Alternative sampling methods for cervical cancer screening: practical perspectives from the laboratory

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Abstract
The coverage of cervical cancer screening in South Africa is inadequate, with an estimated 8.8-million unscreened women who are mainly serviced by the public health sector in lower-resourced areas. Alternative screening options need to be considered. Every step in the screening process needs to be critically evaluated to design a practical programme without a bottleneck, to deliver maximum benefit with limited available resources. Patient self-sampling has been identified as an acceptable method of specimen collection for many women. Patient self-sampling, combined with high-risk human papillomavirus-based testing, has the potential to increase cervical cancer screening coverage, especially in areas where screening is inadequate.

Introduction
The current South African National Department of Health's cervical cancer screening policy was drafted in 2000. The aim was to reduce the incidence of cervical cancer by 67% within the context of limited health resources. The guidelines envisaged a cytology-based screening policy whereby an asymptomatic woman aged 30 years and older could have three free Papanicolaou (Pap) smears in her lifetime. Unfortunately, 13 years later, implementation of this policy seems to be unattainable. An estimated 20% of asymptomatic women in South Africa have never had one or more Pap smears. Cytology-based prevention programmes have proved to be difficult to implement sufficiently, and to maintain in middle and low-resource countries. The main obstacle is not necessarily the cost of the screening test, but rather the cost, complexity and shortage of the infrastructure needed for implementation. Examples of infrastructure needed include trained healthcare providers who are educated to understand the benefits of screening and who can take proper samples, healthcare equipment required to conduct speculum examinations, sample transport, and public sector laboratories with adequately trained personnel who can screen, diagnose and guarantee rigorous quality control. A data information system must be able to deliver the result back to the patient so that healthcare providers can ensure the necessary follow-up and interventions by trained healthcare providers.

Additional identified difficulties in South Africa include competing healthcare priorities, like programmes for the management of human immunodeficiency virus, tuberculosis and maternal and child health, as well as poor system follow-up and referral. Misinformation and lack of knowledge with regard to cervical cancer and cervical cancer prevention opportunities for women and healthcare workers is also of concern. An estimated 8.8-million of the 11-million South African women who are eligible for cervical cancer screening have not yet been screened even once in their lifetime. The National Department of Health has set a goal for 70% (approximately 7.7-million) of eligible women to be screened by 2014. It is time to consider alternative screening options to reach the millions of unscreened women who are mainly serviced by the public health sector in lower-resourced areas. Every step in the screening process needs to be thoroughly considered by a multidisciplinary team of experts, including primary healthcare doctors and nurses, public health specialists, pathologists and gynaecologists. Cervical cancer screening assays must be reproducible and sufficiently sensitive and specific to detect high-grade precursor lesions, i.e. cervical intraepithelial neoplasia (CIN) II or higher.
This article will focus on available sampling options for cervical cancer screening, including collection, transportation to the laboratory, sample preparation, as well as considerations that impact on sample testing and result interpretation.

Method

Sample collection methods

Screening uptake by women is mainly determined by awareness, access and acceptability. The collection of cervical smears is time consuming, labour intensive and needs healthcare infrastructure, such as specula (disposable or properly disinfected) spatulas, glass slides, a working light source, and an examination couch and room or screened-off area. A shortage of any of these leads to a limit in the number of women who can be screened per day at a particular site. By contrast, patient self-collected sampling is only limited by the amount of sample devices available, and the availability of a private area in which the woman can collect the sample, which could be her home or a private area in a clinic. Studies in developed and developing countries have shown that self-sampling is generally preferred to healthcare provider sampling. Thus, patient self-collected sampling can decrease the burden on the healthcare system associated with healthcare provider-collected specimens, and can also boost screening acceptability in women, ultimately allowing more women to be screened.

Evidence shows that high-risk human papillomavirus (HPV) DNA tests using self-collected patient samples are at least as sensitive to the detection of CIN II+ as cytology. Traditionally, the cytological examination of exfoliated cells scraped from the transformation zone of the cervix by a healthcare provider during a speculum examination was considered to be the gold standard. Self-collected patient samples for cytological screening are not an effective alternative for healthcare provider-collected screening, mainly because of major limitations of low sensitivity and low negative predictive value when the sample is not collected from the cervical transformation zone.

It seems that specimen collection from the transformation zone is not as essential with newer screening methodologies based on high-risk HPV detection. Generally, a self-collected sample collects cervical and vaginal cells. High-risk HPV-infected cells are shed in the vagina, and have a similar affinity for vaginal and cervical epithelium. Therefore, self-collected samples seem to be representative of cervical high-risk HPV status. It has now been proven by several studies that cervical cancer screening that detects high-risk HPV DNA using patient-self collection devices is at least as sensitive to the detection of preinvasive high-grade cervical disease and invasive cervical cancer as cytological testing on healthcare provider-collected cervical smears. Some studies have reported a lower, but acceptable positive predictive value. However, it is important to note that self-collection devices are not equal, and recovery of high-risk HPV DNA is dependent on where the sample was collected, as well as the cellular yield.

It was reported that high-risk HPV was detected in 98.3% of cervical samples, compared to 86.2% vaginal, 62.1% vulvar and 44.8% of urine samples, in a review by Sellors et al. Sensitivity to the detection of CIN II+ was progressively lower as the sampling distance from the cervix increased. Vaginal tampons had the highest cellular yield, with more than 99% of samples being adequate for the diagnosis of high-risk HPV, although only 12% of these contained endocervical cells, compared with 77% of healthcare provider-collected samples. Although urine specimens are generally easy to collect, their low sensitivity makes them unsuitable for high-risk HPV screening. Generally, urine samples have a 50-fold lower HPV viral load compared to that of the cervix, as well as inaccurate or invalid results in up to 20% of specimens because of an inadequate cellular yield and/or the presence of inhibitors.

Different sampling devices include smear devices, such as wooden spatulas, sponges, tampons, as well as cervicovaginal lavage devices. A cervicovaginal lavage rinses the vagina with saline to collect cells in suspension. The acceptability of these to women and healthcare providers may vary in urban and rural women, and in different cultures and age groups, and according to personal preferences. Generally, urban women prefer tampon-based self-collectors, while this may be an unfamiliar concept to rural women. Cervical lavage collection devices using saline may be difficult to use. Healthcare providers in clinics report it to be “messy”. Multiple samples show inadequate collection, with a low cellular yield.

Additional considerations when selecting a collection method include the size of the sampler and ease of labelling, as these factors may lead to logistical problems with transport and laboratory processing.

Sample transport methods

Samples can be transported dry, or in collection fluid. HPV DNA is double stranded, which makes it stable. It can withstand prolonged periods of drying. Traditionally, viral RNA, being mostly single stranded, is considered to be less stable than viral DNA. However, E6 and E7 messenger RNA (mRNA) seem to be more stable than initially thought. Evidence from our experience, as well as that in published studies, suggest that E6 and E7 mRNA are detectable in stored clinical samples,
probably because of the cell-associated nature of these markers.18

Dacron or cotton swabs, cervical brushes and broom-like instruments can be transported dry or in transport media used for healthcare provider-collected samples. The advantages of dry samples are ease of collection and transport, the smaller size and reduced costs. The disadvantage is that generally dry samples can only be tested once using a single high-risk HPV DNA detection method, without the option for repeat, reflex or triage testing with alternative assays. Phosphate-buffered saline is the most affordable and widely available type of buffer, and although not advocated by commercial companies, may be an option that should be evaluated in resource-limited settings. Other available transport media include liquid-based cytology, as well as manufacturer-specific media. Transporting liquid-based samples can be troublesome in that the fluid may leak. Sample containers should be sealed properly, and users cautioned to securely close the containers, and to transport them upright, or at least in a separate sealable bag. It is important not to leave the string outside of the container when tampons are used as this can cause the specimen to dry out, contaminate other specimens and poses a safety hazard.

**Pre-analytical sample preparation**

Primary collected samples which can be loaded directly onto a high throughput analytical analyser without any additional specimen preparation steps are ideal when taking into consideration the practical workflow in the laboratory. This reduces hands-on labour time and staff needed, as well as minimising the risk of a possible specimen mix up or carry-over contamination. Dry swabs may be inexpensive and constitute a practical collection method. However, generally they need laborious and time-consuming placement of the swab tip into a tube with disposable forceps and an incubation step with added proteinase K. There is also the risk of carry-over contamination and specimen mix up. Tampon specimens may need 2-3 washing steps before molecular testing, which is labour intensive, time consuming and may also lead to specimen mix up or carry-over contamination.19 Generally, collected brush- or broom-like specimens which are rinsed or inserted into the relevant collection tube immediately after collection, or after receipt in the laboratory, need the least pre-analytic manual preparation time.

There is growing interest in the use of liquid-based cytology media for primary high-risk HPV screening, where positive specimens can be reflexed to cytology if needed. Recovery of HPV DNA and RNA are largely unaffected by exposure to methanol-based ThinPrep® PreservCyt® Solution (Hologic, Bedford, USA). PreservCyt® has sufficient volume for use with most HPV molecular detection assays, and is also compatible for testing with most of these assays. Historically, it was reported that the storage of cells in BD SurePath® (Becton Dickinson, Franklin Lakes, USA) resulted in significantly reduced yields with between 104- and 106-fold reduction, depending on the extraction technique.20 SurePath® contains formaldehyde, which is known to cross-link nucleic acids and protein. This cross-linking must be removed by an additional proteinase K digestion and/or heat step before molecular testing can be performed for certain molecular assays, making it more labour intensive, time consuming and not ideal.21,22 However, some newer-generation platforms have been validated on both PreservCyt® and SurePath®, adequately addressing the denaturation required. Using SurePath® may be a more affordable option in the South African setting, if combined effectively with the correct molecular test.

Manufacturer-specific buffers optimise testing on the specific assay by inhibiting growth of other organisms and stabilising viral nucleic acid, but may also limit further triage or testing, if needed. Some of these media can produce foam, which may be a cause of specimen carry-over contamination. The use of media that is validated on more than one screening and triage assay may be preferable over manufacturer-specific buffers, depending on the algorithm used for testing.

**Results**

**Sample analysis and result interpretation**

High-risk HPV testing gives an objective result, and is less labour intensive when using high-throughput automated platforms. The most commonly used molecular assays that detect HPV DNA can be used on swabs, brush- and broom-like samples, tampons and lavage samples.19 Newer mRNA assays can also be used on tampons and lavage samples, as well as brush samples transported in media (unpublished data). No self-collection for high-risk HPV testing has yet been approved by the US Food and Drug Administration. It is difficult to directly compare studies because of the great variation in the collection devices and high-risk HPV detection assays used. However, data from systematic reviews have demonstrated good overall agreement of high-risk HPV prevalence between healthcare provider, and patient self-collected, samples.8,10,23 Low-risk HPV types are more commonly detected in self-collected patient samples.10 High-risk HPV assays which cross-react with low-risk HPV types may give increased false positive screening results on self-collected patient samples.

The low sensitivity of cytological screening in detecting CIN II+ necessitates shorter screening intervals to optimise the efficacy of a screening programme.24
Data from meta-analyses and large screening studies estimate that the sensitivity of a single cervical cytology test to detect CIN II+ to be between 49% and 57%. The clinical utility of using high-risk HPV-based tests to improve the sensitivity, negative predictive value and reproducibility of cervical screening has been firmly established. Several tests are commercially available. Follow-up data from large-scale randomised trials have shown that HPV-based screening provides 60-70% better protection against invasive cervical carcinomas than cytology. These data also support high-risk HPV-based screening in women from 30 years of age, and the extension of screening intervals to at least five years. Generally, high-risk HPV testing on patient self-collected specimens has a higher sensitivity, and thus lower false-negative rates, than cytology. However, self-collected high-risk HPV testing has a lower clinical specificity and positive predictive value. The challenge facing testing on self-collected patient samples, as a strategy for cervical cancer prevention in low-income countries, is identification of the most effective triage or management plan for high-risk HPV-positive women. An increase in the false-positive rate will add to the number of follow-up or triage tests, as well as colposcopy referrals, and this may lead to potential overtreatment. It is of utmost importance that these alternative collection methods are clinically validated with the relevant high-risk HPV DNA or mRNA test before widespread implementation. The combination of collection method, transport method, specimen preparation and assay used, should be evaluated in clinical trials to show adequate sensitivity in identifying women at risk of CIN II+, yet satisfactorily specific to avoid too many unnecessary interventions. The relative importance of non-detection of CIN II+ lesions will have to be compared to the disadvantages of over-referral or overtreatment in the context of limited screening accessibility, the opportunity for an asymptomatic woman to have three cervical smears every 10 years, and limited resources.

Conclusion

When implementing a nationwide screening policy that involves testing a high volume of samples, every step in the process will have to be critically evaluated, so as to not create a bottleneck. The ideal sample and testing method should be affordable, acceptable to women and healthcare providers, easy to collect and transport, stable during transport, clearly identifiable with a unique patient identifier, and involve minimum pre-analytical sample preparation steps in the laboratory. Self-collected patient samples have been identified as an acceptable, and sometimes preferred, method of specimen collection for many women, and have the potential to increase cervical cancer screening coverage. The immediate aim should not be to replace well functioning cytology-based screening programmes, but rather to expand cervical screening with the implementation of high-risk HPV-based screening in areas where screening is inadequate. Patient self-collection of samples tested with high-risk HPV-based assays may be the solution in areas with limited access to health care, or with a shortage of healthcare provider and healthcare infrastructure in South Africa. However, more comprehensive study data are needed before this can be widely implemented.

Conflict of interest

There is no conflict of interest to declare.

Declaration

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