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Methylation testing and self sampling: Filling a gap in cervical cancer screening programs

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Cervical screening remains important, despite the long-term promise of HPV vaccination. HPV testing is more sensitive than cytology for detecting cervical precancer and cancer, providing increased reassurance and permitting extended screening intervals (1). Nonetheless, the pace and manner of implementing primary HPV testing has varied considerably. In the US, HPV testing is recommended in conjunction with cytology while in the Netherlands, it is recommended as a single primary test (2;3). In many places, it is not yet used at all.

The successful introduction of HPV testing into cervical cancer screening requires more than a sensitive primary screening test (Table 1). New programs must be built around HPV testing. The major goal of cervical screening *programs* is to find precancers that can be treated to prevent invasive cancers. A diagnosis of precancer requires colposcopic evaluation with cervical biopsies. Most HPV infections are transient; an HPV-positive woman has a low risk of progressing to precancer and cancer. Thus, if HPV testing is used, secondary (triage) tests are required to decide who among the HPV-positives needs to be referred to colposcopy. A common suggestion is to move cytology into the role of triage. New molecular assays such as p16/Ki-67 cytology have higher sensitivity at comparable specificity to cytology (4). Additional molecular markers such as host methylation and HPV methylation are also being evaluated (5–7).

Regardless of which screening and triage tests are chosen, the critical problem of nonparticipation remains. A substantial proportion of cervical cancers in developed countries arise in women who participate in screening irregularly, or not at all (8). Previous trials from the Netherlands have shown that offering self-sampling for HPV testing to non-responders, instead of an office visit, can increase participation (9). As with all HPV testing, women found to be HPV-positive in self-sampling require a triage test to decide who needs to go to colposcopy. However, self-collected samples are not suitable for cell-based assays, such as cytology or p16/Ki-67, so that an additional collection is required.

In this issue of *Lancet Oncology*, Verhoef et al. report the results of a randomized trial addressing this gap in their screening program (6). They enrolled initially non-participating

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women, found to be HPV-positive upon self-sampling, who were followed with two different triage strategies: 1. Cytology from physician-collected samples or 2. Methylation testing of two genes, MAL and mir-124, from the self-collected sample. They found that the clinical performance of methylation testing from the self-collected specimen was equivalent to physician-collected cytology. Since the assay was run from the same specimen collected at baseline among HPV-positive women, it avoided an additional office visit for the majority of women.

The Dutch investigational team should be commended as pioneers in creating an integrated HPV-based cervical cancer screening program. The approach described by Verhoef et al. further improves the safety net of their program. As one possible caveat, the participants in the trial generally reported being screened before, and had a very high compliance after they were included in the study, suggesting that they were “soft” refusers. The self-sampling strategy might not apply as well to the firmer refusers who have never been screened before, putting them at highest risk.

How do these results apply to cervical cancer screening in other places? A recent meta-analysis showed that self-sampling has slightly lower sensitivity compared to physician-collected samples (10); in most resource-rich places, self-sampling is currently not approved as a first-line alternative. Offering self-sampling for non-responders is particularly attractive for organized screening settings, but is difficult to implement in countries with opportunistic screening like the US. Moreover, the methylation assay used in the current study has not been approved for clinical use, is currently not available as a kit, and has not been evaluated outside of the laboratory involved in the Dutch screening trials.

Our now-remarkable understanding of HPV and cervical cancer natural history has brought great tools for cervical cancer prevention, including vaccines for primary prevention, HPV testing for screening, and various molecular assays, including methylation markers, for detection of cervical precancers. Presented with multiple HPV-related preventive options, high-resource countries are considering a variety of combinations; no single “winning strategy” has emerged. However, low-resource countries cannot afford the complex programs established or under development in industrialized countries. A triage test that can be performed out of self-sampling material like the methylation assay described here could extend the options for cervical cancer screening in low resource settings, where cytology programs rarely exist and colposcopy capacity is very limited. However, development of a robust, low cost methylation assay is paramount to achieve this goal.

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Table 1

Options for cervical cancer screening programmes

	Cytology	HPV	Co-testing (cytology and HPV)
Technology	Pap smear or liquid based cytology; manual or computer-assisted assessment	Clinician collected or self-collected	Co-collection or separate collection
Relative repeat interval for negative screen	Shortest (lowest negative predictive value)	Longer (greater negative predictive value)	Longest (greatest negative predictive value)
Triage test needed	For equivocal cytology results	For all positive results	For all HPV-positive, cytology-negative results
Triage test options	HPV or repeat cytology or p16/Ki-67	Cytology or HPV genotyping or p16/Ki-67 or methylation	Repeat co-test or HPV genotyping or p16/Ki-67 or methylation
Triage test sampling	Reflex triage or separate collection	Reflex triage or separate collection	Reflex triage or separate collection
Diagnostic test	Colposcopy and biopsy	Colposcopy and biopsy	Colposcopy and biopsy

The three major primary screening options are cytology, HPV testing, or a combination of the two (cotesting). Not considered here is visual inspection, a screening method under consideration for low-resource regions.