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|  | MANUAL | HH/MAN-004 |
| | CYTOLOGY SAMPLING MANUAL | Version No: 3.0 |
| | Compiled By: ANINA RADEMAN; MAURITZ HILL; JOAN PETERSEN | Implementation date: 30/06/2023 |
| | Approved By: ANINA RADEMAN | Page 1 of 21 |

CYTOLOGY SAMPLING MANUAL

LABORATORY CONTACT DETAILS:

Physical address:

Block D, Lagoon Beach Business Park, Lagoon Beach Drive, Milnerton

Tel. 021 553 4006 (all hours)

Fax: 021 553 4072

Email: requests@holmlab.co.za

OPERATING HOURS:

08:00 – 18:00 Monday to Friday

FOR CLINICAL ADVICE:

During work hours: 021 553 4006 and ask to speak to any of the Pathologists

After hours: 021 553 4006 - follow prompts to be transferred to Pathologist on call

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Purpose:

To serve as reference to our users and to ensure that the correct procedure is followed for taking, preserving, and transporting cytology samples.

We require the following information:

- Name of patient
- ID no / Date of birth
- Gender
- Address and medical aid details
- Date of collection of specimen
- Referring doctor
- Nature and origin of specimen
- Adequate history including previous treatment e.g. Previous radiotherapy
- Previous histology and cytology (diagnosis)
- Clearly labelled / identifiable specimen container / slides (instructions below)

In addition, gynaecological cases (Pap smears) also need the following information supplied:

- Specimen type ea. Cervical smear or Vault smear, LBC or Conventional smear
- Date of last menstrual period (LMP)
- If patient is currently pregnant
- If patient is post-menopausal
- Hormones / Contraceptives
- Appearance of the cervix
- Relevant clinical history ea. previous procedures ea. Total or subtotal hysterectomy, cone biopsy, radiation treatment and date of these procedures or previous conditions ea. atypia, LSIL, HSIL or carcinoma

URGENT REQUESTS

- For **urgent cases** make sure that the request form is clearly labelled as “URGENT” and contains a **contact number and e-mail address** where possible as well as a **time /date** when the result is required. It is also advisable to contact the laboratory and alert them of the urgent nature of the specimen in order for

- the laboratory to, where possible, make arrangements for collection and transport to ensure prompt receipt and processing of specimen.
- Request forms, slides, slide holders, spray fixative, Thin prep[®] vials and brushes (both “combi” and “cervex”) are available from the drivers or the laboratory on request.

***Please take note of the following when labeling and transporting**

A. SLIDES

- Please label slides in presence of patient to ensure correct patient identification.
- Please label with **NAME, SURNAME and DATE OF BIRTH**
- Please use standard slides with **frosted end** for labelling.
- Please label with **PENCIL ONLY** on frosted end (use diamond pen to scratch name on non-frosted slides).
- **NEVER use STICKERS or INK** to label slides as they do not withstand the staining process.
- Name and smear should be on the **same side** of the slide.
- Please **do not** send an **unlabelled slide** in a **labelled container** [If it is taken out of the container it is no longer labelled and poses a risk for misidentification]
- Please transport slides in the plastic (or cardboard) slide holders (mailers) provided.
- Please **do not** use the Cytology request form to **directly wrap** the slides, as this poses an infection risk. (Admin staff handles forms without gloves.)
- Discard all collection material into appropriate waste containers.

B. FLUID SPECIMENS

- Please label specimen in presence of patient where possible to ensure correct patient identification.
- Apply patient label containing patient details on both the specimen container and request form or write details clearly in allocated space, ensuring that both the request form and specimen are labelled correctly and that details correspond
- Please label with **NAME, SURNAME, DATE OF BIRTH and SPECIMEN ORIGIN/TYPE**

- Ensure that the lid is securely tightened to avoid leakage and if a partitioned bag is provided, place the specimen container in the sealable pocket and the accompanying request form in the adjacent pocket.
- There is no specific storage or transport requirements for fixed fluids.
- For storage and transport instructions of unfixed fluids, please refer to the specific specimen/test requirements in this document.
- Discard all collection material into appropriate waste containers.

Specimen Rejection criteria

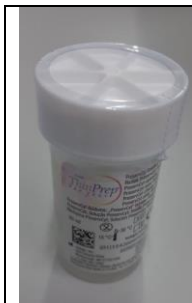
- Unlabelled or improperly / incorrectly labelled slide / specimen
- Request form received without critical information mentioned on p.4 e.g. patient name and surname, date of birth or ID number, requesting doctor and specimen type or origin.
- Specimen without a request form or request form without a specimen
- Patient information on specimen do not match that on the request form.
- Specimen has leaked out of container or slides received irreparably broken.
- Sample too little to perform the test
- Sample type unsuitable for the test requested or incorrect fixative used.

Cytological Tests According to Organ System


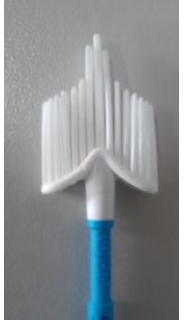
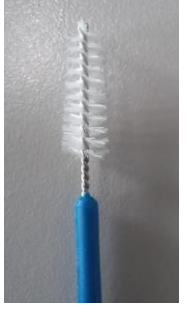
Female genital tract

1. Cervical smear (VCE)
2. Vault / vaginal smear
3. Vulvar smear
4. Endometrial sampling/smear


LBC sampling



For LBC sampling use Thinprep vial containing PreservCyt solution

| | |
|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <p>Rovers Cervex-brush® (Combi brush) has an elongated central spine with fine bristles to improve endocervical sampling is the preferred brush to use routine cervical smears.</p> <p>(Combination of the Broom and Endocervical brush)</p> |
|  | <p>Rovers Cervex brush®(Broom) may be used during pregnancy as it is better tolerated by the patient because of the soft and flexible bristles in the centre</p> |
|  | <p>Craig brush (Endocervical brush) not recommended as it causes bleeding and obscures / traumatise cellular morphology and thereby preventing accurate assess of cellular material.</p> |

Conventional cervical sampling

| | |
|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|  | <p>Please use standard glass slide with frosted end for labelling using graphite pencil and fix immediately (withing 5 seconds) using cytologic spray fixative.</p> <p><i>*Please see above notes on slide labelling, Addendum I for proper fixation technique and Addendum III for notes on how to take an optimal pap smear (both LBC and conventional)</i></p> |
|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

What about sampling during menses?

Menstrual blood can obscure significant findings and prevent optimal sampling therefore it is preferable to avoid sample collection during menses. The ideal to schedule an exam two weeks after the first day of the last menstrual period (i.e. mid-cycle). (*ACOG and CLSI guidelines*)

What about the use of lubricant gel?

The pap smear should be taken before the PV examination / ultrasound is done. Lubricant gel prevents optimal sampling as it forms a slippery film over the cervical surface. It also prevents optimal processing, blocks filters and cause cellular clumping that prevents optimal visualization of the already sparse cellular material. In addition, lubricants are found to cause invalid PCR tests.

Carbomer-free lubricants (e.g. KY Jelly, Beta gel etc.) should only be used when necessary and applied sparingly onto the exterior blades of the speculum, taking care that none of the gel gets transferred onto the cervix. For patients who do not need lubricant, lukewarm water may be used to lubricate the speculum.

Respiratory system

1. Sputum
 2. Bronchial brushings
 3. Bronchiolar-alveolar lavage (BAL)
 4. Bronchial washings
 5. Tracheal aspirates
 6. Pharyngeal brushings
 7. Antral aspirates /sinus washings
 8. Nasal smears
- Please submit sputum after an **early morning deep cough** to ensure that sputum, and not saliva, is collected.
 - Please **fix** these fluids with an **equal amount of 50% or 70% alcohol**.
 - Please make sure that if multiple specimens were collected by use of different techniques or from different sites – the **specimen type is clearly marked** on the container.

Fluids

Body cavity fluids:

1. Pleural
2. Peritoneal
3. Pericardial
4. Peritoneal washings

Please ensure that the fluids reach our laboratory as soon as possible – in case of a delay where specimens would not be processed the same day, please add **equal amount of 50% or 70% alcohol** or **cytological preservative** ea. CytoRiche[®] if available (please indicate if alcohol/preservative was added). Unfixed fluids may also be refrigerated overnight at 4 – 8 degrees C but should NEVER BE FROZEN as it will destroy cellular morphology.

Other fluids:

1. Cerebrospinal fluid *
2. Cyst fluid
3. Hydrocoele
4. All other

Fix with **equal amount of 50% or 70% alcohol** irrespective of delay. Please indicate whether alcohol fixative was added. *[Cells in CSF deteriorates very fast and should be fixed immediately, preferably with 50% alcohol.]

Gastro-intestinal tract

Oesophageal brushing
Gastric brushings
Duodenal brushings
Pancreatic duct aspirates
Bile duct aspirates / brushings
Colonic brushings

It is very important that the slides are **fixed immediately** (within 5 seconds) with cytological spray fixative to prevent degeneration of cells. See Addendum 1 on correct fixation of specimen.

Urogenital tract

Voided urine
Catheterized urine
Ureteric urine
Renal cyst aspirate
Renal pelvis brushings
Urethral smear

Please state clearly if the patient has recently:

- Undergone catheterization
- Undergone cystoscopy
- Undergone retrograde radiography

Cells in urine deteriorate rapidly. Please fix with an **equal amount of 50% alcohol**. **

PLEASE NOTE: NEVER fix URINE in CYTORICH® preservative as it contains a lysing agent and important red blood cell morphology will be destroyed.

Also note that early morning urine and 24h urine collections are unsuitable for cytological diagnosis. (Midstream collection most suitable)

The Breast

Nipple discharge
Nipple smears
Breast aspirate
Cyst aspirates

Spray-fix immediately (within 5 seconds)

Fine needle aspiration (FNA)

Impalpable/ deep / image guided FNAs

Superficial of palpable lesions

- See **Addendum II** for detailed method.
- Spray-fix immediately (within 5 seconds)
- If **more than 2 smears** are made, one could be left unfixed for Giemsa stain, but should be clearly **marked “unfixed” or “air dried” on slide**.

- Needle can also be rinsed in Cytological preservative ea. CytoRich[®] fluid or alcohol. If extra material is available and a cell block preparation needs to be made for immunohistochemical investigation, formalin can be used as fixative. Please indicate what fixative was used (unless it is already indicated on container).

Miscellaneous

Tumour imprints

Lymph node imprints

Skin smears

Tzank smears

Ulcer smears

Tissue imprints

Diaphragm wipes

- Adequate and rapid fixation is essential.
- Please note that material on a swab is not suitable for cytologic investigation.

ADDITIONAL INVESTIGATIONS

PCR Tests currently offered, including Specimen Collection and Transport

1. HPV Genotyping:

HPV28 (19 High-risk Genotypes & 9 Low-risk Genotypes) Detection is an in vitro diagnostic medical screen designed for qualitative detection of human papillomaviruses in cervical specimens self-collected vaginal specimens.

Cervical specimens collected in PreservCyt solution (LBC pap smear)

Co-testing (select HPV PCR) or reflex testing (select HPV PCR if ASCUS/LSIL) may requested on Pap smear request form.

After requests for HPV genotyping may be requested up to 4 months after collection.

*Please complete “after request form” and e-mail request to requests@holmlab.co.za

Estimated TAT – 24hrs

2. STI Essential Assay (7 Pathogens Screened)

STI Essential Assay is a qualitative in vitro test for single or multiple detection of *C. trachomatis* (CT), *N. gonorrhoeae* (NG), *M. genitalium* (MG), *M. hominis* (MH), *U. urealyticum* (UU), *U. parvum* (UP), and *T. vaginalis* (TV) from urine, genital swab, liquid based cytology specimens, semen, oropharyngeal (throat) swab and anorectal swab.

(a) First catch male and female unpreserved urine specimen can be shipped and/or stored at 4°C up to 8 days. (Preferred sample) (Please ask the driver to put specimen in cooler box)

(b) Vaginal swab samples in swab transport reagent tubes are stable for up to 60 days at 2°C to 30°C. (Preferred sample)

(c) Cervical specimens collected in PreservCyt solution (LBC pap smear) can be stored at 2-30°C for 7 days.

STI Essential Assay may be requested on the Pap smear request form. *Please complete “after request form” and e-mail request to requests@holmlab.co.za

Estimated TAT – 3-5 days

3. MTB (Mycobacterium tuberculosis) & Multi-Drug Resistance (MDR):

MTB/MDRe Detection is a qualitative in vitro test for single or multiple detection of *Mycobacterium tuberculosis* and its resistance to first-line anti-tuberculosis drugs (Isoniazid and Rifampicin)

(a) Fresh Sputum (Preferred). Patient should be sitting or standing. Have the patient rinse his/her mouth twice with water. Open the lid on the sputum collection container. Have the patient inhale deeply, cough vigorously, and expectorate the material into the container.

Avoid spills or soiling the outside of the container. Secure the lid on the collection device. Store and transport specimens at 2°C to 8°C prior to processing whenever possible. However, if necessary, the specimens can be stored at a maximum of 35°C for 3 days or less and at 2°C to 8°C for 4 to 10 days.

(b) Fresh tissue – small piece of tissue can be processed.

(c) Biopsy – wax block

Estimated TAT – 3-5 days

4. Vaginitis Screening Assay:

Vaginitis Screening Assay is a qualitative and quantitative in vitro test for the single or multiple pathogen detection of Lactobacillus spp. (Lacto; Lactobacillus crispatus, Lactobacillus gasseri and Lactobacillus jensenii), Gardnerella vaginalis (GV), Atopobium vaginae (AV), Mobiluncus spp.(Mob; Mobiluncus mulieris and Mobiluncus curtisii), Candida albicans (CA), Candida others (CO; Candida krusei, Candida glabrata, Candida dubliniensis, Candida parapsilosis, Candida tropicalis and Candida lusitaniae) and Trichomonas vaginalis (TV).

(a) Genital swab samples in swab transport reagent tubes are stable for up to 60 days at 2°C to 30°C. (Preferred sample)

(b) Cervical specimens collected in PreservCyt solution (LBC pap smear) can be stored at 2-30°C for 7 days. (LBC samples may not be as sensitive as first catch urine or vaginal swab samples but can be used)

Estimated TAT – 3-5 days

5. Ulcer Pathogen Assay

Ulcer Pathogen Assay is a qualitative in vitro test for single or multiple detection of Herpes simplex virus type 1 (HSV-1), Herpes simplex virus type 2 (HSV-2), H. ducreyi (HD), Cytomegalovirus (CMV), Lymphogranuloma venereum (LGV, C. trachomatis Serovar L), T. pallidum (TP), and Varicella-zoster virus (VZV) from urine, genital swab, and liquid based cytology specimens.

(a) First catch male and female unpreserved urine specimen can be shipped and/or stored at 4°C up to 8 days. (Preferred sample) (Please ask the driver to put specimen in cooler box)

(b) Vaginal swab samples in swab transport reagent tubes are stable for up to 60 days at 2°C to 30°C. (Preferred sample)

(c) Cervical specimens collected in PreservCyt solution (LBC pap smear) can be stored at 2-30°C for 7 days.

Estimated TAT – 3-5 days

SAFETY FIRST:

Protect the patient

- The patient's well-being, safety and rights are our main concern.

- When wearing gloves, change them after each patient and wash or disinfect hands.
- Place specimen collection equipment, e.g. needles away from the patient, especially from children or psychiatric patients

Protect yourself

- Always wear gloves and protective clothing when handling body fluids or blood
- Wash hands frequently; at least after each patient
- Dispose of all used items in the appropriate waste containers
- Dispose of needles directly after use. Do not bend, break or re-cap needles as it may pose a risk of accidental needle stick injury or may splash contents
- Clean up bloody or body fluid spills immediately using a 10% chlorine or other institution accepted disinfectant solution, as it may pose a risk of infection to yourself and others.

In case of a needle stick injury:

- Stay Calm
- Dispose of contaminated needle and gloves in the appropriate waste containers
- Squeeze the puncture site to promote bleeding
- Wash the area well with soap and water
- Take down the name and ID number of the patient
- Follow the protocol for needle stick injury for your institution

GENERAL CLIENT INFORMATION**A. Complaints and compliments**

Should you want to lodge a complaint about any aspect of the laboratory service you may:

Verbally:

- by calling 021 553 4006 and speaking to any staff member who will take sufficient information to be able to accurately complete all the necessary documentation

In writing:

- E-mailing the complaint to the requests@holmlab.co.za mailbox
- Faxing the complaint to the laboratory - 021 553 4072

- The Relationship Manager will acknowledge complaints in writing or telephonically (date and time will be recorded) within 24 hours after receipt of a complaint.
- When a complaint is acknowledged, the complainant will be informed of the estimated time it will take to resolve the complaint – usually within 48 hours.
- The complaint will be resolved, and the final outcome of the investigation conveyed to the complainant within a target time frame of 5 working days from the date the complaint was received.
- Any suggestions as to how we could improve our service to our users will be welcomed and considered.

B. Patient confidentiality

The laboratory shall be responsible, through legally enforceable agreements, for the management of all patient information obtained or created during the performance of laboratory activities. Management of patient information shall include privacy and confidentiality. The laboratory shall inform the user and/or the patient in advance, of the information it intends to place in the public domain. Except for information that the user and/or the patient makes publicly available, or when agreed between the laboratory and the patient (e.g. for the purpose of responding to complaints), all other information is considered proprietary information and shall be regarded as confidential.

Addendum I

PROPER FIXATION TECHNIQUE

1. Air-drying of a specimen causes distortion and loss of cytoplasmic density. Crisp nuclear chromatic patterns are lost, and the cytoplasm cannot be coloured properly. Hence **rapid fixation** is a vital step in cytological preparations.
2. All prepared slides should be sprayed **immediately** (within 5 seconds) using a **cytological fixative** to prevent specimen degeneration.
3. Check expiry date on spray fixative.
4. The aerosol spray fixative must be held 17-22cm away from the glass slide to avoid material being blown off the slide.
5. Keep slides horizontal to prevent run off.
6. Allow slides to dry before packing it for transport.
7. Please use slides with a ground glass edge to prevent traumatising of cells.
8. Needle / brush/ sampling device can also be rinsed in Cytological preservative ea. CytoRich[®] fluid or alcohol. If extra material is available and a cell block preparation needs to be made for immunohistochemical investigation, formalin can be used as fixative. Please indicate what fixative was used (unless it is already indicated on container).

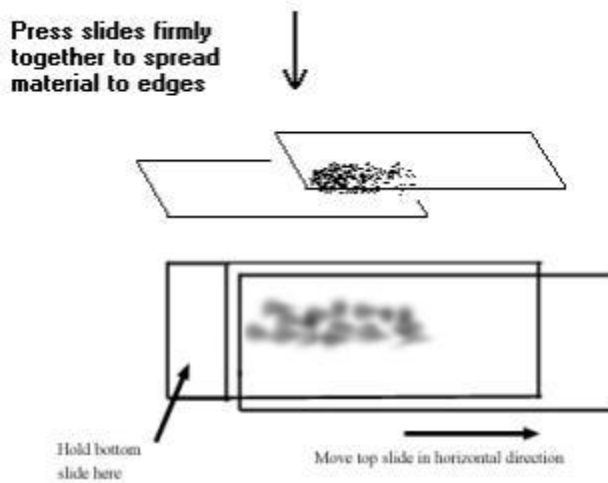
Addendum II

A. METHOD OF FNA OF PALPABLE LESION:

1. Palpate the lump and define its boundaries.
2. The lesion is immobilized between the fingers of the non-dominant hand and the skin cleaned with alcohol swab.
3. The syringe plunger must be fully in. The needle is introduced into the lesion. The plunger is withdrawn to the 2-5ml mark and kept there.
4. The needle is moved slightly sideways and slightly in and out in a fan-like movement without the tip of the needle exiting the skin, while maintaining constant suction. It is important to keep the lesion firmly immobilised between the two fingers throughout the procedure.
5. Before withdrawing the needle allow the plunger to return gently to the resting state.
6. Gently withdraw the needle.

B. METHOD OF PREPARATION OF THE SMEARS:

1. Detach the needle from the syringe.
2. Fill the syringe with air.
3. Attach the needle again and squirt the content of the needle onto the surface of the labelled glass slide.
4. A second glass slide is GENTLY placed face down parallel to the bottom slide and both slides pulled apart in separate directions. (DO NOT APPLY PRESSURE. This is to avoid traumatization of cellular material / smearing artefact)



1. Fixed slides immediately using cytologic spray fixative (within 5 seconds). The aerosol spray fixative must be held 17-22cm from the glass slide.
2. Should more than two slides be prepared, one slide may be left to air dry for a Giemsa stain. The slides should be clearly marked as to which is fixed and which is air-dried.
3. Please label all slides in pencil with the patient's name and DOB. It is best to label slides beforehand, but care should be taken to remove the name on the slides should all slides not be used.
4. The needle can be rinsed in appropriate cytological preservative for additional investigations. For cytological investigation a cytological preservative ea. CytoRich[®] fluid or alcohol is suitable. If extra material is available and a cell block preparation needs to be made for immunohistochemical investigations, formalin can be used as fixative. Please indicate what fixative was used (unless it is already indicated on container).

NOTES:

1. **Non aspiration technique** can be used for small or vascular lesions. This results in reduced blood in highly vascular lesions, such as the thyroid. This method is done by using the needle without an attached syringe or without withdrawal of plunger.
2. **Small lesions** are often mobile; therefore, stabilize the movable target by walking the lesion to an immobile position under the skin using the forefingers and middle fingers, pulling the skin taut over the lesion. In patients with small lesions in small breasts, always place the needle parallel to the chest wall to prevent causing a pneumothorax.
3. In **necrotic and fibrotic lesions** always aim for the periphery of the mass.
4. In **small subcutaneous or dermal nodules** use a 25-gauge needle and ample material will be aspirated.
5. If **bloody aspirates** are obtained, express the blood onto a slide. Place the second slide on top of this slide and gently apply pressure. Gently tilt the slides, with the edge on absorbent paper. The excess blood will run off, leaving the cellular material behind. A smaller gauge needle may be used for the subsequent needle passes.
6. **Normally 2 needle passes** (4 slides – 2 fixed and 2 air-dried) are sufficient for most lesions. **Three** needle passes are routinely performed in **breasts showing peau-de-orange**.

C. PRECAUTIONS:**Lesions requiring special caution.**

Upper inner quadrant of breast, axillary and supraclavicular nodes must be sampled carefully to avoid pneumothorax.

When aspirating highly vascular organs such as the thyroid gland, a 25-gauge needle should be used, and if possible, the non-aspiration technique applied. Pressure from a haematoma secondary to aspiration of the thyroid may temporarily impair the recurrent laryngeal nerve. Having an assistant apply pressure to the aspirated site can minimize this.

In any patient presenting with stridor due to an enlarged thyroid, do not perform the aspirate unless the clinician is at the bedside and resuscitation equipment is

immediately available. A small haematoma may precipitate acute airway obstruction requiring immediate tracheostomy.

Do not aspirate any pulsatile mass: these may be A-V malformation, aneurysms or carotid body tumours. Carotid body tumours occur adjacent to the common carotid artery and adherent to it. Characteristic clinical features of carotid body tumours are a mass that is mobile horizontally but not vertically and may be pulsatile, with a bruit.

Do not perform an aspirate on a patient who is uncooperative and/or confused. This may result in a needle-stick injury.

Deep organ aspirates must preferably be done under radiological guidance.

Addendum III

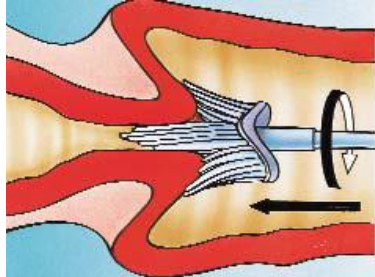
HOW TO TAKE AN OPTIMAL PAP SMEAR

A. Conventional pap smear

1. Get everything ready
2. Label slide and form
3. **Do smear first (*before PV/ultrasound*)**
4. Spread labia
5. Insert speculum (*see notes on use of lubricant gel below, p.14*)
6. Visualise external os
7. Swab cervix free of blood / discharge / gel
8. Scrape full circumference firmly
9. Lay spatula (sampling device) flat on the side
10. Spread along the length of the slide
11. Should you make use of a cervibrush® (in addition):
 - Insert into os
 - Turn clockwise 360°
 - Roll onto slide
12. Spray-fix immediately (within 5 secs)
13. Allow slide to dry (after fixation) before packing for transport.

B. Liquid Base Cytology (LBC) (ThinPrep®)

1. Follow steps 1-7 for a conventional pap smear



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



3. Rinse the broom as quickly as possible into the PreservCyt solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. **Discard the collection device – do not leave the brush tip in the vial**



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



5. Record the patient's name, ID number (or DOB) and name of requesting dr. on the vial, and the patient information and medical history on the cytology requisition form.

AMENDMENT RECORD:

| Proposed by: | Section: | Change |
|--------------|--------------------------------|-------------------------------------------------------------------------------------|
| A Rademan | Additional investigations P.10 | After requests may be requested up to 4 months after collection instead of 6 months |
| A Rademan | Additional investigations P.10 | Updated PCR tests with new panel of PCR tests available |

DOCUMENT REVISION HISTORY

| Date reviewed: | Reviewed by: | Action: |
|----------------|---------------|-------------------------------------------------------------------------------------|
| 20/12/2022 | ANINA RADEMAN | NO ACTION |
| 29/06/2023 | ANINA RADEMAN | After requests may be requested up to 4 months after collection instead of 6 months |
| 08/12/2023 | ANINA RADEMAN | Updated PCR tests with new panel of PCR tests available |