens in cytopreservative solution is acceptable.

Directions:

VOIDED URINE

Place specimen in clean container within a biohazard bag, and include a properly completed cytology requisition. Bring specimen to laboratory immediately. If unable to deliver immediately, place specimen in refrigerator until it is brought to the laboratory.

CATHETERIZED URINE

If possible, freshly voided urine should accompany this specimen. Otherwise, place catheterized urine in a clean container within a biohazard bag, and include a properly completed cytology requisition. Bring specimen to laboratory immediately. If unable to deliver immediately, place specimen in refrigerator until it is brought to the laboratory.

BLADDER WASHINGS

Use saline or a balanced salt solution for bladder washings. Place specimen in clean container within a biohazard bag, and include a properly completed cytology requisition. Bring specimen to laboratory immediately. If unable to deliver immediately, place specimen in refrigerator until it is brought to the laboratory.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Gastrointestinal Tract

Gastrointestinal tract specimens include oral, esophageal, gastric, intestinal, rectal, and anal specimens. The use of endoscopic retrograde cholangiopancreatography (ERCP) guided technique is useful in retrieving samples from the biliary tract or pancreatic head. Listed below are techniques to guarantee optimal specimen collection, and ultimately an accurate cytologic interpretation.

ORAL/ANAL LESIONS

Liquid-based Pap smear containers may be utilized for anal specimens (contact laboratory for supplies if needed). Advantages in utilizing these containers are: increased specimen satisfaction rate, more viable diagnostic cells, and ability to perform HPV DNA testing from remaining specimen in vial (after cytologic processing).

Directions:

1. Use the Cervex-Brush or moistened Dacron swab to sample the oral cavity, or five to six centimeters into anal canal.
2. Apply pressure to the sampling device as it is rotated circumferentially around the oral/anal wall.
3. Detach sample end into the SurePath fixative vial.
4. Specimen will be **rejected** if Cervex-Brush's white sampler end or Dacron swab end is not in the vial. This is an FDA requirement, and no exceptions will be made. Additional sampling made by other brushes is acceptable.
5. Secure lid and label with patient's name and date of birth.
6. Place vial in biohazard bag and seal. Place completed cytology requisition in outer pouch and send to laboratory.

Use the following method if a liquid-based vial is not available.

Directions:

1. Direct sampling using a wooden or plastic spatula, tongue blade, or brush is also acceptable, but not preferred.
2. Smear the sample onto a slide.
3. Immediately spray fix slides.
4. If no fixative is available, drop the slides in slide containers containing 95% alcohol immediately. DO NOT allow the slides to air dry.
5. Obtaining multiple slides is recommended.
6. The sampling tool can also be agitated in a saline container.
7. Suction is also acceptable. Place the specimen in saline solution.
8. Label the specimen container with patient's name, specimen type, and date.
9. Send the specimen to the laboratory immediately, or refrigerate until able to bring to laboratory.

GASTROINTESTINAL SPECIMENS OBTAINED BY ENDOSCOPY

This applies to esophageal, gastric, and intestinal specimens obtained through endoscopy procedures. It is recommended that a Cytotechnologist is present to assist in properly collecting and preparing the specimen; however, if a cytotechnologist is unavailable, the specimen should be placed on a slide and the *pull-apart* technique should be performed by following these directions:

Directions:

1. Label slides used with patient name, date of birth, specimen source, and pass number.
2. Place specimen in the center of one slide with label up.
3. Invert another slide over the specimen; ensure that the proper surface of the slide will be in contact with the specimen.
4. As the specimen spreads gently, pull the two slides apart horizontally.
5. Immediately spray fix slides.
6. If no spray-fixative is available, place slides in slide container jars containing 95% alcohol immediately. DO NOT allow the slides to air dry.
7. Obtaining multiple slides is recommended.
8. Any viable tissue should be placed in 10% formalin.
9. Send the specimen to the laboratory immediately, or refrigerate until able to bring to laboratory.

ESOPHAGEAL BRUSHINGS

Use the following touch-prep technique for esophageal brushings.

Directions:

1. Take the brush and gently touch the upper portion of the slides three times.
2. Next, roll the brush 360° at the lower portion of the slide. Make as many slides as possible and spray fix immediately.
3. If no spray-fixative is available, place slides in slide container jars containing 95% alcohol immediately. DO NOT allow the slides to air dry.
4. Placing entire specimen in a cytology preservative agent or saline is acceptable.
5. Send the specimen to the laboratory immediately, or refrigerate until able to bring to laboratory.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Gastrointestinal Tract

Liquid-Based Pap smear containers may be utilized for anal specimens. Advantages in utilizing these containers are: increased specimen satisfaction rate, more viable diagnostic cells, and ability to perform HPV DNA testing from remaining specimen in vial (after cytologic processing).

Sampling of Anal Lesion using Liquid-Based collection method:
DIRECTIONS

1. Use the Cervex-Brush or moistened Dacron swab to sample five to six centimeters into anal canal.
2. Apply pressure to the sampling device as it is rotated circumferentially around the anal wall.
3. Detach sample end into the SurePath fixative vial.
4. Specimen will be REJECTED if Cervex-Brush's white sampler end or Dacron swab end is not in the vial. This is an FDA requirement, and NO exceptions will be made. Additional sampling made by other brushes is acceptable.
5. Secure lid and label with patient's name and date of birth.
6. Place vial in biohazard bag and seal. Place completed cytology requisition in outer pouch and send to laboratory.

****NOTE: all slides and container must be labeled with patient's name, specimen type and date*****

Skin Lesions

DIRECTIONS

1. Scrape the lesion with a wooden or plastic spatula.
2. Smear the specimen onto the slides, making sure the smear area on glass slide is not too thick.
3. Apply spray fixative to the slides immediately.
4. If spray fixative is unavailable, immediately place slides in slide container jars containing 95% alcohol, and send to laboratory.
5. Placing the entire specimen in saline container is acceptable.
6. Forward the specimen to the laboratory immediately, or refrigerate until able to transport to laboratory.

If a different lesion is sampled, a separate requisition form is required.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Cerebral Spinal Fluid

Obtaining Cerebral Spinal Fluid (CSF) is invasive and painful. Therefore, obtaining the specimen correctly and submitting the correct information is imperative. Submitting accurate information is vital in reaching a valid diagnosis. The following information should be included when CSF is submitted to the laboratory:

1. Source of CSF
2. Clinical impression
3. Symptoms and clinical information
4. Recent results of any additional testing regarding central nervous system
5. Information concerning previous therapy, including intrathecal medication, radiation treatment to head/spinal cord, etc.
6. Surgical history (presence of shunts, FNA or brain biopsy, cyst drainage, etc.)
7. Information regarding prior lumbar taps, invasive procedures, or surgical interventions – all of which could provoke a reactive cellular response.

Directions:

1. Submit CSF to laboratory within 30 minutes following the procedure for optimal cellular preservation and diagnostic viability.
2. Submit specimen in clean container/tube within a biohazard bag, and include a properly completed cytology requisition.
3. If unable to bring to laboratory immediately, refrigerate specimen until it can be delivered.

NOTE: If clinically suspicious of lymphoproliferative disorder (leukemia/lymphoma), it is recommended that 2-5cc of fluid be placed in RPMI for flow cytometry. Contact laboratory for a vial of RPMI and for any additional information.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Fine Needle Aspiration Biopsy

Fine Needle Aspiration Biopsy (FNAB) can be defined as a diagnostic procedure used to investigate superficial (just under the skin) lumps or masses, or lesions on deep-seated organs. In this technique, a thin, hollow needle is inserted into the mass/lesion for sampling of cells that, after being stained, will be examined under a microscope. FNAB is performed on palpable organs or deep-seated organs using a fine needle. Deep-seated FNAB is obtained through the assistance of CT or ultrasound.

There are different techniques in aspirating cells from a mass. Any questions about how to aspirate a mass should be directed to a pathologist for assistance. To ensure an optimal number of adequate cells on palpable organ or deep-seated organ biopsy, the presence of a cytopathologist or cytotechnologist is recommended for assistance; however, if there is no assistance, follow the directions below.

Directions:

1. Label slides with patient's name, date of birth, specimen type, and pass number (two slides are used for each pass).
2. Place specimen in the center of a glass slide with the labeled side up.
3. Invert another glass slide over the specimen; ensure the proper side of the inverted side will contact the specimen.
4. As the specimen spreads gently, pull the two slides apart horizontally.
5. Allow one slide to air dry.
6. Apply spray fixative to the other slide, or place the slide in a slide container containing 95% alcohol for fixation.
7. Please make between 4-10 slides only (2-5 passes).
8. After each pass, perform a needle rinse into a conical tube containing saline solution (contact laboratory if any materials are needed); this material undergoes histological processing to create a cell block.
9. Send properly labeled specimens to the laboratory immediately, along with a properly completed cytology requisition.

NOTE: For Lymph Nodes that are clinically suspicious for lymphoproliferative disorder (leukemia/lymphoma), it is recommended that 2-5cc of fluid be placed in RPMI for flow cytometry testing. Contact the laboratory for a vial of RPMI and for any additional information.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******

Gynecological (PAP Smears) Specimens

In order to have an accurate interpretation on the correct patient, it is important to maintain reliable and consistent procedures in submitting Pap smears to the cytology laboratory. There are two types of Pap smears, liquid-based (preferred) and conventional, that can be submitted. Using the liquid-based Pap smear has numerous advantages; these advantages include an increased satisfactory rate, more viable diagnostic cells, and the ability to perform HPV DNA testing from remaining specimen after cytology processing is complete.

Directions for liquid-based preparations:

1. Using the Cervex-Brush, obtain gynecological sample. Detach white sampler end into the SurePath fixative vial. Specimen will be REJECTED if Cervex-Brush's white sampler end is *not* in vial. This is an FDA requirement and no exceptions will be made. Additional sampling made by other brushes is acceptable.
2. Secure lid and label with patient's name, date of birth, and collection date.
3. Place vial in biohazard bag and seal. Include a properly completed cytology requisition.
4. See *Cytology Specimen Requisition Procedure* for further instructions regarding requisition details.

Directions for conventional preparations:

1. Using a pencil, label frosted end of microscope with patient's full name and date of birth.
2. Obtain an adequate specimen using approved procedures and smear onto the slide.
3. IMMEDIATELY fix the specimen with the fixative spray. Do not allow slide to dry before spraying.
4. After applying spray fixative, allow the fixative to dry completely.
5. Place slide in cardboard mailer and send in a sealed biohazard bag; include a properly completed cytology requisition.
6. See *Cytology Specimen Requisition Procedure* for addition instruction regarding requisition details.

******NOTE: All slides and specimen containers must be labeled with at least two unique patient identifiers (use patient name and patient date of birth); in addition, specimen container must indicate specimen source and collection date *******