1.0 TITLE: Procedure for Gyne Specimen

2.0 PROCESSING OF GYN SPECIMENS ON SUREPATH (LBC):
A method for preparing cytological specimens—in particular from the cervix—for microscopic evaluation in which the patient specimen is suspended in a liquid fixative and processed in semi-automated machine. The PrepStain System is a liquid-based thin layer cell preparation process. The PrepStain System produces the SurePath slides that are intended as replacements for conventional gynecologic Pap smears. SurePath slides are intended for use in the screening and detection of cervical cancer, pre-cancerous lesions, atypical cells and other cytologic categories as defined by The Bethesda System for Reporting Cervical/Vaginal Cytologic Diagnoses. The PrepStain system is based on a semi-automated procedure for the preparation of liquid based cervical cell samples. It converts a liquid suspension of a cervical cell sample into a discretely stained homogenous thin-layer of cells while maintaining diagnostic cell clusters within a 13 mm. diameter circle.

Precautions
Wear PPE when using the PrepStain Processor. For best results, follow the instructions in the PrepStain Processor Operator’s Manual exactly. There are a number of notes, cautions, and warnings that appear in the Manual. If in doubt, refer to the specific section in the manual for more information.

2.1 Patient preparation:
- The patient should not douche for 24 hours before gynecologic sample is obtained.
- It is preferable that gynecologic sample should not be taken during menstrua bleeding because of blood contamination, endometrial debris and histiocytes.
- Cervix brushes must not be used on patients who are ten weeks pregnant and onwards.
- The clinician or any qualified medical personnel is responsible to instruct the patient before specimen collection is done.

2.2 Specimen collection:
Specimen Type:
- Cervical, endocervical, and vaginal specimens Handling Condition:
- Using a broom type of sampling device or an endocervical brush/plastic spatula combination with detachable head, the gynecologic sample is collected. For best results, sampling should include posterior fornix, and exocervix with wooden or plastic spatula, after removing excess mucus. For the endocervix, obtain a commercially available endocervical brush/broom, or moistened cotton tip applicator.

After the collection, the head is placed in a preservative fluid. The vial is capped, labeled and submitted to the laboratory for
processing and analysis.

2.3 Equipment and materials
2.3.1 Equipment:
- Centrifuge
- Microscope
- Vortex mixer
- Prep stain slide processor
- Vacuum pump
- Computer

2.3.2 Materials:
- Centrifuge tubes
- Surepath Glass slides
- Sure Path Preservative Collection Vial
- Cervical Sampling Device with Detachable Head
- PrepStain Syringing Pipettes
- PrepStain Settling Chambers
- Cytology Stain Kit
- Slide and Tube Racks
- Disposable Transfer and Aspirator Kits
- Deionized Water (pH 7.5 to 8.5)
- Isopropanol and Reagent Grade Alcohol
- Plastic Coplin Jars or Slide Tank
- Clearing Agent, Mounting Media and Glass Coverslips
- Tissue paper (Kimwipes)

2.4 Reagents:
- Hematoxylin Stain 0.75%
- EA-50/ Orange G Combo Stain
2.5 **Reagent Preparation:**

2.5.1 **Buffered De-ionized Water:**
To 10 ml of Tris Buffer concentrate (PrepStain) add 500 ml of de-ionized or distilled water. Cap and invert gently to mix.

2.5.2 **Cleaning Solution: Clorox 5%**

2.6 **Storage and Stability:**

2.6.1 The storage condition for SurePath Preservative Fluid without cytological samples is up to 36 months from date of manufacture at room temperature (15-30 degrees C).

2.6.2 The storage limit for SurePath Preservative Fluid with cytological samples is 6 months at refrigerated temperature (2-10 deg. C) or 4 weeks temperature (15-30 degrees centigrade). Other reagents are stored as specified on their labels.

2.7 **Calibration:** Calibration and verification of performance is performed only by a TriPath authorized service personnel. Centrifuge calibration is performed annually by Biomed Department.

2.8 **Quality control:**

2.8.1 Control, Standards and Calibrators: Tachometer for centrifuge

2.8.2 Quality Control Procedure:

2.8.2.1 Re-screening of those cases with discrepancy between Cytology and Histology reports.

2.8.2.2 A complete review of all slides should be done when the final result does not conform to the cytological diagnosis.

2.8.2.3 Cases with history of abnormalities are re-screened when the initial tests are negative.

2.8.2.4 Records of recognized false positives and false negatives must be kept accordingly.

2.8.2.5 For every subsequent screening, a copy of previous cytology result must be provided for each patient.

2.8.2.6 A cross-reference file should be maintained to allow easy retrieval of data for statistical evaluation.

2.8.2.7 Reports should be stored for 10 years and slides for 10 years. It must be readily accessible whenever needed.
2.8.2.8 Participation of the cytotechnologist in continuing education, programs, trainings, and seminars within the hospital and outside with emphasis on audio-visuals and slide studies should be enhanced.

2.9 Limitations:
2.9.1 Gynecologic specimen should be collected using the broom-type sampling device or an endocervical brush/plastic spatula combination with detachable head(s) as provided by the manufacturer. Endocervical brush/plastic spatula combinations which are not detachable should not be used with the PrepStain System.
2.9.2 Training by authorized persons is a prerequisite for the production and evaluation of SurePath slides. Training will include proficiency examination, and for the laboratory customers, the use of instructional slide and test sets. It will also provide assistance in the preparation of training slides from each customer's own patient population.
2.9.3 Proper performance of the PrepStain requires the use of supplies indicated by the manufacturer and should be disposed of properly in accordance with institutional and government regulations.
2.9.4 All supplies are intended for single use only and cannot be reused.

2.10 Step by step Procedure for Slide Preparation and Staining:
2.10.1 Pre-coat slides are properly labeled.
2.10.2 Label specimen vials, centrifuge tubes accordingly and vortex for 15-20 seconds after checking the right volume of each.
2.10.3 Place the specimen vials and centrifuge tubes in the Prepmate rack to transfer the specimen from the vial to the rack. Confirm that each tube matches its corresponding vial.
2.10.4 Density Reagent is added to the tubes, making sure that each vial has a corresponding disposable syringe. The Prepmate is run and adjusted according to the specifics of the samples being tested.
2.10.5 The tubes are removed from the rack and centrifuged using Program# 1 (to separate the cells from the supernatant) and Program# 2 (to concentrate the diagnostic components into a cell pellet).
2.10.6 Remove the tube racks from the centrifuge and decant the supernatant in a single rapid motion at 180 degrees so as not to disturb the cell pellet. While inverted, carefully blot the mouth of all the tubes with absorbent paper and turn upright.
2.10.7 The pellets in the tubes are re-suspended by means of a Vortex, positioned corresponding to their numbers or labels and checked that they are properly seated in the PrepStain Slide Processor.
2.10.8 The previously coated slides are securely positioned and locked together with the settling chamber in the PrepStain Slide Processor the corresponding labels of the coated slides should match with those of the centrifuge tubes.
2.10.9 Before running the PrepStain Slide Processor, the following should be taken into consideration:
2.10.9.1 Reagents should be adequate and each labeled intake tubing going all the way to the bottom of the reagent bottle.
Disposable tips on the DiTi are adequate and pressed down firmly on the plastic tip holder so that the front and back tabs snap into place on the station securely.

As the PrepStain system is turned on, the computer automatically runs the GYN software application. Select "Slide Preparation and Staining" from the Main Menu of the computer and follow the instrument prompts.

An alarm sounds as each slide rack has completed the process.

Immediately remove the slide rack from the PrepStain instrument and invert to decant the residual alcohol. Before turning to an upright position, blot the excess alcohol from the settling chambers.

Taking one slide at a time, remove and discard the settling chamber and coverslip the slide after a few dips in 95% Isopropanol and two changes in Xylene to clear.

The remaining specimen can be stored at room temperature up to 4 weeks by adding 2.0 ml. of preservative and capped. It can also last up to 6 months if refrigerated at 2-10 degrees centigrade.

Cleaning of the machine after each use is a must. The procedure is indicated in the Main Menu of the computer under the "Clean up System". When this is completed, the screen will return to the Main Menu. Select quit, turn off the power to the PrepStain, and the vacuum pump.

Reprocessing in cases of instrument downtime or failure:

In case of instrument downtime, the manual method of preparation is adapted.

- The sample is centrifuged in a conical tube with the supernatant being poured-off after centrifuging.
- An aliquot of specimen is dropped on a slide and spread.
- Spraycyte fixative is sprayed on the spread specimen.
- Stain and coverslip.

In case of instrument failure during the processing of specimen.

a. Resuspend each cell pellet with 4 ml of buffered DIH2O.
b. Mix each sample 8 times & transfer 800µl of cell suspension to the chamber.
c. Allow 10 minutes for gravity sedimentation to occur.
d. Decant fluid from slide rack and blot excess liquid on absorbent paper.
e. Rinse each setting chamber with 95% alcohol, decant. Repeat and blot.
f. Remove each settling chamber and place slide in 95% alcohol.
g. Stain and coverslip.

PROCEDURE GYNAECOLOGY CYTOLOGY (CONVENTIONAL PAP-SMEARS)

3.1 Sampling:
Specimen from cervix is obtained at the clinics by Ayer’s spatula through scraping. In addition endometrial aspirate, and vaginal vault smears can also be obtained if needed and fixed with spray fixative immediately or dip in 80% isopropanol/95% ethanol.

3.2 **Reagents:** Papanicolaou stains

3.3 **Reception and Registration**
Specimen labels are checked against data on the Request Form for accuracy. All request forms are given a unique sequential number, then logged in a register as well as in the computer. If a patient has a previous cytology or histology report the laboratory number and the result are noted.

3.4 **Smear Processing**
The smears are stained by a standard Papanicolaou staining technique. If the slide is fixed with spraycyte, the carbowax content of the fixative is removed from smears in step 1 of the staining protocol by soaking the smears in 80% isoprophyl alcohol. Cover slips are adhered to the smear with synthetic mountant. Each smear has a label showing laboratory number and medical record number. The smears are examined as soon as possible.

3.5 **Microscopic Examination**
Essentially, cells on the smears are examined for nuclear changes, which suggest an existing or a premalignant lesion or other disorders. The whole smear is examined with care by experienced Cytotechnologists. Any smear, which shows suspicious nuclear abnormalities, is marked for further reference. Bethesda System 2001 for reporting cervical/vaginal cytologic diagnoses is followed.

3.6 **Reporting:**
The Cytopathologist is responsible for all reports issued from the Cytology laboratory. He reviews all reports before they are issued and also selected smears including those, which the Cytotechnologist has marked as abnormal. Typed reports are issued to, Clinics and Wards after signature/approval from the Cytopathology Consultant. Copy of reports is filed in cytology.

4.0 **BETHESDA SYSTEM 2001 FOR REPORTING PAP SMEAR**

4.1 The 2001 Bethesda System (TBS) provides a uniform format and standardized lexicon for cervical/vaginal cytopathology reports emphasizing communication of clinically relevant information. TBS has received general support from professional societies, and has gained widespread acceptance in laboratory practice.
4.2 The general format for laboratory reports has three elements: a statement regarding the adequacy of the specimen for diagnostic evaluation; a general categorization of the diagnosis (an optional element); and a descriptive diagnosis.

4.3 Box 2 summarizes the The 2001 Bethesda System classification.

4.4 SATISFACTORY FOR EVALUATION indicates that the specimen has all of the following:

4.4.1 Appropriate labeling and identifying information.

4.4.2 Relevant clinical information.

4.4.3 For LBC preparation, a minimum of 5,000 well-visualized, well-preserved squamous epithelial cells. For conventional preparation, an adequate smear contains between 8,000 - 12,000. A quantitative guideline is provided (computer-generated reference images) for the estimation of cellularity.

4.4.4 Some specimens are satisfactory but limited due to blood, inflammation and or mucous partially obscuring cellular details.

4.5 UNSATISFACTORY SPECIMEN indicates that the specimen has:

4.5.1 Seventy five percent (75%) or more of the cellular components are obscured by inflammation, blood, bacteria, mucus, or artifact that precludes cytologic interpretation of the slide.

4.5.2 Less than 5,000 well-visualized, well preserved squamous epithelial cells for LBC preparation.

4.5.3 Less than 8,000 well-visualized, well- preserved squamous epithelial cells for conventional preparation.

Note: Specimen adequacy is evaluated in all cases. However, any epithelial abnormality is of paramount importance, and must be reported regardless of compromised specimen adequacy. If abnormal cells are detected, the specimen is never categorized as unsatisfactory. Such cases may be considered satisfactory.

Bethesda System 2001

Specimen type
Indicate conventional smear (Pap smear) vs. liquid-based vs. other.

Specimen adequacy
- Satisfactory for evaluation (Describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g. 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)

General categorization (optional)
- Negative for intraepithelial lesion or malignancy
Epithelial cell abnormality: See “Interpretation/result”
(Specify ‘squamous’ or ‘glandular’ as appropriate.)
Other: See “Interpretation/result”
(e.g. endometrial cells in a woman > 40 years of age)

**Interpretation/result**

**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.)

**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

**Other non-neoplastic findings**
(optional to report; list not inclusive):
- Reactive cellular changes associated with inflammation (includes typical repair)
- Radiation
- Intrauterine contraceptive device (IUD)
- Glandular cells status post hysterectomy
- Atrophy

**Other**
- Endometrial cells (in a woman > 40 years of age)
(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)**

**Educational notes and suggestions (optional)**
Suggestions should be concise and consistent with clinical follow up guidelines published by professional organizations (references to relevant publications may be included).

5.0 **Responsibility:**
  Applies to clinicians or laboratory staff responsible for handling the specimens.

6.0 **Attachment:**
  Pap Staining MonitoringForm01
7.0 Distributions:
- LMD Administration
  - Cytology Laboratory
  - All Clinical Departments

8.0 References
- Cytology- Diagnostic Principles and Clinical Correlates Third Edition. By Edmund S. Cibas and Barbara S. Ducatman