The purpose of a cytological examination is generally for the cytological detection of malignancy. However, if other tests are requested e.g. detection of TB, fungi etc, these must be clearly indicated on the form.

Some cytological specimens require immediate fixation while others need to be fresh/unfixed specimens. All fresh/unfixed specimens should be sent to the laboratory immediately, if possible.

The sample container or glass slides (as in the case of prepared smears) must be clearly marked with the patient’s name/initials and NRIC number. If the frosted end of a glass slide is used, write with a lead pencil. Unlabelled slides will be returned to sender.

**CYTOLOGY: CERVICAL (PAP) SMEAR**

**INTRODUCTION**

The cervical (PAP) smear is a screening technique to aid in the detection of cancer and cancer precursors of the uterine cervix. It is not a diagnostic procedure. Both false-positive and false-negative results have been experienced with PAP smears. Accordingly, any lesion detected on screening should be biopsied. The PAP smear should not be used as the sole means to diagnose or exclude malignant and premalignant lesions.

**SPECIAL INSTRUCTIONS ON SPECIMEN COLLECTION AND HANDLING**

1. **Essential Patient Information**
   Complete a Test Request form including:
   - Patient’s name and age (or date of birth)
   - Date of specimen collection
   - Source of material submitted (cervical, endocervical, vaginal, other body site)
   - Submitting physician’s name and contact number

   Additionally, the following pertinent clinical information must be supplied:
   - Last menstrual period (LMP)
   - Hormonal status (e.g. post-menopausal, gravid)
SECTION 4: SAMPLE COLLECTION & HANDLING – SPECIAL INSTRUCTIONS & LAB TESTS

1. Exogenous hormone therapy (including birth control pills, treatment for endocrine-responsive malignancy, estrogen creams)
2. Use of intrauterine device (IUD)
3. DES exposure
4. History of abnormal cytology and gynaecological disorders
5. History of systemic chemotherapy, pelvic radiotherapy, gynaecologic surgery, cryosurgery, electrocautery, or laser surgery;
6. Any current abnormal clinical findings or patient symptoms; and
7. Risk factors for cervical cancer (e.g. multiple sexual partners, sexually transmitted diseases including human papillomavirus [HPV], sexual activity at an early age, and smoking) if obtainable.
8. Date of last gynaecological smear, if any.

It is imperative that these instructions be strictly adhered to, omission of which may result in delay of reporting.

2. Sample Collection
   - A single slide technique is strongly recommended.

PATIENT PREPARATION

Proper patient preparation encompasses the following:
- Ideal sampling date is two weeks after the first day of the LMP.
- Discourage sampling during normal menses
- Avoid use of vaginal medication, vaginal contraceptives, or douches for 48 hours prior to examination.

CERVICAL (GYN) PAP SMEAR: COLLECTION PROCEDURE

GENERAL CONSIDERATION

Successful methods of specimen collection include the following:
- Before sample collection, label the frosted end of the glass slide with the patient’s initial and NRIC number. The identifiers should be legibly printed on the frosted end of the slide using a hard lead pencil. If the specimen consists of more than one slide, it is mandatory that the source of the specimen be indicated.
- It is important to obtain a smear that is not obscured by blood, mucus, or inflammatory exudate.
- During smear taking, clinician may wipe away excess mucus plug at the cervical os with ring forceps holding a folded gauze pad.
- Inflammatory exudate may be removed by placing a piece of gauze over the cervix and peeling it away after it absorbs the exudate.
- Visually inspect the cervix for abnormalities. Identify the transformation zone, if visible, and direct sampling efforts to encompass this area.
- If there is overlying mucus and exudate or necrotic material, these should be removed prior to sampling underlying lesion.
- Choose the contoured end of the spatula that best conforms to the anatomy of the cervix and the location of the transformation zone. A plastic spatula is recommended. Rotate the spatula at least 360° about the circumference of the cervical os and ectocervix, while maintaining firm contact with the epithelial surface. (see Figure 1)
Note: A clockwise rotation beginning and ending at 9 o'clock (or counter-clockwise rotation from 3 o'clock to 3 o'clock) will position the spatula so that the collected material is retained on the upper horizontal surface as the instrument is removed.

**Figure 1 – Sampling of the Cervix with Three Different Instruments: Spatula, Brush, and “Broom”**

- If a quantitative maturation index is requested, a portion of the specimen must be taken from the lateral vaginal wall and smeared on a separate slide.

**SINGLE SLIDE TECHNIQUE** (recommended method for Cervical/Pap Smears)

1. Place the material collected near the frosted end of the slide.
2. Spread the material collected evenly over the glass slide with a single, smooth stroking motion along the entire length of the slide within a few seconds.
3. Apply spray fixative immediately (see Figure 6). Allow fixative to dry before closing the slide container. Alternatively, specimen may be fixed in 95% alcohol.

**Figure 2 – To transfer material from the spatula, smear the sample with a single stroking motion using moderate pressure to thin out clumps of cellular and mucus material. Avoid excessive force or manipulation, which will damage cells.**

**Figure 3 – To transfer material from the brush, roll the bristles across the slide by rotating the brush handle.**

**Figure 4 – To transfer material from the broom, smear the sample with a painting action, using both sides of the broom.**
SECTION 4: SAMPLE COLLECTION & HANDLING – SPECIAL INSTRUCTIONS & LAB TESTS

CYTOBRUSH TECHNIQUE

1. Introduce an extended-tip spatula into the endocervix. Rotate the spatula through 360° pivoting at the os. Withdraw the spatula and put aside temporarily, keeping the material on the spatula.
2. Introduce an endocervical brush device into the cervix. Rotate the brush 90°–180°. (A brush is not recommended in pregnancy, cervical stenosis, or other clinical conditions indicated by the manufacturer).
3. Spread the spatula specimen onto the left side of the slide and fix while covering the right side.
4. Roll the cytobrush sample over of the right side of the slide and fix.

Figure 5 – Spread the spatula sample over the left side of the slide and fix while covering the right side. Roll the brush over the right side of the slide and fix.

QUALITY INDICATORS

INADEQUATE SMEARS

1. A satisfactory smear should show well-preserved and well-visualised squamous cells covering at least one-third of the area of a regular glass slide surface.
2. If fewer than these are seen because of paucity of cells, poor fixation, air-drying artefact, thick smearing, or covering of blood, inflammatory exudate or other contaminants, the smear is consider unsatisfactory.
3. A smear comprising mainly endocervical cells is also considered unsatisfactory, unless the smear was intended to specifically evaluate the endocervical canal.

ENDOCERVICAL CELL / TRANSFORMATION ZONE (EC/TZ) COMPONENT

1. At least 10 well preserved endocervical columnar cells or squamous metaplastic cells qualifies as an EC/TZ component.
2. If < 10 cells are seen, the EC/TZ component is reported as absent.
3. In the presence of atrophy, only definite squamous metaplastic or endocervical cells count as an EC/TZ component.

HORMONAL EVALUATION

A separate slide must be taken from the lateral vaginal wall for optimum reliability. This request should be clearly stated in the request form.

TURNAROUND TIME

100% of cases are reported within 7 working days

DAY(S) TEST SET UP

Daily
GENERAL INSTRUCTIONS

SMEAR PREPARATION
1. Write the NRIC number in pencil on the frosted end of each prepared slide.
2. Submit 4 slides of material from any source that can be evaluated cytologically.
3. Fix slides immediately with cytology spray fixative before air-drying occurs or immerse in 95% ethyl alcohol for 20 minutes.
4. Allow fixative to dry thoroughly before packaging slides in the appropriate slide container for transport.
5. If air-dried smears (for indications, see specific sites), one to be submitted together with wet fixed smears; the slides should be labelled ‘wet-fixed’ or ‘air-dried’ as appropriate.

FLUID
1. Submit 20 to 50 mL of fluid fresh to the Cytology Lab within 1 hour of collection. If delay is anticipated, refrigerate at 4ºC. Exceptions include CSF and urine, which degenerate within one hour, even with refrigeration and should be sent immediately to the laboratory.
2. Place fluid in a tightly capped, appropriately labelled container.

THE REQUEST FORM
All specimens should be accompanied by the appropriate request form. The request form must include:
- Patient’s name, age, sex and identification number
- The hospital and ward number, or name of clinic and telephone number, as this facilitates despatch of reports
- Summary of clinical history
- Operative findings
- Type of sample and body site
- Provisional diagnosis
- Name of physician/surgeon in charge of case
- Previous biopsy number or date of previous operation

Failure to provide all the above information will delay turnaround time for the Cytology report.

When more than one specimen is sent from the same patient at the same operation, use only one form.

SLIDE FIXATION
Fixatives are agents that are used on smear preparation to prevent cell distortion and to maintain true morphologic structure. Distortion due to improper fixation nearly always prevents proper and accurate evaluation of the cell population.

After the specimen has been spread evenly on the slide, the slide should be fixed immediately (within seconds). The slide(s) must not be allowed to air dry.
TYPE OF FIXATIVES

COATING SURFACE FIXATIVES

Coating fixatives (alcohol with polyethylene glycol) are those that cover the surface of the prepared smears. When coating or spray fixatives are used, the nozzle of the spraying apparatus should be held at the appropriate distance from the slide per the manufacturer’s instructions. (See Figure 6) Holding the pump-spray fixative container too close to the slide can result in the development of cellular artefacts, while holding the spray fixative container too far from the slide may result in drying artefacts or uneven fixation. Holding the spray fixative too close to the slide can also result in flooding the slide and washing or blowing away the cells. (The use of commercially available hairspray as a fixative is not recommended.)

Figure 6 – Fixation of the Spread Sample Using a Pump Spray

ETHANOL (WET FIXATION)

An alternative cellular fixative for smears is 95% ethanol. Place 95% ethanol in an appropriate container and immerse the freshly prepared smear immediately into the fixative. If the fixative is to be reused, it should be filtered.

AIR DRYING

Unintentional air-drying of smears produces artefactual changes that hamper cytologic evaluation. This can be minimised by rapid application of fixative (mentioned above).

Intentional air-drying of smears and the subsequent applications of a Romanovsky type stain are useful for evaluation of fine needle aspirates and effusions.

See following pages for site-specific instructions.
ALPHABETICAL TEST LISTING – CYTOLOGY

<table>
<thead>
<tr>
<th>Source</th>
<th>Submission Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cyst aspiration</td>
<td>If aspirate is scanty, fluid may be smeared one drop at a time on clean, dry slides and fixed immediately. If aspirate is abundant, collect in a clean tube and send fresh to the Cytology Lab. Indicate the volume aspirated.</td>
</tr>
<tr>
<td>Breast secretions</td>
<td>Drops of fluid from the nipple are smeared directly on (Nipple Discharge) clean glass slides and fixed immediately with spray fixative or immersed in 95% alcohol for 20 minutes. Submit 4 slides whenever possible, half of the smears should be left air-dried without fixative.</td>
</tr>
<tr>
<td>Bronchial brushings</td>
<td>Roll brush(es) over clean, dry slide. Fix immediately with 95% ethyl alcohol or spray fixative. The brush(es) used to prepare bronchial brushing slides may be swished in a container of 70% ethyl alcohol to dislodge remaining specimen. Submit slides and liquid together with one requisition.</td>
</tr>
<tr>
<td>Bronchial washings</td>
<td>Collect in a clean tube and send fresh to the Cytology Lab.</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>A minimum of 1 mL of CSF should be collected; 2 to 3 mL are preferred. Collect specimen in a clean tube and send immediately to the Cytology Lab. (See also Non-gynaecological smear and fluid – General Instruction)</td>
</tr>
<tr>
<td>Colonic washings</td>
<td>Collect in a clean tube and send fresh to the Cytology Lab.</td>
</tr>
<tr>
<td>Effusions</td>
<td>Collect in a clean tube and send fresh to the Cytology Lab.</td>
</tr>
</tbody>
</table>
| Fine needle aspiration         | 1. Fix 2 slides immediately (within a few seconds) using cytology spray fixative (allow fixative to dry thoroughly before packaging slides for transport) or immersed in 95% alcohol for 20 minutes. Leave 2 slides to dry without fixative.  
2. If fluid is obtained with a needle pass, it should be expressed into a clean container. Submit the liquid specimen with the fixed slides using one request form.  
3. Clinical information is required for the pathologist to render a diagnosis. Please indicate on the request form the specific site, clinical diagnosis, whether the lesion is solid or cystic and gross appearance of the aspirate if applicable. |
| Gastric brushings              | Roll brushes over two-thirds of a fully frosted slide. Fix immediately with 95% ethyl alcohol or spray fixative. The brush(es) used to prepare slides may be swished in a container of 70% ethyl alcohol to dislodge remaining cells. Submit slides and liquid together with one requisition. |
| Gastric washings               | Collect in a clean tube and send fresh to the Cytology Lab. (See also Non-gynaecological smear and fluid – General Instruction) |
| Joint aspirates for crystals   | Collect in a clean tube and send fresh to the Cytology Lab.                              |
| Oesophageal brushings          | Roll brush(es) over clean, dry slide. Fix immediately with 95% ethyl alcohol or spray fixative. The brush(es) used to prepare slides may be swished in a container of 70% ethyl alcohol to dislodge remaining cells. Submit slides and liquid together with one requisition. |
Oesophageal washings: Collect in a clean tube and send fresh to the Cytology Lab.

Pelvic washings: Collect in a clean tube and send fresh to the Cytology Lab.

Pericardial, peritoneal pleural fluids: Collect in a clean tube and send fresh to the Cytology Lab. (See also Non-gynaecological smear and fluid – General Instruction)

Pneumocystis carinii: Bronchoalveolar lavage or washings are preferred specimens. Bronchial brushings or sputum may be submitted, but the diagnostic yield is less. Send fresh specimens (see General Instruction). Fix submitted smears with cytology spray fixative or immerse in 95% ethyl alcohol for 20 minutes. Write “Evaluate for Pneumocystis carinii” on the Test Request Form.

Viral skin lesions (Tzanck Smear): Remove crust or dome from lesion. Scrape the base of the ulcer with a curette. Spread the material on alcohol-moistened slide. Spray-fix immediately or fix slides in 95% ethyl alcohol.

Sputum: Submit early morning deep-cough specimen prior to the ingestion of any food. Have the patient rinse mouth with plain water. Collect separate specimens on 3–5 consecutive mornings. Do not pool specimens. Send specimens fresh within one hour of collection. (See also Non-gynaecological smear and fluid – General Instruction)

Urine (bladder, kidney, voided): Submit all specimens fresh in a clean tube. This includes urine sent for eosinophil examination. Mark on the Test Request Form “Voided” or “Catheterised” as appropriate. (See also Non-gynaecological smear and fluid – General Instruction)

Urgent samples: Arrange with Cytology Lab at Tel: 6321 4954. Urgent cytology specimens should be indicated accordingly in the request form and delivered immediately, by hand to the Cytology Lab.

Turnaround time: 90% of cases reported within 2 working days and 100% of cases reported within 7 working days

Day(s) test set up: Daily

Other services:
(a) One-Stop Thyroid FNA Cytology Clinic
Available every Tuesday (morning only) and Wednesday within Outram Campus. Cases will be reported within a few hours of receipt. Please contact the laboratory for details.

(b) One-Stop Breast FNA Cytology Clinic
Available every Thursday within Outram Campus. Cases will be reported within a few hours of receipt. Please contact the laboratory for details.

(c) FNA Procedure Performed by a Pathologist
Available every Tuesday and Thursday at Specialist Outpatient Clinic, Room K20. Booking is required. Please contact the laboratory for details.

(d) EUS FNA – Cytotechnical Assistance
Available every Tuesday, Thursday at Endoscopy Centre, Block 6, level 2.

(e) Radiologically Guided Biopsy/Aspiration – Cytotechnical Assistance
Available every Monday and Wednesday afternoon at Diagnostic Radiology Department.