CERVICAL CYTOLOGY

PRACTICE GUIDELINES

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Prepared by the Hong Kong Society of Cytology

Cervical Cytology Practice Guideline Group

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1. INTRODUCTION

Cervical cytology has been perhaps the most successful cancer screening technique of the 20th century[1,2]. Practice guidelines for participants of the cervical screening program are important to better define and communicate standards of performance and to ensure delivery of quality health care[3-6]. The present **Cervical Cytology Practice Guidelines** are intended for use in Hong Kong by cytologists (pathologists and cytotechnologists) who conduct cervical cytology analyses and issue reports for use by clinicians. The guidelines aim to set achievable standards and targets for laboratory practice to improve the quality of cervical cytology service. The emphases of the guidelines are on practical aspects of specimen procurement, analysis, reporting as well as laboratory management. Specific microscopic diagnostic criteria, clinical management and recommendation are not included in the current guidelines. In light of the rapidly evolving science and technical methods, these guidelines will require timely review and revision. It must be understood that the cervical smear test, like any other screening test, has some inherent limitations and cannot be 100% effective in detecting abnormalities later proven to be present on the cervix. Furthermore, the unavailability of the cervical cytology test to women remains the biggest obstacle to the success of screening.

2. SPECIMEN COLLECTION

A quality sample is fundamental to ensure reliability of test and minimize false negative rates[7-9]. The following highlight some practical aspects of sampling and fixation.

2.1 Timing of Taking Cervical Sample

The sample should best be taken around mid-cycle. It is important to avoid taking a sample when the patient is menstruating, as excessive blood will obscure the smear. The patient should be instructed not to use vaginal douche, pessary or any type of lubricant 24 hours prior to sampling. Samples taken a few days prior to the onset of next menstrual period may show marked cytolysis due to large numbers of Doderlein bacilli, which are the normal vaginal flora at this time of the cycle.

2.2 Exposing the Cervix

Most pre-malignant and malignant lesions of the cervix arise in the transformation zone (squamo-columnar junction). For a sample to have maximum diagnostic value, the sample taker has to ensure that the whole circumference of the transformation zone has been sampled and sufficient well-preserved cells are collected. The cervix must be visualized with the speculum in-situ under adequate lighting. A lubricant should not be used for insertion of the speculum. Bimanual examination should be carried out after sampling to prevent lubricant contamination, trauma or dislodgment of diagnostic cells.

2.3 Collection Instrument

The collection instrument plays an important role in sample adequacy. A variety of devices such as the wooden or plastic spatula, endocervical brushes and the "broom-

type" samplers are available for obtaining conventional cervical samples. The use of cotton-tipped swab is not recommended.

In woman with a visible transformation zone, a single sample obtained with either a spatula or "broom-type" brush is probably adequate. In postmenopausal woman and woman with previous cervical treatment, the transformation zone is often anatomically higher and not visualized. In these circumstances, the addition of an endocervical brush is recommended. It must be noted that an endocervical brush alone is not an adequate examination and an ectocervical sample should always be submitted as well. In pregnant woman, an endocervical brush may be contraindicated. One should always refer to the manufacturer's recommendations. The choice of a particular device is dependent on variations in the size and shape of the cervix and clinical conditions. The sample taker should consider factors that may change the features of the cervix when choosing a collection device.

2.4 Conventional Papanicolaou Smear

2.4.1 Obtaining Cellular Sample

When using the spatula to collect sample, it should be applied with some degree of firmness and rotated around the full circumference of the cervix. For "broom-type" sampler, it should be rotated 5 times in a clockwise direction with the central longer bristles in the canal. Slight bleeding is to be expected and will not interfere with interpretation. Similarly, a small amount of mucus does not affect interpretation. However, if a large plug of mucus obscures the external os, it can be removed gently with a swab prior to sample collection. If an endocervical brush sample is required, this should follow the spatula sample as it may cause slight bleeding. The brush is gently inserted into the endocervix and rotated one half to one full turn.

2.4.2 Smear Preparation

The spatula with the sample should be rapidly but lightly stroked, thinly and evenly across the surface of the slide without any delay. Cells obtained with the endocervical brush may be placed on the glass slide by a rolling action. If a "broom-type" sampler is used, the sample is transferred using a firm paint stroke motion along the long axis of the slide. The broom is then turned over and the paint stroke motion is repeated over the same area. In case where use of two applicators is indicated, both the spatula/ "broom-type" sampler and endocervical brush samples may be placed on one slide. One half of the slide near the labeled end is covered with thin cardboard and the spatula/ "broom-type" sampler sample is spread and spray fixed. The cardboard is then removed and the endocervical brush specimen is rolled along the remaining half of the slide and spray fixed. Avoid using slides that have been stored for long period as there may be fungal overgrowth that can affect proper interpretation.

2.4.3 Smear Fixation

The smear must be fixed rapidly by immersion in 95% alcohol or spraying with fixative to prevent air-drying artifact which can occur within seconds. Pre-fixation air-drying results in degenerative changes with loss of cellular features compromising proper interpretation and diagnosis. It should be noted that smears from postmenopausal patients and blood stained smears dry very rapidly. For wet fixation

with alcohol, the volume of the alcohol must be sufficient to cover the slide and that the alcohol is changed regularly. Spray fixation with an aerosol fixative is a good alternative and is generally considered more convenient. However, only good quality cytology spray fixatives should be used. When applying spray fixative, one should follow the manufacturer's instructions. Generally, it should be 15-25 cm from the glass slide when applied. If positioned too close, cells may be washed away or the spray fixative may freeze the cells causing great distortion artifacts. Do not use spray fixative beyond expiry date. Hair spray should not be used.

2.5 Specimen Collection for Liquid-based Preparation

2.5.1 ThinPrep[10]

The transformation zone of the cervix is sampled by using a broom-type sampler specified by the manufacturer. The broom is pushed gently into the endocervical canal until it reaches deep enough for the shorter bristles to contact the ectocervix. It is then rotated in a clockwise direction 5 times to obtain the sample. After the sampler is removed, it is then pushed into the bottom of the appropriate vial forcing the bristles to spread apart for about 10 times. Finally, the broom is swirled vigorously to further release material into the preservative solution. The broom is then discarded (not into the vial). The vial is then capped, labeled and sent with appropriate request form to the laboratory. The manufacturer's instructions may be revised and must be referred to and followed.

2.5.2 Autocyte Prep[11]

The transformation zone of the cervix is sampled by using a broom-type sampler specified by the manufacturer. The longer central bristles of the broom are inserted into the endocervical canal. The sampler is then gently pushed until it reaches deep enough for the shorter side bristles to fully contact the ectocervix. The sampler is then rotated in one direction 5 times to obtain the sample. Do not rotate back and forth. After the broom is removed from the cervix, the entire detachable head of the broom is removed from the handle of the sampler and placed into the appropriate vial. The vial is capped, labeled and sent with appropriate request form to the laboratory. The manufacturer's instructions may be revised and must be referred to and followed.

2.6 Schedule for Repeats

In general, all repeats should not be performed within 6 – 8 weeks. It is because the scraped surface may not have re-epithelialised and the chance of a false negative result is increased. If colposcopic investigation is performed within this period upon a report of possible or definite abnormality, a concurrent cytology sample is not recommended.

3. SPECIMEN SUBMISSION

All specimens should be submitted to the laboratory accompanied with completed cytology request forms. The glass slide or specimen vial must be labeled with the patient's name and one other unique identifier. Unique identifier refers to information that can uniquely identify the patient, e.g. identity card number, clinic/hospital number, passport number etc.

The request form accompanying the specimen should be completed which should include patient's name, one other unique identifier, age/date of birth, date of collection of specimen, type of specimen and name of requesting physician. Pertinent clinical details are essential for reliable cytological evaluation. Important data such as date of last menstrual period, whether the patient is pregnant, postnatal or postmenopausal as well as use of oral contraceptive, other hormonal therapy and intrauterine contraceptive device usage should be given. History of previous abnormal cervical cytology, history of cancer, radiotherapy, chemotherapy and gynecologic surgery should be mentioned. Relevant clinical signs or symptoms such as abnormal cervical assessment, post coital bleeding or contact bleeding are essential.

All specimens should be packaged carefully to prevent breakage or leakage and transported to the laboratory for processing.

4. LABORATORY PROCESSING

Laboratories should have Standard Operating Procedures (SOP) for sample processing starting from receipt of specimens to delivery of the stained slides for microscopic examination. The integrity of specimens must be maintained throughout processing and the principles of universal precautions should be followed. The technologist-in-charge should ensure that the SOP are followed and reviewed when necessary.

4.1 Specimen Reception

Upon receipt of specimen, the following should be checked.

(1) Specimen label: Every smear or vial for liquid-based preparation should be individually labeled with patient's name and one other unique identifier. Information on the specimen label should match exactly with corresponding information on the request form.

(2) Specimen type: The specimen appearance should be consistent with the specimen type indicated on the request form.

(3) Specimen integrity: Glass slide should be checked for breakage and vial for liquid-based preparation should be checked for leakage.

4.2 Specimen Rejection

Unlabeled specimen, identity mismatch between specimen and form, empty vial or irreparably broken slide should be returned to the requesting physician for rectification. The reason for rejection should be clearly specified by a note attached to the rejected specimen. The rejected specimen should be marked to facilitate identification upon return.

A logbook should be kept for all rejected specimens. Information that should be recorded includes:

(1) Date and time of specimen rejection
(2) Patient's name
(3) Patient's Hong Kong identity card number or other unique identifier
(4) Specimen type
(5) Destination for returning the specimen
(6) Reason(s) for specimen rejection

(7) Name and signature of laboratory personnel who handles the rejection

4.3 Specimen Accessioning

Specimen accessioning involves the assigning of every accepted specimen with a unique laboratory (accession) number. The laboratory number is used for identification of the specimen in the laboratory. No specimen should be processed without a laboratory number. The same laboratory number should never be assigned to more than one specimen. Specimen accessioning should be performed immediately after verification of its identity. In order to avoid mixing up of specimen, specimen should always be accessioned one after another.

4.4 Liquid Based Specimen

Liquid-based specimen should be processed according to the manufacturer's instructions for transfer of cells from the liquid medium to a glass slide[10,11].

4.5 Staining Procedure

The modified Papanicolaou method is recommended for the staining of cervical cytology slides[12]. It uses a standard nuclear stain, hematoxylin and two cytoplasmic counterstains (OG-6 & EA). A progressive or regressive method may be used for nuclear staining. Several automatic programmable stainers are available. Each laboratory should have a staining protocol for manual or automated staining methods that result in optimal staining of specimen. In order to maintain good staining quality, staining solutions should be filtered and changed on a regular schedule. The quality of stain should be monitored daily. To minimize cell loss, slides should be handled gently. To minimize cross-contamination, cervical smears are usually stained separately from non-gynecologic smears or staining solutions be changed or filtered between gynecologic and non-gynecologic samples.

4.6 Dehydration, Clearing & Coverslipping Procedure

The slide is dehydrated in a series of graded alcohol and clearing agent such as xylene. Slides should be removed from the clearing agent (xylene) one at a time to avoid drying artefacts. Care should be taken to limit exposure to xylene by using a fume absorber or fume hood. Mounting medium that is compatible with the clearing agent, transparent and with a refractive index similar to glass slide and specimen should be used for binding the coverslip. Most commercially available mountant have refractive indices ranging from 1.49-1.57, which is compatible with that of glass slide. Sufficient but not excessive mounting medium should be applied to form a protective seal. Coverslips can be placed manually or by automatic coverslippers. The mounting medium should be allowed to dry before the slide is being reviewed.

4.7 Checking Procedures before Distribution of Slides

An experienced technologist should check each batch of stained slides and request forms before distributing them out for screening and reporting. The following items should be inspected:

(1) The slides prepared are correctly labeled with the right laboratory (accession) numbers.

(2) The appearance of the slides is concordant with the specimen type.

(3) The number of slides is correct.
(4) The staining quality is optimal.
(5) The dehydration and clearing processes are properly done.
(6) The slides are properly coverslipped.
In case that serious error is detected, the whole batch of slides should be withheld from screening until investigation and rectification work is completed.

5. CERVICAL CYTOLOGY ANALYSIS

5.1 Qualification and Training Requirements

5.1.1 Pathologists

Histopathologist who has completed a training program in Anatomical Pathology (including cytology) of the Hong Kong College of Pathologists or equivalent and holding FHKAM (Pathology) or FHKCPath; or, equivalent qualifications is qualified to issue cytology reports. For those with training in AP/CP program, documentation of ample training in cytology is required. Medically qualified individuals in other specialty should have cytology training equivalent to that required by the Hong Kong College of Pathologists with continuous working experience after completion of training with no lapse of over 2 years and with evidence of participation in continuous medical education in cytology/pathology. Additional certification in cytology is desirable.

5.1.2 Cytotechnologists

Cytotechnologists should have special training in screening, general cytological techniques and basic laboratory management including concepts of quality assurance and laboratory safety. They should have two years of training under the supervision of a qualified trainer* in a suitable training center# (see below). A cumulative experience of screening at least 9,000 cases of cervical cytology slides under supervision is expected[2]. A record of training is recommended. A qualified screener should have Part I / II registration with the Medical Laboratory Technologist Board and certification by passing IAC examination or equivalent with continuation of work in cytology after certification with no lapse of over 2 years and/or with evidence of participation in continuous cytology education.

*A qualified trainer should be a qualified screener with at least 3 years of post-qualification full time working experience in cytology; or, a qualified pathologist with working experience in cytopathology i.e. FHKAM (pathology), FHKCPath or equivalent.

#A training center should have a minimum workload of 10,000 gynecological cytology cases per year, under the supervision of a qualified pathologist and supported by correlation with histology and regular review sessions. Laboratories with less than the recommended workload but with good teaching materials may be suitable.

5.2 Workload

Workload limits must be set for each cytology screener based on his/her capability. The actual amount of time spent on analyzing one slide is influenced by the sample preparation, cellularity, obscuring factors, clinical history and personal experience, etc [13]. Each cytotechnologist may examine up to a maximum of 100 slides (including screening and rescreening) per 24-hours (average 12.5 slides/hour) and in not less than 8 hours[3]. Pathologists who are doing primary screening and rescreening are also limited by this ceiling.

5.3 Screening Process

Screening should begin with a check of slide identification against the laboratory form and review of the available clinical history. The screening process should start with low power scan to assess cellularity and background. The actual screening is usually performed with a 10x objective and 10x magnification eyepieces. Higher magnification is used for more detailed analysis of abnormal cells and areas. The slide should be screened in a systematic and thorough way. The location of abnormal cells should be marked in a consistent pattern by all cytologists within the same laboratory to facilitate review. Care should be taken to avoid obscuring significant cytological abnormalities by the marks.

5.4 Reporting Results

Each cytologist should record his/her findings accurately and legibly for future review. All slides with epithelial cell abnormalities must be referred to a qualified pathologist for final interpretation and report.

5.5 Automated Screening

Automated screening device relies on computer analysis of digitized images to triage cervical slides for subsequent identification of pre-malignant and malignant changes. One device is at present approved by FDA for primary screening. One should refer to the Product Insert and Operator's Manual for details[14,15].

6. CERVICAL CYTOLOGY REPORTING

As the cervical cytology report is a medical consultation, a medically qualified pathologist should normally be available (preferably on site) for consultation in relation to reporting cases. The objective of the report is to convey the results from laboratory to clinician in a meaningful and unambiguous way. It should include the information provided on the request form. All specimens must be reported using descriptive nomenclature. The use of a numerical reporting system alone is unacceptable. Several reporting systems are in use. Most laboratories in Hong Kong are currently using either The Bethesda System (TBS) of cervical cytology or its modified forms for reporting cervical cytology[16,17]. TBS was first developed in 1988 and updated in 1991 and 2001.Other reporting systems including WHO, BSCC[4,18] , Walton[19], etc., may be used as long as the partner at the receiving end has a complete understanding of the meaning of the report, with regards to prognostic significance and therapeutic options. However, one standardized reporting system is preferred for better communication of cervical cytology diagnoses among different participants in

the screening program[20].

Recommendations on schedule of repeating smear, clinical management of abnormal smear results or educational explanatory notes are optional. Medical literature in these areas does not indicate a consensus approach. Some clinical professional organizations have issued guidelines for follow-up of abnormal test reports. One may refer to the guidelines published by the Hong Kong Society of Obstetricians & Gynaecologists[21,22]. The attending clinician should consider all known circumstances and apply appropriate standards of care to their decision to follow, reject or modify the recommendation for an individual patient [23-25].

7. QUALITY CONTROL AND ASSURANCE

7.1 General Principle

Quality assurance is defined as systematic monitoring of quality control results and quality practice parameters to ensure that all systems are functioning in a manner appropriate to excellence in health care delivery[26]. Laboratories should regularly follow their Standard Operating Procedures for all aspects of laboratory procedures, from collecting and processing of samples, to screening and interpreting smears, delivery of reports and policies of staff training. Problems/errors should be identified, investigated and corrected. All quality assurance processes must be described and documented in a quality assurance program in the laboratory.

7.2 Monitor Specimen Adequacy

Laboratories should develop and document sample adequacy criteria, monitor the adequacy of cervical cytology samples and provide feedback to sample taker [27,28].

7.3 Quality Control of Screening

7.3.1 Laboratories should ensure that screeners have sufficient time to screen every slide completely.

7.3.2 Pathologists should confirm significantly abnormal findings noted by cytotechnologists.

7.3.3 Peer review of difficult and borderline cases helps promote optimal patient care and uniformity in diagnoses.

7.3.4 Retrospective review of previous negative cases in patients with newly diagnosed high-grade lesions and cancers is mandatory.

7.3.5 Rescreening of negative cases is an essential part in the quality control of screening. It can be done in three ways[29-33].
(1)The most widely used is the rescreening of 10% negative cases including target risk group.
(2)Rapid rescreening of 100% cases at low magnification (x10 objective) may be used.
(3) Automated rescreening may be done by computerized equipment.

7.3.6 Laboratories should establish a system to follow-up all high grade pre-malignant and malignant cervical cytology reports by correlating with relevant histology, if available.

7.3.7 It is recommended that all laboratories participate in external quality assurance program.

7.4 Management of Incident

Policies to handle complaints, problems and errors should be developed and implemented to improve delivery of quality service.

7.5 Evaluation of Individual

Evaluation of individual cytotechnologist is recommended by continuous assessment of daily performance and proficiency evaluation by internal (laboratory) and/or external quality assurance program[34,35].

7.6 Measurement of Screening Performance

The cervical cytology test, like any other screening test, has some inherent limitations and can never be 100% accurate. However, there are objective data and performance indicators that can help laboratories to assess their own work for continuous quality improvement and compare with their peer[36,37]. These may include proportion of unsatisfactory specimen, proportion of negative, atypical cellular changes, low grade and high grade lesions, atypical to low grade lesion ratio, proportion of woman with high grade lesion but with recent negative cytology, an estimate of false negative and false positive rates, and turnaround time for reporting, etc.

7.7 Continuing Education

Ongoing education is a requirement for proficiency in cytology. This can be fulfilled by reading relevant journal and textbook, by participation in slide review sessions, workshops and symposia, reading teaching sets or proficiency programs that are available such as CheckSample, CheckPath, and STAR from American Society of Clinical Pathologists, Cytology Quality Assurance Program of Royal College of Pathologist of Australasia, etc.

7.8 Accreditation

Different countries have different policies and requirements for laboratory assessment. Accrediting agencies or accrediting programs may be at national or state level or by peer-review. In USA, there is a minimum requirement stipulated in CLIA ’88 guidelines. In Australia, the standards are set by the National Pathology Accreditation Advisory Committee[38]. In Hong Kong, accreditation for laboratory practice is not yet mandatory. One may follow the USA or Australia accreditation programs before the local accreditation program is established.

8. MANAGEMENT

8.1 Storage and retrieval of glass slides

Cytology laboratories are required to retain all cervical cytology slides regardless of diagnoses for a minimum of 5 years from the date of specimen reception[3]. The period of retention is considered as a minimum and individual laboratories can store slides more than the specified period. A system is needed to ensure that the slides are stored in an orderly way for easy retrieval. It is recommended that the slides are stored in the laboratories or within the institutional premises. If they are stored elsewhere, it is necessary to ensure that retrieval can be done within reasonable time. The integrity of all original slides should be maintained. Broken slides should be repaired in every possible way. Irreparably damaged slides should be documented. Policies for retrieval of slides should be implemented. The purpose of retrieval, personnel involved, transfer and receipt of material for consultation and legal proceeding should be recorded.

8.2 Record/Report Storage and Retrieval

Laboratory should have a system to record and retrieve information on specimens and cytology reports. The system may be manual or automated. Manual methods may include log and card files organized by date, patient's name, specimen number or other patient identifiers. It is preferable to have an automated system, often referred as the laboratory information system (LIS). Records and cytology reports are preferably stored in the laboratory or within the institutional premises. If stored elsewhere, it is necessary to ensure that retrieval can be achieved within reasonable time.

The following are recommendations on period of storage. They represent the minimum requirement.

Material	**Recommendation**
Laboratory accession records	3 years
Quality Assurance records	3 years
Equipment Manual/maintenance records	3 years after discontinuous use
Laboratory methods/procedures	3 years after discontinuous use
Referring doctors' request forms	3 years
Cytology reports	10 years

8.3 Discarding slides and Records

Slides and records of patients may be discarded when they have been kept beyond the required retention period. Patients' identifiers must be destroyed when discarding these materials. If materials are kept for research or teaching purposes, patient

confidentiality must be maintained.

8.4 Security of data

All laboratory records and patients' reports are confidential. Procedures should be set up to restrict access of these materials to authorized persons only. Paper records and reports should be stored in a secure place. Security codes are needed for electronic systems. Copies of reports and records for teaching or research purposes should be destroyed after use. If the reports are stored in the laboratory information system, it is necessary to have backup copies.

8.5 Safety management

Each laboratory should have a clear and comprehensive safety policy depicted in its laboratory SOP. One should follow the guidelines provided by the Hospital Authority[39]. The laboratory should have a documented policy to record any laboratory accident. The record should include the nature of the accident, personnel involved and any remedial action.

9. VARIABILITY OF PRACTICE

These practice guidelines only reflect our current understanding and would be updated and revised as and when necessary in accordance with changes in practice and scientific advancement. There may be variations in cytological technical methodologies, analyses, reporting and laboratory management. Differences may reflect practice variations that are dependent upon individual laboratory resources, physician needs and patient population. Individual laboratory may elect to use methodologies that differ from those described in this document, if conducted in accordance with appropriate regulatory and professional knowledge, can be considered as reasonable practice. Such variations in practice should be documented in the laboratory procedure manual.

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