(GEN.40016,GEN.40032,COM.06000,COM.06100,COM.06300,493.1242(A)(7),493.1251(B)(2),493.1274(E)(1), 493.1451(C)(4))

CYTOLOGY FIXATION AND COLLECTION PROCEDURES:

GENERAL INFORMATION

These procedures are designated for specimens collected in the hospital setting, clinic, or private physician office. Recommended fixatives and collection procedures are very important in order to properly preserve cells for microscopic study and render the best possible diagnosis.

All specimens should be properly labeled with at least two acceptable identifiers: patient name, date of birth, hospital number, social security number, requisition number, or accession number. All specimens should be accompanied by a cytology requisition form which has been properly completed by the patient's physician.

The following procedures are those of specimens most frequently collected for cytologic studies. However, studies can be done on many other types of specimens. Questions concerning procedures, including information not listed in this manual, should be directed to the Tulsa Medical Laboratory Cytology Department @918-481-7854.

FEMALE GENITAL TRACT

FIXATION FOR PAP TESTS:

1. Liquid-Based Pap Tests - PreservCyt Container. (Check expiration date on vial)
2. Spray Fixative.
3. Immersion into a vial of 95% alcohol.

COLLECTION INSTRUCTIONS FOR LIQUID-BASED PAP TESTS (TWO OPTIONS):

OPTION (1): Combo Collection Method (Endocervical Brush and Plastic Spatula) - Obtain adequate sampling from the ectocervix and rinse in PreservCyt Solution by swirling the spatula vigorously in vial (10) times. Discard the spatula. Obtain adequate sampling from the endocervix using endocervical brush device. Insert brush into cervix with only bottom-most fibers being exposed. Slowly rotate 1/4 or 1/2 turn in one direction. Do not over rotate. Rinse as quickly as possible in PreservCyt Solution by rotating the device in the solution (10) times and pressing against the sides of the fixative vial. Swirl vigorously the brush to further release material. Discard the brush.

OPTION (2): Broom-Like Device - Obtain an adequate sampling from the cervix using a broom-like device. Insert central bristles of the broom into the endocervical canal deep enough to allow shorter bristles to fully contact ectocervix. Push gently and rotate the broom in a clockwise direction (5) times. Rinse the broom as quickly as possible into the PreservCyt fixative vial by pushing the broom into the bottom of the vial (10) times forcing the bristles apart. As a final step swirl the broom vigorously to further release material. Discard collection device.

Tighten the cap of the vial so the black torque line on the cap passes the black torque line on the vial. Always record the patients name and a second identifier on the vial. Record the patient's name, medical history, LMP, ordering physican, molecular tests ordered, date of birth, date of collection, and insurance information on the Cytology Requisition or electronic order. Place printed copy of requisition in the outer side pocket of biohazard bag. Place vial inside center of biohazard bag and seal in case of leakage.

HANDLING: Maintain at room temperature.
REJECTION CRITERIA:

1. Specimens that have leaked from the container.
2. Specimen container without two patient identifiers.
3. Check the expiration date on the vial. No molecular test will be performed.

RETENTION: Liquid specimen - six weeks. If a molecular test has been performed the vial is discarded in one week. Slides are retained for five years.

COLLECTION INSTRUCTIONS FOR CONVENTIONAL PAP TESTS:

Note: Slides should be labeled with the patient's name before smears are taken. In addition, the speculum should not be moistened with surgical lubricating jelly before insertion into the vagina. If needed, moistening the speculum with warm tap water will aid in insertion and will not interfere with subsequent cytologic examination.

METHOD NO.1:

- Insert pointed cervical end of spatula a high as possible into the external cervical os.
- Rotate the spatula completely around the canal (360 degrees) thus sampling the entire transition zone.
- Quickly spread the material in a lengthwise motion on a previously labeled glass slide. Fix immediately. Do not contaminate specimen with surgical lubricant.

METHOD NO.2:

- Follow the directions as stated above, but in addition to the scraper - insert a cytology cervical brush into the endocervical canal.
- Rotate the brush clockwise and counterclockwise (360 degrees) to ensure proper sampling of area.
- The material can be placed on the same slide as the cervical scraping. One area of the slide can be reserved for the cervical scraping material and another portion can be reserved for the cytobrush material.
- Spray fix material immediately. Do not contaminate specimen with surgical lubricant.

METHOD NO.3:

- A sample containing ectocervical material and endocervical canal material can be obtained simultaneously by utilizing a Unimar Cytology Cervical Brush. Call the TML Cytology Department if you would like to try this particular type of cytology brush.
- Insert the Unimar Cytology Brush into the endocervical canal until the shorter bristles are flush with the ectocervix. Rotate the brush 360 degrees, first in a clockwise motion 3-4 turns, then in a counter clockwise motion another 3-4 turns.
- Spread the material on a pre-labeled slide in a paintbrush motion - one smooth stroke. Turn the brush over and follow the same motion.
- Spray fix immediately. Do not contaminate specimen with surgical lubricant.
- If smeared properly, endocervical cells will be centered directly in the middle of the slide. The ectocervical cells will be on both sides of the endocervical cells. This facilitates the detection of endocervical material which denotes a proper cervical smear.

HANDLING: Maintain at room temperature.

REJECTION CRITERIA:

1. Slides that are broken but repairable, with 50% of the slide missing, will be repaired and processed as usual with an appropriate comment issued with the final report; slides with greater than 50% missing will be rejected.
2. The technologist/pathologist may decide material is insufficient for proper evaluation. Severe air drying artifact and/or mechanical distortion may render the smear unsatisfactory for evaluation of cell morphology. Unsatisfactory smears include acellular smear, severe obscuring inflammation, lubricant or blood, and/or extensive air drying artifact.

RETENTION: Five years.

COLLECTION INSTRUCTIONS FOR VAGINAL POOL SMEAR:

Sampling the Vaginal Pool (Posterior Vaginal Fornix) is less valuable than direct cervical scraping for detection of early cancer of the cervix. However, it is an excellent method for detection of endometrial lesions and extrauterine neoplasms, as well as for evaluation of radiation responses, and may be included as part of a routine cytologic evaluation for malignancy. In addition, it is the ideal method for following post-hysterectomy patients.

Note: Slides should be labeled with the patient's name before smears are taken. In addition, the speculum should not be moistened with surgical lubricating jelly before insertion into the vagina. If needed, moistening the speculum with warm tap water will aid in insertion and will not interfere with subsequent cytologic examination.

- Vaginal pool material is obtained with a spatula or by pipette aspiration and placed as a thick drop on the slide near the label end.
- Spread the material across the slide in a smooth even lengthwise motion with the spatula.
- Fix immediately in 95% alcohol or with spray fixative.

HANDLING, REJECTION, RETENTION: Same as for a conventional pap test.

COLLECTION INSTRUCTIONS FOR "FAST" SMEAR (COMBINED CERVICAL, VAGINAL, ENDOCX):

For routine use, the "fast" smear technique offers a high rate of general gynecological cancer detection, yet it is fast and simple to prepare clinically. Mixing the pancervical scraping with the vaginal pool material from serous, inflamed cervices is better than with a simple cervical scrape smear.

- Obtain a sample of the vaginal pool mucus and place it as a thick drop on the slide near the label end. Do not spread the material.
- Insert the pointed end of the cervical scraper into the external cervical os as high as can be reached and scrape completely around the canal (360 degrees). Thus, the entire transition zone will be sampled (Endocervical Canal, External Os, and Ectocervix).
- Mix the material from the pancervical scraping(Utilization of the cytobrush can enhance the collection procedure) with the vaginal pool material on the slide.
- Using the cellular spreader portion of the cervical scraper make two lengthwise light strokes, resulting in a smear that is evenly distributed on the slide. Do not smear with spatula in a circular motion.
- Fix immediately in 95% alcohol or with a suitable spray fixative.

HANDLING, REJECTION, RETENSION: Same as for a conventional pap test.

COLLECTION INSTRUCTIONS FOR HORMONAL EVALUATION SMEAR:

When taken correctly, the vaginal smear for hormonal evaluation can be a valid and reliable asset for monitoring estrogen, progesterone, and androgen levels.

Note: The patient should be instructed not to douche, use intravaginal medication, diaphragm, or contraceptive cream for at least 24 hours before smear collection.

- Expose the vaginal walls with an unlubricated speculum.
- Using the rounded end of a cervical scraper, collect the material from the lateral wall of the middle third of the vaginal wall. Use several gentle vertical strokes.
- Smear collected material on a glass slide, previously labeled with patient's name and site of specimen.
- Fix immediately in 95% alcohol or with a suitable spray fixative.

Note: Accurate hormonal evaluation cannot be made if the specimen is collected when there is vaginal bleeding or infection present. Although formal maturation indexes (M.I.) cannot be performed on ThinPrep Pap Test specimens, estimated estrogen effect can be performed which may provide clinically useful information.

HANDLING, REJECTION, RETENTION: Same as for a conventional pap test.

COLLECTION INSTRUCTIONS FOR VULVAR OR VAGINAL LESION SMEAR:

The best specimens are obtained from the growing margins of the lesion, not from the necrotic center.

- For best results, soak the margins of the lesion with saline, then remove and discard the necrotic and keratinized debris
- Abrade the growing margins of the lesion with the rounded end of a wooden scraper.
- Smear the collected cellular material on a previously labeled glass slide.
- Fix immediately in 95% alcohol or with a suitable spray fixative.

Note: Cellular specimens from direct lesion scrapings dry out rapidly. Thus, rapid fixation is critical if optimum results are to be obtained.

HANDLING, REJECTION, RETENTION: Same as for a conventional pap test.

GYNECOLOGICAL COLLECTION PROTOCOL FOR PATIENTS WITH MULTIPLE SITES SAMPLED:

1. If a patient with a routine pap test has an additional site sampled for cytologic studies such as a vaginal lesion, perineal lesion, and/or vulvar lesion - please label each subsequent specimen vial with the appropriate source/site. This will alleviate any confusion as to the source of the specimen if any discrepant issue arises.
2. Please fill out a separate cytology requisition for each site and possible description of the lesion observed. Any clinical information relevant to the cytologic examination will be greatly appreciated.
3. Please place all of the same patient's vials (such as the routine pap test vial and any other vial (s) with different cytologic source/sites) in the same biohazard bag along with the requisitions and the laboratory will determine whether one or more accession numbers will be assigned. Please remember to place vial (s) in the center compartment of the biohazard bag and the requisition in the outer compartment.

PLEASE NOTE THE FOLLOWING CONDITIONS OR LIMITATIONS WHEN COLLECTING GYNECOLOGICAL SPECIMENS:

1. The patient should schedule her pap smear for mid-cycle of her menses. If the patient is experiencing her menses at the scheduled time of her pelvic exam, the patient should be instructed to call the doctor's office and reschedule the examination. The blood may obscure the evaluation of the cells or limit the amount of cellularity.
2. The patient should not douche for 24 hours prior to the collection of the pap smear. She should also refrain from sexual intercourse for 24 hours prior to the collection of the pap smear.
3. The doctor should refrain from using surgical lubricant prior to the pap smear collection. The lubricating jelly will markedly interfere with the cytologic examination.

PURPOSE:

When a pulmonary lesion is suspected, a complete sputum series should be examined. The complete sputum series consists of a SINGLE early morning deep cough specimen EACH DAY for three to five consecutive days. A post-bronchoscopy specimen may be included in the series. The complete sputum series increases the detection of primary bronchogenic carcinoma from 45% (one specimen) to 95% (five specimens). Do not submit 24-hour specimens!

SCOPE

All TML clients including hospitals and doctors offices.

RESPIRATORY TRACT (SPUTUM) PROCEDURE:

1. Give the patient a clean sputum collection receptacle containing Cytolyt the preceding day and instruct he or she not to use until the following morning. Cytolyt is normally clear so the addition of food coloring may be utilized to forewarn the patient to not drink the solution. The patient should be made aware that the Cytolyt fixative solution is poisonous.
2. Instruct the patient to cough DEEPLY (from the diaphragm) upon awakening and encourage the patient to expectorate only secretions coughed up from the bronchial tree - not saliva which accumulates in the mouth or post-nasal accumulation in the pharynx. It is important that the patient rid his mouth of saliva, food or other material before expectorating by spitting or rinsing his mouth with water: for patients with scanty sputum, it may take 15-30 minutes of intermittent coughing before an adequate sample can be obtained. Time spent in patient instructions is most important.
3. The properly labeled specimen with the source on the container, patient name, and a second identifier (MRN, SS#, DOB, etc.), accompanied by a complete cytology requisition, should be taken to the laboratory.
4. Post-bronchoscopy sputum should be collected an hour or so after the bronchoscopy procedure in the same manner as outlined above. Send the properly labeled specimen and cytology requisition IMMEDIATELY to the laboratory.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must be a deep cough specimen with alveolar macrophages present to be considered satisfactory.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure the proper collection of bronchial aspirates or washings.

SCOPE:

All TML clients including hospitals and doctors offices.

BRONCHIAL ASPIRATES OR WASHINGS PROCEDURE:

1. Optimum cellular detail is obtained when bronchial lavage specimens are collected using BALANCED ELECTROLYTE SOLUTION. DO NOT USE SALINE SOLUTION.
2. After collection, label the specimen container and cytology requisition as to the site of procurement and send the specimen IMMEDIATELY to the hospital laboratory or outpatient clinic area responsible for fixation procedures.
3. Upon arrival at the hospital laboratory or outpatient clinic area, CYTOLYT (cytology fixative), should be added to the bronchial washing specimen immediately. 30cc's of Cytolyt should be added to the specimen (equivalent to one full depression of the plunger apparatus).
4. The specimen should be placed in the specified retrieval area for TML couriers.

BAL-PEDS SPECIMEN REQUIREMENTS

1. Specimens should be labeled as BAL-PEDS.
2. Cytology will need as much of the specimen as possible.
3. Specimens must remain unfixed. Please send to TML without fixation. These specimens will deviate from the normal protocol due to the request for lipid-laden macrophages.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the lung.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

This procedure is to provide guidance for cytology specimen fixation, collection, rejection, and retention for Bronchial Brush Specimens.

SCOPE:

All TML clients including hospitals and doctors offices.

BRONCHIAL BRUSHING PROCEDURE:

1. All bronchial brushings collected for cytologic studies should be submitted with the brush tip clipped and placed directly into an appropriately labeled CytoLyt container.
2. The container must be labeled with the appropriate source descriptors such as Rt., Lt., RUL, RML, RLL, LUL, LLL, etc., along with the patient's name and a second identifier (DOB, MRN, SS#, etc.)

No preparation of slides will be necessary. Utilizing this method, superior cellular morphology can be attained in addition to the preparation of a cell block to further enhance the architectural evaluation of the cells. The cell block preparation will also allow for possible Immunohistochemical stains to further determine the origin of abnormal cells if necessary.

Special Instructions for Multiple Clipped Brush Tips:

1. If more than one brush is utilized to brush the same area or site, then the multiple brush tips that have been clipped can be placed in the same CytoLyt container and only one order will be placed or one requisition completed.
2. If multiple areas or sites are brushed, then separately labeled CytoLyt containers must be utilized with the appropriate corresponding sites written on patient identification label and corresponding orders/requisitions for the different areas or sites.

Special Note: If a physician chooses to rinse a brush in between sampling, please use a balanced electrolyte solution such as Plasma-Lyte® or Polysol®. Utilize no more than 15cc's of this solution. When the procedure is complete, please add the 15cc's of Plasma-Lyte® or Polysol® to the CytoLyt container that contains the clipped brush tip. There is no need for two separate containers in this instance.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the lung.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure proper fixation, collection, handling, rejection, and retention of all body cavity fluids (includes pleural, peritoneal, pericardial, synovial/joint fluid and cyst fluid).

SCOPE:

All TML clients including hospitals and doctors offices.

BODY CAVITY FLUID PROCEDURE:

1. If microbiology and/or chemistry tests are ordered (in addition to cytology studies), please extract the required amount of specimen for these particular tests. Send the remainder of the specimen - without the addition of Heparin - to the laboratory. It is encouraged to send as much of the specimen as possible for cytologic evaluation.
2. Send the properly labeled specimen container and cytology requisition slip IMMEDIATELY to the hospital laboratory or clinic for appropriate fixation procedures.
3. For specimens under 100cc's add 30cc's of cytolyt (equivalent to one full depression of the plunger apparatus). Heparin should not be added. Refrigerate specimen.
4. NO FIXATIVE IS REQUIRED ON SPECIMENS OF 100cc's OR MORE. Do not add Heparin. Please refrigerate immediately. Time delays of 24 hours or more are discouraged. Delivery to the laboratory as soon as possible will greatly benefit the examination of the cellular detail during microscopic evaluation.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure the proper collection of all gastrointestinal tract specimens.

SCOPE:

All TML clients including hospitals and doctors offices.

GASTROINTESTINAL TRACT PROCEDURE:

Because of the nature of the gastrointestinal tract, ADEQUATE PREPARATION OF THE PATIENT IS ESSENTIAL in order to obtain satisfactory specimens. If possible, cytology procedures should be performed prior to barium examinations (since barium contamination of the cellular sediment makes microscopic interpretation difficult). Otherwise, wait 48-72 hours after a barium examination before attempting a cytologic study.

FOR ESOPHAGEAL AND GASTRIC SPECIMENS: The patient should fast overnight and withhold antacids and other medication. Clear fluids may be consumed until one hour before the procedure. If obstruction is present, the patient should be on liquid diet the day before the procedure and all residue should be removed from the stomach by washing with normal saline prior to obtaining the cytologic sample.

FOR COLONIC SPECIMENS: The patient should be placed on a low residue diet as soon as the procedure is planned. Two (2) to three (3) cleansing enemas should be given prior to obtaining the cytologic specimen.

A. Gastrointestinal Tract Aspirations and Washings

    1. Aspirated secretions or lavage specimens should be mixed IMMEDIATELY with 30cc's of Cytolyt. The washings/lavages should be obtained prior to biopsy.

       NOTE: Due to the enzymatic nature of the gastrointestinal tract, rapid cellular lysis and degeneration occurs unless the specimen is properly fixed until it can be processed in the laboratory.
    2. The specimen container and cytology requisition should be labeled as to the SPECIFIC site of the procurement.

B. Gastrointestinal Tract Brushing
    1. All gastrointestinal tract brushings collected for cytologic studies should be submitted with the brush tip clipped and placed directly into an appropriately labeled CytoLyt container.
    2. The container must be labeled with the appropriate source descriptors such as esophageal brushing, duodenal brushing, common bile duct brushing, etc., along with the patient's name and a second identifier (DOB, MRN, SS#, etc.).

No preparation of slides will be necessary. Utilizing this method, superior cellular morphology can be attained in addition to the preparation of a cell block to further enhance the architectural

evaluation of the cells. The cell block preparation will also allow for possible Immunohistochemical stains to further determine the origin of abnormal cells if necessary.

Special Instructions for Multiple Clipped Brush Tips:

1. If more than one brush is utilized to brush the same area or site, then the multiple brush tips that have been clipped can be placed in the same CytoLyt container and only one order will be placed or one requisition completed.
2. If multiple areas or sites are brushed, then separately labeled CytoLyt containers must be utilized with the appropriate corresponding sites written on patient identification label and corresponding orders/requisitions for the different areas or sites.

Special Note: If a physician chooses to rinse a brush in between sampling, please use a balanced electrolyte solution such as Plasma-Lyte® or Polysol®. Utilize no more than 15cc's of this solution. When the procedure is complete, please add the 15cc's of Plasma-Lyte® or Polysol® to the CytoLyt container that contains the clipped brush tip. There is no need for two separate containers in this instance.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure the proper collection of urine specimens.

SCOPE:

All TML clients including hospitals and doctors offices.

VOIDED OR CATHETERIZED URINE PROCEDURE:

Urine may contain cells exfoliated from all areas of the genito-urinary system. Multiple specimens increase the sensitivity of the procedure. Female patients should be catheterized to avoid contamination of the urine by vaginal secretions. The following technique will ensure representative specimens of adequate volume, which will contain well-preserved cells.

1. Have the patient drink one (1) glass of water every fifteen (15) minutes for two (2) hours.
2. At the end of two (2) hours, have the patient void, or catheterize bladder and discard the urine sample.
3. One (1) hour later, have the patient void, or catheterize bladder and save this urine sample.
4. Fix the specimen with 30cc's of Cytolyt (equivalent to one full depression of plunger apparatus). Please place on the cytology requisition that Cytolyt was added.
5. Take the properly labeled specimen and cytology requisition to the laboratory or call for courier pick up.

NOTE: Due to the enzymatic nature of agents present in urine, cells degenerate rapidly. Therefore, prompt fixation is necessary for satisfactory microscopic interpretation.

URINARY BLADDER WASH OR BARBOTAGE PROCEDURE:

Barbotage or washing of the urinary bladder, ureters, and renal pelvis is utilized quite frequently. It is a popular technique for differential diagnosis of urinary lesions.

1. The specimen is collected during cystoscopy by gently washing the selected area with 100-300cc's of normal saline or Ringer's solution.
2. The selected area should be washed twice and labeled #1 and #2 respectively on the appropriate container.
3. After the specimens are collected, it is imperative that the properly labeled specimen containers be fixed with 30cc's of Cytolyt, cytology fixative, IMMEDIATELY. Each container would receive the same allotment of Cytolyt: one full depression of the plunger for each specimen.

Urinary detection of Cytomegalic Inclusion Disease:

1. Collect freshly voided urine and IMMEDIATELY transport the specimen to the laboratory.
2. DO NOT FIX. Fixation will interfere with subsequent processing of the specimen.

3. Urine that has been collected over a long period of time, or specimens that have been allowed to stand at room temperature following collection, are unsatisfactory for cytologic study since the CMV affected cells degenerate rapidly.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure proper collection of urinary tract brushing specimens (bladder, ureteral or renal pelvis).

SCOPE:

All TML clients including hospitals and doctors offices.

BLADDER, URETER, OR RENAL PELVIS BRUSHING PROCEDURE:

1. All bladder, ureter, or renal pelvis brushings collected for cytologic studies should be submitted with the brush tip clipped and placed directly into an appropriately labeled CytoLyt container.
2. The container must be labeled with the appropriate source descriptors such as Rt Ureter Brushing, Lt. Ureter Brushing, Rt. Renal Pelvis Brushing , etc., along with the patient's name and a second identifier (DOB, MRN, SS#, etc.)

No preparation of slides will be necessary. Utilizing this method, superior cellular morphology can be attained in addition to the preparation of a cell block to further enhance the architectural evaluation of the cells. The cell block preparation will also allow for possible Immunohistochemical stains to further determine the origin of abnormal cells if necessary.

Special Instructions for Multiple Clipped Brush Tips:

1. If more than one brush is utilized to brush the same area or site, then the multiple brush tips that have been clipped can be placed in the same CytoLyt container and only one order will be placed or one requisition completed.
2. If multiple areas or sites are brushed, then separately labeled CytoLyt containers must be utilized with the appropriate corresponding sites written on patient identification label and corresponding orders/requisitions for the different areas or sites.

Special Note: If a physician chooses to rinse a brush in between sampling, please use a balanced electrolyte solution such as Plasma-Lyte® or Polysol®. Utilize no more than 15cc's of this solution. When the procedure is complete, please add the 15cc's of Plasma-Lyte® or Polysol® to the CytoLyt container that contains the clipped brush tip. There is no need for two separate containers in this instance.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

To ensure proper collection of breast nipple discharge smears and/or direct lesion scrapings.

SCOPE:

All TML clients including hospitals and doctors offices.

BREAST NIPPLE DISCHARGE PROCEDURE:

NIPPLE DISCHARGE SMEARS:
Immediate fixation is of vital importance since mammary cells spread in a single layer on a glass slide rapidly undergo air-drying and degeneration. Serous or bloody material in the secretion does not protect the cells.

Preparation of six (6) smears usually gives optimum diagnostic results. The first few smears collected display scanty, degenerated cellular material, while the latter obtained slides exhibit superior samples due to freshly obtained cells with clear nuclear and cytoplasmic detail.

The following technique should be followed:

NOTE: It is extremely important that both the slides and the fixative container be labeled with the patient name, date of birth, and the source (Rt. Breast, Lt. Breast). Positively charged slides should be utilized for best results.

1. Have the patient hold an opened bottle of fixative (95% alcohol - the green fixative supplied by TML) below her breast.
2. Gently compress and milk the areolar area and nipple with the thumb and forefinger, first vertically, then in a clockwise direction, to include the total area. When a mass is palpable, a very gentle milking of the space between the mass and the areolar area is performed.
3. Allow only a drop the size of a pea to accumulate on the apex of the nipple.
4. Supporting the areolar and nipple with one hand, use the other hand to touch the slide to the drop of expressed secretion and draw the previously labeled slide quickly, laterally across the nipple.
5. IMMEDIATELY DROP THE SLIDE INTO THE BOTTLE OF FIXATIVE.
6. Repeat procedure using the next previously labeled and numbered slide until all secretion obtainable from the nipple is utilized.

NIPPLE DISCHARGE DIRECTLY INTO CYTOLYT:

1. Use the above procedure except instead of placing on a slide place the fluid directly into Cytolyt Solution.

BREAST DIRECT LESION SCRAPING PROCEDURE:

1. When there is nipple erosion or ulceration, the specimen usually consists of fluid oozing from the lesion. In such instances, saline can be added to the erosion fluid. Gently mix with the lesion to exfoliate cells and prepare a smear as described in the section on nipple discharge smears.
2. More satisfactory, however, is a cotton swab which has been soaked in sterile saline. The swab serves as an excellent abrader of the lesion and can then be used to transfer and spread the material onto slides. FIX IMMEDIATELY in 95% alcohol (green TML fixative) or with a suitable spray fixative.
3. NOTE: It is extremely important that the slides are labeled with the patient name, date of birth, and the source (Rt. Breast, Lt. Breast). Positively charged slides should be utilized for best results.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen slide/container must be labeled with two identifiers.
4. Slides broken beyond repair.

RETENTION: The slide is kept for five years.

PURPOSE:

Cytology examination of oral smears is primarily directly toward the diagnosis of incipient, cryptic or unsuspected carcinoma. In addition, it should be used when it is not practical to subject a patient to repeated biopsies, as with post-irradiation examinations. Oral smears are not meant to be a substitute for biopsy, but should be used as an adjunct to biopsy.

SCOPE:

All TML clients including hospitals and doctors offices.

BUCCAL CAVITY DIRECT LESION SCRAPING PROCEDURE:

1. Using a pencil, write the patient's name and date of birth on frosted end of a positively charged slide.
2. Using gauze moistened with saline or tap water, wipe away any saliva, debris or slough over the site to be scraped. The top layers of keratinized or crusted lesions must be removed with either dry gauze or a rotating stone or a curette. Soften lip lesions by applying wet gauze for 15 minutes prior to obtaining the specimen.
3. Support the area to be scraped firmly, and then scrape VIGOROUSLY with a moistened tongue blade or cytobrush applicator several times in the same direction.
4. Spread RAPIDLY on a previously labeled glass slide and FIX IMMEDIATELY in 95% alcohol (green TML fixative) or with a suitable spray fixative. Alternate fixative procedure involves rinsing brush or tongue blade in Cytolyt fixative. Specimen is sent to laboratory for processing.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen slide/container must be labeled with two identifiers.
4. Slides broken beyond repair.

RETENTION: The slide is kept for five years.

PURPOSE:

To ensure proper collection of cerebrospinal fluid (CSF). In addition to examination for malignant cells, cellular studies of cerebrospinal fluid (CSF) have become increasingly important in both the diagnostic and management phases of leukemic disorders and infectious disorders. Accurate evaluation of the cerebrospinal fluid is dependent upon proper collection technique.

SCOPE:

All TML clients including hospitals and doctors offices.

CEREBROSPINAL FLUID COLLECTION PROCEDURE:

1. The final tube collected during the spinal tap is best for cytologic interpretation. One (1) cc of fluid is adequate for examination; however, two (2) cc's or more are preferred. Since cellular deterioration is rapid - immediate fixation is required.
2. The specimen should be immediately fixed with 15cc's of Cytolyt (equivalent to one half of a depression of the plunger apparatus).
3. The appropriately labeled specimen container with two patient identifiers and cytology requisition should be sent to the laboratory as soon as possible.

HANDLING: Maintain at room temperature if Cytolyt has been added. Refrigerate specimen if no fixative has been added.

REJECTION: The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

Microscopic examination of eye secretions is a rapid method for diagnosis of certain inflammatory diseases, primarily viral and chlamydial. Tumors, although rare, can also be diagnosed by cytologic evaluation of eye material.

SCOPE:

All TML clients including hospitals and doctors offices.

EYE SECRETION COLLECTION PROCEDURE:

1. Using a cotton swab moistened with sterile water or saline, gently, but vigorously, scrape the margins of a conjunctival or corneal lesion.
2. Quickly spread the sample on a previously labeled glass slide and FIX IMMEDIATELY in 95% alcohol (green TML fixative) or a suitable spray fixative.
3. Send the properly labeled specimen container and cytology requisition immediately to the laboratory.

   NOTE: For keratinized lesions, energetic scraping of the margins of the lesion with a metal or plastic spatula after local anesthesia will offer the best results.

4. Fine needle aspiration of the anterior or posterior chambers and from the vitreous body should be fixed with 30cc's of Cytolyt, cytology fixative. The specimen should be sent to the laboratory immediately.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen slide/container must be labeled with two identifiers.
3. Slides broken beyond repair.

RETENTION: One week for the liquid specimen. The slide is kept for five years.

PURPOSE:

This procedure is to provide guidance for cytology specimen fixation, collection, rejection, and retention for Anal Pap Tests.

SCOPE:

All TML clients including hospitals and doctors offices.

ANAL PAP TEST COLLECTION PROCEDURE:

The anal canal is a 3-4 cm long tubular structure which is surrounded by smooth muscle. The canal extends from the anal verge to the rectal mucosa and is delineated by the anal-rectal transformation zone. Samples should be taken from the entire canal to ensure proper sampling.

1. Label a PreservCyt vial with patient name and date of birth.
2. Use a Cytobrush or a moistened Dacron Swab to obtain sample from the entire anal canal including keratinized and nonkeratinized squamous epithelium and anorectal transformation zone.
3. Rinse Dacron Swab or Cytobrush in vial.
4. Close vial securely to prevent leakage.

HANDLING: Maintain at room temperature.

REJECTION CRITERIA:

1. If insufficient material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation. According to Bethesda 2001 for an anal pap to be considered satisfactory it should contain 2000-3000 nucleated squamous cells.
2. Obscuring material including fecal material, bacteria, inflammation, mucus, and blood may hinder microscopic evaluation and be considered unsatisfactory for evaluation.
3. The specimen container must be labeled with two identifiers.

RETENTION: Liquid specimen is retained six weeks. The slide is retained for five years.

PURPOSE:

To ensure proper collection for fine needle aspiration of any site.

SCOPE:

All TML clients including hospitals and doctors offices.

FINE NEEDLE ASPIRATION COLLECTION PROCEDURE:

- To prevent errors in identification of the patient, the site, and the procedure a time out must take place prior to collection of specimen to confirm.

1. Properly fill out the cytology requisition form. Information MUST include anatomic site of aspiration, description of mass being aspirated, and clinical history of patient to include history of previous malignancy (if applicable) and current therapy.
2. Label the colored end of the slide with a No. 2 lead pencil with the patients name and the specimen source (FNA Rt. Thyroid, FNA Parotid, etc.). Label the two fluid filled containers with the patient name, date of birth, and source of specimen.
3. Procure the specimen in the routine manner of aspiration.
4. Once the specimen has been obtained, disperse the contents of the sampling device into the Cytolyt container. If multiple passes are made through the same mass, the cellular material from each pass may be placed into the same Cytolyt vial. If a separate mass is aspirated, a second clearly distinguished Cytolyt vial should be used.
5. Aspirate a small amount of the Cytolyt fixative into the syringe to rinse the remaining material from the needle and syringe.
6. Using a clean needle each pass, aspirate mass again to dispense contents onto the slide. Place another slide face down on top of the aspirated slide and gently pull the two slides apart in a horizontal direction. Immediately place the white slide into the green liquid fixative container and allow the blue slide to air dry.
7. Repeat step 5 and 6 with the remaining two white end slides and place them in the green alcohol fixative vial. Securely fasten the lid. Place the blue slide in the plastic slide holder once it is dry.
8. Securely fasten the top on the Cytolyt vial to prevent leakage.
9. Place the Cytolyt vial into the biohazard bag and insert into the sponge opening in the FNA kit (if you are using the preassembled kit) along with the green fixative vial and plastic slide holder. Fold the requisition slip and insert the requisition into the FNA kit. Do not write on the outside of the box.
10. Send the vial and requisition slip or hospital order to the laboratory in a biohazard bag (if not using the preassembled kit).

The vials of Cytolyt will be available to the clinician. Phone requests to the number listed below. Please do not hesitate to call the TML cytology staff at 918-481-7854 if there are any questions regarding this procedure.

HANDLING: Maintain at room temperature.
REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen container must be labeled with two identifiers.

RETENTION: One week for the liquid specimen. The slide is kept for ten years.

PURPOSE:

This procedure is to provide guidance for the collection and evaluation of Tzanck Smears.

SCOPE:

This procedure is intended for use by physicians and laboratory personnel.

TZANCK SMEAR PROCEDURE:

1. Label the slides with the patient's first and last name, DOB, and specimen source in pencil on the frosted end of the slide.
2. Place in a container filled with 95% ethyl alcohol (green fixative) so the slides are completely covered.
3. Gently scrape the area of abnormality. If the abnormality is a vesicle, remove the covering and scrape both at the base of the vesicle and around the rim.
4. Remove one of the slides from the fixative and quickly and evenly smear the collected material on one of the glass slides.
5. Immediately re-immerse the slide in fixative. Repeat the process with the second slide, if necessary, for better diagnostic yield.
6. After collection, leave the slides in the 95% alcohol for 10 minutes and then air dry.
7. Submit the specimen and the completed requisition to Tulsa Medical Laboratory.

UNACCEPTABLE CONDITIONS:

1. Air dried smears. Refer to above collection procedure.

PREPARATION:

1. Prepared slides are stained with the pap stain after laboratory accessioning.

MICROSCOPIC EVALUATION:

1. The Cytotechnologist will evaluate the stained slides to look for typical cytopathic changes in epithelial cells. The cells become enlarged, with intranuclear inclusions, often with the formation of multinucleated cells. They will send the slide to the Pathologist for final signout and reporting.

LIMITATIONS:

1. This test can be performed when an urgent test is needed and no alternative test is immediately available, but it does not negate the need for follow-up testing of all negatives with a more sensitive test.

HANDLING: Maintain at room temperature.

REJECTION:

1. If insufficient cellular material is present, the technologist/pathologist may decide the material is insufficient for proper evaluation.
2. The specimen must contain cells representative of the source.
3. The specimen slide must be labeled with two identifiers.

RETENTION: The slide is kept for five years.

PREPARED BY:

COLLEEN JOPLIN, CT

_____

Signature

DATE: _____

DEPARTMENTAL DIRECTOR (OR DESIGNEE):

TAMARA L CHANEY, MD, MEDICAL DIRECTOR

_____

Signature

DATE: : _____

IMPLEMENTATION DATE:

DATE: _____

DEPARTMENTAL DIRECTOR (OR DESIGNEE) ANNUAL REVIEW

Medical Director or Designee	Medical Director or Designee Signature	DATE
TAMARA CHANEY, MD		

EMPLOYEE REVIEW

EMPLOYEE NAME	EMPLOYEE SIGNATURE	DATE