



Long Beach Memorial Pathology Medical Group

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SPECIMEN COLLECTION MANUAL

TABLE OF CONTENTS

Quick Reference Guide	3
How to Send a Specimen to LBM Pathology Medical Group	4
Surgical Pathology	
How to complete a requisition	5
Supplies	6
Specimen Labeling	7
Criteria for Acceptable Specimens	7
Specimen Collection by Tissue Type	
Breast core biopsies	8
Calculi	8
CLO test for H. pylori	8
Frozen Section	9
Lymph node biopsy	10
Muscle biopsy	10
Prostate biopsies	10
Radiation Safety Hazards	10
Routine Pathology with Microbiology (Culture) Studies	10
Special handling of tissue specimens	11
Uterine contents for genetic testing (chromosome analysis)	11
Non-gynecological Cytopathology	
How to complete a requisition	12
Supplies	13
Specimen Labeling	15
Criteria for Acceptable Specimens	15
Specimen Preparation by Specimen Type	
Brushings	16
Fine Needle Aspirations (FNA)	16
Other Fluid Specimens	16
Nipple Discharge	17
Urine	17
Minimum Non-Gyn Cytology Specimen Quantities	18
Gynecological Cytology	
How to complete a requisition	19
Supplies	20
Criteria for Acceptable Specimens	21
Patient Information and Preparation	21
Lubricants	22
Specimen Collection and Handling	23
Ancillary Studies	26
Specimen Labeling	26

ThinPrep Quick Reference	27
Molecular Diagnostics	
Affirma VPIII Test for Vaginitis/Vaginosis	29
High-risk HPV	30
Chlamydia trachomatis/Neisseria gonorrhoeae	31-32
Bone Marrow Aspiration and Biopsy: Contact Special Hematology (562) 933-0760 or call Client Services at (562) 989-5858	
Muscle Biopsy	Contact Client Services at (562) 989-5858

SPECIMEN COLLECTION QUICK REFERENCE

Tissue Type	Procedure	Fixative	Special Instructions
Breast	Core biopsy/excision/mastectomy	10% buffered formalin	Always indicate date and time specimen was removed from patient
Calculi or Stones	Removal	Fresh no fixative	
CLOtest for H. Pylori		CLOtest	See procedure below
Fluids (nipple discharge, FNA, breast cyst)		CytoLyt	
Frozen Section/OR consult		Fresh, preferably sterile	
Lymph Node	Biopsy	Fresh no fixative	
Muscle	Biopsy	Fresh sterile	Notify LBMPMG 48 hours before procedure. Bring fresh to LBMPMG
Pap smear		PreservCyt	
Prostate	Biopsy	Zinc formalin	
Microbiology culture		Fresh sterile	Bring to TOPA immediately upon removal, Cultures not performed by LBM will be sent to our reference lab.
Uterine contents for genetic testing		Fresh	Call LBM for further instructions
Urine	Void	Fresh	Immediately call LBM for pick up. Specimen needs to be picked up no later than an hour after specimen is collected. Patient may also come directly to TOPA for specimen collections.

HOW TO SEND A SPECIMEN TO TOPA:

- **Use LBM requisition.**
- **Place specimen in proper fixative.**
- **Label specimen bottle with two patient's identifiers, i.e., name and date of birth. Also include specimen source, physician name & collection date.**
- **Call LBM Client Services courier for pick up at (562) 989-5858**

If you are already on the LBM courier's daily route, place specimen in your designated pick-up area or in the lockbox for after-hours pick up.

If your office is on a will-call basis, please call the LBM courier at (800) 873-3444 Client #1584 give to Apollo customer services to schedule a pickup.

If you need a lock box or would like daily specimen pick up, please call LBM Client Services, at (562) 989-5858

SURGICAL PATHOLOGY (biopsies and other surgically removed tissue)

How to complete a LBM requisition

Please use the requisition most appropriate for the tissue type:

- LBM tissue/ Gyn Cytology

All specimens must be accompanied by a LBM requisition. Please fill out the form completely.

All requisitions should include the following:

1. Patient's name, first and last (any name change should be noted)
2. Medical record # (if applicable)
3. Patient's address
4. Date of birth
5. Gender
6. Pertinent clinical history
7. Pre-op diagnosis including ICD-10 code
8. Specimen source
9. Type of fixative (as appropriate)
10. Collection date and time (when appropriate, e.g. breast biopsies)
11. Type of examination requested, if other than routine
12. Requesting physician (name, address, phone number, fax number)
13. All billing information including patient face sheet (demographics) from patient's chart, copy of insurance card. Include patient's social security number.

The patient's insurance information section does not need to be filled out if you provide a copy of the insurance card (front and back) as well as a copy of the patient's face sheet.

Each LBM requisition is customized with your doctor's name, address, and phone and fax numbers. To order more requisitions. Please use the Client Supply Order form. You may print a copy of this form and fax it to LBM at (562) 989-5858 or LBMPATH.com

Supplies

Formalin and zinc formalin bottles are supplied in several sizes.

Order supplies using the Client Supply form. Please fax a completed copy to LBM at (562) 989-5858

Please refer to “Specimen Collection and Submission” for types of fixatives required for specific tissue types.

Example of formalin bottles



Formalin containers for tissue biopsies should always be labeled “10% neutral buffered formalin.”

Zinc formalin is used for prostate core biopsies and labeled accordingly.

For plastic surgery: Large empty containers with lids and gallon jugs of formalin are provided to be used for very large specimens. Place specimen in container and add formalin to generously cover the specimen. Place in large zip-lock plastic bag for transport.

Specimen Labeling:

The provider must properly label specimen containers at the time of collection with the following information:

- Patient name (first and last)
- A second patient identifier, i.e., date of birth
- Specimen source
- Physician name
- Collection date and time
 - Note: Breast biopsies require time of collection and time place in formalin.

Glass slides must be labeled with the patient's name, using a solvent-resistant pen or pencil, on the frosted end of the slide.

Please attach a copy of the patient's insurance card (front & back) and a copy of the demographics face sheet (if available).

If any information is missing, LBM personnel will call your office in an attempt to obtain the missing information. LBM reserves the right to return a specimen due to inadequate information or specimen preservation.

Criteria for Acceptable Specimens

1. Properly labeled specimen container, ThinPrep vial or slides.
2. Concordant information between specimen label and requisition.
3. Unbroken slides (or not broken beyond repair).
4. Adequate clinical information.
5. Tightly sealed specimen vial/container with no evidence of leakage.
6. Sufficient volume (fluid specimen).
7. Specimen greater than 12 hours old without preservative or refrigeration cannot be accepted.
8. Specimen submitted by authorized source.
9. No evident contamination of outside surface of container.
10. Specimen submitted with correct fixative or media.

It is imperative that specimens be submitted appropriately and according to the specimen requirements outlined below. LBM provides clients with specimen requirements for all samples (see below). Upon receipt, all specimens are evaluated for appropriateness of container, preservatives, sample size/volume, and accuracy and completeness of patient/specimen identification. Specimens judged unsuitable for testing are rejected.

General Instructions:

Specimens not requiring immediate examination by the pathologist, culture, or special handling for other purposes (see below), must be placed in 10% formalin.

Routine tissue specimens are to be placed immediately into fixative, using 10-20 times as much fixative solution as the bulk of the biopsy specimen.

Specimen Collection and Submission by Tissue Type:

Breast Specimens

Core biopsies should be immersed in fixative within one hour of the procedure. Excisional biopsies and resection specimens should be delivered immediately to LBM, so that the specimen can be appropriately dissected and placed in fixative within one hour of the procedure.

Indicate the date; time specimen was removed from the patient and the time the specimen was placed in 10% NB (neutral buffered) formalin on

3. If the biopsy specimen appears to be very small, it may be worthwhile taking a second biopsy and inserting both specimens into the CLOtest. Be careful not to contaminate the second specimen with blood from the first biopsy site.

CLO Test Procedure:

1. After removing the CLOtest rapid urease slide from refrigeration, lift the label far enough to expose the yellow gel. For faster test results, allow the gel to reach room temperature before inserting the biopsy (usually between 7-10 minutes). Before use, the CLOtest should be inspected to make sure that the well is full and is yellow in color.
2. With clean applicator device (i.e. Toothpick, etc.) push the entire sample from the forceps beneath the surface of the gel to expose as much of the specimen to the gel as possible. Make sure that the biopsy specimen is completely immersed in the gel.
3. Re-seal the pressure-sensitive label on the slide and record the patient name, date, and time the biopsy sample was inserted.

Frozen Section / O.R. Consultation:

Frozen Section Scheduling Information & Forms:

Frozen sections should be scheduled with the pathologists in advance. Please use the appropriate Frozen Section Scheduling form for your office location, or go on website form Frozen Section Form-LBMPATH.COM

Frozen Section Specimen Submission:

Specimens to be examined by a pathologist during surgery are to be submitted fresh (not in formalin) and preferably sterile. Sterility is required if microbiological cultures, tissue cultures or clonogenic assays are also requested or indicated. These specimens are best submitted in capped sterile containers.

Please provide a telephone number on the requisitions, so that pathologist may speak directly to surgeon.

Frozen section specimens received from physicians' offices but also requiring microbiology Physician/surgeon submits fresh specimen to LBM for frozen section and/ or other appropriate studies (studies done by LBM). It is the responsibility of the physician's office to deliver the specimen to LBM. Microbiology and other studies not performed by LBM will be sent to our reference laboratory. The reference lab will bill for their services separately.

You may preschedule Frozen Sections- contact Client Services at (562) 989-5858

Lymph Node Biopsy:

Lymph nodes removed for diagnostic reasons must be submitted to the pathologist as for frozen section so that testing may be performed as indicated. These may include special fixation in zinc formalin (done by pathologist), microbiological cultures, T and B cell studies, lymphoma work, etc.

If lymphoma is suspected, send fresh specimen immediately to LBM. If there will be a delay in transport, place fresh specimen in small amount of sterile saline or in sterile saline-soaked gauze. If multiple needle biopsies are obtained, place one core in RPMI for flow cytometry.

Please call the LBM pathologist as early as possible to assure proper handling of specimen.

Muscle Biopsy:

Please notify LBM at least 48 hours prior to the procedure. Bring fresh specimen to LBM in sterile container immediately upon removal.

Prostate biopsies are to be placed in zinc formalin.

Bouin's solution is provided for testicular biopsies. It will not be a cause for specimen rejection, however, if these types of specimens are submitted in 10% formalin.

Zinc formalin, 10% formalin and Bouin's solution are toxic substances and must be handled with caution.

Radiation Safety Hazards:

For any Specimen that represents a radiation safety hazard, please call LBM at (805) 373-8582 for handling instructions.

Routine Pathology & Microbiology (culture) Studies:

If microbiology (culture) studies are required in addition to routine pathology, physician/surgeon submits fresh specimen to LBM in sterile container. It is the responsibility of the physician's office to deliver the fresh specimen to TOPA immediately upon removal. Microbiology and other studies not performed by LBM will be sent to our reference laboratory. The reference lab will bill for their services separately.

Special handling of tissue specimens:

As noted above, all tissue specimens must be submitted in 10% buffered formalin, 10% buffered zinc formalin, or Bouin's solution unless the specimen is submitted for special studies such as:

1. Immunofluorescence (submitted in Zeus fixative)
2. Drug resistance (clonogenic) assays (submitted in RPMI transport media)
3. Any other special studies

These specimens require special handling by the pathologist. Please call the Pathology Department as early as possible to assure availability of proper fixative for these special tests.

Uterine Contents for Genetic Testing (Chromosome Analysis):

Obtain products of conception using sterile technique. Submit at least 4mm (100mg) sample of sterile tissue. Placental tissue (containing chorionic villi) is preferred. Umbilical cord tissue or fetal skin may be submitted if placental tissue is not available.

Sterile tissue should be placed in RPMI solution and refrigerated. Please call LBM Pathology Medical Group at (562) 989-5858 to arrange same day pick-up. After refrigerated storage during the day, the specimen may be placed in a lock-box for short-term storage (as long as TOPA has been notified, to ensure same day pick-up).

Please note that the specimen should not be placed in formalin. If only formalin-fixed tissue is available, limited genetic testing can be performed; but complete cytogenetic analysis cannot be performed on formalin-fixed tissue.

NON-GYNECOLOGICAL CYTOLOGY

How to complete a LBM requisition

Please use the requisition most appropriate for the tissue type:

- General surgical pathology & non-gyn cytology requisition & gyn cytology

All specimens must be accompanied by a LBM requisition. Please fill out the form completely.

All requisitions should include the following:

1. Patient's name, first and last (any name change should be noted)
2. Medical record # (if applicable)
3. Patient's address
4. Date of birth
5. Gender
6. Pertinent clinical history
7. Pre-op diagnosis including ICD-10 code
8. Specimen source
9. Type of fixative (as appropriate)
10. Collection date and time (when appropriate, e.g. breast biopsies)
11. Type of examination requested, if other than routine
12. Requesting physician (name, address, phone number, fax number)
13. All billing information including patient face sheet (demographics) from patient's chart, copy of insurance card. Include patient's social security number.

The patient's insurance information section does not need to be filled out if you provide a copy of the insurance card (front and back) as well as a copy of the patient's face sheet.

Each LBM requisition is customized with your doctor's name, address, phone and fax numbers. To order more requisitions, please use the Client Supply Order form. You may print a copy of this form and fax it to LBM at (562) 989-5860.

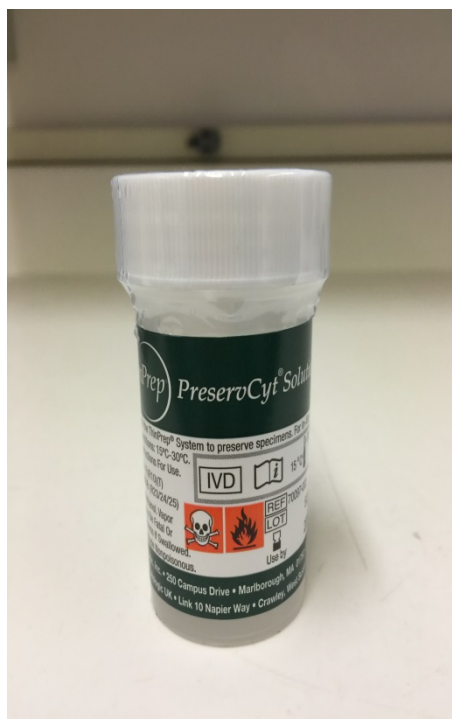
Urology Offices: Please note that the LBM requisition that contains a section for urine cytology and fish studies. This requisition is also used for prostate biopsies. Use the Client Supply Order form for customized, sequentially numbered Urology requisitions with corresponding biopsy bottle labels.

Supplies:

- CytoLyt solution for non-gyn cytology studies (ThinPrep) is provided by TOPA in pre-filled containers (15 ml of solution in a 90 ml container) (see photo).
- PreservCyt solution for urine FISH studies (UroVysion) is provided by TOPA.
- Plastic jars with screw-top lids containing 95% alcohol (see photo).
- Microscope glass slides.
- Spatulas with removable handles (i.e., for nipple discharges).

Order supplies using the Client Supply order form. Please fax completed form to LBM at (562) 989-5860

Examples of non-gyn cytology supplies (photos)



CytoLyt container for non-gyn cytology specimens.

Fluids for cytology are placed in this solution.

If specimen is obtained on a brush or spatula (such as for esophageal brushings or nipple discharge), place portion of brush or spatula with specimen on it directly into the container.



Jar containing 95% reagent alcohol for cytology.

Place labeled slides with smears **immediately** into alcohol. Do not let air dry. Glass slides must be labeled with the patient's name, using a solvent-resistant pen or pencil, on the frosted end of the slide.

Thin-Prep Methodology:

TOPA utilizes ThinPrep non-gyn cytology technology. The ThinPrep non-gyn application ensures optimal cell preservation and specimen integrity. This method provides standardized preparation with true thin –layer technology which reduces clumping and overlapping, preserves cell morphology, enhances nuclear detail, and eliminates air-drying artifact. Adjunctive testing is available with special stains, cell blocks and molecular diagnostic testing.

Specimen Labeling:

The provider must properly label specimen containers at the time of collection with the following information:

- Patient name (first and last)
- A second patient identifier, i.e., date of birth
- Specimen source
- Physician name
- Collection date

Glass slides must be labeled with the patient's name, using a solvent-resistant pen or pencil, on the frosted end of the slide.

Please attach a copy of the patient's insurance card (front & back) and a copy of the demographics face sheet (if available).

If any information is missing, LBM personnel will call your office in an attempt to obtain the missing information. LBM reserves the right to return a specimen due to inadequate information or specimen preservation.

Criteria for Acceptable Specimens

1. Properly labeled specimen container, ThinPrep vial or slides.
2. Concordant information between specimen label and requisition.
3. Unbroken slides (or not broken beyond repair).
4. Adequate clinical information.
5. Tightly sealed specimen vial/container with no evidence of leakage.
6. Sufficient volume (fluid specimen).
7. Specimen greater than 12 hours old without preservative or refrigeration cannot be accepted.
8. Specimen submitted by authorized source.
9. No evident contamination of outside surface of container.
10. Specimen submitted with correct fixative or media.

It is imperative that specimens be submitted appropriately and according to the specimen requirements outlined below. LBM provides clients with specimen requirements for all samples (see below). Upon receipt, all specimens are evaluated for appropriateness of container, preservatives, sample size/volume, and accuracy and completeness of patient/specimen identification. Specimens judged unsuitable for testing are rejected.

Specimen Preparation by Specimen Type:

Brushings (example: esophageal brushings):

Cut brush and immediately place in CytoLyt solution to prevent air drying.

Label container with patient's name, specimen source, date & time collected, and physician's name. Complete LBM requisition.

Fine Needle Aspiration (FNA):

Three to five passes are recommended.

- For the first two passes
 - Prepare one smear for each pass and immediately place each labeled slide in cytology jar containing 95% alcohol.
 - Rinse/inject remainder of material into CytoLyt.
- For all other passes:
 - Inject/rinse material directly into CytoLyt (No smears prepared).

When preparing a slide, place one drop of aspirate on the slide, and lay a second "spreading slide" on top. Spread the sample across the slide. Then place the bottom slide (containing the sample) in alcohol. The spreading slide can be re-used for each pass (wiping off any debris if necessary) and then discarded after the final pass.

Slides should be labeled with patient identifiers prior to the procedure.

PLEASE NOTE: the majority of the air-dying artifact will occur once the drop of aspirate material on the slide has been "spread" across the slide. For this reason, please be prepared to place the slide immediately into fixative as soon as the smear is made.

If molecular testing is requested for thyroid specimens, the third and fourth passes should be submitted in the molecular testing solution. Molecular testing can be ordered as a reflex test (based on cytology results), or at the discretion of the ordering physician. Samples in molecular testing solution will be retained at TOPA for 5 weeks.

For **Flow Cytometry (lymphoma studies)**, please perform one additional pass, and inject/rinse material directly into RPMI

Label containers with patient's name, a second patient identifier (eg. Birth date), specimen source, date and time collected, and physician's name. Complete LBM requisition.

Other Fluid Specimens Breast Cyst, Thyroid Cyst, etc.

Inject (if applicable) or otherwise place entire specimen into CytoLyt; rinse hub of needle in CytoLyt.

Label container with patient's name, specimen source, date & time collected, and physician's name, Complete TOPA requisition.

Nipple Discharge

Using the spatula with removable handle, obtain the nipple discharge on the spatula end. Disconnect the handle and drop the spatula end into CytoLyt.

Urine:

Method- patient voids in physician's office.

For urine cytology and/or urine for FISH (UroVysion):

Label urine container provided by physician's office with patient's name, specimen source, date & time collected, and physician's name. Patient voids into this container.

Urine for Cytologic Evaluation (ThinPrep):

Slightly agitate (swirl) urine and decant a minimum of 15 ml (preferably 35 ml) into container prefilled with CytoLyt (provided by TOPA). Label CytoLyt container with patient's name, specimen source, date & time collected, and physician's name.

Urine for FISH (UroVysion):

Slightly agitate (swirl) urine and decant a minimum of 33 ml into container prefilled with PreservCyt (provided by TOPA). Label PreservCyt container with patient's name, specimen source, date & time collected, and physician's name.

Alternate Method:

Place minimum of 48 ml fresh urine in urine container. Refrigerate and call LBM immediately for pick up that day.

Complete LBM requisition indicating test(s) ordered in Cytology section of form.

Example: _____ Urine for Cytology (ThinPrep)
 _____ Urine for FISH (UroVysion)

Place specimen(s) in zip-lock biohazard bag and insert folded requisition in side pocket. Specimen can be kept at room temperature until courier picks up from your office that day. If specimen is to be picked up after hours, place specimen in lock box. An ice pack is not necessary in the lock box.

Collection hours at physician's office (call LBM for specimen pick up):

Monday through Thursday: Office opens-3:30 p.m.

Friday: Office opens-2:00 p.m.

Minimum Non-Gyn Cytology Specimen Quantities:

Adequate minimum quantity of non-gyn cytology specimen:

Sputum	3 mL
CSF	3 mL
Body fluids	10 mL
Washings	7 mL
Urine for cytology only	15 mL
Urine for UroVysion (FISH) only	33 mL
Urine for cytology & UroVysion	48 mL

GYNECOLOGICAL (GYN) CYTOLOGY**How to complete a LBM requisition**

All specimens must be accompanied by a LBM requisition. Please fill out the form completely.

All requisitions should include the following:

1. Patient's name, first and last (any name change should be noted)
2. Medical record # (if applicable)
3. Patient's address
4. Date of birth
5. Sex of the patient
6. Pertinent clinical history
7. Pre-op diagnosis including ICD-10 code
8. Specimen source
9. Type of fixative (as appropriate)
10. Collection date and time (when appropriate, e.g. breast biopsies)
11. Type of examination requested, if other than routine
12. Requesting physician (name, address, phone number, fax number)
13. All billing information including patient face sheet (demographics) from patient's chart, copy of insurance card.

Requisitions for gynecologic cytology specimens should also include the following:

1. Menstrual status (LMP, hysterectomy, pregnant, post-menopausal, post-partum)
2. Hormone/ contraceptive therapy
3. Relevant clinical findings (eg. Abnormal bleeding, grossly visible lesion, etc.)
4. Previous cervical cytology result or biopsy result
5. Previous treatment or surgical procedures
6. Other relevant clinic information (eg. DES exposure, history of radiation or chemotherapy)
7. Source of specimen (eg. cervical, vaginal)
8. Specific tests requested (eg. HPV reflex, GC/Chlamydia)

The patient's insurance information section does not need to be filled out if you provide a copy of the insurance card (front and back) as well as a copy of the patient's face sheet.

Each LBM requisition is customized with your doctor's name, address, and phone and fax numbers. To order more requisitions, please use the Client Supply Order form.

Supplies:

- ThinPrep Pap Test Solution (PreservCyt) for gyn cytology studies is provided by TOPA in pre-filled containers (15 ml of solution in a 90 ml container) (see attached picture).
- Specimen collection devices:
 - Spatula and cervical brush
 - Broom

Brooms are generally used on pregnant patients since the broom does not typically go up as high into the endocervical canal as the cervical brush.

ThinPrep Pap Test solution (PreservCyt)



Criteria for Accepting a Specimen:

1. Properly labeled specimen container, ThinPrep vial or slides.
2. Concordant information between specimen label and requisition.
3. Unbroken slides (or not broken beyond repair).
4. Adequate clinical information
5. Tightly sealed specimen vial/container with no evidence of leakage.
6. Sufficient volume (fluid specimen)
7. Specimen greater than 12 hours old without preservative or refrigeration cannot be accepted.
8. Specimen submitted by authorized source.
9. No evident contamination of outside surface of container.
10. Specimen submitted with correct fixative or media.

Principle for Obtaining Pap test

The detection of cervical cancer and its precursors as well as other gynecologic abnormalities is the primary purpose of obtaining a cervical cell sample. The following guidelines are referenced from NCCLS Document GP15-A and are recommended in the collection process for obtaining a ThinPrep Pap Test specimen. In general, the guidelines state that it is important to obtain a specimen that is not obscured by blood, mucus, inflammatory exudate or lubricant.

The importance of proper specimen collection and submission cannot be overemphasized. At least one-half to two-thirds of false negatives are the result of patient conditions present at the time of sample collection and submission, and the skill and knowledge of the individual who obtains the specimen.

Patient Information and Preparation for Pap test

The patient should be tested two (2) weeks after the first day of her last menstrual period, and definitely not when she is menstruating. Even though the ThinPrep Pap Test reduces obscuring blood, clinical studies have demonstrated that excessive amounts of blood may still compromise the test and possibly lead to an unsatisfactory result.

Repeat Pap test should not be performed for at least six (6) weeks after previous unsatisfactory specimen to give the cervix time to re-epithelialize.

The patient should not use lubricants, vaginal medication, vaginal contraceptives or other vaginal creams, tampons or douches during the forty-eight (48) hours before the exam. The patient should refrain from intercourse forty-eight (48) hours prior to the exam.

Discussion Regarding Lubricants:

Patient use: Patients should not use any vaginal lubricants or vaginal moisturizers for at least four (4) days prior to their pelvic examination. New, long lasting lubricants/ moisturizers on the market can last up to four days. Some examples are KY Long Lasting®, KY Liquibeads®, KY Silk-E®, and Replens®.

During the exam: Lubricant jellies should not be used to lubricate the speculum. Even though most lubricant jellies are water soluble, excessive amounts of jelly may compromise the test and possibly lead to an unsatisfactory result. If lubricant is necessary due to patient discomfort or use of plastic speculum, it should be applied to the speculum directly using as little as needed to create a thin film on the speculum's surface avoiding the tip. Lubricant on the cervix may interfere with obtaining a representative cervical sample or cause artifact in the alcohol-based transport medium.

Lubricant jellies can adversely affect the cervical cytology collection process in many ways including the following:

- Abundant lubricant on the cervical face will require removal with swabbing of the cervix which theoretically could remove exfoliated diagnostic cells.
- Residual lubricant could interfere with the endocervical brush and spatula or cervical broom in the acquisition of cervical cells.
- Residual lubricant may create a potential immiscible interface in alcohol-based liquid Pap solution leading to potential agglutination and cellular loss.

Cytec Corporation (the makers of the ThinPrep Pap Test) has evaluated a variety of popular lubricants and found that those containing an ingredient known as “carbomers” or “carbopol polymers” are prone to interfere with popular liquid-based Pap Test.

For those situations identified above in which a lubricant must be used, the following lubricants do not contain the interfering substance. Please note that this list is not exhaustive and is merely a starting point provided for your reference.

Suggested Lubricants:

Surgilube®

- PSS World Medical
 - Cardinal Health
 - www.savagelabs.com
- Surgilube is a registered trademark of E. Fougera & Co.

Astroglide®

- PSS World Medical
- www.drugstore.com
- Astroglide is a registered trademark of Biofilm, Inc.

Crystelle®

- Check with local distributors
- Crystelle is a registered trademark of Deltex Pharmaceuticals

If you have any questions, please contact Cytec's Technical Support Department at 1-800-442-9892, option 6.

Specimen Collection and Handling:

Visualization of the Cervix for Collection of an Adequate Sample

Collection of a cervical cytology specimen is usually performed with the patient in the dorsolithotomy position. A sterile or single-use bivalve speculum of appropriate size is inserted into the vagina without lubrication. Warm water may be used to facilitate insertion of the speculum. Refer to “discussion Regarding Lubricants” and “Suggested Lubricants”

The position of the speculum should allow for complete visualization of the os end ectocervix.

The transformation zone is the site of origin for most cervical neoplasia and should be the focus of cytology specimen collection. The transformation zone may be easily visualized or may be high in the endocervical canal. Location varies not only from patient to patient, but in an individual over time. Factors producing variation include changes in vaginal pH, hormonal changes including pregnancy, childbirth, and menopausal status, and hormonal therapy. In postmenopausal patients or women who have received radiation therapy, cervical stenosis may prevent visualization of the transformation zone. It remains important to sample the endocervix in these patients. This may require more extensive clinical procedures. If a patient has had a hysterectomy, a vaginal sample is sufficient, with particular attention to sampling the vaginal cuff.

Remove excess mucus or other discharge present before taking the sample. This should be gently removed with ring forceps holding a folded gauze pad. The excess cervical mucus is essentially devoid of meaningful cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.

Remove inflammatory exudate from the cervical canal before taking the sample. Remove by placing a dry 2 x 2 inch piece of gauze over the cervix and peeling it away after it absorbs the exudate or by using a dry protoswab or scopette. The excess inflammatory exudate is essentially devoid of diagnostic cellular material and when present in the sample vial may yield a slide with little or no diagnostic material present.

The cervix should not be cleaned by washing with saline or it may result in a relatively acellular specimen.

The sample should be obtained before the application of acetic acid.

Collection Devices

There are a variety of collection devices available for sampling the endocervix, transformation zone and ectocervix. They include endocervical brushes, wooden and plastic spatulas, and plastic “broom-type” samplers. Plastic spatulas are preferred over wooden since the wooden spatulas retain cellular material. The use of a cotton-tipped swab is NOT recommended, even if the swab is moistened. Cells adhere to the cotton and do not transfer well to the glass slide, which results in an incomplete specimen.

Analysis of different sampling methods has shown that overall, the cytobrush and spatula together provide the best specimen for cervical cytology. However, the choice of a particular device is dependent on variations in the size and shape of the cervix and the clinical situation. As previously stated, age, parity, and hormonal status of the patient can affect the exposure of the transformation zone. Previous therapy, such as conization, laser therapy or cryotherapy, can also change the features of the cervix. The clinician ought to consider these factors when choosing a collection device. Liquid based methods require the use of collection devices that have been approved by the FDA for use with the particular specimen preparation instrument.

Note: The Manufacturer' (Cytoc) instructions and/or package inserts should be consulted and the recommendations should be followed.

Techniques for Sample Collection

Collection of cervical/vaginal specimens for conventional smear preparations using the spatula and endocervical brush.

- The vaginal fornix and ectocervix should be sampled before the endocervix/transformation zone. First, a sample of the ectocervix is taken using a plastic (or wooden) spatula. The notched end of the spatula that corresponds to the contour of the cervix is rotated 360° around the circumference of the cervical os, retaining the sample on the upper surface of the spatula. Grossly visible lesions, including irregular, discolored or friable areas should be directly sampled and can be placed on a separate slide, especially if the lesion is distant from other collection areas. The spatula is held with the specimen face up while the endocervical sample is collected.
- Sampling of the endocervix requires insertion of the endocervical brush into the endocervical canal until only the bristles closest to the hand are visible. The brush is rotated 45-90° and removed. At this time, the sample on the spatula is spread evenly and thinly lengthwise down one half of the labeled slide surface, using a single uniform motion. The endocervical brush is then rolled along the remaining half of the labeled slide surface by turning the brush handle and slightly bending the bristles with gentle pressure. The brush should not be smeared with force or in multiple directions. The entire slide is then rapidly fixed by immersion or spray and the collection devices are discarded.
- Note: the used of and endocervical brush maybe contraindicated in pregnant patients. Refer to the package insert. If the above-described sampling order is reversed, bleeding secondary to abrasion from the brush may obscure the cellular material.

Collection of cervical/vaginal specimens for liquid-based preparations using the spatula and endocervical brush.

- For liquid based preparations, the ectocervix should be sampled using the same procedure as for conventional Pap smears. However, the spatula with the cellular material is rinsed in the specimen vial and then discarded.
- The endocervical specimen is collected using the same technique as for conventional Pap smears. However, the endocervical brush is rinsed in the vial and then discarded.
- Manufacturer's directions must be followed ---See "ThinPrep Pap Test Quick Reference Guide".

Collections of cervical/vaginal specimens for conventional smear preparation using the broom-like device.

- The ectocervix and endocervix are collected simultaneously with the "broom-like" device. The central bristles of the broom are inserted into the endocervical canal until the lateral bristles bend fully against the ectocervix. The sampling device is rotated 360° in the same direction five (5) times while maintaining gentle pressure.
- The broom is removed and with a single paint stroke motion the cellular sample is transferred down the long axis of the labeled surface of the slide. The broom is turned over and the pain stroke motion is repeated over the same area. The slide is rapidly fixed either by immersion or spray and the device is then discarded.

Collection of cervical/vaginal specimens for liquid-based preparations using the broom-like device.

- The ectocervical and endocervical specimens are collected with the "broom-like" device simultaneously. The central bristles of the device are inserted into the endocervical canal until the lateral bristles fully bend against the ectocervix. Maintaining gentle pressure, the broom is rotated in a clockwise direction 360°; for a total of five (5) times.
- The broom is then rinsed in the specimen vial. Manufacturers' directions vary and must be referred to and followed.

Cell Fixation for Conventional Cervical Cytology

Immediate fixation of the cellular sample, within seconds of specimen collection is necessary to prevent air-drying. Air-drying obscures cellular detail and compromises specimen evaluation. Immersing the slide in alcohol or spraying with fixative can prevent air-drying artifact.

If the specimen is immersed in alcohol, it may remain in the alcohol for transport to the laboratory. Alternatively, the specimen can be immersed in alcohol for 20-30 minutes, removed and allowed to air dry, then placed in a container/mailler for transport to the laboratory. The immersion technique requires use of a separate container for each specimen and changing or filtering the alcohol between specimens.

If a specimen is spray fixed, only quality-controlled cytology fixatives should be used. Hair spray should not be used. Whether using a pump spray, aerosol fixative or single application packet, the manufacturer's instructions on the container and package insert should be followed.

Generally, spray fixatives should be 6-10 inches (15-25 cm) from the glass slide when applied.

Refer to the ThinPrep Pap Test Quick Reference Guide.

Ancillary Studies Offered on ThinPrep Vial:

Additional studies can be performed on the ThinPrep vial specimen (Pap specimen in PreservCyt).

Test choices are (located bottom right of TOPA requisition):

- ThinPrep Pap smear only
- ThinPrep with Chlamydia and GC by PCR
- ThinPrep and HPV
- ThinPrep with HPV reflex on ASCUS
- 4 in 1 Panel, which includes:
 - ThinPrep Pap smear
 - Chlamydia and GC by PCR
 - HPV on ASCUS

Specimen Labeling:

The provider must properly label specimen containers at the time of collection with the following information:

- Patient name (first and last)
- A second patient identifier, i.e., date of birth
- Specimen source
- Physician name
- Collection date

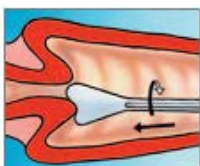
Glass slides must be labeled with patient's name, using a solvent-resistant pen or pencil, on the frosted end of the slide.

Please attach a copy of the patient's insurance card (front & back) and a copy of the demographics face sheet (if available).

If any information is missing, LBM personnel will call your office in an attempt to obtain the missing information. LBM reserves the right to return a specimen due to inadequate information or specimen preservation.

ThinPrep® Pap Test Quick Reference Guide

Endocervical Brush/Spatula Protocol



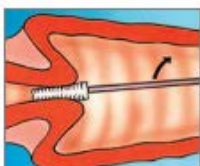
Obtain...

...an adequate sampling from the ectocervix using a *plastic* spatula.



Rinse...

...the spatula as quickly as possible into the PreservCyt® Solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.



Obtain...

...an adequate sampling from the endocervix using an endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate $\frac{1}{4}$ or $\frac{1}{2}$ turn in one direction. DO NOT OVER-ROTATE.



Rinse...

...the brush as quickly as possible in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush.



Tighten...

...the cap so that the torque line on the cap passes the torque line on the vial.



Record...

...the patient's name and ID number on the vial.
...the patient information and medical history on the cytology requisition form.

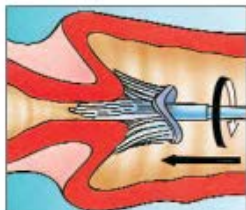


Place...

...the vial and requisition in a specimen bag for transport to the laboratory.

ThinPrep® Pap Test Quick Reference Guide

Broom-Like Device Protocol



Obtain...

...an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.



Rinse...

...the broom as quickly as possible into the PreservCyt® Solution 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 the collection device.



Tighten...

...the cap so that the torque line on the cap passes the torque line on the vial.



Record...

...the patient's name and ID number on the vial.

...the patient information and medical history on the cytology requisition form.



Place...

...the vial and requisition in a specimen bag for transport to the laboratory.

MOLECULAR DIAGNOSTICS

Affirm VPIII Microbial Identification Test (for Vaginitis/Vaginosis)

Preparation of Transport System and Collection of Vaginal Sample-

1. Open the seal on outer plastic pouch of Affirm VPIII Ambient Temperature Transport System and remove all components (each plastic pouch contains enough material for the collection and transport of one vaginal specimen).
2. Tear open the foil pouch and remove the ATTS Reagent Dropper.
3. Break ampule in ATTS Reagent Dropper by firmly squeezing vial with finger and thumb.
Caution: Break ampule close to its center one time only. Do not manipulate dropper any further, as the plastic may puncture and injury may occur.
4. Dispense reagent from ATTS Reagent Dropper into sample collection Tube.
5. Peel wrapper to expose patient swab. Remove swab. Discard wrapper.
6. Collect patient specimen/take sample.
 - Place the patient in position for a pelvic examination. Insert a speculum into the vagina to permit visualization of the posterior vaginal fornix.
 - Using the sterile polyester-tip swab, obtain a sample from the posterior vaginal fornix. Twist or roll the swab against the vaginal walls two or three times, ensuring the entire circumference of the swab has touched the vaginal wall. Swab the lateral vaginal wall while removing the swab.
7. Immediately place the patient swab in the Sample Collection Tube containing the ATTS Reagent.
8. Break swab shaft at pre-scored line just above the top of the tube. Discard remaining shaft into an infectious waste container.
9. Place the Sample Collection Cap over the exposed end of the swab and firmly press the cap onto the Sample Collection Tube. The cap will 'snap' onto the tube when it is properly seated.
10. Label the Sample Collection tube with patient /lab identification information. Include date and time that sample was taken.

Transport and Storage of Vaginal Sample: Send the capped Sample Collection Tube to the lab by sealing it in the emptied outer plastic pouch of the Affirm VPIII Ambient Temperature Transport System. Label plastic pouch with appropriate information.

Human Papilloma Virus (HPV)

TOPA utilizes the Roche Cobas PCR methodology. The advantages of this assay include the following:

1. Fully automated processing for enhanced reliability and reproducibility.
2. Rapid turn-around time.
3. Concurrent genotyping for HPV types 16 and 18, on high-risk HPV-positive samples, providing immediate genotyping results at **no additional cost to the patient**.

Ordering Options:

HR-HPV testing can be ordered in conjunction with cervicovaginal cytology (eg. for women age 30 and above), or can be ordered as a reflex test based on cytologic findings (eg. for a diagnosis of ASCUS).

This assay is designed to detect HPV type 16, HPV type 18, and other high risk HPV types (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). A negative result does not preclude the presence of HPV infection, because results depend on adequate specimen collection, absence of inhibitors and sufficient DNA to be detected.

Indications for testing and clinical significance of results are outlined in recently published guidelines.

References: Am J Clin Pathol 2012; 137: 516-54. CA Cancer J Clin 2012; 62: 147-172.

Current guidelines recommend incorporation of HPV genotyping results into management strategies, as follows:

- Cytology-negative, HPV-positive women aged 30-65: women testing positive for HPV 16/18 should be referred directly to colposcopy; women testing negative for HPV 16/18 should be co-tested in 12 months.
- Cytology-ASCUS, HPV-positive women aged 21 and above: initial management consists of referral to colposcopy regardless of genotyping results. Subsequent management may be influenced by genotyping results, since a positive result for HPV 16/18 is associated with a higher risk for high-grade CIN.
- These are guidelines only; management decisions should be based on the clinical situation of the individual patient. (Am J Clin Pathol 2012; 137: 516-54. CA Cancer J Clin 2012; 62: 147-172).

Cervical specimens for HPV testing must be collected in ThinPrep® Pap Test PreservCyt® Solution, using a broom-type device or Endocervical Brush/Spatula. Specimens can be stored at room temperature (2-30°C) for up to 6months prior to performing the test.

For additional information on the collection of cervico-vaginal samples, see earlier section of this manual (Gynecological Cytology).