DOI:10.1111/j.1365-2303.2007.00464.x

European guidelines for quality assurance in cervical cancer screening: recommendations for collecting samples for conventional and liquid-based cytology*

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Accepted for publication 20 April 2007

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The current paper presents an annex in the second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening. It provides guidance on how to make a satisfactory conventional Pap smear or a liquid-based cytology (LBC) sample.

Practitioners taking samples for cytology should first explain to the woman the purpose, the procedure and how the result will be communicated.

Three sampling methods are considered as acceptable for preparing conventional Pap smears: (i) the cervical broom; (ii) the combination of a spatula and an endocervical brush; and (iii) the extended tip spatula. Smear takers should take care to sample the entire circumference of the transformation zone, to quickly spread the cellular material over a glass slide, and to fix the preparation within a few seconds to avoid drying artefacts. According to local guidelines, one of these three methods may be preferred. Sampling with a cotton tip applicator is inappropriate.

Similar procedures should be followed for sampling cells for LBC, but only plastic devices may be used. The collected cells should be quickly transferred into a vial with fixative liquid according to the instructions of the manufacturer of the LBC system.

Subsequently, the slide or vial and the completed request form are sent to the laboratory for cytological interpretation.

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*The views expressed in the Article are those of the authors and do not necessarily reflect the official position of the European Commission.

Financial support was provided by the European Communities.

Keywords: cervical cancer screening, conventional Pap smear, cytology, European guidelines, liquid-based cytology, sample collection

Introduction

The correct sampling of the cervix with appropriate equipment contributes significantly to the diagnostic value of the Pap test.[1–3] Unsatisfactory samples are an important cause of false negative and false positive results. The purpose of this guideline is to minimize the proportion of unsatisfactory smears and to maximize the accuracy of the screening test. The current guideline is included as a special annex to Chapter 3 of the *European Guidelines for Quality Assurance in Cervical Cancer Screening*.[4] The guidelines aim to bring together good practice in different countries across Europe. However, it is recognized that there may be minor variations from recommendations of local and national programmes.

Facilities

The cervical screening programme will invite well women. It is important that women are satisfied with the service offered to them, or they will not return for rescreening or follow-up tests. Before the sample is even taken, the environment should be suitable. There should be privacy, warmth and a relaxed atmosphere. The woman must be comfortable and there must be an adjustable spotlight to visualize the cervix before the sample is taken.

The equipment required for taking the sample should be available before beginning the examination to minimize the time the woman spends in what some consider to be an embarrassing position. The equipment that should be available will include gloves, a range of specula, sampling devices, slides, fixative, pencil and slide carrier for conventional smears or vials and a ballpoint pen where liquid-based cytology (LBC) is used. Special care should be taken to keep the interval between taking the sample and fixing it as short as possible. The top should already be removed from the fixative dropper bottle or aerosol can, and the can should be checked to ensure it is not blocked or empty. Waste disposal and sterilization facilities will be required for when the examination is concluded.

In addition, there should be leaflets available to give the woman information on a variety of issues that she might raise. The test request form should be properly completed.

Contraindications for cervical screening cytology are: total hysterectomy, cervical amputation (if the surgery was performed for a cervical lesion, a vaginal smear should be performed at the recommended frequency) and the presence of a suspect, macroscopically visible lesion in the area of the cervix. In the latter case, the woman must be referred for colposcopic examination and biopsy.

Factors adversely affecting the quality of a cell sample are:

- menstruation, blood loss, breakthrough bleeding;
- vaginal inflammation/infection;
- sexual intercourse within 24 hours;
- severe genital atrophy (menopause);
- pregnancy, postpartum period and lactation;
- physical manipulation or chemical irritation such as: preceding digital vaginal examination, disinfectant cream or liquid, lubricating jelly, vaginal medication, vaginal douche or spermicidal jelly (less than 24 hours before), prior colposcopy with acetic acid (less than 24 hours before), previous smear (less than 3 weeks before), cervical surgery (less than 3 months before);
- radiotherapy.

It is essential to be aware of these factors and reduce their effect to a minimum. The quality of the preparations may be poor in pregnancy and the early postpartum period due to reactive inflammatory changes. Therefore, taking a smear should be postponed for pregnant women with negative screening histories until 6–8 weeks after delivery unless the last smear was more than 3 years ago and/or compliance for screening is considered likely to be poor. If a previous smear was abnormal and in the interim the woman becomes pregnant then the follow-up smear should not be delayed.

All relevant clinical information must be recorded on the request form.

Preparing to take the sample

- Explain to the woman the aim of taking the sample and what to expect; and give reassurance. Ask about her general health and whether she has any symptoms such as irregular bleeding or discharge. The date of the last menstrual period or of a recent pregnancy should be noted. Follow

any local consent protocols. Inform her that sometimes the examination has to be repeated within 3–6 months, if the smear was not of satisfactory quality. Make a clear arrangement about how the woman will be notified of the laboratory result.

- For conventional smears, label the slide or slides clearly in pencil on the frosted end with the woman's identification data (including at least two parameters such as name, number, date of birth). Other methods of marking may be lost during processing of the slide. For LBC, label the vial with the same information using a ballpoint pen.
- Ensure that the woman is lying comfortably on the examination couch in the dorsal or lateral position and position the light source so as to visualize the cervix clearly. Avoid taking a swab before the cervical sample.
- Select the largest speculum that can be inserted comfortably and bring to body temperature by warming it in the gloved hand or in tepid water. Insert the speculum along the axis of the introitus and, when half way up the vagina, rotate 90° and open when fully inserted. Lubricants are not usually necessary. If required a little tepid water or a small amount of water-soluble lubricant may be used but this must not contaminate the surface of the cervix as this impairs the sample quality. Bring the cervix into view by gentle movement of the speculum encouraging the woman to relax. If this proves difficult, digital examination taking care not to disturb the surface of the cervix, or change in position may be beneficial. The appearance of the cervix should be noted. All those taking samples should be taught to recognize the various normal and abnormal appearances of the cervix and suspicious symptoms. Do not routinely clean the cervix or take a swab before taking the sample.

Sampling the transformation zone

The precursors of cervical cancer arise mainly in the transformation zone (TZ) between the ectocervical multilayer squamous epithelium and the endocervical columnar epithelium.[5–7] Therefore, it is important that cell material be sampled primarily from this zone. The presence of metaplastic squamous cells and endocervical cells, in addition to squamous cells, indicates that the transformation zone has been sampled but cannot provide assurance that its full circumference has been sampled.

In the past, absence of an endocervical component was considered as a reason to repeat the smear.[8] However, longitudinal studies have shown that women with a previous negative smear lacking endocervical cells (EC–) are not at higher risk for future cervical lesions compared to women with a negative EC+ smear.[9–11] Nevertheless, the presence of endocervical and/or metaplastic cells indicates that the target zone has been sampled.

Sampling devices

Cervical screening always requires an endocervical and an ectocervical sample, taken with the appropriate instruments. Sampling the transformation zone may be carried out using wooden or plastic spatulas of various types. Spatulas with extended tips, brooms and brushes are recommended sampling instruments.[12,13] We distinguish two possible ends in the spatula: Ayre (lower part in Figure 1a) and extended tip or Aylesbury end (upper part of Figure 1a). Use of cotton tip applicators is not advised.

Three methods are recommended:

- Cervical broom (Cervex-Brush, Rovers, Oss, the Netherlands) (Figure 1c).
- Combination of a spatula (Figure 1a) for the ectocervical sample and the endocervical brush (Figure 1b) for the endocervical sample.
- Extended tip spatula alone (Figure 1a, upper end).

An endocervical brush should never be used alone.

The cervical broom is best if the woman is pregnant or has a cervix that bleeds easily. The combination method, including the endocervical brush, is best if the squamo-columnar junction is high (often post menopausal), after cervical surgery or if there is extensive ectropion of the columnar epithelium. In the UK, one sample with an extended tip spatula is the recommended first choice.[2,14]

Sampling and preparing a conventional smear

Cervical broom. Endocervical cells and ectocervical cells are sampled simultaneously – the long bristles pick up endocervical cells while the short bristles collect ectocervical cells and are bevelled to collect cells when rotated in a clockwise direction only.

- The long bristles are positioned endocervically (Figure 2).

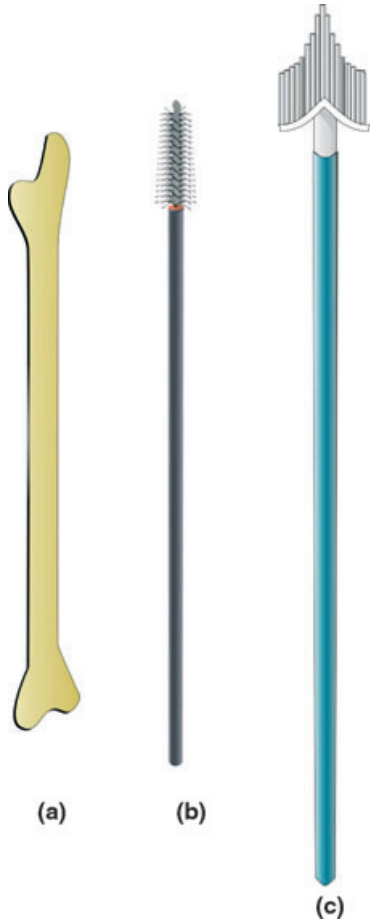

Figure 1. Sampling devices: (a) combined spatula with an *Aylesbury* end (extended tip) above and an *Ayre* end (below); (b) endocervical brush; (c) cervical broom.

Figure 2. Cervical broom: sampling and spreading the sample on the slide.

- Rotate the brush five times over 360° with gentle pressure by rolling the handle clockwise between thumb and forefinger.
- Sweep the broom lengthwise along the slide, turn over and repeat for the other side.
- Fix immediately.

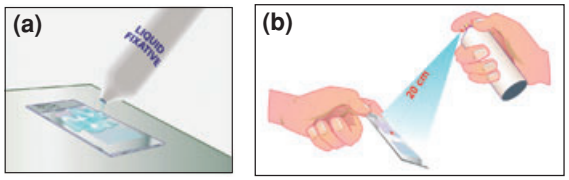

Figure 3. Fixation of the smear by flooding with fixative from a dropper bottle (left) or by spraying (right).

- The fixative of choice is 95% ethyl alcohol but other appropriate fixatives may be used. The smear should be flooded with fixative from a dropper bottle (Figure 3a), sprayed with an aerosol fixative (Figure 3b), or placed immediately in a container of fixative that covers the whole of the cellular area of the slide. The slide should be fixed for at least 10 minutes. It should be removed from the fixative and placed dry in a slide box for transportation.
- If spray fixation is used, the specimen should be fixed immediately by spraying at a right angle from a distance of 20 cm (Figure 3b). If closer, the cells are blown away or frozen, if on a slant, the material is blown into aggregates. Droplet formation should be avoided by not using too much fixative. The BSCC guidelines recommend placing the slide on a flat surface for spray fixation, to avoid uneven fixation.[2] Very fast fixation, within a few seconds, is essential to prevent drying artefacts.
- It is critical that smears are fixed immediately to prevent partial air-drying, which will distort cellular detail. It should be noted that smears from postmenopausal women and blood-stained smears dry very rapidly.

Combination of spatula and endocervical brush.

Spatula sampling

- Use the end of the spatula that is most appropriate to the anatomy of the portio. For nullipara, this is usually the Aylesbury end, for multipara the broader Ayre end. The pointed end of the spatula should be inserted into the cervical os until the inner curved surface is applied to the cervical surface (Figure 4a).
- Rotate the spatula through more than one complete turn while maintaining firm contact with the cervix. When turning clockwise, stop at the 9 'o clock position; or when turning anti-clockwise

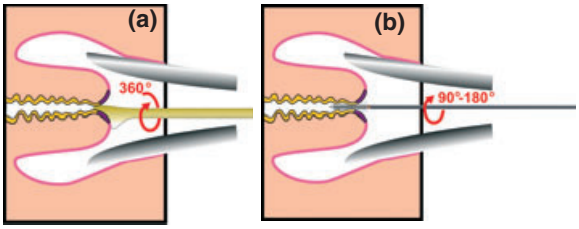

Figure 4. Sampling of cellular material using the spatula (left) and the endocervical brush (right).

stop at the 3 'o clock position, so that the scraped material remains on the upper side when the spatula is in the horizontal position.[15]

- The tip scrapes the os while the less protruding part scrapes the surface of the portio. Take special care to scrape the squamocolumnar junction as fully as possible. If there is extensive ectropion, scrape the outer part of the portio separately using the blunt end of the Ayre spatula.
- Place the spatula on a rack and proceed without delay to take the brush sample. The danger of drying out is slightly less if the cell material and mucus remain in contact with the sampling device.

Endocervical brush sampling

- Insert the endocervical brush for two thirds into the endocervical canal, so that the lower bristles are still visible, and rotate gently 90–180° (Figure 4b).

Transfer of cellular material onto the glass slide

- Roll (not wipe) the endocervical brush immediately over the outer third of the slide in the opposite direction from which it was collected by twirling the handle (Figure 5a). Do the rolling in a single movement (not in a zigzag) and without pressure, in order to obtain a thin and even smear.
- Then spread the material from the spatula as quickly as possible onto the central third (Figure 5b). Use firm longitudinal sweeps ensuring that material from both sides of the spatula is removed.
- An alternative is to transfer the brush material lengthwise over the first half length and the spatula over the other half length of the slide (Figure 5c,d).
- Fix immediately using one of the methods described above. Endocervical cells dry very quickly and a drop of fixative spread on the slide before spreading the cellular sample may aid rapid fixation.[2,14]

For inexperienced smear-takers, it can be difficult to spread the two samples on one slide and to fix both specimens adequately before the first sample dries. In this situation, it may be easier to spread the endocervical brush sample and the spatula sample over two slides. In that case, first fix the spatula sample, before proceeding to endocervical sampling.

Sampling with the extended tip spatula alone. Finally a third option is to collect the cells from the endocervix and exocervix with an extended tip spatula alone (Figure 1a) and to spread one side of the spatula over one half length and the other side over the other half length (Figure 5d).

Preparing a liquid-based cytology sample

A LBC sample is collected from the cervix in the same way as for a conventional smear, but only plastic sampling devices may be used. The manufacturers' instructions for collecting the sample must be followed. Either a single broom-like device or a combination of plastic spatula and endocervical brush are recommended for ThinPrep (Cytyc, Boxborough,

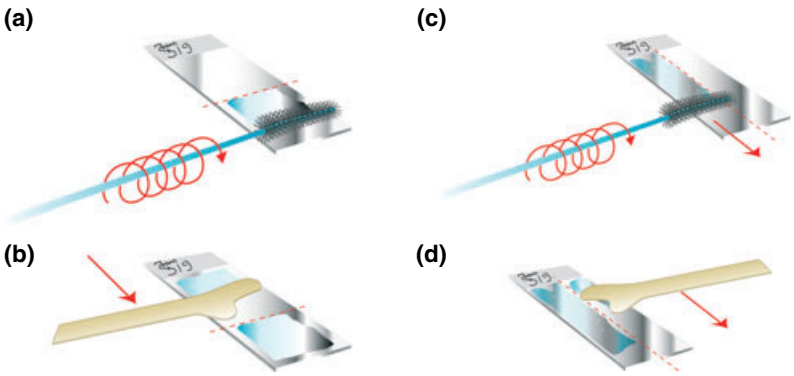

Figure 5. Transfer of cellular material from the sampling device onto a glass slide.

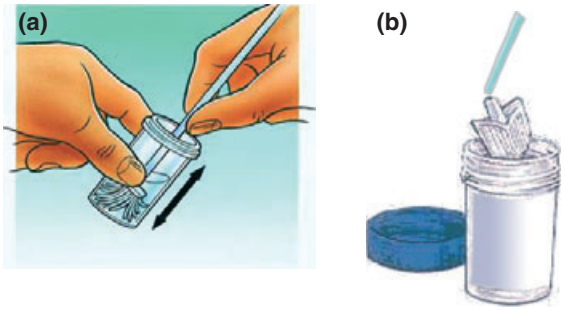

Figure 6. Left: the broom is pressed multiple times vigorously against the bottom of the vial (ThinPrep sample). Right: the head of the broom with the bristles is removed and dropped in the vial with preservation liquid (SurePath sample).

MA, USA) while only a broom with a detachable head is recommended for the SurePath system (TriPath Imaging Inc., Burlington, NC, USA). The protocol for rinsing the sample into the vial of collection fluid depends on the methodology used. In both instances the lid should be removed from the vial before the sampling procedure begins. Procedures for other liquid-based methods should be adapted according to the manufacturers' instructions.

For the ThinPrep system, the broom should be pressed vigorously against the bottom of the vial 15–20 times to remove all the cellular material (Figure 6a). Before discarding the broom, the bristles should be inspected and the procedure for rinsing in the vial repeated if any residual material is seen.

For SurePath samples, the head of the broom is detached into the vial of collection fluid (Figure 6b).

The lid of the vial should be firmly closed to prevent leakage during transportation. The ThinPrep Preservcyt vial has torque lines to facilitate correct sealing. Overtightening of the lid should be avoided since this may impede functioning of the T3000 automated ThinPrep processor.

Removing the speculum from the vagina

In view of the importance of fixing the slides quickly, the speculum may be left in place, having explained to the woman before the procedure the reason for doing so, until the slides have been prepared and fixed. The speculum should be withdrawn gently with the blades apart until the cervix is no longer between them. The speculum should then be allowed to close as it is withdrawn completely.[14]

Complete the request form

After completing the procedure, the request form should be fully filled in with the woman's surname, forename, date of birth and other identifying features clearly written. The number of slides or sampling technique, date of last menstrual period or recent pregnancy, and clinical observations such as irregular bleeding or suspicious looking cervix must be recorded. The sample taker should ensure that the woman has understood the procedure and is aware of when and how she will receive the test result.

Information should be provided on the request form as to whether the result of the examination has to be sent to another physician (for instance to the general practitioner if the sample is taken by a gynaecologist).

Transport to the laboratory

After fixation, the conventional slide should be allowed to dry completely. It should then be placed in a cardboard or plastic container for transport to the laboratory. If it is put in the container too quickly, a wet specimen can stick at the edges. The container must be labelled with identification details matching those on the request form.

LBC samples must be placed in a sealed plastic bag with the request form in a separate compartment of the bag as for other clinical samples.

There may be local and manufacturers' regulations about how specimens of human material should be transported, which should be followed.

Feedback on the quality of the specimen

The cytological report, should use a standard reporting system compatible with the Bethesda System and must include a judgement of the quality of the specimen preferably including information about TZ sampling.[16–18] Moreover, if the sample is unsatisfactory, the reasons should be indicated.[18]

Every practitioner taking samples for cytology should be provided with periodical summary reports of the quality of their samples in terms of detection of cytological abnormality, specimen adequacy and, preferably also TZ sampling. The reports should be compared with those of other practitioners using the same cytology service. This feedback, provided by the laboratory or a central register, is helpful in

improving the average quality of cytological preparations.

Disclaimer

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Acknowledgments

The content of this article is derived from the European Guidelines for Quality Assurance in Cervical Cancer Screening © European Communities, 2007.

The financial support of the European Communities through the European Cervical Cancer Screening Network and the European Cancer Network is gratefully acknowledged. Other funding agencies were: the *DWTC*/SSTC (Federal Service for Scientific, Cultural and Technical Affairs, Brussels, Belgium) and the Gynaecological Cancer Cochrane Review Collaboration (Bath, UK). The assistance of Herman Vanvinckeroye for the creation of illustrations is also gratefully acknowledged. The authors thank Mrs. Ine Van Marsenille (Scientific Institute of Public Health, Brussels), Dr. Lawrence von Karsa and Mr. John Daniel (both form IARC, Lyon, France) for the technical editing of the paper.

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