TEST DIRECTORY:
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TEST DIRECTORY:
SPECIMEN COLLECTION
QUICK REFERENCE GUIDE

TISSUE TYPE	PROCEDURE	FIXATIVE	SPECIAL INSTRUCTIONS
Bone Marrow	Aspiration, smears and core biopsy	Formalin Green top tube Lavender top tube	
Breast	Core biopsy Excision Mastectomy	10% buffered formalin	Always indicate date and time specimen was removed from patient and when placed into formalin
Calculi or Stones	Removal	Fresh, no fixative	
CLOtest for H. Pylori		CLOtest	See separate procedure
Fluids (nipple discharge, breast cyst, FNA)		CytoLyt	
Frozen Section OR Consult		Fresh, preferably sterile	
Lymph node	Biopsy	Fresh, no fixative OR 1 core in RPMI, rest in formalin	
Muscle	Biopsy	Fresh, sterile	Notify TOPA 48 hours before procedure. Bring fresh to TOPA
Pap smear		PreservCyt SurePath solution	
Peripheral Blood		Green top tube Lavender top tube	
Prostate	Biopsy	Zinc formalin	
Microbiology culture		Fresh, sterile	Bring to TOPA immediately upon removal. Cultures not performed by TOPA will be sent to our reference lab
Molecular Testing			See Molecular Quick Reference Guide
Semen Analysis (post-vasectomy only)			See separate procedure
Thyroglobulin rinse	FNA		See separate procedure
Thyroid molecular testing	FNA	Molecular testing media	
Uterine contents for genetic testing		Fresh	Call TOPA for further instructions
Urine	Voided	Fresh	Call TOPA immediately for pick up. Specimen should be picked up no later than an hour after specimen is collected.

- **Use the appropriate TOPA requisition.**
- **Place specimen in proper fixative.**
- **Label specimen bottle with <u>two</u> patient identifiers, i.e., name and date of birth. Also include specimen source, physician name & collection date.**
- **Call TOPA courier for pick up at (805) 373-8582.**

If you are already on the TOPA 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 TOPA courier at (805) 373-8582 to schedule a pickup ask for "Dispatch".

If you need a lock box or would like daily specimen pick up, please call TOPA Client Services, at (805) 373-8582.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
HOW TO COMPLETE A REQUISITION

All specimens must be accompanied by a TOPA requisition.

Please fill out the requisition form corresponding to the appropriate tissue type:

- General surgical pathology
- Breast
- Hematopathology
- Podiatry
- Urology
- Women's Health

Please fill out the form completely and 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 TOPA 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 TOPA at (805) 373-0023.

Urology Offices: Please note that the Urology requisition also 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.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
SUPPLIES

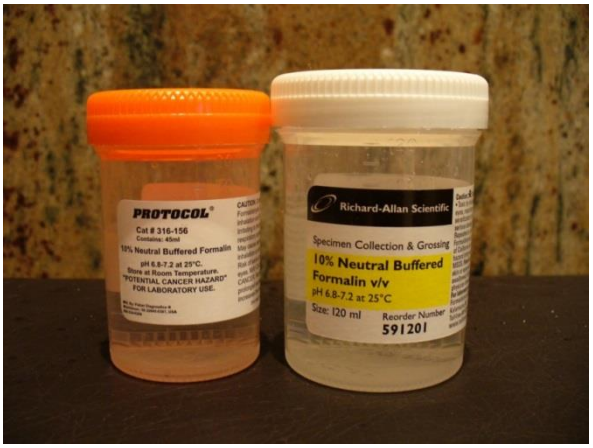
Supplies may be ordered using the Client Supply form

Please fax a completed copy to TOPA at 805-373-0023

Type of container	Specimen
Formalin bottle*	Specimens for routine histology
Sterile container (no fixative)	Specimens for microbiology culture
Zinc formalin bottle*	Prostate biopsy specimens
RPMI bottle	Specimens for flow cytometry
Nail bag	Nail clippings

*Formalin and zinc formalin bottles are supplied in several sizes

Example of formalin bottles



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

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 containers must be labeled at the time of collection with the following:

- 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 placed 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) to the requisition.

If any information is missing, TOPA personnel will call your office in an attempt to obtain the missing information. TOPA 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. TOPA provides clients with specimen requirements for all samples. 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.

Specimens not requiring immediate examination by the pathologist, culture, or special handling for other purposes, 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.

Each specimen must be properly labeled and accompanied by a complete requisition form, demographics/face sheet (if available), and copy of insurance information.

Specimen Source:

- Tissue biopsy
- Tissue excision

Collection Instructions:

- Specimens for routine histology should be placed into formalin bottles immediately after removal
- Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- See table below for specific specimen collection guidelines
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.
- For any specimen that may pose a radiation hazard, please contact TOPA Diagnostics for handling instructions

Specimen Source	Collection Media
Tissue for routine histology	Formalin bottle
Tissue for lymphoma workup	See separate instructions (Lymph node and lymphoma workup)
Tissue for microbiology culture	Fresh specimen in sterile container
Tissue for immunofluorescence	Zeus fixative
Tissue for clonogenic assays (drug resistance)	RPMI tube
Muscle biopsy tissue*	Fresh specimen in sterile container
Nerve biopsy tissue*	Fresh specimen in sterile container

*Please notify TOPA Diagnostics at least 48 hours prior to procedure.

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens without fixative should refrigerated
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.

Methodology: see separate protocols

Specimen Source:

- Tissue biopsy
- Tissue excision

Collection Instructions:

- Frozen sections should be scheduled with the TOPA pathologists in advance
- Please fill out the Frozen Section Scheduling form for your office location (provide phone number so pathologist may directly contact surgeon at the time of the intraoperative consultation)
- Specimens should be submitted fresh, not in formalin, preferably sterile
- Specimens submitted for microbiology cultures or cytogenetics should be submitted in capped sterile containers

Specimen Source	Collection Media
Tissue for intraoperative consultation	Fresh tissue (without fixative)
Tissue for microbiology cultures	Fresh, sterile tissue, in sterile container
Tissue for cytogenetics	Fresh, sterile tissue, in sterile container

Storage Instructions:

- Specimens should be delivered to TOPA Diagnostics as soon as possible during the operative procedure. It is the responsibility of the physician's office to deliver the specimen to TOPA Diagnostics.

Methodology: Specimens will be grossly evaluated, appropriately dissected and, using rapid freezing techniques and H&E staining, examined under the light microscope, if necessary, to provide information to guide intraoperative management. If cultures, cytogenetics or flow cytometry are requested, the pathologist will select appropriate material for those studies and submit them to the appropriate reference laboratories for evaluation, in the proper media. The reference lab will bill for their services separately.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
BREAST SPECIMENS

Specimen Source:

- Breast core biopsy
- Breast excisional biopsy
- Breast lumpectomy
- Breast mastectomy
- Axillary lymph node biopsy

Collection Instructions:

- All breast specimens should be placed into 10% neutral buffered formalin as soon as possible and within 1 hour of removal.
- If a specimen is submitted without fixative, it should be delivered to TOPA Diagnostics immediately, so that it may be dissected and placed into fixative within one hour of removal.
- Record the date and time the specimen was removed from the patient and the time the specimen was placed in 10% neutral buffered formalin on the accompanying TOPA breast requisition where indicated.
- Biopsies from separate locations must be placed into separate containers
- Biopsies for calcifications: Tissue cores with calcifications should be submitted in a separate container than those without calcifications
- Lymph node biopsies for suspected lymphoma cases: one core in formalin, one core in RPMI tube

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens in RPMI should be stored in refrigeration until pickup
- Specimens without fixative should be delivered to TOPA Diagnostics as soon as possible

Methodology: Specimens will be fixed in 10% neutral buffered formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis. Appropriate ancillary testing is performed reflexively in cases of malignancy.

Interpretative Comments: When possible, the pathology results are correlated with the radiologic and clinical impression.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
HEMATOLOGY
Lymph Node and Lymphoma Workup

Specimen Source:

- Lymph node biopsy
- Lymph node excision

Supplies:

- Formalin bottle
- RPMI tube
- Sterile container

Collection Instructions:

Specimen Source and/or Test requested	Collection Media
Lymph node for flow cytometry	RPMI tube
Lymph node for routine histology	Formalin bottle
Lymph node for cultures	Fresh tissue in sterile container
Lymph node for lymphoma workup	Fresh tissue +/- small amount of sterile saline (see below*)

- *If lymphoma is suspected and biopsy or excision is performed, submit all the tissue fresh (place a small amount of sterile saline or a sterile saline-soaked gauze on the tissue to keep it from drying out). The pathologist will properly handle the tissue
- If multiple biopsies are obtained, place one core in RPMI tube for flow cytometry and remainder of tissue in formalin bottle.
- Please call TOPA Diagnostics as soon as possible to arrange for pickup of the specimen.

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens in RPMI should be stored in refrigeration
- Fresh tissue specimens should be stored in refrigeration

Methodology: Specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis. In cases of suspected flow cytometry, specimens submitted in RPMI will be used for flow cytometry analysis. FISH and PCR may be performed on paraffin embedded tissue.

SPECIMEN COLLECTION MANUAL
ANATOMIC PATHOLOGY:
MUSCLE BIOPSY

Specimen Source:

- Muscle biopsy

Collection Instructions:

- Please notify TOPA Diagnostics at least 48 hours prior to procedure
- Collect fresh specimen in a sterile container (may be submitted on saline-moistened gauze
- Specimen should be from belly of affected muscle, ideally 2.5 to 3.0 cm in length and 1.0 cm in width
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.

Specimen Source	Collection Media
Muscle biopsy tissue	Fresh in sterile container

Storage Instructions:

- Fresh specimens should be stored in refrigeration until pick-up
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.

Methodology: Muscle biopsy specimens will be submitted to a reference laboratory for evaluation. The reference lab will bill the insurance company.

Specimen Source:

- Nerve biopsy

Collection Instructions:

- Please notify TOPA Diagnostics at least 48 hours prior to procedure
- Collect fresh specimen in a sterile container (may be submitted on saline-moistened gauze)
- Specimen should be from 4 cm or longer and include the entire thickness of the nerve
- Sural nerve biopsy is sufficient for the diagnosis, however, in the case of pure motor neuropathy, a radial nerve or a branch of peroneal nerve may yield better results
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.

Specimen Source	Collection Media
Nerve biopsy tissue	Fresh in sterile container

Storage Instructions:

- Fresh specimens should be stored in refrigeration until pick-up
- Please call TOPA Diagnostics to arrange for pick-up/delivery of all fresh specimens as soon as possible.

Methodology: Nerve biopsy specimens will be submitted to a reference laboratory for evaluation.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
UROLOGY SPECIMENS

Collection Instructions:

- Specimens for routine histology should be placed into formalin or zinc formalin bottles immediately after removal.
- Prostate biopsy specimens should be placed into zinc formalin bottles. Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- Please use customized urology requisition forms that include sequentially numbered requisitions and corresponding biopsy bottle labels
- Urology requisitions also include a section for urine cytology and FISH studies
- See table below for specific specimen collection guidelines

Specimen Source	Collection Media	Specimen Source	Collection Media
Prostate biopsy	Zinc formalin bottle	**Bladder biopsy**	Formalin bottle
Prostate TURP	Formalin bottle	**Bladder TURBT**	Formalin bottle
Calculus/Stone (bladder, kidney, ureter)	Sterile container without fixative	**Urine for cytology**	CytoLyt, or Sterile container
Testis biopsy/orchiectomy	Bouin's solution or 10% formalin	**Urine for FISH**	PreserveCyt or Sterile container

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

Storage Instructions:

- Specimens in formalin or zinc formalin may be stored at room temperature
- Specimens without fixative should refrigerated

Methodology: Specimens will be fixed in formalin or zinc formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis.

Specimen Source:

- GI biopsy from upper or lower endoscopy

Collection Instructions:

- Specimens for routine histology should be placed into formalin bottles immediately after removal
- Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens without fixative should refrigerated

Methodology: Specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis.

Specimen Source:

- Skin biopsy
- Skin excision

Collection Instructions:

- Specimens for routine histology should be placed into formalin bottles immediately after removal
- Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- See table below for specific specimen collection guidelines

Specimen Source	Collection Media
Skin biopsy for routine histology	Formalin bottle
Skin biopsy for immunofluorescence	Zeus fixative
Skin biopsy for culture	Sterile container
Skin biopsy for lymphoma workup	RPMI bottle

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens without fixative should refrigerated

Methodology: Specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis.

Specimen Source:

- Cervical biopsy
- Cervical LEEP
- Cervical CONE
- Endometrial biopsy
- Endometrial curettage
- Vulvar biopsy
- Vaginal biopsy

Collection Instructions:

- Specimens for routine histology should be placed into formalin bottles immediately after removal
- Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- See specimen collection guidelines under cytology and microbiology for additional specimen sources

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens without fixative should refrigerated

Methodology: Specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis.

Specimen Source:

- Products of conception
- If cytogenetic testing is to be performed, placental tissue (chorionic villi) is preferred; umbilical cord tissue or fetal skin may be submitted if placental tissue is not available)

Supplies:

- RPMI tube if cytogenetics requested
- Formalin bottle for routine histology

Collection Instructions (for cytogenetic testing):

- Specimens should be obtained using sterile technique.
- Submit at least 4 mm (100 mg) sample of sterile tissue.
- Sterile tissue should be placed in RPMI tube.
- If the specimen is placed into formalin, limited genetic testing can be performed, but complete cytogenetic analysis CANNOT be performed on formalin-fixed tissue
- Phone TOPA Diagnostics to arrange for same day pick-up.

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens without fixative or in RPMI should be refrigerated (may be temporarily placed in lock box at room temperature if after hours pick up is scheduled for the same day)

Methodology: Specimens will be grossly analyzed and a portion will be submitted in RPMI solution for cytogenetic analysis. Some of the specimen will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, immunohistochemical stains and DNA ploidy analysis may be employed to make a diagnosis. If cytogenetics is ordered, it will be performed at a reference lab and reported in an addendum report.

CLOtest for Helicobacter pylori

CLOtest Storage Prior to Use:

The unused CLOtest has a shelf life of 18 months when stored at 2º-8º C.

Preparation of the Patient:

Patients should not have taken antibiotics or bismuth salts for at least three weeks prior to endoscopy and should not have taken protein pump inhibitors for at least two weeks prior to endoscopy. These drugs may inhibit the growth of H. pylori.

Taking and Inserting the Biopsy:

1. The recommended area to biopsy is the antrum, at least 2 cm away from the pylorus along the lesser or greater curvature.

2. Biopsy an area of normal-looking tissue rather than an area affected by erosions or ulceration. This is because H. pylori may be present in smaller numbers if the epithelium is eroded or the mucus layer is denuded. The standard biopsy forceps will provide a specimen of sufficient size.

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 devise (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.

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

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

TEST DIRECTORY
ANATOMIC PATHOLOGY:
SPECIAL HANDLING OF TISSUE SPECIMENS

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.

TEST DIRECTORY
NON-GYNCOLOGICAL CYTOPATHOLOGY:
HOW TO COMPLETE OUT A REQUISITION

All specimens must be accompanied by a TOPA requisition.

Please fill out the requisition form corresponding to the appropriate tissue type:

- General surgical pathology
- Non-gynecologic cytology
- Urology (urine cytology, urine FISH, prostate biopsy)

Please fill out the form completely and 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 TOPA 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 TOPA at (805) 373-0023.

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

TEST DIRECTORY
NON-GYNCOLOGICAL CYTOPATHOLOGY: SUPPLIES

Supplies provided by TOPA Diagnostics:

Solutions & Supplies	Specimen Type
CytoLyt solution (ThinPrep)*	Non-gyn cytology
PreservCyt solution	Urine FISH studies
Plastic jars with screw-top lids containing 95% alcohol	Smears on microscope glass slides should be placed in alcohol jars
Microscope glass slides	For smears
Spatulas with removable handles	For nipple discharges
Thyroid molecular testing transport medium (Veracyte, Interpace)	Thyroid FNA
Biohazard bags	Jars containing fluids that may leak/spill
RPMI solution	Lymphoma workup

*Pre-filled containers of CytoLyt solution (15 ml of solutions in a 90 ml container)

Order supplies using the Client Supply Order form. Please fax completed form to TOPA at (805) 373-0023.

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. The majority of the air-drying 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. 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, TOPA personnel will call your office in an attempt to obtain the missing information. TOPA reserves the right to return a specimen due to inadequate information or specimen preservation.

Criteria for Acceptable Specimens

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

It is imperative that specimens be submitted appropriately and according to the specimen requirements outlined below. TOPA 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 Source:

- Brushing (i.e. esophageal brushing)

Supplies:

- Brush
- CytoLyt solution

Collection Instructions:

- Cut brush and immediately place in CytoLyt solution to prevent air drying
- Label container with the patient's name, specimen source, date & time collected and physician's name
- Complete TOPA requisition

Storage Instructions:

- Specimens in CytoLyt may be stored at room temperature

TEST DIRECTORY
NON-GYNOCOLOGICAL CYTOLPATHOLOGY:
FINE NEEDLE ASPIRATION (FNA)
Thyroid (with Smears):

Specimen Source:

- Thyroid FNA

Supplies:

- CytoLyt solution
- Microscope glass slides
- Jar containing 95% alcohol
- RPMI solution (for lymphoma workup)
- Thyroid molecular testing transport media (VeraCyte, Interpace)

Collection Instructions:

- Three to five passes are recommended:
  - For the first two passes:
    - Prepare one smear for each pass and immediately place each labeled slide into 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)

- **Slide Preparation**: 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-drying 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.

- **Molecular testing**: 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.

- **Flow Cytometry (lymphoma workup):** Please perform one additional pass, and inject/rinse material directly into RPMI solution

- Label all containers with patient's name, a second patient identifier (eg. birth date), specimen source, date and time collected, and physician's name
- Complete TOPA requisition and indicate all tests requested

Storage Instructions:

- Specimens in CytoLyt, 95% alcohol, and molecular transport media may be stored at room temperature
- Specimens without solution or in RPMI solution should be refrigerated (specimen may be placed into lock box without ice pack for after-hours pickup)

TEST DIRECTORY
NON-GYNCOLOGICAL CYTOPATHOLOGY
FINE NEEDLE ASPIRATION (FNA):
Thyroid (without Smears):

Specimen Source:

- Thyroid FNA

Supplies:

- CytoLyt solution
- RPMI solution (for lymphoma workup)
- Thyroid molecular testing transport media (VeraCyte, Interpace)

Collection Instructions:

- Three to five passes are recommended:
  - Inject/rinse material from each pass directly into CytoLyt

- **Molecular testing**: 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.

- **Flow Cytometry (lymphoma workup):** Please perform one additional pass, and inject/rinse material directly into RPMI solution

- Label all containers with patient's name, a second patient identifier (eg. birth date), specimen source, date and time collected, and physician's name
- Complete TOPA requisition and indicate all tests requested

Storage Instructions:

- Specimens in CytoLyt molecular transport media may be stored at room temperature
- Specimens without solution or in RPMI solution should be refrigerated (specimen may be placed into lock box without ice pack for after-hours pickup)

TEST DIRECTORY
NON-GYNCOLOGICAL CYTOPATHOLOGY:
FINE NEEDLE ASPIRATION (FNA):
Non-Thyroid

Specimen Source:

- FNA

Supplies:

- CytoLyt solution
- Microscope glass slides
- Jar of 95% alcohol
- RPMI solution (for lymphoma workup)

Collection Instructions:

- Three to five passes are recommended:
  - For the first two passes:
    - Prepare one smear for each pass and immediately place each labeled slide into cytology jar containing 95% alcohol
    - Rinse/inject remainder of material into CytoLyt
  - For all other passes:
    - Rinse/inject material directly into CytoLyt (no smears prepared)

- **Slide Preparation**: 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-drying 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.

- **Flow Cytometry (lymphoma workup):** Please perform one additional pass, and inject/rinse material directly into RPMI solution

- Label all containers with patient's name, a second patient identifier (eg. birth date), specimen source, date and time collected, and physician's name

- Complete TOPA requisition and indicate all tests requested

Storage Instructions:

- Specimens in CytoLyt, 95% alcohol and molecular transport media may be stored at room temperature
- Specimens without solution or in RPMI solution should be refrigerated (specimen may be placed into lock box without ice pack for after-hours pickup)

TEST DIRECTORY
NON-GYNCOLOGICAL CYTOPATHOLOGY:
THYROGLOBULIN RINSE TEST

Specimen Source:
- Lymph Node Washing (1 ml node washings in a sterile transport tube)

Supplies:
- 1 ml FNA transport container

Collection Instructions:
- Please contact TOPA Diagnostics at least 1 week prior to the procedure so appropriate supplies may be delivered to the office
- Preferred Specimen(s):
  - 1 mL fine needle aspirate (FNA)
  - Node washings are acceptable
- Three to six separate passes are performed, each with a new needle
- After collection of the cytology samples, each FNAB needle is then washed with 0.1-0.5 ml of normal saline
- The washes from all needles are pooled (final volume 1 ml) and immediately frozen, then transported to the laboratory directly
- Saline and glass tubes are not acceptable
- Call TOPA Diagnostics for immediate pick up after the specimen has been collected

Storage Instructions:
- Transport temperature: Frozen or shipping refrigerated is acceptable

Clinical Significance:
- Clinically enlarged cervical lymph nodes with a history of thyroid cancer are usually assessed by fine-needle aspiration biopsy (FNAB) followed by a cytology. Thyroglobulin (Tg) is frequently elevated in malignant FNAB needle wash specimens and it's use may possibly augment or replace cytology.

Methodology: Test is sent to Quest Laboratories, test code: 16559X

Specimen Source:

- Miscellaneous Fluid Specimens (i.e. breast cyst, cyst, etc)

Supplies:

- CytoLyt solution

Collection Instructions:

- Inject (if applicable) or otherwise place entire specimen in CytoLyt
- Rinse hub of needle in CytoLyt
- Label container with patient's name, specimen source, date & time collected, and physician's name
- Complete TOPA Diagnostics requisition

Specimen Source:

- Nipple Discharge

Supplies:

- Spatula with removable handle
- CytoLyt solution

Collection Instructions:

- Using the spatula with removable handle, obtain the nipple discharge on the spatula end
- Disconnect the handle and drop the spatula end into CytoLyt solution
- Label container with the patient's name, specimen source, date & time collected and physician's name
- Complete TOPA requisition

Storage Instructions:

- Specimens in CytoLyt may be stored at room temperature

TEST DIRECTORY
NON-GYNECOLOGICAL CYTOPATHOLOGY: URINE

Specimen Source:
- Urine

Supplies:
- CytoLyt solution
- PreservCyt solution
- Sterile specimen container
- Biohazard bag

Collection Instructions:
- Label urine container provided by physician's office with the patient's name, specimen source, date & time collected, and physician's name
- Patient voids into empty, sterile specimen container
- **Urine for cytologic evaluation**: Slightly agitate (swirl) urine and pour 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**: Slightly agitate (swirl) urine and pour 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
- **Urine for Culture and/or Urinalysis**: Send fresh specimen to physician's reference laboratory. Do not send to TOPA Diagnostics.
- **Alternate method**: Place minimum of 48 ml fresh urine in urine container. Refrigerate and call TOPA immediately for pick up that day.
- Complete TOPA requisition indicating test(s) ordered in Cytology section of form
  - Example: _____Urine for Cytology (ThinPrep)
_____Urine for FISH
- Place specimen(s) in zip-lock biohazard bag and insert folded requisition in side pocket.
- Phone TOPA Diagnostics for specimen pickup

Urine Test Requested	Collection Media
Urine for cytology	CytoLyt solution (ThinPrep) OR Fresh without fixative
Urine for FISH	PreservCyt solution OR Fresh without fixative
Urine for culture	Fresh, no fixative
Urine for urinalysis	Fresh, no fixative

Storage Instructions:

- Specimens in CytoLyt may be stored at room temperature
- Specimens without solution (i.e. fresh urine) should be refrigerated (specimen may be placed into lock box without ice pack for after-hours pickup)

TEST DIRECTORY
NON-GYNECOLOGICAL CYTOPATHOLOGY: URINE

Specimen Source:
- Urine

Supplies:
- CytoLyt solution
- PreservCyt solution
- Sterile specimen container
- Biohazard bag

Collection Instructions:
- Label urine container provided by physician's office with the patient's name, specimen source, date & time collected, and physician's name
- Patient voids into empty, sterile specimen container
- **Urine for cytologic evaluation**: Slightly agitate (swirl) urine and pour 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**: Slightly agitate (swirl) urine and pour 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
- **Urine for Culture and/or Urinalysis**: Send fresh specimen to physician's reference laboratory. Do not send to TOPA Diagnostics.
- **Alternate method**: Place minimum of 48 ml fresh urine in urine container. Refrigerate and call TOPA immediately for pick up that day.
- Complete TOPA requisition indicating test(s) ordered in Cytology section of form
  - Example: _____Urine for Cytology (ThinPrep)
_____Urine for FISH
- Place specimen(s) in zip-lock biohazard bag and insert folded requisition in side pocket.
- Phone TOPA Diagnostics for specimen pickup

Urine Test Requested	Collection Media
Urine for cytology	CytoLyt solution (ThinPrep) OR Fresh without fixative
Urine for FISH	PreservCyt solution OR Fresh without fixative
Urine for culture	Fresh, no fixative
Urine for urinalysis	Fresh, no fixative

Storage Instructions:

- Specimens in CytoLyt may be stored at room temperature
- Specimens without solution (i.e. fresh urine) should be refrigerated (specimen may be placed into lock box without ice pack for after-hours pickup)

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	33 mL
Urine for cytology & UroVysion	48 mL

TEST DIRECTORY
CYTOLOGY:
ANAL SAMPLES FOR CYTOLOGY

Specimen Source:
- Anal sample

Supplies:
- ThinPrep (PreservCyt) media
- Cytobrush or Dacron Swab

Collection Instructions:
- Insert cytobrush or Dacron swab approximately 5 cm into anal canal, passing the anal verge, so that the anorectal junction is sampled.
- Rotate the brush/swab against the anorectal wall.
- Carefully withdraw the swab.
- Swirl the swab vigorously, 10 times, in the PreservCyt (ThinPrep) vial.
    - Note: if a brush is used, rotate the brush 10 times while pushing against the PreservCyt vial wall.
- Discard the brush/swab.

Storage Instructions:
- Samples are stable at room temperature for up to 6 weeks.

Methodology: Thin-layer cytology.

Ancillary Studies available:
- High-risk HPV by PCR, with 16/18 genotyping
- Chlamydia/ Gonorrhea by PCR

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
HOW TO COMPLETE A TOPA REQUISITION

All specimens must be accompanied by a TOPA 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, eg. 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 clinical 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 TOPA requisition is customized with your doctor's name, address, phone and fax numbers. To order more requisitions, please use the Client Supply Order form.

- 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)

- SurePath Pap Test Solution for gyn cytology studies is provided by TOPA in pre-filled containers (10 ml of solution) (see attached picture).

- Specimen collection devices:
  - Spatula and cervical brush with detachable heads
  - Broom-type device with detachable heads

SurePath Pap Test Solution

Criteria for Accepting a Specimen:

1. Properly labeled ThinPrep vial, SurePath vial, or slides and specimen container.
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 and SurePath Pap Test reduce 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.

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
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 a 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 solutions leading to potential agglutination and cellular loss.

Cytyc 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 Tests.

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 Cytyc's Technical Support Department at 1-800-442-9892, option 6.

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 and 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 proctoswab 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.

Specifically for SurePath, the broom-type device (Rovers Cervex-Brush) has a detachable head. For SurePath, the Pap Perfect plastic spatula and Cytobrush Plus GT endocervix brush also have detachable head devices which can be snapped free at the red scoring lines.

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 Manufacturers’ (Cytyc) instructions and/or package inserts should be consulted and the recommendations should be followed.

Collection of cervical/vaginal specimens for liquid-based 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. The spatula with the cellular material is rinsed in the specimen vial and then discarded.
- 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. The endocervical brush is rinsed in the vial and then discarded.
- Note: The use of an endocervical brush may be 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.
- Manufacturers' directions must be followed – See "ThinPrep Quick Reference Guide".

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.

Techniques for Sample Collection for Conventional Smear

Collection of cervical/vaginal specimens for conventional smear preparation 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 use of an endocervical brush may be 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 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 360º 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 paint stroke motion is repeated over the same area. The slide is rapidly fixed either by immersion or spray and the device is then discarded.

Using the Broom-Type detachable head device insert the Rovers Cervex-Brush into the endocervical canal. Rotate brush five times in a clock wise direction. Drop the detachable head of the device into the BD SurePath vial (see picture below). Place the cap on the vial and tighten.

Detach head of broom into into vial:

If using the combination of the Pap Perfect plastic spatula and endocervix Cytobrush Plus GT, first insert the contoured end of the plastic spatula to the exocervix surface and rotate 360 degrees around the entire exocervix. Then snap the device handle at the red scoring line and drop the detachable head of the device into the BD SurePath Vial (see picture below). Place cap on vial but do not tighten cap until the cytobrush sample is collected. Then insert the cytobrush into the endocervix until only the bottom-most bristles are exposed at the os. Slowly rotate ¼ to ½ turn in one direction. To reduce unnecessary bleeding, do not over-rotate brush. Then snap the device handle at the red scoring line and drop the detachable head of the device into the BD SurePath Vial (see picture below). Now place the cap on the vial and tighten.

There are alternative methods to detach the heads of the spatula and cytobrush. One is a two-hand snap (see pictures below).

The other is a Cap-Assisted snap (see pictures below).

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/mailer 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 reflex on ASCUS

Please note: HPV testing includes high-risk HPV and 16/18 genotyping (if HR-HPV positive).

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
SPECIMEN LABELING

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

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
ThinPrep Pap Test Quick Reference Guide

Endocervical Brush/Spatula Protocol

Obtain:

- Adequate sampling from the ectocervix using a plastic spatula
- The use of lubricants is not recommended during Pap testing[1]

Rinse:

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

Obtain:

- 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 1/4 or 1/2 turn in one direction. DO NOT OVER-ROTATE.

Rinse:

- 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:

- Patient's name and ID number on the vial
- Patient information and medical history on the cytology requisition form

Place:

- Vial and requisition in a specimen bag for transport to the laboratory

REFERENCES:

www.thinprep.com
1. Papanicolaou Technique Approved Guidelines (NCCLS Document GP15-A) Part No. 85217-001 Rev. H ©2007, Cytyc Corporation

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
ThinPrep Pap Test Quick Reference Guide

Broom-Like Device Protocol

Obtain:

- Adequate sampling from the cervix using a broom-like device
- The use of lubricants is not recommended during Pap testing[1].
- 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:

- 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:

- Cap so that the torque line on the cap passes the torque line on the vial

Record:

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

Place

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

Reference: www.thinprep.com

TEST DIRECTORY
GYNECOLOGIC CYTOLOGY:
BD SurePath Pap Test Quick Reference Guide

STEP 1: COLLECT

- Collect sample with broom-like device or combination of cytobrush and plastic spatula

STEP 2: DROP

- Drop the detachable head device(s) into the BD SurePath vial

STEP 3: SEND

- Place the cap on the vial and tighten. Send the BD SurePath vial to TOPA diagnostics for processing

TEST DIRECTORY
MOLECULAR DIAGNOSTICS:
AFFIRM VPIII MICROBIAL IDENTIFICATION TEST
FOR VAGINITIS/VAGINOSIS

Option 1 (Affirm ATTS transport system):

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.
   - Using the sterile polyester-tip swab, obtain a sample from the posterior vaginal fornix. Twist or roll the swab against the vaginal wall 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.

Option 2 (UTM swab):

- Insert swab into vagina.
- Rotate swab along the vaginal wall for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

<u>Storage Instructions:</u>
Store refrigerated or at room temperature (2-30^{o}C) for up to 3 days for ATTS transport system, or 7 days for UTM samples.

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.

Ordering Options:

- Co-testing (HPV testing in conjunction with cervicvaginal cytology)
- Reflex testing based on cytologic findings (eg. for a diagnosis of ASCUS)
- Primary HPV screening (HPV with reflex to cytology for positive results)

This assay is designed to detect HPV type16, HPV type18, 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 published guidelines. Reference: J Low Genit Tract Dis 2020;24: 102–131.

Cervical specimens for HPV testing must be collected in ThinPrep® PreservCyt® or SurePath Solution. Anal samples must be collected in ThinPrep® PreservCyt solution.

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

Liquid-based Cytology Specimen:

Collection Instructions:

Collect endocervical sample and place in PreservCyt or SurePath media according to instructions for routine pap smears.

Interpretive Comments:

Nucleic acid amplification testing is performed, using the Roche Cobas PCR assay. A negative result does not exclude the possibility of infection, since results are dependent on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.

If the results are positive for Chlamydia trachomatis or Neisseria gonorrheae, please note that a positive result indicates the presence of Chlamydia trachomatis or Neisseria gonorrheae DNA. Viability and/or infectivity cannot be inferred, since target DNA may persist in the absence of viable organisms.

Endocervical Swab Specimen in UTM:

Acceptable Samples:
Vaginal or cervical sample in UTM (universal transport media).
(Note: For samples collected in ThinPrep or SurePath medium, see separate instructions for liquid-based cytology specimens.)

Collection Instructions:

1. Remove excess mucus from the cervix with a separate swab. Discard the swab.
2. Insert the collection swab into the endocervical canal.
3. Rotate the swab clockwise for 10-30 seconds in the endocervical canal. Withdraw the swab carefully.
4. Immediately place the specimen collection swab into the UTM tube.
5. Identifying the scoreline, break the swab shaft against the side of the tube. Discard the top portion of the swab shaft.
6. Leave the swab tip in the UTM tube.
7. Re-cap the UTM tube and tighten the cap securely. Invert the tube 3-4 times. Avoid foaming.
8. Label the UTM tube with the sample identification information, including date of the collection, as required.
9. Samples in Transport Reagent tubes are stable for up to 4 weeks at 2-30^{o}C.

Storage Instructions:
Store refrigerated or at room temperature (2-30^{o}C) for up to 4 weeks.

Methodology:
Nucleic acid amplification testing is performed using the Roche Cobas CT/NG PCR assay.

Interpretive Comments:
A positive result indicates the presence of nucleic acid from the target organism. Detection of viral nucleic acid does not imply that the corresponding organisms are viable, or are the causative agents for clinical symptoms.

A negative result indicates that nucleic acid from the target organism was not detected. False negative results may occur due to interfering substances, antimicrobial therapy, improper specimen collection or handling, or a concentration of organisms below the limit of detection.

Patient-Collected Vaginal Swab Specimen:

Collection Instructions:

1. Open the individual collection package that contains the pink-capped Xpert® Swab Transport Reagent tube and individually wrapped collection swab. Set the tube aside before beginning to collect sample. Discard the larger swab.
2. Open the collection swab wrapper by peeling open the top of the wrapper. Remove the swab, taking care not to touch the tip or lay it down.
3. Hold the swab in your hand, placing your thumb and forefinger in the middle of the swab shaft across the scoreline.
4. Carefully insert the swab into your vagina about two inches inside the opening of the vagina.
5. Gently rotate the swab for 10 – 30 seconds. Ensure the swab touches the walls of the vagina so that moisture is absorbed by the swab. Withdraw the swab and continue to hold it in your hand.
6. Unscrew the cap from the transport tube. Immediately place the collection swab into the transport tube.
7. Identifying the scoreline, break the swab shaft against the side of the tube. Discard the top portion of the swab shaft. Avoid splashing contents on the skin. Wash with soap and water if exposed.
8. Re-cap the transport tube and tighten the cap securely. Invert the tube 3-4 times. Avoid foaming.
9. Label the transport tube with the sample identification information, including date of the collection, as required.
10. Samples in Transport Reagent tubes are stable for up to 4 weeks at 2-30^{o}C.

Interpretive Comments:
Nucleic acid amplification testing is performed using the Roche Cobas CT/NG PCR assay.

A positive result indicates the presence of nucleic acid from the target organism. Detection of viral nucleic acid does not imply that the corresponding organisms are viable, or are the causative agents for clinical symptoms.

A negative result indicates that nucleic acid from the target organism was not detected. False negative results may occur due to interfering substances, antimicrobial therapy, improper specimen collection or handling, or a concentration of organisms below the limit of detection.

Urine Specimens

Collection Instructions:

Unpreserved (Neat) Urine:

1. The patient should not have urinated for at least 1h prior to specimen collection.
2. The patient should collect the first 10-50 mL of voided urine (not midstream) into a sterile, preservative-free specimen collection cup.
3. Cap and label the urine collection cup with patient identification and date/time collected.
4. Unpreserved samples may be stored for up to 24 hours at 2-30^{o}C (room temperature or refrigerated).
5. 5. Transport samples at 2-30^{o}C.

Urine in Transport Media:
Follow instructions in package insert for Cobas PCR Urine Sample Kit for collection and storage.

Interpretive Comments:
Nucleic acid amplification testing is performed using the Roche Cobas CT/NG PCR assay.

A positive result indicates the presence of nucleic acid from the target organism. Detection of viral nucleic acid does not imply that the corresponding organisms are viable, or are the causative agents for clinical symptoms.

A negative result indicates that nucleic acid from the target organism was not detected. False negative results may occur due to interfering substances, antimicrobial therapy, improper specimen collection or handling, or a concentration of organisms below the limit of detection.

Acceptable Specimen:
Preferred Sample Types:

- Cutaneous and mucocutaneous lesion swab samples should be placed in universal transport media (UTM).

Undesirable samples:

- Swab samples in viral transport media with protein stabilizers.
- Calcium alginate swab samples.

Collection Instructions:
Place swab(s) in transport medium. Samples should be stored refrigerated (2-8 C) after collection and during transportation to the laboratory. Samples should be tested as soon as possible, but may be stored refrigerated (2-8^{o} C) up to 7 days prior to testing. Do not freeze samples. Do not store at room temperature.

Interpretive Comments:
Testing is performed using the Illumigene loop-mediated isothermal DNA amplification (LAMP) method. This assay does not distinguish between viable and non-viable organisms. Positive results do not rule out co-infection with other organisms.

False negative results may occur due to improper specimen collection or handling, mutations or polymorphisms in target regions, a concentration below the limit of detection, or interfering substances. This assay is intended to aid in the diagnosis of HSV infection in symptomatic patients. Latent infection is not detectable, since HSV DNA can only be detected in the setting of viral shedding.

Acceptable Samples:
Vaginal or cervical sample in UTM (universal transport media).
PreservCyt (ThinPrep), or SurePath medium.
Urine (unpreserved).

Collection Instructions:
Vaginal sample:

- Insert swab into vagina.
- Rotate swab along the vaginal wall for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

Endocervical sample:

- Remove excess mucus from the cervix with a separate swab, and discard.
- Insert specimen collection swab into endocervical canal.
- Rotate swab for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

ThinPrep/ SurePath samples:

- Collect sample following routine pap smear collection procedures.

Urine (unpreserved):

- The patient should not have urinated for at least 1h prior to specimen collection.
- Patient should not cleanse the genital area prior to collecting specimen.
- The patient should collect the first 15-60 mL of voided urine (not midstream) into a sterile, preservative-free specimen collection cup.
- Cap and label the urine collection cup with patient identification and date/time collected.

Storage Instructions:

UTM sample:

Store refrigerated or at room temperature (2-30^{o}C) for up to 7 days.

ThinPrep/ SurePath samples:

Store at room temperature (15-30^{o}C) for up to 14 days.

Urine:

Store refrigerated (2-8^{o}C) for up to 4 days
Or at room temperature (15-30^{o}C) for up to 4 hours

Methodology:

This test was developed and the performance characteristics determined by TOPA Diagnostics. This test has not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.

Reference Ranges:

- Trichomonas vaginalis: Not detected

Interpretive comments:

- This test is intended to aid in the diagnosis of *Trichomonas vaginalis* infection in symptomatic or asymptomatic patients.
- False negative results may occur due to improper specimen collection or handling, mutations or nucleotide polymorphisms in primer or probe binding regions, or because the number of organisms in the sample is below the limit of detection of the test.

Acceptable Samples:

- Throat swabs
- Acceptable media: eSwab

Unacceptable Samples:

- Collection/transport systems other than those specified above.
- Samples collected with calcium alginate swabs.

Sample Collection:

- Swab the posterior pharynx, tonsils and other inflamed areas following standard clinical methods.

Storage:

- Samples should be stored between 2 C and 25 C for up to 72 hours.

Methodology:

- Real-time polymerase chain reaction (PCR)

Interpretive Comments:

- This assay is a qualitative real-time PCR assay for the detection of *Streptococcus pyogenes* nucleic acids from throat swab specimens obtained from patients with signs and symptoms of pharyngitis.
- A Positive Test indicates that Group A Streptococcus *(Streptococcus pyogenes*) was detected by nucleic acid amplification testing.
- This assay does not distinguish between viable and non-viable organisms, and does not differentiate between carriers and infected individuals.
- A Negative Test indicates that Group A Streptococcus (*Streptococcus pyogenes*) was not detected by nucleic acid amplification testing.
- False negative results may occur due to antimicrobial therapy, improper specimen collection or handling, a bacterial concentration below the limit of detection, or interfering substances.
- Culture is recommended if the result is negative and clinical symptoms persist, or in the event of an acute rheumatic fever (ARF) outbreak.

Sample Collection: Nasopharyngeal swab specimen collection should be performed in accordance with standard procedures for collection of clinical specimens for *Bordetella pertussis* infection.

California Department of Public Health guidelines (February 2011) for collection of nasophayngeal swab specimens:
Procedure:
1. Put on mask and clean gloves.
2. Have patient sit with head against a wall as patients have a tendency to pull away during this procedure.
3. Insert swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met). The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. Do not force swab, if obstruction is encountered before reaching the nasopharynx, remove swab and try the other side.
4. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.
5. Remove swab and immediately place in transport media.

Acceptable Sample Types: Nasopharyngeal swabs.

Acceptable Swab Types: Polyester, Flocked Nylon or Rayon
Unacceptable Swab Types: Calcium alginate swabs.

Acceptable Media: UTM or eSwab.

Sample Storage/Handling Prior To Testing:
Store refrigerated (2-8^{o} C) for up to 7 days.

Methodology:
Real-time PCR.

Reference Ranges:
For each target, the expected result is “Not Detected”.

Interpretive comments:

- The DiaSorin Simplexa Bordetella Direct assay is intended for the qualitative detection and differentiation of Bordetella pertussis and Bordetella parapertussis nucleic acids from nasopharyngeal (NPS) specimens from patients with signs and symptoms of Bordetella infection of the respiratory tract.
- This assay utilizes real-time PCR amplification to detect B. pertussis by targeting the IS481 insertional element, and to detect B. parapertussis by targeting the IS1001 insertional element.
- The IS481 insertional element can also be present in B. holmesii and B. bronchiseptica. Positive results may occur with respiratory infection caused by B. pertussis, B. holmesii or B. bronchiseptica
- Negative results for the Bordetella assay do not preclude Bordetella infection, and positive results do not rule out co-infection with other respiratory pathogens.
- False negative results may occur due to sequence variants in the target genes, improper specimen collection or handling, amplification inhibitors, or a concentration of organisms below the limit of detection.

Influenza or Influenza/RSV (nucleic acid amplification testing)

Samples should be collected and stored using the Cepheid Universal Transport Medium (UTM) System.

- Collect nasopharyngeal swab specimen using the Cepheid swab:
  Procedure:
  1. Put on mask and clean gloves.
  2. Have patient sit with head against a wall as patients have a tendency to pull away during this procedure.
  3. Insert swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met). The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. Do not force swab, if obstruction is encountered before reaching the nasopharynx, remove swab and try the other side.
  4. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.
- Remove swab and aseptically remove cap from tube.
- Insert swab into the UTM tube.
- Break swab shaft by bending it against the tube wall.
- Replace cap on the transport tube and tighten securely.
- Label with appropriate patient information.

Storage and Transportation

Samples can be stored at room temperature for up to 24 hours,
or refrigerated for up to 7 days.
Samples should be transported at 2-8^{o}C.

Acceptable Specimen: Nasopharyngeal Swab

Collection Instructions:
Collect nasopharyngeal swab specimen using standard technique:

1. Put on mask and clean gloves.
2. Have patient sit with head against a wall as patients have a tendency to pull away during this procedure.
3. Insert swab into one nostril straight back (not upwards) and continue along the floor of the nasal passage for several centimeters until reaching the nasopharynx (resistance will be met). The distance from the nose to the ear gives an estimate of the distance the swab should be inserted. Do not force swab, if obstruction is encountered before reaching the nasopharynx, remove swab and try the other side.
4. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells.
5. Immediately place swab specimen in viral transport media (VTM) or ESwab (liquid Amies medium).

Specimen Storage Requirements: Specimens in VTM can be held at room temperature (15-25 ^{o}C) for up to 4 hours, at refrigerator temperature (2-8 ^{o}C) for up to 3 days, or at freezer temperature (< -15 ^{o}C) for up to 30 days.

Methodology:
The FilmArray Respiratory panel is a multiplex nucleic acid amplification test for 21 respiratory pathogens:

- Adenovirus
- Coronavirus (229E, HKU1, NL63, and OC43)
- Human rhinovirus/enterovirus
- Human metapneumovirus
- Influenza A (and subtypes H1, H1-2009, and H3)
- Influenza B
- Parainfluenza virus (types 1-4)
- Respiratory syncytial virus
- Bordetella parapertussis
- Bordetella pertussis
- Chlamydophila pneumonia
- Mycoplasma pneumonia
- SARS-CoV-2

Interpretive Comments:

- Nucleic acid may persist independently of organism viability. Therefore, detection of target organisms does not imply that the organisms are infectious or are the causative agents for clinical symptoms.
- Positive results do not rule out co-infection with other organisms.
- Negative results may occur due to infection with organisms not included in the FilmArray panel or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.
- False negative results may occur due to sequence variants in the target genes, procedural errors, amplification inhibitors, antiviral/antibacterial therapy, or a concentration of organisms below the limit of detection.
- Due to the genetic similarity between Rhinovirus and Enterovirus, the FilmArray assay cannot reliably differentiate them.
- This assay includes two SARS-CoV-2 targets: Spike protein (S) and Membrane protein (M) genes.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

TEST DIRECTORY
MOLECULAR MICROBIOLOGY:
GI Pathogen PCR Panel

Acceptable Specimen: Stool specimen

Collection Instructions:

- Stool specimens should be collected in Cary Blair transport media.
- 200 µL of sample is required for testing.
- **Instructions for Patients are included in the specimen collection kits.**

Storage Requirements:

- Stool specimens in Cary Blair should be processed and tested as soon as possible, although they may be stored at room temperature or under refrigeration for up to four days

Indications for Testing:

For patients with acute infectious diarrhea, indications for molecular testing include:

- Moderate-to-severe disease
- Travel-related diarrhea
- Dysentery
- Symptoms lasting > 7 days
- Situations in which the individual patient is at risk of spreading disease to others (eg. workers who handle food, health-care workers, daycare workers, and residents of institutional facilities, and during known or suspected outbreaks.

Interpretive Comments:

- Nucleic acid may persist independently of organism viability, and some organisms may be carried asymptomatically. Therefore detection of target organisms does not imply that the organisms are infectious or are the causative agents for clinical symptoms.
- Positive results do not rule out co-infection with organisms not included in the FilmArray panel.
- Negative results may occur in the setting of infection with organisms not included in the FilmArray panel or non-infectious etiologies. False negative results may occur due to sequence variants in the target genes, procedural errors, amplification inhibitors, or a concentration of organisms below the limit of detection.
- Diarrheagenic E. coli may contain more than one pathogenic genetic determinant; therefore a single strain may produce positive results with more than one assay (eg. STEC and EAEC).

Methodology:
The FilmArray GI panel is a multiplex nucleic acid amplification test for 22 gastrointestinal pathogens:

- Bacteria
  - Campylobacter
  - Clostridium difficile toxin A/B
  - Plesiomonas shigelloides
  - Salmonella
  - Vibrio species
  - Vibrio cholera
  - Yersinia enterocolitica
  - Enteroaggregative E. coli (EAEC)
  - Enterotoxigenic E. coli (ETEC)
  - Enteropathogenic E. coli (EPEC)
  - Shiga-like toxin producing E. coli (STEC)
  - E. coli 0157
  - Shigella/Enteroinvasive E. coli (EIEC)
- Parasites
  - Cryptosporidium
  - Cyclospora cayetanensis
  - Entamoeba histolytica
  - Giardia lamblia
- Viruses
  - Adenovirus F40/41
  - Astrovirus
  - Norovirus GI/GII
  - Rotavirus A
  - Sapovirus (genogroups I, II, IV, V)

Note: If a sample is PCR-positive for C. difficile, reflex testing for C. difficile GDH antigen and toxin will be performed by enzyme immunoassay.

Acceptable Specimen: Stool Specimen

Testing Options:

- Enzyme Immunoassay for GDH antigen and Toxin A/B.
- Nucleic acid amplification (PCR).
  Note: If PCR is positive for toxigenic C. difficile, reflex testing for GDH antigen and Toxin A/B will be performed.

Collection Instructions:

- Stool specimens should be collected in a clean container.
- **Instructions for Patients are included in the specimen collection kits.**

Storage Requirements:
For PCR Testing:

- Store specimen at 2–25°C.
- The specimen is stable for up to 48 hours when stored at 2–25 °C.

For EIA Testing:

- Store refrigerated or at room temperature (2-30ºC) for up to 5 days.

Comments:

- The DiaSorin Simplexa C.difficile Direct assay is intended for the detection of C. difficile nucleic acids present in liquid or unformed stool samples from individuals suspected of *C. difficile* infection.
- This assay utilizes real-time PCR amplification to detect Clostridium difficile toxin B gene (tcdB).
- C. difficile infection is generally defined as symptomatic disease associated with the presence of toxigenic C. difficile. Asymptomatic carriage may also occur.
- This test is intended for use with liquid or unformed human stool specimens. Performance characteristics of other specimen types have not been established.
- Negative results do not rule out C. difficile infections and should not be used as the sole basis for treatment or other patient management decisions.
- False negative results may occur due to sequence variants in the target genes, improper specimen collection or handling, amplification inhibitors, or a concentration of organisms below the limit of detection.

The PCR assay in use at TOPA is highly sensitive and specific for the presence of C. difficile organisms, but does not distinguish between colonization and active infection. The addition of GDH/Toxin testing, as a supplement to PCR testing, is intended to aid in the distinction between colonization and active infection, and to assist in optimum patient management and the avoidance of inappropriate treatment of colonized patients.

If C. difficile is detected by PCR testing, GDH/Toxin testing by EIA will be automatically performed as a reflex test. GDH/Toxin EIA may also be ordered as a stand-alone test. Interpretation is based on the combination of results, as follows:

For patients with a positive PCR test for C. difficile:

- A positive toxin result (regardless of GDH result) indicates that C. difficile toxin is detected; results are consistent with active C. difficile infection.
- A negative toxin result (regardless of GDH result) indicates that C. difficile toxin is not detected. These results would not exclude active infection, but are most suggestive of asymptomatic colonization.

If PCR testing is not performed:

- Positive results for both GDH and Toxin indicate that C. difficile toxin is detected; results are consistent with active C. difficile infection.
- Positive results for GDH alone indicate that C. difficile toxin is not detected. These results would not exclude active infection, but are most suggestive of asymptomatic colonization.
- Negative results for both GDH and Toxin indicate that C. difficile is not detected.
- Positive results for Toxin alone are considered indeterminate; repeat testing of a new sample is recommended.

TEST DIRECTORY
Molecular Testing:
Bacterial Vaginosis Panel

Acceptable Specimen:

- Vaginal or cervical sample in UTM (universal transport media), PreservCyt (ThinPrep medium), or SurePath medium

Collection Instructions:

Vaginal sample:

- Insert swab into vagina.
- Rotate swab along the vaginal wall for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

Endocervical sample:

- Remove excess mucus from the cervix with a separate swab, and discard.
- Insert specimen collection swab into endocervical canal.
- Rotate swab for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

ThinPrep/ SurePath samples:

- Collect sample following routine pap smear collection procedures.

Storage Requirements:

UTM sample:

Store refrigerated or at room temperature (2-30^{o}C) for up to 7 days.

ThinPrep/ SurePath samples:

Store at room temperature (15-30^{o}C) for up to 14 days.

Methodology:

The Bacterial Vaginosis panel consists of nucleic acid amplification testing for 4 target bacteria:

- Gardnerella vaginalis
- Atopobium vaginae
- Megasphaera-1
- BVAB-2

These tests were developed and the performance characteristics determined by TOPA Diagnostics. These tests have not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.

Reference Ranges:

- Gardnerella vaginalis: Not detected
- Atopobium vaginae: Not detected
- Megasphaera-1: Not detected
- BVAB-2: Not detected

Interpretive comments:

- Bacterial Vaginosis (BV) is a clinical syndrome associated with an alteration in the vaginal flora, and is typically associated with characteristic clinical findings.
- Altered vaginal flora may be found in asymptomatic patients, as well as in patients with clinical findings of BV, so laboratory findings should be correlated with clinical findings.
- Molecular characterization of the vaginal flora may support a diagnosis of bacterial vaginosis and help to distinguish this diagnosis from other causes of vaginal discharge.
- Erroneous results may occur from improper specimen collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test.
- A positive result does not imply that the organisms are viable, or are causative of the patient's symptoms.
- Mutations in primer/probe binding regions may affect detection using this assay.
- Results from this assay should be used as an adjunct to clinical observations and other information available to the physician.
- Interpretive guidelines:
    - 2 or more organisms detected: Positive (supportive of bacterial vaginosis).
    - 1 organism detected: Equivocal for bacterial vaginosis.
    - No organisms detected: Negative for bacterial vaginosis.

Acceptable Specimen:

- Vaginal or cervical sample in UTM (universal transport media), PreservCyt (ThinPrep medium), or SurePath medium.

Collection Instructions:

Vaginal sample:

- Insert swab into vagina.
- Rotate swab along the vaginal wall for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

Endocervical sample:

- Remove excess mucus from the cervix with a separate swab, and discard.
- Insert specimen collection swab into endocervical canal.
- Rotate swab for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

ThinPrep/ SurePath samples:

- Collect sample following routine pap smear collection procedures.

Storage Instructions:

UTM sample:

Store refrigerated or at room temperature (2-30^{o}C) for up to 7 days.

ThinPrep/ SurePath samples:

Store at room temperature (15-30^{o}C) for up to 14 days.

Methodology:

The Candida panel consists of nucleic acid amplification testing for 3 target species of Candida:

- C. albicans
- C. glabrata
- C. krusei

These tests were developed and the performance characteristics determined by TOPA Diagnostics. These tests have not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.

Reference Ranges:

- C. albicans: Not detected
- C. glabrata: Not detected
- C. krusei: Not detected

Interpretive comments:

- Vulvovaginal Candidasis is a common cause of vaginitis.
- Approximately 10-20% of women with vulvovaginal Candidasis have complicated disease, defined by one or more of the following:
    - Recurrent disease
    - Severe disease
    - Non-albicans species
    - Underlying comorbidity, such as diabetes, debilitation, or immunosuppression.
- Historically, approximately 90% of cases of vulvovaginal Candidasis have been caused by C. albicans, but the proportion of non-albicans Candida has increased, possibly related to use of OTC anti-fungal drugs.
- The proportion of non-albicans Candida is also increased in cases of recurrent vulvovaginal Candidasis.
- Identification of non-albicans species may have clinical significance, due to differences in antimicrobial susceptibility.
- Candida species may be identified in approximately 10-20% of asymptomatic patients, so laboratory findings should be correlated with clinical findings.
- Erroneous results may occur from improper specimen collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test.
- A positive result does not imply that the organisms are viable, or are causative of the patient's symptoms.
- Mutations in primer/probe binding regions may affect detection using this assay.
- Results from this assay should be used as an adjunct to clinical observations and other information available to the physician.

TEST DIRECTORY
Molecular Testing:
Mycoplasma/ Ureaplasma Panel

Acceptable Specimen:

- Vaginal or cervical sample in UTM (universal transport media), PreservCyt (ThinPrep medium), or SurePath medium.
- Urine (unpreserved).

Collection Instructions:

Vaginal sample:

- Insert swab into vagina.
- Rotate swab along the vaginal wall for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

Endocervical sample:

- Remove excess mucus from the cervix with a separate swab, and discard.
- Insert specimen collection swab into endocervical canal.
- Rotate swab for 10-30 seconds; then remove the swab.
- Place the swab in the UTM collection tube, and break the swab shaft (at the score line) against the side of the tube.
- Leave the swab tip in the collection tube, and submit for testing.

ThinPrep/ SurePath samples:

- Collect sample following routine pap smear collection procedures.

Urine (unpreserved):

- The patient should not have urinated for at least 1h prior to specimen collection.
- Patient should not cleanse the genital area prior to collecting specimen.
- The patient should collect the first 15-60 mL of voided urine (not midstream) into a sterile, preservative-free specimen collection cup.
- Cap and label the urine collection cup with patient identification and date/time collected.

Storage Requirements:

UTM sample:

Store refrigerated or at room temperature (2-30^{o}C) for up to 7 days.

ThinPrep/ SurePath samples:

Store at room temperature (15-30^{o}C) for up to 14 days.

Urine:

Store refrigerated (2-8^{o}C) for up to 4 days
Or at room temperature (15-30^{o}C) for up to 4 hours

Methodology:
The Mycoplasma/ Ureaplasma Panel consists of nucleic acid amplification testing for 4 target organisms:

- Mycoplasma hominis
- Mycoplasma genitalium
- Ureaplasma urealyticum
- Ureaplasma parvum

These tests were developed and the performance characteristics determined by TOPA Diagnostics. These tests have not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.

Reference Ranges:

- Mycoplasma hominis: Not detected
- Mycoplasma genitalium: Not detected
- Ureaplasma urealyticum: Not detected
- Ureaplasma parvum: Not detected

Interpretive comments:

- Mycoplasma and Ureaplasma species have been associated with female genital tract infection, but may also be found in asymptomatic women.
- M. genitalium has been associated with cervicitis, endometritis, and salpingitis.
- M. hominis and U. urealyticum have been associated with salpingitis, bacterial vaginosis, and pre-term birth.
- U. parvum is commonly found in symptomatic and asymptomatic women, and may also be associated with pre-term birth.
- Erroneous results may occur from improper specimen collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the specimen is below the analytical sensitivity of the test.
- A positive result does not imply that the organisms are viable, or are causative of the patient's symptoms.
- Mutations in primer/probe binding regions may affect detection using this assay.
- Results from this assay should be used as an adjunct to clinical observations and other information available to the physician.

TEST DIRECTORY
Molecular Testing:
Group B Streptococcus (GBS)

TOPA DIAGNOSTICS

Acceptable Specimen:

- GBS colonization status should be determined by collecting both vaginal and rectal specimens at 35-37 weeks' gestation
- A single combined vaginal-rectal specimen can be collected

Collection Instructions:

CDC Guidelines (2010) specify the following:

- Swab the lower vagina (vaginal introitus), followed by the rectum (i.e., insert swab through the anal sphincter) using the same swab or two different swabs. Cervical, perianal, perirectal or perineal specimens are not acceptable, and a speculum should not be used for sample collection.
- Place the swab(s) into a nonnutritive transport medium (e.g., Stuart's or Amies with or without charcoal). GBS isolates can remain viable in transport media for several days at room temperature; however the recovery of isolates declines over one to four days, especially at elevated temperatures, which can lead to false-negative results. When feasible, specimens should be refrigerated before processing.
- Specimen requisitions should indicate clearly that specimens are for group B streptococcal testing. For patients who are allergic to penicillin and are determined to be at high risk for anaphylaxis, susceptibility testing should be ordered.

Storage Requirements:

- Store at room temperature for up to 24 hours, or refrigerated for up to 4 days.

Methodology:

- Nucleic acid amplification (PCR method, following 18-24 hours incubation in Lim broth).

Interpretive comments:

- Vaginal or rectal colonization with GBS is found in 10-30% of pregnant women.
- Screening for GBS is recommended at 35-37 weeks gestation
- According to 2010 CDC guidelines:
    - For non-allergic patients, penicillin is the drug of choice for intra-partum prophylaxis.
    - Antimicrobial susceptibility testing should be performed in penicillin-allergic patients at high risk for anaphylaxis.

    - If intrinsic clindamycin resistance or inducible clindamycin resistance is identified, penicillin-allergic patients at high risk for anaphylaxis should be treated with vancomycin.
- Erroneous results may occur from improper specimen collection and handling, mutation in the target gene, or because the number of organisms in the specimen is below the analytical sensitivity of the test.
- A positive test result does not necessarily indicate the presence of viable organisms. It is, however, presumptive for the presence of Group B *Streptococcus* DNA.

TOPA DIAGNOSTICS

TOPA Molecular Quick Reference

Target Organism	Specimen Source	Collection Media	Storage
Bordetella PCR Panel	Nasopharyngeal swab	viral transport media (VTM) or e-Swab	Refrigerated – 7 days Room Temp – 5 days
Group A Streptococcus	Throat swab	e-Swab	Room Temp or Refrigerated 3 days
Group B Streptococcus	Vaginal and Rectal sample swab	liquid Stuart's or liquid Amies with or without charcoal	Refrigerated – 4 days Room Temp – 24 hours
Influenza / RSV	Nasopharyngeal swab	Viral transport media	Refrigerated – 7 days Room Temp – 24 hours
FilmArray Respiratory panel	Nasopharyngeal swab	viral transport media (VTM)	Refrigerated – 3 days Room Temp – 4 hours
COVID-19 (SARS-CoV-2)	Nasopharyngeal swab	viral transport media (VTM)	Refrigerated – 5 days Room Temp – 2 days
Chlamydia/Gonorrhea	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated 4 weeks
		SurePath vial	Room Temp or Refrigerated 2 weeks
		ApexDx UTM	Room Temp or Refrigerated 4 weeks
	Throat	ApexDx UTM	Room Temp or Refrigerated 7 days
	Rectal/Anal	ApexDx UTM	Room Temp or Refrigerated 7 days
	Urine	Unpreserved	Refrigerated – 8 days Room Temp – 24 hours
Bacterial vaginosis panel Candida panel	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated 7 days
		SurePath vial	
		ApexDx UTM	
Mycoplasma/ Ureaplasma Panel	Vaginal or endocervical swab	Thinprep Vial	Room Temp or Refrigerated 7 days
		Surepath Vial	
		ApexDx UTM	
	Urine	Unpreserved	Refrigerated – 4 days Room Temp – 4 hours
Trichomonas	Vaginal or endocervical swab	Thinprep Vial	Room Temp or Refrigerated 7 days
		Surepath Vial	
		ApexDx UTM	
	Urine	Unpreserved	Refrigerated – 4 days Room Temp – 4 hours
Affirm Vaginosis/ vaginitis panel	Vaginal swab	ApexDx UTM	Room Temp – 7 days
HSV 1,2	Cutaneous and mucocutaneous lesion swab samples	ApexDx UTM	Refrigerated – 7 days
HPV	Cervico-vaginal pap sample	ThinPrep vial	Room Temp or Refrigerated 6 weeks
		SurePath vial	Room Temp or Refrigerated 2 weeks
	Anal	ThinPrep vial/ SurePath vial	Room Temp or Refrigerated 6 weeks
	Oral	Scope and sterile cup	Room Temp – 7 days
FilmArray GI panel (22 targets)	Stool sample	Cary Blair transport media	Room Temp or Refrigerated 7 days
C. difficile PCR	Stool sample	Clean container without preservative	Room Temp or Refrigerated 2 days
C. diff GDH and Toxin EIA	Stool sample	Cary Blair or clean container without preservative	Room Temp or Refrigerated 5 days
Pneumonia Panel	Sputum sample	Sterile container	Refrigerated – 24 hours
Nail Panel	Nail Clippings	Sterile container or Nail Bag	Room Temp – 14 days

Nasopharyngeal Samples:

Viral Transport Media ***(VTM)***
Nasopharyngeal

Test for:
Bordetella Panel
Respiratory Panel
Influenza / RSV
COVID-19

Mouth/Throat Samples:

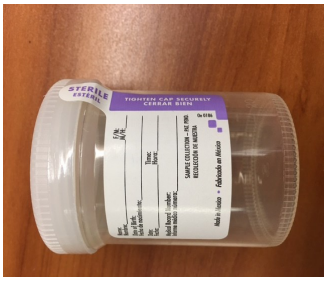
Group A Strep
Throat

Test for:
Group A. Strep

Scope mouthwash
Sterile container
Oral rinse

Test for:
HPV

Urine:



Sterile/Non-Sterile Cup
Urine
Test for:
Trichomonas
CT/GC
Mycoplasma/Ureaplasma

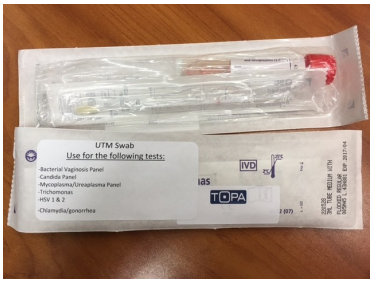
Gynecological Samples:

ThinPrep
SurePath
Cevico-vaginal sample
Anal sample

Test for:
HPV
Chlamydia
Gonorrhea
Trichomonas

Affirm
Vaginal

Test for
Affirm
Vaginosis/Vaginitis
Panel



ApexDx UTM

Test for:
Bacterial Vaginosis Panel
Candida Panel
Mycoplasma/Ureaplasma Panel
Trichomonas
HSV 1 & 2
Chlamydia/Gonorrhea
Affirm Vaginosis/Vaginitis Panel

Stool Samples:

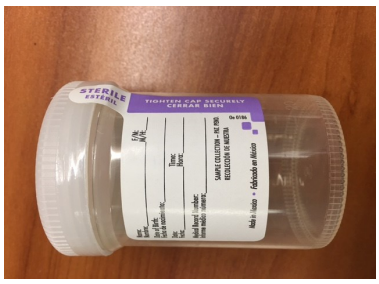
Cary Blair
Stool

Test for:
GI Panel
C. diff GDH/toxin EIA

Clean Container
Stool

Test for:
C. Difficile PCR
C. diff GDH/toxin EIA

Sputum:



Sterile Cup
Sputum
Test for:
Pneumonia Panel

Refer to our website for complete collection instructions:

[Test Directory – TOPA Diagnostics (topathology.com)]

Gynecological Molecular Testing Quick Reference

TOPA DIAGNOSTICS
Phone: (805) 373-8582

Target Organism	Specimen Source	Collection Media	Storage
Chlamydia/gonorrhea	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated – 4 weeks
		SurePath vial	Room Temp or Refrigerated – 2 weeks
		ApexDx UTM	Room Temp or Refrigerated – 4 weeks
	Urine	Unpreserved	Refrigerated – 8 days Room Temp – 24 hours
		Xpert collection kit	Refrigerated – 45 days Room Temp – 3 days
Bacterial vaginosis panel	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated – 7 days
		SurePath vial	
		ApexDx UTM	
Candida panel	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated – 7 days
		SurePath vial	
		ApexDx UTM	
Trichomonas	Vaginal or endocervical swab	**Thinprep Vial**	Room Temp or Refrigerated – 7 days
		Surepath Vial	
		ApexDx UTM	
	Urine	Unpreserved	Refrigerated– 4 days Room Temp – 4 hours
Mycoplasma/Ureaplasma Panel	Vaginal or endocervical swab	ThinPrep vial	Room Temp or Refrigerated – 7 days
		SurePath vial	
		ApexDx UTM	
Affirm Vaginosis / Vaginitis panel	Vaginal swab	Affirm ATTS transport media	Room Temp– 3 days
		ApexDx UTM	Room Temp–7days
HSV 1,2	Cutaneous and mucocutaneous lesion swab samples	ApexDx UTM	Refrigerated– 7 days
HPV	Cervico-vaginal pap sample	ThinPrep vial	Room Temp or Refrigerated – 6 weeks
		SurePath vial	Room Temp or Refrigerated – 2 weeks
Group B Streptococcus	Vaginal and rectal sample swab	Liquid stuart or liquid Amies with or without charcoal	Refrigerated – 4 days Room Temp – 24 days

ThinPrep SurePath
Cevico-vaginal sample

Test for:
HPV
Chlamydia
Gonorrhea
Trichomonas

ApexDx UTM
Test for:
Bacterial Vaginosis Panel
Candida Panel
Mycoplasma/Ureaplasma Panel
Trichomonas
HSV 1 & 2
Chlamydia/Gonorrhea
Affirm Vaginosis/Vaginitis Panel

Sterile/Non-Sterile Cup
Test for:
Trichomonas
Chlamydia
Gonorrhea

Xpert CT/NG
Urine

Test for:
Chlamydia
Gonorrhea

Extragenital Swab Specimens:

Acceptable Samples:
Rectal or Throat sample in UTM (universal transport media).

(Note: For rectal samples collected in ThinPrep medium, see separate instructions for anal cytology specimens.)

Collection Instructions:

Rectal samples:

1. Insert the collection swab approximately 3-5 cm into the rectum.
2. Rotate the swab against the rectal wall; then withdraw the swab carefully.
3. Immediately place the specimen collection swab into the UTM tube.
4. Identifying the scoreline, break the swab shaft against the side of the tube. Discard the top portion of the swab shaft.
5. Leave the swab tip in the UTM tube.
6. Re-cap the UTM tube and tighten the cap securely. Invert the tube 3-4 times. Avoid foaming.
7. Label the UTM tube with the sample identification information, including date of the collection, as required.

Throat samples:

1. Using a sterile swab, swab the posterior pharynx and tonsils.
2. Remove swab and insert into a vial containing 1-3 ml of UTM media.
3. Break the swab handle at scored breakpoint line.
4. Leave the swab tip in the UTM tube.
5. Label the vial with appropriate patient information.

Storage Instructions:
Store refrigerated or at room temperature (2-30^{o}C) for up to 4 weeks.

Methodology:
Nucleic acid amplification testing is performed using nucleic acid amplification (PCR) methodology.

Interpretive Comments:
A positive result indicates the presence of nucleic acid from the target organism. Detection of viral nucleic acid does not imply that the corresponding organisms are viable, or are the causative agents for clinical symptoms.

A negative result indicates that nucleic acid from the target organism was not detected. False negative results may occur due to interfering substances, antimicrobial therapy, improper specimen collection or handling, or a concentration of organisms below the limit of detection.

Methodology:
The pneumonia panel is a multiplex nucleic acid amplification test for the detection of 18 bacteria, 8 viruses, and 7 antimicrobial resistance genes in lower respiratory samples:

Bacterial Targets
- Acinetobacter calcoaceticus-baumannii complex
- Enterobacter cloacae complex
- Escherichia coli
- Haemophilus influenzae
- Klebsiella aerogenes
- Klebsiella oxytoca
- Klebsiella pneumoniae group
- Moraxella catarrhalis
- Proteus spp.
- Pseudomonas aeruginosa
- Serratia marcescens
- Staphylococcus aureus
- Streptococcus agalactiae
- Streptococcus pneumoniae
- Streptococcus pyogenes

Atypical Bacteria
- Chlamydia pneumoniae
- Legionella pneumophila
- Mycoplasma pneumoniae

Viruses
- Adenovirus
- Coronavirus
- Human Metapneumovirus
- Human Rhinovirus/Enterovirus
- Influenza A
- Influenza B
- Parainfluenza Virus
- Respiratory Syncytial Virus

Antimicrobial Resistance Genes:
- CTX-M (ESBL; Extended spectrum beta lactamase)
- IMP (Carbapenem resistance)
- KPC (Carbapenem resistance)
- mecA/C and MREJ (Methicillin resistance)
- NDM (Carbapenem resistance)
- OXA-48-like (Carbapenem resistance)
- VIM (Carbapenem resistance)

Acceptable Specimens:

- Bronchoalveolar lavage (BAL)-like specimens
  - Including BAL and mini-BAL collected according to standard technique
- Sputum-like specimens
  - Including induced and expectorated sputum as well as endotracheal aspirate (ETA) collected according to standard technique

Minimum Sample volume:

- Approximately 0.2 mL (200 µL) of specimen material will be captured by the Sample Swab for transfer into the test.

Storage and Transportation:

- Specimens can be held refrigerated for up to 1 day (2-8 °C)

Collection Instructions:

- Samples should be collected in accordance with routine procedures for collection of sputum and BAL samples for culture.
- Sputum samples should be produced from a deep cough (Note: samples will be evaluated for adequacy, and rejected if excessive oropharyngeal contamination is observed).
- The sample should be collected in a sterile leak-proof container.

Reference Ranges:

- For each target, the expected result is “Not Detected”.

Interpretive comments:

- Nucleic acids may persist regardless of organism viability. Detection of nucleic acid targets does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.
- Negative test results may occur due to sequence variants, amplification inhibitors, or infection by an organism not detected by the panel. Test results may be affected by concurrent antiviral/antibacterial therapy or levels of organism below the limit of detection. Negative results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
- Concomitant culture of specimens is recommended. Culture is needed for recovery of isolates and antimicrobial susceptibility testing, as well as further speciation.
- Due to the genetic similarity between human rhinovirus and enterovirus, the FilmArray Pneumonia Panel cannot reliably differentiate them.
- Bacterial targets are reported quantitatively as copies/mL (which is generally greater than the number of CFU/mL determined by quantitative culture). Quantitation may be useful in evaluating the clinical significance of an organism, but there is no cut-off value that reliably distinguishes colonization from infection.
- The numerical concentrations may be descriptively categorized as:
  - 10^4 copies/ mL = Low
  - 10^5 copies/ mL = Low-Intermediate
  - 10^6 copies/ mL = Intermediate-High
  - ≥ 10^7 copies/ mL = High
- Antimicrobial resistance can occur via multiple mechanisms. A negative result for antimicrobial resistance gene assays does not indicate antibiotic susceptibility.
- mecA/C and MREJ genes are only reported if Staph aureus is detected.
- The other antimicrobial resistance genes are only reported if relevant gram-negative bacteria are detected.

Methodology:
The nail panel consists of 2 multiplex PCR assays for 7 fungal targets, intended to aid in the evaluation of infected nails:

Nail Panel 1	Nail Panel 2
Pan-fungus	C. albicans
Pan-dermatophyte	C. glabrata
T. rubrum	C. krusei
	C. tropicalis/ parapsilosis

Acceptable Specimen:
- Nail sample in sterile container or nail bag

Collection Instructions:
- Clean nail with 70% alcohol
- Collect clippings in sterile container

Storage Requirements:
Store at room temperature for up to 14 days.

Reference Ranges:
- For each target, the expected result is “Not Detected”.

Interpretive comments:
- These tests were developed and the performance characteristics determined by TOPA Diagnostics. These tests have not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.
- This assay has been validated for use with nail samples only.
- Results from this assay should be used as an adjunct to clinical observations and other information available to the physician.
- Nucleic acid may persist independently of organism viability. Therefore detection of target organisms does not imply that the organisms are infectious or are the causative agents for clinical symptoms.
- Positive results do not rule out co-infection with other organisms.
- Negative results may occur due to infection with organisms not included in the panel.
- False negative results may occur due to sequence variants in the target genes, amplification inhibitors, antiviral/antibacterial therapy, or a concentration of organisms below the limit of detection.
- When fungal organisms are detected, this assay does not distinguish between colonization and infection.

Methodology:
Testing is performed using a real-time RT-PCR assay.

Intended Use:
This assay is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and nasal swab samples from individuals suspected of COVID-19 by their healthcare provider.

Acceptable Specimens:
- Nasopharyngeal swab samples
- Nasal swab samples
- Samples must be placed in universal transport media (UTM) or viral transport media (VTM)

Minimum Sample volume:
- Swab sample in 1-3 mL of UTM or VTM.

Storage and Transportation:
- Specimens can be held at Room temperature (2-25ºC) for up to 48 hours; or Refrigerated (2-8ºC) for up to 5 days.

Collection Instructions:
- Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.
- Nasopharyngeal Swab Collection Procedure
  - Insert the swab into either nostril, passing it into the posterior nasopharynx.
  - Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
  - Place the swab into a transport tube. Break swab at the indicated break line and cap the specimen collection tube tightly.
- Nasal Swab Collection Procedure
  - Collect the sample using standard technique.
  - Place the swab into a transport tube. Break swab at the indicated break line and cap the specimen collection tube tightly.
- **Note: Wear gloves when handling viral transport media (VTM) or Universal Transport Media (UTM) specimens. If gloves come in contact with the specimen, immediately change them to prevent contamination of other specimens.**

Reference Range:

- "Not Detected"

Interpretive comments:

- This assay is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal and nasal swab samples from individuals suspected of COVID-19 by their healthcare provider.
- Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.
- Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.

Attachments:

- **Fact Sheets for Healthcare Providers**
- **Fact Sheets for Patients**

FACT SHEET FOR HEALTHCARE PROVIDERS

Simplexa™ COVID-19 Direct assay – DiaSorin Molecular LLC **Updated: June 4, 2020**

Coronavirus Disease 2019 (COVID-19)

This Fact Sheet informs you of the significant known and potential risks and benefits of the emergency use of the Simplexa COVID-19 Direct assay.

The Simplexa COVID-19 Direct assay is authorized for use on using respiratory specimens collected from individuals suspected of COVID-19 by their healthcare provider.

All patients whose specimens are tested with this assay will receive the Fact Sheet for Patients: Simplexa COVID-19 Direct.

What are the symptoms of COVID-19?
Many patients with confirmed COVID-19 have developed fever and/or symptoms of acute respiratory illness (e.g., cough, difficulty breathing). The current information available to characterize the spectrum of clinical illness associated with COVID-19 suggests that symptoms include cough, shortness of breath or dyspnea, fever, chills, myalgias, headache, sore throat or new loss of taste or smell. Based on what is known about the virus that causes COVID-19, signs and symptoms may appear any time from 2 to 14 days after exposure to the virus. Based on preliminary data, the median incubation period is approximately 5 days, but may range 2-14 days.

Public health officials have identified cases of COVID-19 infection throughout the world, including the United States, which may pose risks for public health. Please check the CDC webpage for the most up to date information.

What do I need to know about COVID-19 testing?
Current information on COVID-19 for healthcare providers is available at CDC's webpage, *Information for Healthcare Professionals* (see links provided in "Where can I go for updates and more information" section).

- The Simplexa COVID-19 Direct assay can be used to test nasopharyngeal swabs (NPS), nasal swabs (NS), nasal wash/aspirate (NW) or bronchoalveolar lavage (BAL) specimens.

This test is to be performed only using respiratory specimens collected from individuals suspected of COVID-19 by their healthcare provider.

- The Simplexa COVID-19 Direct assay should be ordered for the detection of COVID-19 in individuals suspected of COVID-19 by their healthcare provider.
- The Simplexa COVID-19 Direct assay is only authorized for use in laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high and moderate complexity tests.

Specimens should be collected with appropriate infection control precautions. Current guidance for COVID-19 infection control precautions are available at the CDC's website (see links provided in "Where can I go for updates and more information" section).

Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of having COVID-19 as outlined in the CDC *Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)*. For additional information, refer to CDC *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) (*see links provided in "Where can I go for updates and more information" section).

What does it mean if the specimen tests positive for the virus that causes COVID-19?
A positive test result for COVID-19 indicates that RNA from SARS-CoV-2 was detected, and the patient is infected with the virus and presumed to be contagious. Laboratory test results should always be considered in the context of clinical observations and epidemiological data in making a final diagnosis and patient management decisions. Patient management should follow current CDC guidelines.

The Simplexa COVID-19 Direct assay has been designed to minimize the likelihood of false positive test results. However, in the event of a false positive result, risks to patients could include the following: a recommendation for isolation of the patient, monitoring of household or other close contacts for symptoms, patient isolation that might limit contact with family or friends and may increase contact with other potentially COVID-19 patients, limits in the ability to work, the delayed diagnosis and treatment for the true infection causing the symptoms, unnecessary prescription of a treatment or therapy, or other unintended adverse effects.

All laboratories using this test must follow the standard testing and reporting guidelines according to their appropriate public health authorities.

What does it mean if the specimen tests negative for the virus that causes COVID-19?
A negative test result for this test means that SARS-CoV-2 RNA was not present in the specimen above the limit of detection. However, a negative result does not rule out COVID-19 and should not be used as the sole basis for treatment or patient management decisions. A negative result does not exclude the possibility of COVID-19.

When diagnostic testing is negative, the possibility of a false negative result should be considered in the context of a patient's recent exposures and the presence of clinical signs and symptoms consistent with COVID-19. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation indicate that COVID-19 is likely, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If COVID-19 is still suspected based on exposure history together with other clinical findings, re-testing with an alternative method should be considered by healthcare providers in consultation with public health authorities.

Risks to a patient of a false negative include: delayed or lack of supportive treatment, lack of monitoring of infected individuals and their household or other close contacts for symptoms resulting in increased risk of spread of COVID-19 within the community, or other unintended adverse events.

What is an EUA?
The United States (U.S.) FDA has made this test available under an emergency access mechanism called an Emergency Use Authorization (EUA). The EUA is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to justify the emergency use of in vitro diagnostics (IVDs) for the detection and/or diagnosis of the virus that causes COVID-19.

An IVD made available under an EUA has not undergone the same type of review as an FDA-approved or cleared IVD. FDA may issue an EUA when certain criteria are met, which includes that there are no adequate, approved, available alternatives, and based on the totality of scientific evidence available, it is reasonable to believe that this IVD may be effective in the detection of the virus that causes COVID-19.

The EUA for this test is in effect for the duration of the COVID-19 declaration justifying emergency use of IVDs, unless terminated or revoked (after which the test may no longer be used).

Where can I go for updates and more information?

CDC webpages:

General: https://www.cdc.gov/COVID19
Healthcare Professionals: https://www.cdc.gov/coronavirus/2019-nCoV/guidance-hcp.html
Information for Laboratories: https://www.cdc.gov/coronavirus/2019-nCoV/guidance-laboratories.html
Laboratory Biosafety: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
Isolation Precautions in Healthcare Settings: https://www.cdc.gov/coronavirus/2019-ncov/infection-control/control-recommendations.html
Specimen Collection: https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
Infection Control: https://www.cdc.gov/coronavirus/2019-ncov/infection-control/index.html

FDA webpages:

General: www.fda.gov/novelcoronavirus
EUAs:(includes links to patient fact sheet and manufacturer's instructions) https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations

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FACT SHEET FOR PATIENTS

Simplexa™ COVID-19 Direct assay – DiaSorin Molecular LLC **Updated: June 4, 2020**

Coronavirus Disease 2019 (COVID-19)

You are being given this Fact Sheet because your sample(s) was tested for the Coronavirus Disease 2019 (COVID-19) using the Simplexa COVID-19 Direct assay.

This Fact Sheet contains information to help you understand the risks and benefits of using this test for the diagnosis of COVID-19. After reading this Fact Sheet, if you have questions or would like to discuss the information provided, please talk to your healthcare provider.

- **For the most up to date information on COVID-19 please visit the CDC Coronavirus Disease 2019 (COVID-19) webpage:**

- **https://www.cdc.gov/COVID19**

What is COVID-19?
COVID-19 is caused by the SARS-CoV-2 virus. The virus can cause mild to severe respiratory illness and has spread globally, including the United States. The current information available to characterize the spectrum of clinical illness associated with COVID-19 suggests that symptoms include cough, shortness of breath or difficulty breathing, fever, chills, muscle pain, headache, sore throat or new loss of taste or smell.

What is the Simplexa COVID-19 Direct?
The test is designed to detect the virus that causes COVID-19 in respiratory specimens, for example nasal or oral swabs.

Why was my sample tested?
You were tested because your healthcare provider believes you may have been exposed to the virus that causes COVID-19 based on your signs and symptoms (e.g., fever, cough, difficulty breathing), and/or because:

- You live in or have recently traveled to a place where transmission of COVID-19 is known to occur, and/or
- You have been in close contact with an individual suspected of or confirmed to have COVID-19.

Testing of the samples will help find out if you may have COVID-19.

What are the known and potential risks and benefits of the test?

Potential risks include:

- Possible discomfort or other complications that can happen during sample collection.
- Possible incorrect test result (see below for more information).

Potential benefits include:

- The results, along with other information, can help your healthcare provider make informed recommendations about your care.
- The results of this test may help limit the spread of COVID-19 to your family and others in your community.

What does it mean if I have a positive test result?
If you have a positive test result, it is very likely that you have COVID-19. Therefore, it is also likely that you may be placed in isolation to avoid spreading the virus to others. There is a very small chance that this test can

- **Where can I go for updates and more information?** The most up-to-date information on COVID-19 is available at the CDC General webpage: **https://www.cdc.gov/COVID19.** In addition, please also contact your healthcare provider with any questions/concerns.

give a positive result that is wrong (a false positive result). Your healthcare provider will work with you to determine how best to care for you based on the test results along with medical history, and your symptoms.

What does it mean if I have a negative test result?
A negative test result means that the virus that causes COVID-19 was not found in your sample. For COVID-19, a negative test result for a sample collected while a person has symptoms usually means that COVID-19 did not cause your recent illness.

However, it is possible for this test to give a negative result that is incorrect (false negative) in some people with COVID-19. This means that you could possibly still have COVID-19 even though the test result is negative. If this is the case, your healthcare provider will consider the test result together with all other aspects of your medical history (such as symptoms, possible exposures, and geographical location of places you have recently traveled) in deciding how to care for you.

It is important that you work with your healthcare provider to help you understand the next steps you should take.

Is this test FDA-approved or cleared?
No. This test is not yet approved or cleared by the United States FDA. When there are no FDA-approved or cleared tests available, and other criteria are met, FDA can make tests available under an emergency access mechanism called an Emergency Use Authorization (EUA). The EUA for this test is supported by the Secretary of Health and Human Service's (HHS's) declaration that circumstances exist to justify the emergency use of in vitro diagnostics for the detection and/or diagnosis of the virus that causes COVID-19. This EUA will remain in effect (meaning this test can be used) for the duration of the COVID-19 declaration justifying emergency of IVDs, unless it is terminated or revoked by FDA (after which the test may no longer be used).

- **Where can I go for updates and more information?** The most up-to-date information on COVID-19 is available at the CDC General webpage: **https://www.cdc.gov/COVID19.** In addition, please also contact your healthcare provider with any questions/concerns.

TEST DIRECTORY
ANATOMIC PATHOLOGY:
HEMATOLOGY

Specimen Source:

- Bone marrow aspiration and smears
- Bone marrow core biopsy

Supplies (bone marrow collection kit):

- 2 bottles of 10% formalin
- 2 5-slide cassette containers containing glass slides
- 1 Lavender top tube (EDTA)
- 2 Green top tubes (heparin)

Collection Instructions:

Specimen Source/Test requested	Collection Media/Specimen Requirements
Bone marrow aspiration (clot)	Formalin bottle
Bone marrow smears	Glass slides, air dried
Bone marrow core biopsy	Formalin bottle
Bone marrow for flow cytometry	Green or lavender top tube (1-2 mL)
Bone marrow for FISH	Green top tube (1-2 mL)
Bone marrow for PCR	Lavender top tube (1-2 mL)
Bone marrow for cytogenetics	Green top tube (1-2 mL)
Peripheral Blood	Smear OR Lavender top tube

- Bone marrow aspirate smears:
  - Should be made within thirty minutes of the procedure
  - Place thoroughly dried slides into 5-slide cassette container and place into foam insert in the space provided
- Bone marrow aspirate for flow cytometry, cytogenetics, FISH or PCR:
  - Place in green and lavender top tubes
- Bone marrow biopsy:
  - Place in one of the formalin jars
- Bone marrow clot:
  - Place in one of the formalin jars
- Peripheral blood:
  - Provide a copy of the most recent CBC as well as the peripheral blood smear OR peripheral blood tube (lavender top).
- Please call TOPA Diagnostics as soon as possible to arrange for pickup of the specimen
- All smears, blood tubes and formalin jars must be properly labeled with two patient identifiers. Never send the needle or syringe to the laboratory.

Storage Instructions:

- Specimens in formalin may be stored at room temperature
- Specimens in RPMI should be stored in refrigeration
- Fresh tissue specimens should be stored in refrigeration
- Specimens in red and green top tubes should be stored in refrigeration

Methodology: Aspirate smears are stained with Wright-Giemsa stain and reviewed with light microscopy. Bone marrow clot and core biopsy sections are fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining as well as special stains such as iron, PAS and reticulin will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, immunohistochemical stains may be employed to make a diagnosis. Flow cytometry and cytogenetic analysis is routinely performed on all bone marrow specimens. FISH and PCR may be performed, if applicable.

Specimen Source:

- Lymph node biopsy
- Lymph node FNA
- Lymph node excision

Supplies:

- Formalin bottle
- Sterile container
- RPMI tube
- CytoLyt bottle (FNA)
- Microscopic glass slide with 95% alcohol bottle (FNA)

Collection Instructions:

Specimen Source and/or Test requested	Collection Media
Lymph node for flow cytometry	RPMI tube
Lymph node for routine histology	Formalin bottle
Lymph node FNA for cytology	CytoLyt
Lymph node for cultures	Fresh tissue in sterile container
Lymph node for lymphoma workup	Fresh tissue +/- small amount of sterile saline (see below)

- Lymph Node Core Biopsy for Lymphoma workup:
  - Place 1 core in RPMI and the remainder of the cores in formalin
- Lymph Node Excision for Lymphoma workup:
  - Submit all tissue FRESH without fixative to the lab (may place a small amount of sterile saline or sterile saline-soaked gauze with specimen to keep it from drying out)
- Lymph Node FNA for Lymphoma workup:
  - 3-5 passes are recommended
  - For the first two passes:
    - Prepare one smear from each pass and immediately place the 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)
  - For lymphoma studies, place one pass in RPMI
  - 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.

- Please call TOPA Diagnostics as soon as possible to arrange for pickup of the specimen.

Storage Instructions:

- Specimens in formalin or CytoLyt may be stored at room temperature
- Specimens in RPMI should be stored in refrigeration
- Fresh tissue specimens should be stored in refrigeration

Methodology: Tissue biopsy/excision specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis. FNAs of the lymph node will be processed using ThinPrep technology. A cell block will be made. Smears will be reviewed if submitted. All material is then reviewed under the light microscope to render a diagnosis. In cases of suspected lymphoma, specimens submitted in RPMI will be used for flow cytometry analysis. FISH and PCR may be performed, if applicable.

Specimen Source:

- Peripheral blood

Collection Instructions:

Specimen	FISH	Flow Cytometry	PCR	Cytogenetics
Peripheral blood	Green top tube 5-10 mL	Green or Lavender top 5-10 mL	Lavender top 5-10 mL	Green top tube 5-10 mL

- Provide a copy of the most recent CBC as well as the peripheral blood smear OR peripheral blood tube

Storage Instructions:

- Specimens in purple and green top tubes should be stored in refrigeration

Methodology: A smear will be made and stained with Wright-Giemsa stain and reviewed under light microscopy in order to render a diagnosis. In some cases, flow cytometry, FISH and PCR may be performed.

Specimen Source:

- Nail
- Skin or soft tissue biopsy
- Bunion
- Crystal (gout/pseudogout) analysis
- Joint and non-joint fluid

Collection Instructions:

- Specimens for routine histology should be placed into formalin bottles immediately after removal
- Each site should be placed into a separately labeled bottle
- Specimens should be submitted with the appropriate requisition, demographics and insurance information
- See table below for specific specimen collection guidelines

Specimen Source	Collection Media	Special Instructions
Tissue (skin, bone, soft tissue)	Formalin container	Adequate for histology including fungal stain
	100% Alcohol container	Adequate for histology and crystal (gout) analysis
Nail	Dry nail bag	Adequate for both fungal culture and histology with fungal stain (PAS)
	Formalin	Adequate for histology with fungal stain (PAS)
Joint fluid	Lavender top tube	Adequate for crystal analysis (gout) and cell count
	Culture tube	If culture desired *For specimens requiring culture and crystal analysis, split specimen into lavender and culture tubes
Non-joint fluid	CytoLyt container	Adequate for cytology
	Lavender top tube	If crystal (gout) analysis desired
	Culture tube	If culture desired
Microbiology cultures	Swab or culture tube	For culture only

Storage Instructions:

- Specimens in formalin, CytoLyt or alcohol may be stored at room temperature
- Nails may be stored at room temperature
- Tissue specimens without fixative should refrigerated

Methodology: Specimens will be fixed in formalin, processed and embedded into paraffin blocks. Hematoxylin & eosin staining will be employed in order to examine the tissue under the light microscope in order to render a diagnosis. In some cases, special stains and immunohistochemical stains may be employed to make a diagnosis. PAS stain to rule out fungus is performed on all nail biopsy specimens. Polarized light microscopic examination is performed on all tissue/fluids for crystal analysis in possible cases of gout/pseudogout.

TEST DIRECTORY
Molecular Testing:
Nail PCR Panel

Methodology:
The nail panel consists of 2 multiplex PCR assays for 7 fungal targets, intended to aid in the evaluation of infected nails:

Nail Panel 1	Nail Panel 2
Pan-fungus	C. albicans
Pan-dermatophyte	C. glabrata
T. rubrum	C. krusei
	C. tropicalis/ parapsilosis

Acceptable Specimen:
- Nail sample in sterile container or nail bag

Collection Instructions:
- Clean nail with 70% alcohol
- Collect clippings in sterile container

Storage Requirements:
Store at room temperature for up to 14 days.

Reference Ranges:
- For each target, the expected result is “Not Detected”.

Interpretive comments:
- These tests were developed and the performance characteristics determined by TOPA Diagnostics. These tests have not been cleared or approved by the FDA. Testing is performed using PCR technology on the BD MAX system.
- This assay has been validated for use with nail samples only.
- Results from this assay should be used as an adjunct to clinical observations and other information available to the physician.
- Nucleic acid may persist independently of organism viability. Therefore detection of target organisms does not imply that the organisms are infectious or are the causative agents for clinical symptoms.
- Positive results do not rule out co-infection with other organisms.
- Negative results may occur due to infection with organisms not included in the panel.
- False negative results may occur due to sequence variants in the target genes, amplification inhibitors, antiviral/antibacterial therapy, or a concentration of organisms below the limit of detection.
- When fungal organisms are detected, this assay does not distinguish between colonization and infection.