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The purpose is to provide recommendations for optimal Cytology & Histolog specimen collection and transportation.

**PROCEDURE - CYTOLOGY**

The primary purpose of Cytology is to detect abnormalities, but the method is also appropriate for the detection of inflammation. Cytology is the study of the structure and function of cells. Fixation and staining techniques are used to preserve cells and prepare them so that they can be examined for abnormalities. Cytology is divided between gynaecological (conventional and liquid based cervical smears) and non-gynaecological specimens.

Non-gynaecologic specimens must be processed in such a manner as to maximise potential for cell preservation, cytological detail, adequacy and diagnosis.

**PROCEDURE - HISTOLOGY**

Histology is the study of the microscopic anatomy of cells and tissues. It is performed by examining cells and tissue by sectioning and staining, followed by examination under a microscope. Proper pathologic interpretation requires immediate fixation in 10% formalin for routine specimens.

**PROCEDURE – IMMUNOHISTO/CYTOCHEMISTRY (IHC)**

An IHC antibody detects an antigen present in a specimen, which is contained within intact or histologically sectioned cells or tissue.

IHC testing makes it possible to categorise tumours more accurately. It is a technique whereby an antigen reacts with an antibody specific to the antigen. The specific antibody is labelled with a suitable marker that will allow identification.
### CYTOLOGY & HISTOLOGY TESTS PERFORMED

#### A Gynaecological specimens:
- Pap smear
- Vulval smears
- Vaginal smears
- Posterior fornix smears
- Endopap
- LBC

#### B Non – gynaecological specimens:
- Urine
- Sputums
- Bronchial washings/brushings/BAL
- Bronchial lavage
- CSF
- Body Fluids (e.g. pleural, peritoneal, pericardial & synovial fluids)
- Fine Needle Aspirations (e.g. breast, lung, soft tissue) Scrapings (e.g. nipple)

#### C Histology:
- Routine Histology – H&E stain
- Renal Biopsies
- Rectal Biopsies – Meier-Rouge
- Skin Biopsy for Immunofluorescence
- Foetus for autopsy
- Bone Marrow Trephines
- Muscle biopsies
- Fish
- EM

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The patient's surname and initials must be written on the slides or specimen container for proper identification and prompt processing. A fully completed requisition form must accompany the specimen to the laboratory, e.g. patient and doctor details, type of specimen, collection date, time and relevant clinical history.
TIME LIMITS FOR ADDITIONAL AFTER REQUESTS

Cytology:
Non-Gynae: If the specimen is fixed, additional requests can be done within a week.

LBC:
Specimens are stored for 2 to 4 weeks, (can vary from lab to lab) and any after requests can be done within this period.

Histology and Immunohistochemistry:
Blocks are stored for 30 years; any additional requests can be done at any time during the 30-year period.
If any additional requests need to be done on the tissue, it needs to be done within one month, depending on the relevant laboratory.

STORAGE TIMES (PathCare's Recommended Guidelines)

Cytology:
Non – Gynae specimens are stored for 1 week at the relevant laboratory.

LBC:
Specimens are stored for 2 to 4 weeks.

Histology
Specimens are stored for a 4 week period after the final report is released.
# The Importance of Clinical Information for Cytology Pathology test

<table>
<thead>
<tr>
<th>Clinical Information</th>
<th>Why it is important?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Menstrual status</strong></td>
<td>The different stages of the menstrual cycle have different smear patterns. Absence of the LMP status can lead to unnecessary diagnosis of inflammatory changes.</td>
</tr>
<tr>
<td><strong>Intermenstrual-, contact- and post coital bleeding</strong></td>
<td>The causes of bleeding are important to make the cytotecnologist aware of a possible lesion and to interpret the cytology cell picture.</td>
</tr>
<tr>
<td><strong>Pregnant and post-partum</strong></td>
<td>Large amounts of glycogen can cause intermediate cells to have a boat-shaped appearance (navicular cells), with haloes around the nuclei. These cells can be mistaken for HPV cells if the clinical information is unknown.</td>
</tr>
<tr>
<td></td>
<td>The presence of endometrial cells in a pregnant patient is of utmost importance to the clinician and in cases where the clinical information is not known, it may influence the follow-up of the patient.</td>
</tr>
<tr>
<td></td>
<td>During the post-partum period the cells can appear very atrophic, inflamed and atypical. Parabasal cells in varying stages of degeneration may mimic HSIL.</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td>In the absence of clinical history of the use of hormonal treatment, various changes can be interpreted as reactive or atypical.</td>
</tr>
<tr>
<td><strong>IUCD</strong></td>
<td>The IUCD can cause changes in the metaplastic, endocervical and endometrial cells, for instance the string of the IUCD apparatus and infections (Actinomyces) can cause reactive changes in the endocervical cells. Endometrial cells may exfoliate at any time of the cycle as a result of irritation.</td>
</tr>
<tr>
<td></td>
<td>These reactive changes may simulate adenocarcinoma and therefore indicating the presence of an IUCD is very important.</td>
</tr>
<tr>
<td><strong>Age, especially postmenopausal patients</strong></td>
<td>Severe atrophy can cause cellular and nuclear degeneration that could be a source of a false positive result. Nuclear abnormalities seen in atrophic vaginitis can closely mimic carcinoma.</td>
</tr>
<tr>
<td><strong>Radiation and chemotherapy</strong></td>
<td>This causes bizarre cytological changes. Cells show nuclear and cytoplasmic enlargement, multinucleation, vacuolisation, are “blown-up” and can look very atypical. This may lead to misinterpretation if the clinical information is unknown. These changes may persist for years after treatment.</td>
</tr>
<tr>
<td><strong>Laser, biopsy and surgery</strong></td>
<td>Changes are observed within one week after treatment. Bizarre cells can be mistaken for malignant cells.</td>
</tr>
</tbody>
</table>

**In conclusion:**
In view of the above it is important that all relevant clinical information is made available to ensure accurate diagnosis, which will then result in the successful treatment of the patient.
PathCare Cervical Screening Protocol 2016

Cervical screening is changing, with HPV typing now an integral component in the screening protocols of all major authorities in the world. In addition, there is a worldwide trend for HPV typing to be the preferred cervical primary screening test in certain age groups. We believe that South Africa will soon adopt a similar position.

In this transitional phase, we will be guided by our supporting clinicians' preferred screening algorithm. In addition to liquid-based and conventional cytology, PathCare offers both hr HPV DNA and hr HPV mRNA E6/E7 typing. These molecular tests include routine testing for “highest-risk” HPV types (HPV 16 & 18 and HPV 16 & 18/45 respectively). The preferred hr HPV typing method, i.e. DNA versus mRNA, must be indicated on the pathology request form.

Three possible screening scenario’s exist, depending on the preference of the clinician, age of the patient and clinical history:

- LBC as 1st screening, with hr HPV genotyping as triage*;
- LBC and hr HPV as co-testing;
- hr HPV as 1st screen, with cytology as triage*.

*If a triage test is indicated, this will be performed routinely, and will affect the cost. Please advise the patient accordingly.

<table>
<thead>
<tr>
<th>Age</th>
<th>Recommended screening method</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 years and 1 year after initial sexual intercourse</td>
<td>Screen with Cytology (cervical smear) every 2 - 3 years.</td>
<td>HPV testing should not be used for primary screening or as a component of co-testing or in the management of ASC-US in this age group.</td>
</tr>
<tr>
<td>25 - 29 years</td>
<td>Screen with Cytology and HPV testing (co-testing) every 2 - 3 years.</td>
<td></td>
</tr>
<tr>
<td>30 - 65 years</td>
<td>Screen with HPV-testing as primary screen every 3 - 5 years, Or \ Screen with Cytology and HPV testing (co-testing) every 3 - 5 years, Or \ Screen with Cytology every 2 - 3 years if no history of abnormal smears and prior adequate negative screening [1].</td>
<td></td>
</tr>
<tr>
<td>&gt; 65 years</td>
<td>No screening following prior adequate negative screening [1].</td>
<td>Women with a history of CIN II+ should continue routine screening for at least 20 years.</td>
</tr>
<tr>
<td>Post hysterectomy</td>
<td>No screening</td>
<td>Applies to women without a cervix and without a history of CIN II+ diagnosis in the past 20 years, or any history of cervical cancer ever. Evidence of adequate negative prior screening is not required.</td>
</tr>
<tr>
<td>HPV vaccinated</td>
<td>Screening practices should not change on the basis of HPV vaccination status</td>
<td></td>
</tr>
</tbody>
</table>

1) The above guidelines recommend the screening from the age of 21 years.
2) This screening protocol is for HIV/AIDS negative individuals. Those living with HIV/AIDS require more frequent screening.
3) Adequate negative screening is defined as: 3 Consecutive negative cervical smears or 2 consecutive negative HPV tests, tested within 10 years of stopping, and the most recent within the last 5 years.

Information adapted from ASCCP
To identify patients who have cellular changes that put them at risk for the development of cervical cancer. Patients should be advised to schedule a gynaecological examination two weeks after the first day of the last menstrual period, if possible.

Cervical samples should be taken from the transformation zone where 90% of lesions that eventually lead to invasive cancer of the cervix originate.
Bethesda reporting system for Cervical Cytology:

The Bethesda System for Reporting Cervical Vaginal Cytologic Diagnosis (TBS) was developed at the National Cancer Institute (NCI) in December 1988 to provide uniform diagnostic terminology that would facilitate communication between the laboratory and the clinician. The Bethesda System was designed to be flexible so that it could evolve in response to changing needs in cervical cancer screening as well as to advances in the field of cervical pathology. PathCare laboratories are using the Bethesda reporting system. See below Bethesda reporting classification.

The Pap Test and Bethesda 2014/Nayar and Wilbur

TABLE 1. THE 2014 BETHESDA SYSTEM SPECIMEN TYPE

Indicate conventional smear (Pap smear), liquid-based preparation (Pap test) vs other

- Satisfactory for evaluation (describe presence or absence of endocervical / transformation zone component and any other quality indicators, eg, partially obscuring blood, inflammation, etc)
- Unsatisfactory for evaluation (specify reason)
  - Specimen rejected/not processed (specify reason)
  - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)
- Negative for intraepithelial lesion or malignancy
- Other: see Interpretation/Result (eg, endometrial cells in a woman aged 45 years)
- Epithelial cell abnormality: see Interpretation/Result (specify “squamous” or “glandular,” as appropriate)

Negative for Intraepithelial Lesion or Malignancy
(When there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report—whether or not there are organisms or other non-neoplastic findings)

Non-Neoplastic Findings (optional to report)
- Non-neoplastic cellular variations
  - Squamous metaplasia
  - Keratotic changes
  - Tubal metaplasia
  - Atrophy
  - Pregnancy-associated changes
- Reactive cellular changes associated with:
  - Inflammation (includes typical repair)
  - Lymphocytic (follicular) cervicitis
  - Radiation
  - Intrauterine contraceptive device (IUCD)
- Glandular cells status posthysterectomy
Organisms
- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp.
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp.
- Cellular changes consistent with herpes simplex virus
- Cellular changes consistent with cytomegalovirus

- Endometrial cells (in a woman aged 45 years) (Also specify if “negative for squamous intraepithelial lesion”)

Epithelial Cell Abnormalities
Squamous Cell
- Atypical squamous cells
  - Of undetermined significance (ASC-US)
  - Cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL)
  (Encompassing: HPV/mild dysplasia/CIN-1)
- High-grade squamous intraepithelial lesion (HSIL)
  (Encompassing: moderate and severe dysplasia, CIS; CIN-2 and CIN-3)
  - With features suspicious for invasion (if invasion is suspected)
  - Squamous cell carcinoma
Glandular Cell
- Atypical
  - Endocervical cells (NOS or specify in comments)
  - Endometrial cells (NOS or specify in comments)
  - Glandular cells (NOS or specify in comments)
- Atypical
  - Endocervical cells, favor neoplastic
  - Glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - Endocervical
  - Endometrial
  - Extrauterine
  - Not otherwise specified (NOS)

Other Malignant Neoplasms (specify)

Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician

If case examined by an automated device, specify the device and result

(optional)

Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included)
### When to have a Pap smear taken:
- Ideal time for Pap smear: 5 days after menstrual period has ended (mid cycle).

### Cervical Screening Recommendations
(adopted from ACS, ACOG, ASCCP updated guidelines 2013)

<table>
<thead>
<tr>
<th>AGE</th>
<th>Recommended screening method</th>
<th>Comments</th>
<th>Rationale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger than 21 years and 1 year after initial sexual intercourse</td>
<td>Screen with Cytology (cervical smear) every 3 years.</td>
<td>HPV testing should NOT be used for as a component of co-testing or primary screening or in the management of ASC-US in this age group</td>
<td><strong>Rationale for avoiding HPV Test:</strong>&lt;br&gt; - Prevalence of carcinogenic HPV approaches 20% in teens and early 20's.&lt;br&gt; - Most carcinogenic HPV infections resolve without intervention. Identifying carcinogenic HPV that will resolve leads to repeated call-back, anxiety, interventions without benefit.</td>
</tr>
<tr>
<td>21 – 29 years</td>
<td>Screen with Cytology (cervical smear) every 3 years.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 – 65 years</td>
<td>Screen with Cytology and HPV testing (co-testing) every 5 years&lt;br&gt;Or&lt;br&gt;Screen with Cytology every 3 years if no history of abnormal smears and prior adequate negative screening*</td>
<td>Screening by HPV testing alone is currently not recommended for most clinical settings.</td>
<td><strong>Rationale for co-testing:</strong>&lt;br&gt; - Increased detection of prevalent CIN III.&lt;br&gt; - Decreased risk of CIN III in subsequent screening rounds.&lt;br&gt; - Achieves risk of CIN III equal to Cytology alone @ 1-3 year intervals.&lt;br&gt; - Enhances detection of adenocarcinoma/AIS&lt;br&gt; - Minimise increased number of colposcopies, thus reducing harm.</td>
</tr>
<tr>
<td>Older than 65 years</td>
<td>No screening following prior adequate negative screening*.</td>
<td>Women with a history of CIN II+ should continue routine screening for at least 20 years.</td>
<td><strong>Rationale for stopping:</strong>&lt;br&gt; - CIN II is rare after age 65.&lt;br&gt; - HPV risk remains 5 – 10%&lt;br&gt; - Incident HPV infection unlikely to lead to cancer within remaining lifetime.</td>
</tr>
<tr>
<td>Post hysterectomy</td>
<td>No screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV vaccinated</td>
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</table>

1) These guidelines are based on the 2012 ACS/ASCCP/ASCP recommendations.
2) The above guidelines recommend the screening from the age of 21 years.
3) Adequate negative screening* is defined as: 3 Consecutive negative cervical smears or 2 consecutive negative HPV tests, tested within 10 years of stopping, and the most recent within the last 5 years.

For the complete ASCCP updated Consensus Guidelines on Management of Women with Abnormal Cervical Cancer Screening tests go to: www.ASCCP.org/Guidelines

The ASCCP Algorithms Mobile App can be downloaded from the iTunes & Google Play Stores.

Contact
PathCare Histo- and Cytopathologists: | References
ASCCP Updated Consensus Guidelines
Equipment:
- Adequate light source
- Speculum (Lubricant e.g. KY jelly)
- Gloves
- Spray fixative
- Glass slide labelled in pencil with the patient’s surname, initials and date of birth.
- Requisition form
- Slide holder or vial for liquid based cytology
- Sampling instruments, e.g. cytobrush, spatula, cervix brush, combi-brush.

Sampling devices:

i) Cytobrush:

- Usually gives a good endocervical cell sample, the collected cells gather between the bristles and easily leave the brush when it is rolled over the slide. Not an adequate ectocervical sample.

ii) Aylesbury Spatula:

- The mucus and cells adhere to the wooden spatula, considered one of the best sampling techniques.

iii) Cervix brush:

- Its shape makes it possible to simultaneously collect ecto- and endocervical cells and thus sample the transformation zone where most of the abnormalities originate.
iv) Combi-Brush

- The Rovers® Cervex-Brush® Combi is a new patented product capable of delivering higher cellular yield of endocervical cells than the traditional Rovers® Cervex-Brush®.

Advantages of Combi-brush

- Only 2 rotations needed.
- 2 - 3 times more endocervical cells
- For liquid-based as well as conventional cytology

Procedure for taking a conventional cervical smear:

- The patient must undress and be given a gown to put on.
- Ensure that the woman is lying comfortably on the examination couch, knees bent and feet apart.
- Keep the patient covered as much as possible at all times.
- Explain each step of the procedure as it is being done.
- Select a speculum that can be inserted comfortably, insert the speculum along the axis of the introitus and when half way up the vagina rotate 90 degrees and open when fully inserted.
- Bring the cervix into view by gentle movement of the speculum, encouraging the patient to relax.
- It is essential that the cervix is clearly seen otherwise satisfactory smears cannot be taken.
- Note any significant features or abnormalities of the cervix.
- Choose a sampling instrument which best suits the shape of the cervix and os. See below for conventional cervical smears.

Cervix-Brush:

- Insert the central bristles 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.

Cytobrush:

- Insert the endocervical brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate 1/4 or 1/2 turn in one direction.
Ayre’s spatula:

- Rotate spatula 360 degrees.

Transfer cervical cell sample onto a marked glass slide with a thin, even spreading motion.

Fix immediately with spray fixative, holding container approximately 30 cm from the slide.

Once the smear has been taken, remove the speculum and let the patient get dressed.

Leave the slide to dry for approximately 10 minutes before transportation to the laboratory.

Complete the laboratory requisition form and mark the glass slide in pencil on the frosted end with patient details.

Special handling:

- Any delay in smearing specimen onto slides, or delay in fixation, can result in the specimen rapidly drying out and being unsuitable for accurate cytological examination.

- Fix immediately with spray fix, holding container approximately 30 cm from the slide.

- Slides must be allowed to dry and then be transported in such a way that cells from one patient cannot be transferred to the slide of another.

- The liquid based container must be sealed properly and sent to the nearest Cytology laboratory.
HANDLING OF LIQUID BASED SAMPLE (LBC)
Surepath method.

Contra-indications: The Rovers® Cervex-Brush® Combi should not be used during pregnancy. (Use plastic Aylesbury Craig Spatula or Cervix Brush)

Please follow these directions to be assured of the best performance from the Rovers® Cervex-Brush® Combi.

- Insert the central bristles of the brush into the endocervical canal.
- Apply gentle pressure on the cervix until the lateral bristles bend against the ectocervix.
- Maintain the gentle pressure and rotate the Rovers® Cervex-Brush® Combi two times in a clockwise direction by rolling the stem between thumb and forefinger.
- The head of the brush is detached or removed and placed into the vial with preservative fluid. Reseal the vial.

**LEAVE THE BRUSH HEAD IN THE SURE PREP VIAL.**

Use the interior rim of the new BD Surepath™ Collection Vial to pull off the head of the broom-like device or snap the heads of the brush and spatula into the large opening in the collection vial.
NON-GYNAECOLOGICAL SPECIMENS

50% alcohol - equal amounts of 95 - 100% alcohol and distilled water.

URINE

The source of the specimen and the method of collection should be indicated on the requisition form, as well as treatment such as chemo or radiotherapy since it causes cellular changes which may mimic carcinoma. Urine can be obtained by catheterisation or by voiding.

Purpose

The major role of urinary tract cytology is to detect malignancy arising in the bladder, ureters or renal pelvis.

Patient preparation:

In female patients vaginal contaminants are common and therefore the patient should be instructed to wash the labia, separate them and try to pass urine without labial contamination. Urine should be voided directly into the container.

Method:

Voided urine:
- Specimen is collected by patient. Be sure all specimens are collected “clean – catch” and in properly labelled containers.
- A mid-stream sample of the second, subsequent specimen in the morning is the preferred specimen. Adult patients may collect their own sample if well instructed. Explain the procedure carefully using words the patient can understand. Ensure that the patient has correctly understood the instructions.

Equipment needed:
- Sterile / surgically clean gauze
- Sterile / surgically clean kidney dish
- Sterile urine container with lid
**Procedure**

Patients are to wash their hands and moisten the gauze with clean water.

**Females:**
- Spread the labia with one hand and while keeping them open and using the gauze moistened with water, clean the urethral meatus and labia from front to back. Some female patients find it easier to sit back to front on the toilet and use the top of the toilet cistern to rest their equipment on.

**Males:**
- Male patients are to clean the tip of the penis with gauze moistened with water. If they have not been circumcised, they are to retract the foreskin before cleaning.
- Patients are then to begin voiding into the toilet. (Females are to hold the labia apart with one hand.)
- After the first amount of urine has been passed into the toilet pan, the patient collects ±25 ml of urine directly into the container. The container must not come into contact with the genitalia or clothing.
- The patient completes emptying their bladder into the toilet.
- Screw the lid back onto the container and label.
- Send the specimen to the laboratory within 2 hours. If a delay is anticipated, place the specimen in the fridge.

**Catheterised urine:**
- Specimen is collected by doctor or nursing staff in a clean, properly labelled container.
- Catheter specimens are only to be collected from the specimen port on the catheter.
- They must not be collected from the drainage bag or by disconnecting the catheter from the drainage tubing.
- Clamp the catheter for 30 minutes.
- Clean the specimen port with 70% alcohol (methanol/ethanol) and allow drying before using a sterile needle and syringe to aspirate ± 25 ml of urine.
- Transfer the urine into a sterile urine container.
- Indicate clearly on the specimen label and the requisition form that the specimen has been collected from an indwelling catheter.
Renal Pelvic and Bladder washings:

- Using normal saline, the washing specimen is collected by a doctor in a clean specimen container.
- Label container with the specific body site, e.g. L (left) or R (right)

**Specimen requirements:**

**Amount of specimen:**
- 10 - 30 ml of urine is usually adequate and large volumes are not required.

**Fixation:**
- Equal volume of 50% ethanol/methanol or Surepath fixative for preservation can be added and may be stored in the fridge prior to processing.
  Do not add alcohol if Microbiology and Cytology are requested and there is only one sample. It is advisable to collect a second sample or send the specimen to the laboratory, as soon as possible, to be split.

**Transport:**
- Urine specimens without fixative should be transported directly to the laboratory or refrigerated if any delay is anticipated.
### SPUTUMS

#### Purpose
- Sputum specimens are useful for detecting malignant cells of the lower respiratory tract and also to diagnose infectious conditions in immunocompromised patients. Central lesions are more readily detected than peripheral lesions.
- It is mandatory to get a good representative sample of the lower respiratory tract – this is evidenced by the presence of carbon-laden macrophages or bronchial mucosal cells in the sputum. Many sputum specimens are poorly collected samples and contain mainly salivary material.

#### Collection timing:
- An early morning deep cough (not saliva) specimen before breakfast is recommended on 3 consecutive days.

#### Patient preparation:
- Patient rinses mouth with water.

#### Method:
- To reduce oropharyngeal flora and food particle contamination, patient may rinse mouth and gargle with sterile water just prior to collecting the specimen. Commercial mouthwash preparations are not to be used.
- Use a wide mouthed sterile container.
- Instruct the patient to hold the container just below the lower lip, cough deeply and spit into the container.
- The patient must understand the meaning of “sputum” as opposed to “saliva” (spit). The specimen must be sputum and not saliva.
- Patients experiencing difficulty in producing a sputum specimen can be nebulized using a saline solution.
- Label and send specimen to the laboratory as soon as possible

#### Specimen requirements:

<table>
<thead>
<tr>
<th>Amount of specimen:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three consecutive early morning deep cough specimens are recommended.</td>
</tr>
<tr>
<td>Minimum amount required: 1 ml preferred.</td>
</tr>
</tbody>
</table>
Fixation:

- Sputums are NOT PRESERVED IN ALCOHOL as this causes severe mucolytic changes.

Special Handling:

- If processing is to be delayed, refrigerate specimens. Sputum samples should be stored in the fridge until transported to the laboratory for analysis. This helps prevent cell degeneration and overgrowth by organisms.

Transport:

- Fresh sputum specimens should be transported immediately to the laboratory.

**BRONCHIAL WASHINGS / BRUSHINGS**

Purpose:

- To detect and classify neoplasms, involving the bronchial tree. Useful for peripheral lung lesions.

Method:

Washings:

- Specimen is collected by doctor in a clean `specimen trap`. Label container with exact body site.

Brushings:

- Insert the brush into a specimen container with 50% methanol / ethanol or Surepath fixative or roll the contents of the brush onto a clean, labelled glass slide and fix immediately with spray fixative.

Specimen requirements:

**Amount of specimen:**

- Washings: 10 ml preferred.
Fixation:

- Equal volumes of 50% ethanol/methanol or Surepath fixative for preservation can be added and may be stored in the fridge prior to processing. Do not add alcohol if Microbiology and Cytology is requested on one sample. It is advisable to collect a second sample or send specimen to the laboratory as soon as possible to be split.

Transport:

- Bronchial washings should be transported to the laboratory immediately.

**BRONCHO-ALVEOLAR LAVAGE (BAL)**

**Purpose:**

- It is useful in the diagnosis of opportunistic infections in immunocompromised hosts; it is also helpful to diagnose interstitial lung disease, granulomatous disease and evaluation of transplant rejection. Malignancies and certain other diseases can also be diagnosed.

**Method:**

- Specimen is collected by doctor in a sterile, labelled container. BAL specimens are obtained by wedging a sub segmental bronchus with the bronchoscope and lavaging the area with saline or balanced salt solution.
Specimen requirements:

**Amount of specimen:**
- 10 ml preferred.

**Fixation:**
- Equal volumes of 50% ethanol/methanol or Surepath fixative for preservation and may be stored in the fridge prior to processing. Do not add alcohol if Microbiology and Cytology is requested on one sample. It is advisable to collect a second sample or send the specimen to the laboratory as soon as possible, to be split.
- For Diff Count: Add equal amount of formalin and deliver immediately, on ice, to cytology laboratory.

**Transport:**
- Specimen should be transported immediately to the laboratory.

**BODY FLUIDS – Peritoneal (ascites), Pleural, Pericardial, Cyst, etc.**

When body fluids are tapped, they should always be submitted for cytological examination. Large effusions, when initially tapped, may not be adequate due to degenerative changes or a poor cellular yield. Cells lying in free fluid for too long a period of time often undergo severe degenerative changes, which limit accurate cytology diagnosis.

If fluids need to be tapped repeatedly, re-accumulation of the fluids often yield more cells which are better preserved. It is therefore recommended that each time an effusion is tapped, that it is sent for cytological evaluation if a definitive diagnosis has not been made previously.
Purpose:

- Body cavity fluids are usually collected for the diagnosis of malignant neoplasms.

Collection timing:

- Washings are usually obtained at the time of surgery.

Method:

- Specimen is collected by doctor in a clean, properly labelled container.

Specimen requirements:

Amount of specimen:

- Minimum of 10 ml – laboratory will accept up to 1000 ml.

Fixation:

- Equal volume of 50% ethanol/methanol or Surepath fixative for preservation and may be stored in the fridge prior to processing.

Transport:

- Specimens should be transported directly to the laboratory. Cells remain interpretable in body fluids for several days after refrigeration.
GASTROINTESTINAL TRACT

Gastrointestinal cytology is used primarily in diagnosing symptomatic or high risk patients and is generally more useful for diagnosing visible lesions than screening patients. The most useful cytologic technique is usually direct brushings of visible lesions.

**Special handling:**
- Washings: Deliver on ice immediately to the laboratory.
- Brushings: Brushes should be rapidly rolled on a glass slide. The slides should be fixed immediately or brushes can be transported to the laboratory in 50% methanol/ethanol or Surepath fixative.

**Specimen storage:**
- Specimen will be stored for at least one week in a fridge in Cytology Department.

CEREBROSPINAL FLUID

Do not use a blood tube containing gel/anticoagulant.

**Purpose:**
- CSF is usually examined to detect malignant cells, infections, vascular disorders, trauma and organisms. CSF specimens are always considered to be URGENT.

**Method:**
- Specimen is collected by doctor in a sterile properly labeled **plastic** container as cells adhere to glass.

**Amount of specimen:**
- 1 ml preferred.

**Fixation:**
- Fresh, unfixed specimen or 50% methanol/ethanol or Surepath fixative

**Special handling:**
- The specimens must be transported to the laboratory immediately. Specimens should be processed as soon as possible and kept at body temperature. If processing will be delayed, it should be fixed with 50% methanol/ethanol or Surepath fixative or kept in the fridge to prevent bacterial growth.

**Transport:**
- Specimen should be transported immediately to the laboratory.
### General:
- Surface area is scraped with a spatula or brush and the material is smeared or rolled directly onto a glass slide, which should be fixed immediately with cytology fixative to avoid air-drying artifact and maintain preservation.

### Amount of specimen:
- At least 4 slides.

### Fixation:
- Fix immediately with spray fixative, hold container approximately 30 cm from glass slide and apply for one to two seconds. Send slides to laboratory.

### Transport:
- No time limit for transport of fixed slides.

### Nipple discharge:
**Purpose:** To detect malignant cells in a patient with a nipple discharge.

**Method:**
- Label at least 6 clean slides. Number in numerical order.
- Gently massage the sub-areolar area and nipple, using the thumb and forefinger.
- When a secretion occurs, allow a small drop to accumulate on the apex of the nipple.
- Support areola and nipple with other hand.
- Place the slide on the nipple, touching the drop which will spread laterally, then draw the slide quickly across the nipple.
- Place two slides together and gently pull the slides apart.
- Fixation: Spray fix the smears by holding the can 30 cm away from the slides.
- Unfixed slides are prepared for Giemsa stain.
- When no nipple secretion can be obtained, but nipple erosion or ulceration is present, touch slide directly onto the nipple.
- Carefully label the slides e.g. Right or Left Breast / Nipple.
**Tzanck smear:**

Purpose: To confirm the diagnosis of vesicular diseases secondary to Herpes virus infections.

**Method:**

- Identify a fresh typical vesicle.
- Unroof the vesicle.
- Scrape the margin of the vesicle with a scalpel blade.
- Spread the cells and debris adherent to the blade on a clean, labelled glass slide.
- Fix immediately with spray fixative, hold container approximately 30 cm from glass slide and apply for one to two seconds.
- Send slides to the laboratory.
Fine needle aspirates are usually done on more solid masses in the body. These may be superficial or deep. The deeper ones are often done under radiologic guidance, either under ultrasound or CT scan and hence the majority are done in the radiology department. The most common superficial masses aspirated are soft tissue masses, breast masses and thyroid masses. It should be noted that only palpable masses are to be aspirated.

The FNA sampling procedure is highly operator dependent and formal training should be received in this procedure.

**Choice of needles**

- Generally, FNA utilizes 22-25 gauge needles (not larger than 22 gauge)
- Thyroid aspiration: Bloodstained material, which makes microscopic evaluation more difficult, is frequently seen in cases where aspiration was performed with thicker needles
- Larger gauge needles work well on lesions with high density of epithelial cells and minimal stroma.
- Smaller gauge needles are superior for highly fibrous lesions.
- Very small 26 - 27 gauge needles are useful for intracutaneous lesions and, sometimes, for very small targets.
- The length of the needle is a function of the size and depth of the target. Generally, the needle should be significantly longer than necessary to sample the target.
Aspiration guidelines:

- Attached the needle to a 5/10 ml syringe.
- Aim needle for the central portion of the mass.
- Immobilise the lesion, introduce the needle and apply suction.
- Move the needle in and out in a sewing cutting motion, until material is present in the hub of the needle. This motion is required to obtain an adequate sample.
- Release suction and withdraw the needle.
- Remove needle, introduce air into syringe, attach needle again and express material onto a slide. Place second slide parallel to first, apply gentle pressure and pull slides apart or rinse needle in Surepath fixative.
- Slides can be either be fixed with Cytology fixative or left to airdry.

**Needle capillary method:**

This method can be used for performing FNA of the Thyroid. The needle is used with no syringe attached but may have a syringe attached, without applying suction. The FNA is performed without applying suction and the contents of the needle are then expressed on to a slide.
HANDLING OF DIFFERENT HISTOLOGY SPECIMENS

Fixatives:

Glutaraldehyde

- Glutaraldehyde is recommended for fixation of tissues for electron microscopy as it fixes very quickly. The glutaraldehyde must be cold and buffered and the tissue must be as fresh as possible. The standard solution is a 2.5% buffered glutaraldehyde.

Formalin

- This is used for all routine Histology specimens.
- Formalin penetrates tissue well, but is relatively slow. The standard solution is 10% neutral buffered formalin.

Zenker’s fixatives

- This is recommended for bone marrow. Zenker’s fixes nuclei very well and gives good detail. However, the mercury deposits must be removed before staining as black deposits will result in the sections.

Rectal biopsies for Hirschsprung’s disease and chromosome studies

- Fresh tissue, transport on ice or fix in formalin as per lab’s preferences.
- If a specimen is received in incorrect fixatives, the referring doctor must be contacted to discuss the tests requested and fixative uses.
<table>
<thead>
<tr>
<th>TYPE OF SPECIMEN</th>
<th>FIXATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine specimens; e.g. colon, breast and uterus</td>
<td>• Specimens must be submitted in 10% buffered formalin immediately.</td>
</tr>
<tr>
<td></td>
<td>• Containers must be adequately filled with Formalin, with volume:specimen ratio = 4 times. (submerge the entire specimen in formalin)</td>
</tr>
<tr>
<td></td>
<td>• Ensure that containers are labelled properly with proper identification.</td>
</tr>
<tr>
<td></td>
<td>• The uterus must be cut open to ensure proper fixation of the endometrium.</td>
</tr>
<tr>
<td>Urate crystals</td>
<td>50% alcohol</td>
</tr>
<tr>
<td>Bone marrow trephines</td>
<td>Zenker’s - must be freshly made up</td>
</tr>
<tr>
<td>Rectal biopsy for Meier-Rouge studies</td>
<td>Saline on ice or formalin depending on lab preference</td>
</tr>
<tr>
<td>Renal biopsies</td>
<td>• One piece in 10% formalin for routine histology</td>
</tr>
<tr>
<td></td>
<td>• One piece in 2.5% buffered glutaraldehyde for electron-microscopy, pH 7.2 – 7.4.</td>
</tr>
<tr>
<td></td>
<td>• One piece in transport medium for Immuno-fluorescent studies. The glutaraldehyde and transport medium specimen are kept at 4°C until requested for send away</td>
</tr>
</tbody>
</table>
RECTAL BIOPSIES, MEIER-ROUGE STUDIES AND MUSCLE BIOPSIES
(Processing and reporting performed by a referral laboratory)

Rectal and muscle biopsies are regarded as urgent and delicate and must be kept sterile. Notify the laboratory, a day before the procedure.

A PathCare send away form together with full clinical details must accompany the specimen.

Handling of muscle biopsy specimen sent to Red Cross Hospital

- The biopsy should measure at least 10x5x5mm if possible. The biopsy may be placed longitudinally on a spatula then dampened gauze wrapped around it, but otherwise just wrapped in dampened gauze is sufficient. Gauze must be moistened with saline. The specimen should be kept moist, but not drenched in saline.

- If some tissue is going to be sent in formalin and glutaraldehyde please ensure that the largest piece is sent in saline dampened gauze and that it is still the minimum size as mentioned above.

Handling of muscle biopsy specimen sent to Potchefstroom

- Contact the local Histopathologist for handling guidelines.

NB: It is very important for the specimen to be kept cold at all times. It is therefore recommended that the saline be cooled before use.

SKIN OR RENAL BIOPSIES - IMMUNOFLUORESCENCE

Contact the pathologist on duty for information on the fixative and notify the laboratory that a specimen is on its way.

Before the specimen can be frozen on to the CO₂ chuck, the specimen needs to be washed in a special washing buffer.
**RENAL BIOPSIES**

A tru-cut needle biopsy is taken. It is preferable to request 3 cores of tissue.

Tissue site: Only tissue from the cortex of the kidney is selected – this is confirmed by examining the tissue under a dissecting microscope. The cores are then put into the appropriate solution.

**TRANSPORTATION OF A FOETUS**

Death of the foetus

≤ 26 Weeks
Non-viable

- Karyotyping and microbiology: Placenta must be fresh or in saline.
- Histology: 10% formalin

Foetus - place in 10% formalin.

≥ 26 Weeks *
Viable

- Place foetus in linen.
- Contact UCT Laboratory at 021 - 404 3000 or Prof Wainwright at 021-404 5258
- Keep foetus in fridge until time of transportation.
- Do not freeze.
- Use a cooler box for long distance transportation.
- Put the placenta in 10% formalin or transport it fresh.

Arrange transportation and costs with the local Funeral undertaker.

According to the Births and Deaths Registration Act, No 51 of 1992, stillbirth with regards to an infant means that the foetus had at least 26 weeks of intra-uterine existence, but showed no signs of life after complete birth.

A doctor who was present at the stillbirth, or who examined the baby and is convinced that the baby was stillborn, must complete the death notification form (BI-1663) accordingly. If no medical practitioner was present at the stillbirth, or no doctor examined the baby, any person who was present at the stillbirth can make a statement to that effect, and the (death of the) stillborn baby can be registered. A burial order is then issued to bury the stillborn baby.

When completing the BI-1663, the first line of Section A “Particulars of Stillborn Child” should be ticked, as opposed to “Particulars of Deceased individual”. The weight of the stillborn baby should be indicated in Section G. Otherwise the form is completed as per usual.

If a maternal condition has caused or initiated the stillbirth, it should be indicated as the underlying cause of death, while some foetal conditions may result from it, being the intermediate and immediate causes of death. “Prematurity” should never be entered without explaining the etiology of prematurity.

A Guide for completing the Death Notification Form (DNF) – BI-1663 (www.mrc.ac.za)
BONE MARROWS

Bone marrows are performed by PathCare pathologists. An appointment for a bone marrow biopsy can be made with the local pathologist or via the local laboratory.

50% alcohol - equal amounts of 95 - 100% alcohol and distilled water.
HISTOLOGY & CYTOLOGY LABORATORIES

<table>
<thead>
<tr>
<th>Location</th>
<th>Phone Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bethlehem</td>
<td>058 303 4961</td>
</tr>
<tr>
<td>Bloemfontein</td>
<td>051 401 4600</td>
</tr>
<tr>
<td>Claremont, Cape Town</td>
<td>021 670 5700</td>
</tr>
<tr>
<td>East London</td>
<td>043 701 5900</td>
</tr>
<tr>
<td>George</td>
<td>044 803 8200</td>
</tr>
<tr>
<td>Klerksdorp</td>
<td>018 468 9000</td>
</tr>
<tr>
<td>Goodwood, Cape Town</td>
<td>021 596 3666</td>
</tr>
<tr>
<td>Mossel Bay</td>
<td>044 691 1399</td>
</tr>
<tr>
<td>Port Elizabeth</td>
<td>041 391 5700</td>
</tr>
<tr>
<td>Somerset West</td>
<td>021 852 3144</td>
</tr>
<tr>
<td>Vereeniging</td>
<td>016 440 6300</td>
</tr>
<tr>
<td>Windhoek</td>
<td>00264 (61) 431 3000</td>
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Other:

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<tr>
<th>Service</th>
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<tbody>
<tr>
<td>Results / Helpdesk:</td>
<td>021 596 2130</td>
</tr>
<tr>
<td>Transport:</td>
<td>021 596 3669</td>
</tr>
<tr>
<td>Consumables / Stock:</td>
<td>021 596 3679</td>
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<tr>
<td>Account enquiries:</td>
<td>0860 100 442</td>
</tr>
<tr>
<td>Client Services:</td>
<td>021 596 3800</td>
</tr>
</tbody>
</table>

REFERENCES

2. Diagnostic Cytology by Koss
3. Red Cross SOP for muscle biopsy sampling
4. Theory and Practice of Histology Techniques by Bancroft
5. BD Brochure

Compiled by: Wybrand van Wyk, June 2016, 2nd Print