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Acting on evidence to change the current paradigm

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EVIDENCE REVIEW AND REPORT

Introduction of molecular HPV testing as the primary technology in cervical cancer screening: Acting on evidence to change the current paradigm

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Preamble on knowledge translation plan

A report intended to educate and create a common understanding among government policy makers, cancer agencies, health care professional associations, clinicians, researchers and patient groups about the state of evidence for HPV-based screening for cervical cancer and to spark a dialogue about why a paradigm shift for cervical cancer screening is needed. It will be widely distributed through multiple channels by Santis Health (the sponsor) and the co-authors. Organizations are encouraged to share the document through their channels.

Preface

The evidence supporting the introduction of molecular testing for human papillomavirus as the primary technology in cervical cancer screening (also known as HPV primary screening) is now overwhelming. We have taken important steps to prepare for the transition to this new screening modality in Canada. In 2012, Ontario updated its guidelines recommending HPV primary screening for women over 30, and large Canadian studies (CCCaST in Quebec and Newfoundland, HPV-FOCAL in BC, and VASCAR in Quebec) have concluded unequivocally that HPV primary screening detects more potentially cancerous lesions than the Pap test and is more cost-effective. A year ago, the Pan-Canadian Cervical Cancer Screening (PCCSN) Network concluded that the HPV test should be the primary screening modality in Canada.

Countries around the world including the U.S., the Netherlands, Italy, Turkey, Mexico, and others are in various stages of adopting primary HPV screening and tens of millions of women have been screened. In contrast, Canada has taken a relatively tentative and go-slow approach to implementation. While important questions about the most appropriate age groups for screening, screening interval and triage approach continue to be worked out, these are not obstacles to the implementation of HPV primary screening.

As a group of clinical experts and researchers in cervical cancer prevention from across Canada, we have jointly authored this comprehensive examination of the evidence and readiness to implement HPV primary screening in Canada. It is our belief that a number of provinces are ready to move forward on implementing HPV primary screening now. In fact, the opportunity for changing the core technology is a major incentive for implementing organized screening across Canada.

Our intention with the present report is to educate and create a common understanding among policy makers, agencies, clinicians, researchers and all interested Canadians about the evidence about HPV primary screening and to spark a constructive dialogue about how to move from analysis to implementation in Canada. This paper also makes a clear statement that clinical leaders and scientists from across Canada are committed to working with government and agencies to support this crucial but challenging paradigm shift. We would like to acknowledge Santis Health for providing an independent grant to support the development of this paper.

We hope that readers will find this paper instructive as Canada continues to adapt to evidence and provide the most appropriate cervical cancer screening and care for its female citizens.

Sincerely,

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EXECUTIVE SUMMARY

It is well established that persistent infection with human papillomavirus (HPV) is a requirement for cervical cancer.[1] While Pap cytology (or the Pap test) has had unequivocal success in reducing the cervical cancer burden since its introduction in the 1940s, this technology has many limitations in comparison with tests that screen for HPV.

Rationale for shifting from the Pap test to testing for HPV in cervical cancer screening

Evidence from over a decade of large-scale clinical trials, feasibility studies and real-world experience in countries that have adopted testing for HPV as the primary cervical screening method overwhelmingly prove that the benefits for shifting to HPV primary screening far outweigh potential or perceived harms. HPV testing has proven to be clinically superior to the Pap test in cervical cancer screening, without increasing the costs.

- **HPV testing is much more sensitive in detecting high-grade precancerous lesions than the Pap test.**
    - Numerous clinical trials including the Canadian Cervical Cancer Screening Trial (CCCaST) [2] have shown that HPV screening is much more sensitive, demonstrating sensitivity as high as 95% for HPV primary screening versus 55% for conventional Pap cytology (based on alcohol fixed direct smears).
    - Results from the HPV FOCAL RCT in B.C.[3] and the VASCAR (community based) demonstration project in Montreal[4] indicate that HPV testing followed by Pap triage leads to greater detection of precancerous lesions.

- **A negative HPV test provides greater and longer reassurance to women that they are at very low risk of developing cervical cancer.**
    - In a recent US study (n=1,011,092; representing the largest and longest experience with routine HPV testing in clinical practice), investigators reported much lower risks associated with a negative HPV test compared with a negative cytology test result.[5] The five-year risk of high-grade cervical precancer associated with a negative HPV test is lower than the three-year risk associated with a negative cytology test.

- **HPV testing with Pap triage has been shown in Canadian and international studies to be much more effective and less expensive compared with primary screening using the Pap test**.

- **HPV testing has efficiency and quality benefits** - HPV testing is objective, highly consistent, can be automated and centralized, and allows for rapid quality assurance of a high volume of tests.

- **HPV testing offers the opportunity for self-sampling, which could help reduce disparities and increase screening rates among some populations.**

- **HPV testing offers greater protection against cervical adenocarcinoma.**

- **HPV testing is a more logical technology for screening women in the HPV vaccination era.**

The need for the most effective prevention and screening strategy

In Canada, there were an estimated 1,450 cases of cervical cancer and 380 deaths in 2014. Cervical cancer takes a heavy toll on Aboriginal Canadians and recent immigrants.[6,7,8] Fortunately, most cervical HPV infections clear spontaneously and only a small proportion of infections (10-30%) persist beyond two years. However, because precancerous lesions are asymptomatic, screening is essential to detect and treat high-grade lesions.

Current HPV vaccines do not protect against all HPV genotypes. Cervical screening will continue to be recommended for vaccinated and unvaccinated populations for the foreseeable future.

The urgency to shift to HPV primary screening is increased by the fact that, by 2015, the first cohort of Canadian girls vaccinated against HPV 16 and 18 will be 21 years old - the age when routine cervical screening typically begins. As an increasing number of vaccinated females move into the screening target population, ensuring the most appropriate screening protocol that maximizes benefits and minimizes potential harms for both vaccinated and unvaccinated women becomes even more pressing.

Cost-effectiveness

In Canadian and international cost-effectiveness analyses, HPV primary testing was much more effective and less expensive compared with cytology primary screening.[9]

The fact that women will require fewer lifetime screens will also contribute to the cost-effectiveness of HPV primary screening. Investigators for B.C.'s FOCAL study found that, although HPV testing may initially increase referrals for colposcopy (compared with Pap cytology primary screening), cumulative colposcopy rates over the long-term would be similar for women 30 years of age and above.

HPV self-testing may be an acceptable option to women who do not participate in regular screening programs.[10] In an Argentinian study, offering women the opportunity to self-collect a specimen for cervical screening led to a four-fold increase in screening uptake within six months.

HPV test accepted as a superior primary screening method

Health Canada approved the first HPV test for primary cervical cancer screening in 2011; others have been approved subsequently.

The Ontario Cervical Screening Guideline Working Group (in conjunction with the Program in Evidence-based Care; an initiative of Cancer Care Ontario) now recommends stand-alone HPV primary testing every five years for women aged 30 to 65 years, with Pap cytology triage. These new guidelines for Ontario were published in 2012.[11] Cancer Care Ontario's Ontario Cancer Plan 2015-2019 identifies that one of the initiatives is to "pilot the human papillomavirus (HPV) test as the primary screening mechanism for the Ontario Cervical Screening Program".[12] However, the Plan does not specify in which year this pilot will be initiated.

In November 2014, the Canadian Partnership Against Cancer (CPAC) hosted a Pan-Canadian Cervical Cancer Screening Network (PCCSN) Expert meeting to formulate options for optimal cervical cancer screening.[13] Cancer prevention and control experts from across Canada reached "a general consensus that a change in screening protocols is necessary and that the primary screening modality should be the HPV test."

Despite the weight of evidence in support of HPV primary screening and showing that Pap cytology is an inadequate mainstay of cervical cancer screening, HPV testing has not become a frontline strategy in cervical cancer screening in Canada. Some of the hesitation comes from the mistaken perception that cervical cancer screening must first be fully organized before technological changes can be made. In fact, the opportunity for changing the core technology is a major incentive for implementing organized screening in Canada. This has certainly been the case in other jurisdictions that are implementing HPV primary screening.

International experience with Implementing HPV Primary Screening

Other countries have moved more quickly than Canada to adopt HPV primary screening. Mexico recently became the first country to introduce stand-alone HPV primary testing. The program has been implemented in all 32 states and targets women 35 to 65 years of age. To date, over six million women have been screened for HPV.[14,15]

Turkey also recently introduced HPV primary screening. Its goal is to screen 13.5 million women in the next five years. All HPV testing is being consolidated and centralized into two major laboratories, which should improve the quality of testing, ensure standardized processing, and reduce costs.

To date, HPV primary screening programs have been introduced in nine provinces in Italy, with >175,000 women tested each year. The Netherlands, Sweden and Scotland are also currently planning to introduce primary HPV screening within the next few years.

Implementation in Canada is imperative and highly feasible

With the rapid pace of technological changes and new discoveries, uncertainty will almost always exist in cervical cancer screening. However, questions surrounding the best triage strategy for referring HPV positive women to colposcopy, or the most appropriate age groups and interval to screen women should not be viewed as obstacles to the implementation of HPV primary screening in Canada. Moreover, policymakers should not postpone decisions assuming that randomized controlled trials will answer all of these questions. In a recent report, separate from this current evidence review, the PCCSN describes in detail a number of concrete steps that can be taken in Canadian provinces now to implement primary HPV screening.

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Introduction

It is now well established that persistent infection with high-risk human papillomavirus (HR-HPV) is a requirement for cervical cancer to develop (1). This discovery – made several decades following the introduction of Papanicolaou (Pap) cytology as the primary cervical cancer screening test – offers tremendous new opportunities for primary and secondary prevention of cervical cancer, i.e., immunization to prevent infection with HR-HPV types and molecular-based screening technologies, respectively. Despite the unequivocal success of screening programs based on Pap cytology in reducing cervical cancer burden in most developed countries, this technology has many limitations in comparison with molecular approaches to detect HPV as the basis for cervical cancer screening and for alignment of screening practices with risk status.

There are many compelling reasons to consider introducing HPV testing as a replacement for Pap cytology as the anchor technology in cervical cancer screening: 1) HPV testing is more sensitive in detecting high-grade precancerous lesions, 2) a negative HPV test provides greater and longer reassurance against cervical precancer and cancer because HPV infection is an event that occurs more "upstream" in the carcinogenic process, 3) HPV testing will be more cost-effective because of lengthened screening intervals, projected lower costs due to continued market expansion and high-volume testing, 4) HPV testing is not subjective and more reproducible, can be automated, centralized and better quality controlled with a high throughput, 5) it offers the opportunity for self-sampling, which could help reduce income/regional disparities and screening coverage among non-responders, 6) it is effective in detecting cervical adenocarcinoma (AC) precursor lesions, and 7) its performance (sensitivity, specificity and positive predictive value) is expected to be less adversely affected as a consequence of reduced lesion prevalence due to effective HPV vaccination programs. Evidence to support these reasons is summarized in Table 1.

In this report, we discuss the advantages and opportunities provided by introduction of HPV testing as the primary technique for cervical cancer screening (hereinafter referred to as 'HPV primary screening'). In addition, we also present screening algorithms that have been proposed and evaluated, important lessons from population-based HPV cervical screening trials and local demonstration projects, current guidelines and professional societies' recommendations, including knowledge gaps related to implementation of HPV primary screening. Despite the existing knowledge gaps, most experts are in agreement that the evidence to support the introduction of HPV primary screening has now become overwhelming. Therefore, the final section of this report, which focuses on the global experience and how to practically implement HPV primary screening in Canada, may be the most important for readers concerned with Canadian policies in cervical cancer prevention and control.

Table 1. Reasons to introduce HPV testing as a replacement for Pap cytology in primary cervical screening

Reason	Evidence/Rationale (key references)
• HPV testing is more sensitive in detecting high-grade precancerous lesions.	• Sensitivity of a single Pap test is only slightly higher than 50% whereas for a single HPV test it is approximately 95% (61, 96).
• HPV infection is an event that occurs upstream in the carcinogenic pathway, and therefore, a negative HPV test provides greater and longer reassurance against cervical precancer and cancer.	• In a study of > 1 million women, risk of precancer and cancer was lower five years following a negative HPV test than it was three years following a negative Pap test (111).
• HPV testing will be more cost-effective.	• Savings could result from lengthened screening intervals and projected lower costs due to continued market expansion and high-volume testing (111).
• HPV testing is not subjective and can be automated, centralized and better quality controlled with a high throughput.	• HPV results are highly reproducible because the test is less dependent on the training of laboratory personnel (104, 105).
• HPV testing could help reduce income/regional disparities and screening coverage among non-responders.	• HPV primary screening offers the opportunity for self-sampling, and the performance of HPV testing for self-collected versus clinician-collected specimens is only slightly lower (125).
• HPV testing may be more effective in detecting cervical AC precursor lesions.	• Pap cytology screening has had little success in reducing cervical AC rates, while HPV testing is proven to be very effective in preventing cervical AC (84-87, 106).
• Performance of HPV testing (sensitivity, specificity, and positive predictive value) is expected to be less adversely affected as a consequence of reduced lesion prevalence due to HPV vaccination.	• Although positive predictive value of both tests will decline as a result of reduced lesion prevalence in the population, a recent modelling study suggests that for cytology, it could fall below 10% due to the subjective nature of this test (93).

AC, adenocarcinoma

Epidemiology and Burden of HPV infection and Cervical Cancer

In 2008, there were an estimated 530,000 new cases of cervical cancer and 270,000 deaths from this disease, globally (2). Nearly 90% of cases and deaths occur in developing countries, where the annual incidence of cervical cancer frequently exceeds 50 cases per 100,000 women, whereas in developed countries, incidence is below 10 cases per 100,000 women (2). Pap cytology screening has had remarkable success in reducing the burden of cervical cancer or in preventing its resurgence in many developed countries (3-5) but the high cost and infrastructure required to ensure adequate quality, coverage, and follow-up of precancerous cervical lesions are the primary reason that cervical cancer has now become a sentinel disease of economic inequality. In Canada – a country with successful cervical cancer screening activities and programs – there were an estimated 1,450 cases of cervical cancer and 380 cervical cancer deaths in 2014, most of which (~75%) were squamous cell carcinoma (SCC) cases. The age-standardized incidence and death rates in 2014 were 7 and 1.6 per 100,000 women, respectively (6).

Cervical cancer takes a particularly heavy toll among Aboriginal Canadians and recent immigrants in Canada, groups that experience cervical cancer rates that are comparable to those in high-risk developing countries (7-9). Despite the availability of a universal health care system that covers the cost of pelvic exams, Pap cytology screening, and associated follow-up care, women living in the poorest neighbourhoods and from rural/small towns are at higher risk for cervical SCC (10). Similarly, other studies have shown that Aboriginal Peoples, recent Canadian immigrants, those living in rural or remote settings, older women, and those living in low-income areas or households are generally less likely to be screened or receive adequate follow-up care for Pap cytology-detected abnormalities (7, 9, 11-13). In Canada and other Western countries with effective Pap cytology-based cervical screening programs in place, most cases of cervical cancer occur among females with inadequate screening history or who have never been screened (14).

HPV is one of the most common sexually transmitted infections worldwide (15-17). In fact, most individuals (>75%) who have intercourse will at some point become infected with the virus (15, 18). More than 40 different anogenital HPV types exist; however, only 13 types (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are currently classified as definite or probable carcinogens (groups 1 or 2a) by the International Agency for Research on Cancer. Carcinogenicity is based on their frequent association with cervical cancer and its precursor squamous cell lesions known as high-grade cervical intraepithelial neoplasia (CIN of grades 2 or

3) or more recently high-grade squamous intraepithelial lesion (SIL) (19-21)[1]. These HPV types are also causally associated with the development of cervical AC and its precursor, adenocarcinoma in situ (AIS) (1, 22).

In descending order, the most common HPV types implicated in cervical cancer globally are: 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56 (23). The first seven types listed here (i.e., HPVs 16, 18, 58, 33, 45, 31, 52) are responsible for up to 90% of cervical cancer cases worldwide (24-28) and are included as part of Merck's new nonavalent vaccine (Gardasil 9®, Merck and Co., Whitehouse Station, NJ, U.S.A) along with two low-oncogenic risk types, 6 and 11 (29), which cause a large proportion of genital warts (acuminate condylomata) and low grade SIL (22, 30, 31). Although the order of HPV types implicated in cervical cancer varies across regions, HPV16 followed by HPV18 consistently rank at the top – responsible for approximately 70% of cervical cancers globally (24).

Over the years, multiple cohort studies demonstrated that the risk of high-grade SIL and cervical SCC is strongly linked to persistent infection with HR-HPV types (32-38). This eventually led to the conclusion and acceptance that persistent infection with one or more HR-HPV types is a key intermediate step in the etiologic pathway[2]. Fortunately, most cervical HPV infections clear spontaneously without ever causing lesions and only a small proportion of infections (10-30%) will persist beyond two years – mainly with HR-HPV types (32-38). Among females in whom HR-HPV types do not clear, the process of carcinogenesis entails a disruption of the normal maturation of the transformation zone epithelium of the cervix, leading to low- or high-grade SIL. The latter, if left untreated, can grow and traverse the basement membrane separating the epithelium from the adjacent connective tissue, thus becoming invasive, i.e., cervical cancer. However, except for the last step, this entire process is reversible. Only about 1% of low-grade SIL (CIN1) and 12-30% of high-grade SIL (CIN2 and CIN3 combined) will progress to become invasive (39, 40). Because these precancerous lesions are asymptomatic, screening is required to detect and treat high-grade lesions, i.e., those with lower probability of regressing on their own, before they become invasive and grow to reach blood and lymphatic vessels and metastasize.

[1] The designation SIL is not preferred over CIN.
[2] Although not the scope of this review, this epidemiologic evidence is coherent with the wealth of experimental studies demonstrating the carcinogenicity of HPV infection via its ability to interfere with cellular mechanisms, such as mitotic cycle and apoptosis.

Current Primary Cervical Cancer Prevention Strategy

HPV Vaccination

HPV vaccination is an important primary prevention strategy that may influence how screening is done in the future. Figure 1 illustrates opportunities for cervical cancer prevention along the pathway from HPV exposure up until the development of pre-invasive lesions, including vaccination and screening. Models indicate that vaccinated females have a lower lifetime risk of developing cervical cancer, and that eventually vaccinated populations will experience lower rates of cervical cancer and precancerous lesions if they have not already. This is expected to have a tremendous impact on important screening test parameters, which is described in the next section.

Prior to 2015, the approved HPV vaccines in Canada were Gardasil® (Merck & Co., Whitehouse Station, New Jersey) and Cervarix® (GlaxoSmithKline, London, United Kingdom) both of which target HPV 16 and 18, two of the most important high-risk types (41, 42). While additional time is needed to definitively address lingering concerns about duration of protection, it is plausible to assume that the two licensed vaccines will provide protection for longer than 10 years (43-45). Based on favourable safety and efficacy results comparing Merck's new nonavalent vaccine (Gardasil-9®) with its existing quadrivalent vaccine (Gardasil®) (29), Gardasil-9® was approved by Health Canada in 2015 for use among females aged 9-45 for protection against cervical, vulvar and vaginal cancers caused by HR-HPV types and associated precancerous lesions caused by the vaccine-targeted HR- and LR-HPV types, and genital warts caused by LR-HPV types; and for use in males and females aged 9-26 for protection against anal cancer caused by HR-HPV types and precancer caused by HR- and LR-HPV types, and genital warts in males caused by LR-HPV types. Gardasil-9® offers protection against five additional HR-HPV types, responsible for up to 20% more cervical cancer cases worldwide, above the 70% of cases caused by HPVs 16 and 18, totalling 90% (24, 25, 27, 28)[3].

Current HPV vaccines are exclusively prophylactic; therefore, it is generally recommended that routine vaccination be administered to pre-teen girls prior to the onset of sexual activity (aged 9-12 years in Canada; 11-12 years in the United States), with "catch-up" vaccination for females

[3] Assuming that the cost of this vaccine does not exceed $11 per dose (compared with its predecessor, Gardasil®), a recent Canadian modelling study suggests that it may be more cost-effective based on the endpoint of quality-adjusted life-years gained (46). The most cost-effective approach to reduce the burden of HPV among both genders remains to attain high vaccine coverage among females (aged 9-13) and rely on herd immunity to protect heterosexual males (47, 48).

aged 13-26 years (25, 49, 50). Considering the long latency between HR-HPV infection and development of cervical cancer we expect that it will be close to 20 years before a reduction in cervical cancer mortality is observed in Canada as most provincial vaccine programs are only targeting pre-teen girls, with some provincial "catch-up" programs extending coverage to females up to grade 12 (Ontario and the Northwest Territories) or <18 years of age (Quebec). British Columbia recently funded a limited time program, offering free vaccination to females up to age 26 (initiated in 2012) but this program ended in 2015. Since being introduced in 2007, Australia has offered free HPV vaccination to females up to 26 years of age, which has led to high vaccine uptake and early observed reductions in the rate of high-grade precancerous lesions (51). Recently, declines in the high-grade cervical lesions or dysplasia have also been reported among certain vaccinated populations in Canada (British Columbia, Manitoba and Ontario) (52-54) and in the United States (Connecticut) (55).

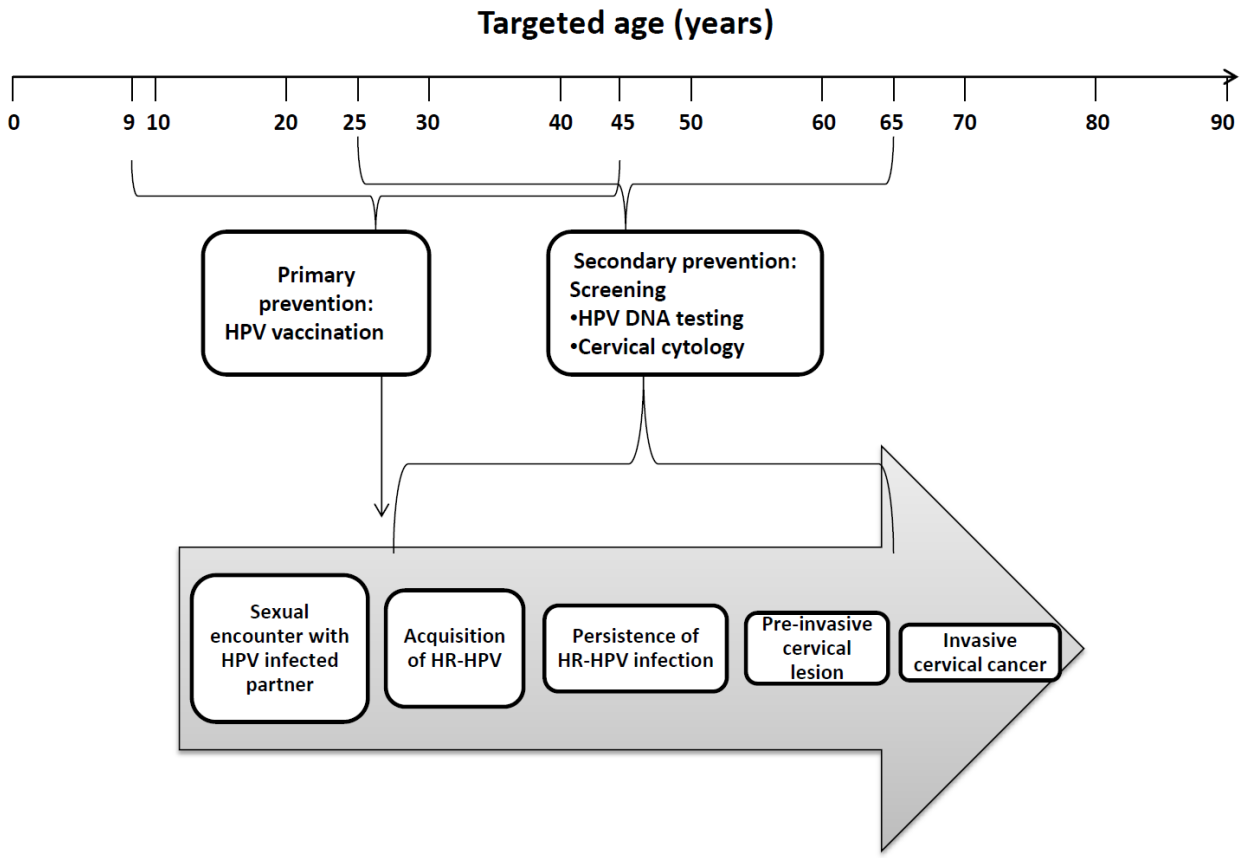


Figure 1. Opportunities for cervical cancer prevention at different time points along the pathway from exposure, acquisition and persistence of HPV, to development of pre-invasive lesions. In the absence of screening, only a very small fraction of pre-invasive lesions would be expected to progress to invasive cervical cancer. The approved age range for HPV vaccination of females in Canada is 9-45 years. Current Canadian guidelines recommend screening of females 25-65 years of age. Adapted with permission from reference 117 (Tota et al., Current Oncology 2015).
HPV, human papillomavirus; HR-HPV, high oncogenic risk HPV.

In Canada, the highest vaccine coverage rates among girls have occurred in Atlantic provinces and Quebec (generally above 85% for the first dose), whereas in other parts of the country, HPV vaccination rates remain lower. In 2010, Ontario had one of the lowest coverage rates in Canada at 59% (for three doses) among grade 8 girls targeted in school systems (56); however, uptake has now increased to 80% and it is hoped that it will continue to rise in all provinces to improve herd immunity, e.g., >90% coverage has already been achieved in Newfoundland and Labrador (57).[4]

Current Secondary Cervical Cancer Prevention Strategy

Pap cytology and colposcopic management

The Pap test (named after the Greek pathologist, Georgios Nicholas Papanicolaou) was described to be a useful approach to detect uterine cancer in 1941 (60). Today, Pap screening is the main reason that most high-income countries have witnessed major reductions in cervical cancer mortality (3, 4).

For decades, screening guidelines in high-income countries have recommended annual Pap testing starting at age 18, or shortly after becoming sexual active. The rationale for encouraging a woman to "Get your annual Pap!" was to compensate for the test's low sensitivity and to achieve an acceptable level of safety. Studies summarized in the Duke Report that were free of verification bias (61) and a more recent pooled analysis of European and Canadian studies (62) report the sensitivity of a single Pap test for detection of CIN2/3 to be between 51% and 53%, but with very high specificity ranging from 96% to 98%. This implies that roughly half of women with cervical lesions will erroneously be classified as negative. The low reassurance offered by a single negative Pap exam in the context of cervical cancer screening has traditionally been the reason for recommending testing at periodic intervals.

In addition to the high costs associated with Pap screening at frequent regular intervals, treatment of detectable high-grade precancerous lesions using cryotherapy, laser vaporization or conisation, loop electrosurgical excision procedure (LEEP), or cold-knife conisation may lead

[4] Nurses and physicians play an important role in addressing concerns and providing reassurance regarding safety, i.e., communicating the fact that the rate of reported adverse events is comparable between HPV vaccine and placebo recipients and within expected background rates in the general population (58). However, anti-vaccine activism (propagated mainly through the internet) is an important issue that limits the ability to achieve higher coverage and that must be addressed (59). Continued monitoring of safety (including rare adverse events) and vaccine effectiveness is needed, and may be achieved through greater linkage with disease registries, and eventually through screening registries.

to future adverse pregnancy outcomes, including pre-term birth and second trimester miscarriage (63). Recently, the Canadian Task Force on Preventive Health Care (CTFPHC) guidelines were updated, recommending that Pap testing be repeated every 3 years, starting at age 25 (64). This revision reflects the desire to avoid overtreatment of young women and our knowledge of the natural history of cervical cancer and progression rates, i.e., within three years of a negative Pap test the development of high-grade cervical abnormalities or worse are rare, and that among females less than 25 years of age, precancerous lesions prompting treatment are common but unlikely to progress to cervical cancer quickly (not before age 25) and most will regress on their own (65-73).

Despite updated guidelines in Canada (64, 74) and the United States (75) recommending Pap screening at extended intervals (every three years) and not among females aged less than 21 (74-76) or 25 years (64), moving away from annual screening has not been universally accepted by patients or by clinicians (77, 78). Furthermore, in the US – where litigation and malpractice lawsuits are common and healthcare delivery is very different from that in Canada – Pap tests being ordered for females less than 21 years of age ranks among the top five most wasteful procedures in primary care medicine, amounting to nearly 50 million dollars annually (79). Meanwhile, in Ontario, there has already been a dramatic drop in the volume of Pap tests since issuance of the new guidelines and support by the Ministry of Health and Long-Term Care, which aligned provider fee schedules with the new recommendations – not reimbursing primary care physicians for Pap tests performed during the time interval between recommended screens among asymptomatic women (9).

Acknowledging the limitations of conventional Pap cytology, there has been some effort to improve its performance over the years. A good example is the development of liquid-based cytology (LBC), which does not require the Pap test sample taker to spread exfoliated cell samples onto glass slides. Instead, samples are placed into a liquid fixative solution and subsequently via automation, uniformly smeared, thin cell preparations on glass slides are stained. Reading these monolayer slides is easier and aided by the reduced density of extraneous materials such as red blood cells and inflammatory cells on the slide, which can obscure the identification of abnormal cells. The leftover cell suspension permits immediate or delayed ancillary molecular testing. ThinPrep® (Hologic, MA, USA) and SurePath™ (Becton, Dickinson and Company, NJ, USA) are two of the most common LBC tests that have received regulatory Health Canada and FDA approval, including for use in conjunction with other commonly used commercial HPV tests. The screening of LBC slides may also be automated, ultimately making it less costly for high-volume laboratories. There are numerous studies comparing the diagnostic performance of LBC to conventional cytology. Most showed marginal increases in sensitivity and decreases in specificity with LBC, and more recent studies including one meta-analysis (80) and two large randomized controlled trials (RCT) (81, 82) suggest that it

offers no clinical performance advantage over conventional Pap smears for detection of high-grade precancerous lesions. Given their equivalence in performance, the decision by laboratories to incorporate LBC is generally taken in light of cost-effectiveness analysis, practicality to laboratories and cytotechnologists and ability to serve as a platform for molecular testing.

Pap cytology screening has had tremendous success in reducing the incidence and mortality from cervical SCC in most developed countries (83); however, it is less effective in detecting and preventing AC and its precursors (84-87). In Ontario, this explains the lack of association observed between income (a variable strongly linked to Pap test screening) and AC risk, despite a strong association with SCC (10). The issue is related more to sampling the AC and precursor lesions rather than under-screening slides with abnormal cells. The arrival of new samplers for dual collection of material from the ectocervix and endocervical zones (i.e., using both a spatula and endocervical brush for collection of glandular cells) that are compatible with both LBC and conventional cytology is thought to have contributed to declines in cervical AC rates observed in some settings, including in Ontario, in recent years (88).

The majority of efforts devoted to improving cervical screening have focused on evaluation of new primary screening tests, and triage tests to guide referral to colposcopy. But ultimately it is the biopsy result that determines how a patient should be managed. We will not describe the recommended management strategies in detail, but all females with a diagnosis of CIN3 regardless of age and females aged 25 years or above with a diagnosis of CIN2+ should be treated according to Canadian guidelines (89). Due to the higher rate of regression of CIN2 among females under age 25 (65-72) and the elevated risk of pregnancy related adverse events among treated individuals (63), repeat colposcopy every six month (for up to two years and then treatment with ablative methods or LEEP if the lesion persists) is the preferred management strategy for younger women (89). To improve the sensitivity of colposcopy for detection of high-grade SIL, recent studies demonstrate that taking multiple biopsies is beneficial (improvement from 61-68% with single biopsy to 96% after three biopsies) (90, 91), suggesting that the added cost/potential harms of taking more than one biopsy could be outweighed by the opportunity for earlier management, fewer referrals for additional colposcopies, and greater reassurance associated with negative results (91).

Anticipated impact of HPV vaccination on cytology screening performance

Current vaccines do not offer protection against all of the HR-HPV types that cause cervical cancer and therefore cervical screening will continue to be recommended among vaccinated populations to detect lesions caused by the remaining HR-HPV types. The lower prevalence of squamous abnormalities in vaccinated cohorts is expected to have major negative

consequences on the accuracy and efficiency of current Pap cytology screening programs (92, 93).

The positive predictive value (PPV) is a useful measure for clinicians because it provides a probabilistic value concerning the action (in this case, colposcopy) prompted by a positive test result. A recent modelling study suggests that with lower prevalence of cervical lesions in the post-vaccine era (up to a 90% reduction, based on the protection offered by the newest nonavalent vaccine) there will be a substantial drop in PPV of cervical cytology (29, 93). For cytotechnologists responsible for evaluating the smears, the reduced prevalence of abnormalities that are serious enough to warrant slide review (e.g., from 10% to 1%) will likely lead to fatigue (given the expectation that abnormalities will be rare) and as a result smears may be under-screened leading to more false-negatives and consequent reduced sensitivity. Estimates of Pap sensitivity (as low as 35%) from low-risk settings in Canada (e.g., Newfoundland and Quebec) provide anecdotal evidence to support this prediction (94, 95). On the other hand, although there will be fewer women with smears containing squamous abnormalities the prevalence of inflammation and reactive atypias will remain the same, thus leading to a reduced signal-to-noise ratio in discriminating true squamous lesions from other benign conditions. Fear of missing relevant abnormalities may lead to more overcalls and consequent reduced specificity with more unnecessary referrals for colposcopy (93). Incorporating reduced sensitivity (high of 70% to a low of 30%) and specificity (high of 98% to low of 95%) into models led to PPV estimates less than 10% (Figure 2), therefore suggesting that in the post-vaccine era Pap cytology will no longer function as a suitable test for primary screening (93). However, the Pap test has the potential to perform with adequate accuracy in the context of a triage test for women found to be HR-HPV positive on primary screening, which will be discussed later.

HPV vaccine uptake in Ontario has already translated into a significant decline in the incidence of cervical dysplasia in this province (5.70 fewer cases per 1,000 girls, equivalent to a 44% reduction) (53), reflecting the urgency to adopt an alternative primary screening test. The foremost alternate test based on performance and efficiency is molecular HPV testing.

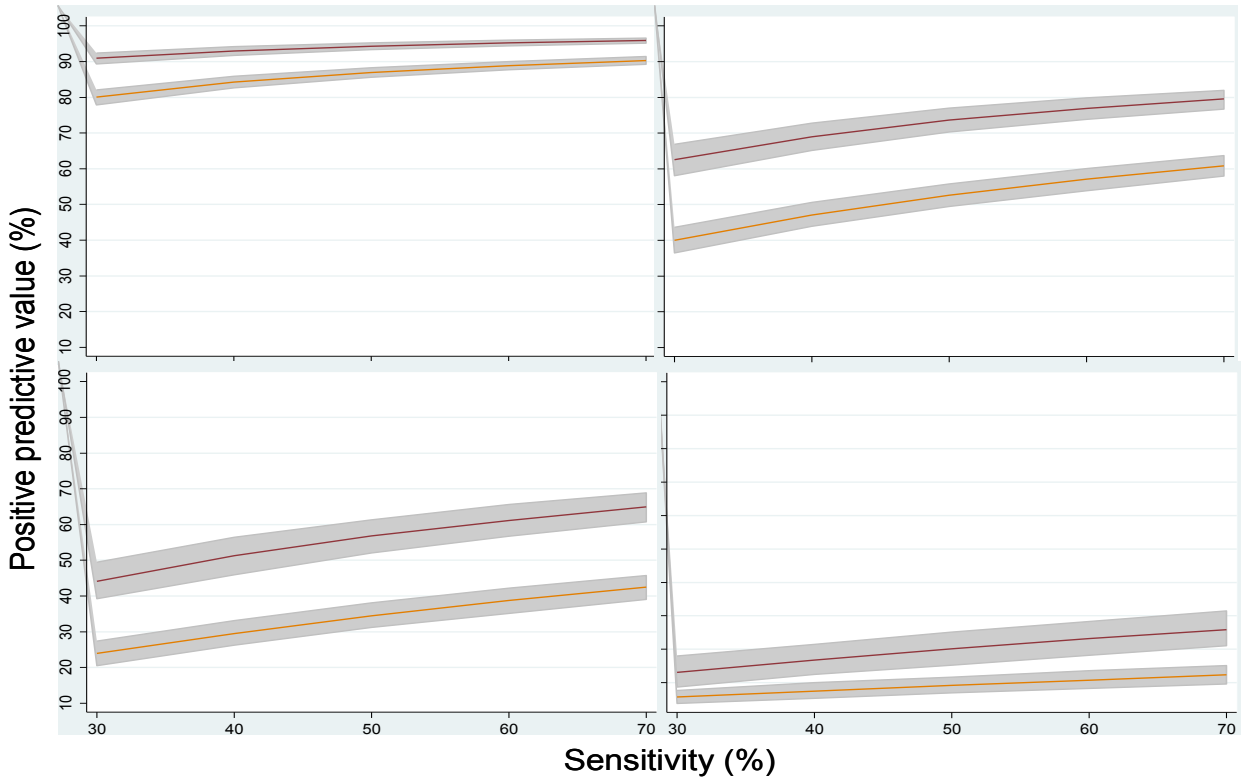


Figure 2. Positive predictive value of cytology screening based on varying lesion prevalence, sensitivity, and specificity. The prevalence rate in the upper left graph (40%) represents the situation found in triage following an initially positive HPV test, whereas the prevalence rates in the other three graphs illustrate situations found in Pap cytology (primary) screening in different settings as well as the ones anticipated post-vaccination (upper right=10%, lower left=5%, and lower right=1%). The two curves in each graph represent different specificity estimates (red line=98%, orange line=95%), and the grey bands surrounding each curve represent the 95% credibility intervals. Adapted with permission from reference 93 (Franco et al., Archives of Medical Research 2009).

HPV Testing: The Best Evidence-Based Option for Cervical Cancer Screening

Following the realization that HPV infection is a necessary cause of cervical cancer and associated high-grade precancerous lesions (1) investigators immediately proceeded with trials to compare the screening performance of HPV DNA testing and Pap cytology. The Canadian Cervical Cancer Screening Trial (CCCaST) (96), which focussed on females aged 30-69 years, was the first North American RCT to demonstrate the superior sensitivity of HPV DNA testing over conventional Pap cytology for the detection of high-grade precancerous lesions (95% versus 55%) and found that it had a slightly lower specificity (94% versus 97%). Other RCTs of HPV testing conducted in Europe have also found HPV testing to perform better (compared with cytology) for detection of high-grade lesions (97-101). In an RCT conducted in rural India, investigators reported a significant reduction in cervical cancer mortality associated with a single round of HPV testing, compared with a single round of cytology screening (102). Results from randomized and non-randomized trials comparing the performance of HPV testing versus Pap cytology were recently meta-analyzed by Richardson and colleagues, showing greater sensitivity (Ratio=1.29, 95% CI 1.18-1.39) but lower specificity (Ratio=0.94, 95% CI 0.92-0.96) for detection of high-grade (CIN2+) lesions (103). In addition to being much more sensitive, HPV testing is now automated, making it less prone to human error and more reproducible compared with cytology, which relies on cytotechnologists and pathologists interpretation for smear evaluation (104, 105). In the context of a cervical screening program, it may also be centralized to ensure sufficient quality control for high specimen throughput, which would make HPV primary screening much more efficient.

Recently, a study that included follow-up data from four European RCTs (97-100) revealed a significantly lower rate of invasive cervical cancer in the HPV testing arm compared with the cytology arm (106). The reduction was larger for AC than for SCC (rate ratio: 0.31 versus 0.78, respectively), which suggests that introducing HPV testing as the primary cervical screening test could lead to further reductions in cervical cancer mortality, due in part to greater reductions in cervical AC (106). A larger proportion of AC than SCC cases are attributable to HPVs 16 and 18 (85% versus 70%, respectively), which also has important implications for vaccination in the primary prevention of cervical cancer (107, 108). The improved ability of HPV testing to detect atypical glandular cells and AIS should be considered a major advantage of switching to HPV primary screening because (as discussed previously) cytology screening has had little success in reducing cervical adenocarcinomas (84-87).

A negative HPV test provides much greater reassurance to women that high-grade cervical precursors are absent relative to the same situation when considering immediate risks after a negative Pap test. But because incident infection with oncogenic HPV type(s) is an event that occurs much more “upstream” in the carcinogenic pathway, it also provides longer reassurance – thus enabling a longer safety margin for screening intervals (109) . There have been several

large trials (106, 110-113) comparing risk of high-grade cervical precancer and invasive cancer following a negative HPV or Pap test (Table 2).

Table 2. 3- and 5-year risk of CIN3+ and invasive cervical cancer following negative HPV or cytology test results

Population/Study (Author, Year)	N	Negative Test at Baseline	3-y CIN3+	5-y CIN3+	3-y Cancer*	5-y Cancer*
7 European studies (Dillner, 2008)	24,295	HPV	0.12	0.25		
		Cytology	0.51	0.83		
KPNC (Katki, 2011)	331,818	HPV	0.063	0.17	0.012	0.019
		Cytology	0.17	0.36	0.018	0.037
KPNC (Gage, 2014)	1,011,092	HPV	0.07	0.14	0.011	0.017
		Cytology	0.19	0.31	0.02	0.031
4 European trials (Ronco, 2014)	176,464	HPV			0.0046	0.0087
		Cytology			0.0154	0.036
ATHENA (Wright, 2015)	42,209	HPV	0.34			
		Cytology	0.78			

ATHENA, Addressing the Need for Advanced HPV Diagnostics; CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; KPNC, Kaiser Permanente Northern California
The population in the 2011 KPNC study is also included in the updated 2014 KPNC study. The Swedish Swedescreen study is included in both Dillner and Ronco's analyses.
*The analysis by Ronco et al. presented cancer risk estimates at 3.5 and 5.5 years.

Recently, two important studies comparing this risk were conducted among a population of females participating in the KPNC cervical screening program (part of a large integrated health delivery system in the United States) (110, 111). Between 2003 and 2012, females in this program received concurrent Pap and high-risk HPV testing (Hybrid Capture 2, Qiagen, Germantown, MD) at approximately 3-year intervals. In the most recent analysis (n=1,011,092; representing the largest and longest experience with routine HPV testing in clinical practice), investigators reported much lower risks associated with a negative HPV test compared with a negative cytology test result (111). Specifically, 3- and 5-year risk of CIN3 or cancer following a negative HPV test at entry was 0.07% and 0.14%, compared with 0.19% and 0.31% following a negative cytology test, respectively. Similarly, risk of cervical cancer at 3- and 5-years was 0.011% and 0.017% in the HPV negative group, compared with 0.020% and 0.031% in the

cytology negative group, respectively (111). A key observation from this study is that the 5-year risk of high-grade cervical precancer associated with a negative HPV test is lower than the 3-year risk associated with a negative cytology test (0.40% versus 0.48% for CIN2+ and 0.14% versus 0.19% for CIN3+, respectively). In an earlier report published in 2011, focusing on the same KPNC population (n=331,818) with follow-up through 2009, investigators reported lower 5-year versus 3-year risk of CIN2+ (0.53% versus 0.96%) and identical 5- and 3-year risk of CIN3+ (0.17%) following negative HPV and Pap tests, respectively (110).

Sufficient evidence now exists to support the safety of the adoption of HPV primary screening and of extended screening intervals, e.g., from 3- to 5-years with similar/lower risk compared with cytology. Fewer required lifetime screens suggests that this approach may also be more cost-effective. Once funding is in place for HPV testing, we would also expect there to be a reduction in individual test costs resulting from market expansion and high volume testing, i.e., additional manufacturers bringing their molecular HPV tests for validation and regulatory approval, and the opportunity to negotiate lower prices with manufacturers based on high-volume testing and the agreement to purchase many units. To ensure safety, it is important that the selected HPV test(s) have been approved by regulatory agencies such as Health Canada and the US Food and Drug Administration (FDA), and that the labs performing these tests are accredited with mechanisms in place to validate performance of testing, i.e., precision, accuracy, reproducibility, reference range, sensitivity and specificity.

An Additional Step after HPV Screening: Triage of HPV Positive Women

One important concern related to the safety and efficiency of implementing HPV primary screening is the increased number of colposcopy referrals that may result, unless a specific triage test/approach is applied. In the ATHENA trial, designed to evaluate the cobas® HPV Test (Roche Molecular Systems, Pleasanton, CA) as the primary screening method for cervical cancer (112), genotyping (types 16 and 18) and cytology (atypical squamous cell of undetermined significance (ASC-US) threshold) were the evaluated triage approaches. In combination, they yielded significantly more colposcopies per case detected but fewer missed cases of cervical cancer or high-grade precancerous lesions (CIN2 or CIN3) than cytology-based screening.

In the post-vaccine era we expect HPV screening would lead to fewer colposcopy referrals as a result of successful vaccination programs aimed at reducing the burden of disease (i.e., HR-HPV infections and associated cancerous/precancerous lesions). Nonetheless, this particular approach (genotyping and cytology triage), despite the high number of colposcopy referrals, was still found to be the most cost-effective compared with other strategies in a recent modeling study, including cytology screening (with reflex HPV testing for management of ASC-US), HPV and cytology co-testing, and HPV primary screening (with reflex to cytology) (114). The last strategy (primary HR-HPV screening with cytology triage) takes advantage of the

desirable properties of both tests (i.e., high sensitivity of HPV and the high specificity of cytology testing) (115-117) and is currently being evaluated in a population based RCT in British Columbia (HPV FOCAL study) (118), as well as a feasibility study within a university-affiliated public hospital in Montreal (Viral Testing Alone with Pap Triage for Screening Cervical Cancer in Routine Practice (VASCAR study)) (119). In a Canadian cost-effectiveness analysis comparing alternative cervical screening strategies (cytology with/without HPV testing for ASC-US triage, HPV testing with/without Pap triage, and HPV/cytology co-testing), HPV testing with Pap triage required fewer colposcopies compared with HPV testing alone (55-59% less), and was much more effective and less expensive compared with primary cytology screening (120). Cost savings of this approach were attributed to the lengthened screening interval and fewer cases of invasive cervical cancer requiring treatment. Other well designed cost-effectiveness analyses of cervical screening in Europe and Mexico (121-124) have produced similar results, i.e., under most scenarios HPV primary screening of women >30 years of age is most cost-effective.

HPV Screening Can Be Done in Self-collected Samples

Not surprisingly, Aboriginal Peoples, recent immigrants, those living in rural or remote settings, older women, and those living in low-income areas or households are less likely to be screened and thus experience a higher risk of cervical cancer than the majority of Canadian women (7, 9, 11-14). Reaching these underserved women can be facilitated by the introduction of HPV primary screening, a technology that can be performed on self-collected samples.

In a recent meta-analysis comparing the accuracy of HPV testing on self-collected versus clinician-collected samples (including data from 36 studies and 154,556 women), sensitivity and specificity for detection of CIN2+ was slightly lower (ratio=0.88, 95%CI 0.85-0.91; and 0.96, 95%CI 0.95-0.97, respectively) suggesting that sampling by a clinician (in the context of a HPV primary screening program) should be the recommended method but that self-collection may be an acceptable option to reach women that do not participate in the regular screening program (125). The performance of self-collected versus clinician-collected specimens for HPV primary screening was recently evaluated in a vaccine trial in Costa Rica and was found to be similar (90% agreement with similar sensitivity and specificity for detection of CIN2+), with improved ability to detect disease earlier compared with cytology. In an Argentinian study, offering women the opportunity to self-collect a specimen for cervical screening (during a home visit by a community health worker) led to a four-fold increase in screening uptake within 6 months (compared with the advice to attend a health clinic for cervical screening), showing that this strategy works to improve coverage in this type of setting.

Soon the Netherlands will switch from cytology to HPV primary screening, with self-sampling devices being sent to women that do not respond to their initial invitation to have a cervical specimen collected by their physician (126). In an effort to reach women living in remote

settings or who do not comply with regular screening (for whatever reason); this approach could also be adopted as part of a HPV primary screening program to increase coverage in Canada (126-129). However, women who do not participate in regular screening may also be less willing to self-collect a specimen for HPV testing or to comply with further follow-up if they are HPV-positive. To maximize the cost-effectiveness of this alternative self-sampling strategy, it will be important to address these and other potential issues. For example, in a study nested within a cohort of women participating in the Canadian HPV FOCAL trial, the most important factor associated with a woman's intention to self-collect a cervical specimen for HPV testing was her knowledge and attitude concerning the procedure, which reinforces the important role of education in improving interventions aimed at HPV and cervical cancer prevention (130).

Merging Vaccination and Screening: Clinical Management based on Net Risk

Considering the high additional cost from newly introduced HPV vaccination programs, it is important to be pragmatic in deciding how a second public prevention program (cervical screening) targeted at preventing cervical cancer should be redesigned. As indicated earlier, persistent infection with HR-HPV types is a necessary cause of cervical cancer (1); however, not all HPV infections present the same level of risk for progression to high-grade cervical precancer or cancer (131). Similarly, not all HR-HPV infections will progress to cause disease at the same rate, e.g., females with persistent HPV16 infection are at much higher risk of developing CIN3 (or cervical cancer) within the next five to 10 years, compared with those infected with other HR-HPV types (131). This discovery has important implications for screening females who have been vaccinated with the new nonavalent vaccine that protects against HPV16 and certain other HPV types that are more likely to progress quickly to CIN3+. For example, considering the low risk of developing cervical cancer before age 30 (attributable to HR-HPV types not included in the current nonavalent vaccine formulation) there is the possibility that screening initiation may be safely delayed. Furthermore, HPV genotyping could enable more individualized risk-based screening (i.e., equal management of women at equal cancer risk) (132) for benchmarking cervical cancer risk and applying similar management based on different combinations of tests, including genotyping. This concept of 'benchmarking' risk could be incorporated into future screening guidelines following the successful development and evaluation of this type of risk-based screening strategy (133, 134). Finally, introduction of HPV primary screening could allow for linkage between vaccination and screening registries to provide a low cost method to monitor vaccine effectiveness (including type replacement, cross-protection, and protection duration) (117, 135) and also provide valuable information on risk of cervical cancer among vaccinated individuals to inform future recommendations for screening among this low-risk group.

Development and Introduction of HPV Testing: Application for Primary Screening and Triage

Before it was firmly established that HPV is a necessary cause of cervical cancer, studies had already been initiated to evaluate the possible role of HPV testing in stratifying risk of women with abnormal cytology (136, 137). The uncertainty surrounding proper management of women with low-grade SIL and equivocal ASC-US diagnoses is eventually what motivated investigators from the National Cancer Institute to launch the ASCUS-LSIL Triage Study (ALTS): a multi-centre RCT designed to evaluate three alternative methods of management (immediate colposcopy, cytologic follow-up, and triage by HPV testing) (138). Early results from this trial revealed the benefit of HPV testing in the triage of women with ASC-US but not LSIL (139-141), which contributed to the 2001 consensus guidelines decision recommending that women with ASC-US (based on screening using LBC) be tested for HPV prior to colposcopy referral, as the preferred management strategy (141).

All HPV assays developed for screening detect only HR-HPV genotypes, e.g., the Hybrid Capture® 2 (HC2) assay (Qiagen, Gaithersburg, MD) is capable of detecting 13 HR-HPV genotypes from cervical specimens as a group but does not indicate which type individually is detected. In the last 15 years, several commercial HPV assays have been approved by the US FDA and Health Canada for use as an adjunct test for triage of ASC-US cases, and for co-testing with cytology. Recently, the cobas® test, capable of detecting HPVs 16 and 18 individually along with 12 other HR-HPV types collectively (112), became the first HPV test approved by the FDA for primary screening. The different commercial HPV tests that have received regulatory agency approval in Canada and the United States and how the tests can be used are listed in Table 3. Of the eight commercial tests, six are approved for ASC-US triage, and two are approved for primary screening.

Table 3. Commercial HPV tests that have received FDA approval and status in Canada

Year	Test Name and Manufacturer	FDA Approval
1988*	ViraPap® Test, Life Technologies	Premarket approval for use as an adjunct for clarifying equivocal/uncertain Pap results
1991*	ViraType® Test, Life Technologies	Premarket approval for use as an adjunct for clarifying equivocal/uncertain Pap results
1995*	Hybrid Capture® Tube Test (HCT), Digene	Approved as adjunct test for clarifying equivocal/uncertain Pap results
1999	Hybrid Capture® 2 Test (HC2), QIAGEN	Approved as adjunct test for clarifying equivocal/uncertain Pap results **
2003	Hybrid Capture® 2 Test (HC2), QIAGEN	Approved for co-testing with Pap **
2009	Cervista® HPV HR Test, Hologic	Approved as an adjunct, and for co-testing with Pap **
2009	Cervista® HPV 16/18 Test, Hologic	Approved as an adjunct, and for co-testing with Pap (to be used alongside or as a follow-up to the Cervista® HPV HR test) **
2011	cobas® 4800 HPV Test, Roche	Approved as an adjunct, and for co-testing with Pap **
2011	APTIMA® HPV Assay, Gen-Probe (now produced by Hologic)	Approved as an adjunct, and for co-testing with Pap **
2015	cobas® 4800 HPV Test, Roche	Approved for primary screening **

*ViraPap, ViraType, and HCT are no longer available. They are shown here for historical interest.
** Health Canada approved tests
Note: Hybrid Capture® 2 Test (HC2), QIAGEN was licenced for primary screening by Health Canada in 2011.

Proposed HPV Primary Screening Algorithms

It is now well established that HPV testing is much more sensitive than Pap cytology for detection of high-grade cervical precancer and cancer, and offers other advantages as well. Therefore, our knowledge of HPV natural history and pathogenesis towards cervical cancer should be utilized (142). Rather than assign HPV testing an ancillary role in cervical screening (e.g., in the management of ASC-US diagnoses), or along with cytology for co-testing[5], it may

[5] Historically, the first clinical applications of molecular HPV testing were for triage of equivocal (i.e., ASC-US) Pap smears (in the mid to late 90's) and then as a complement to Pap tests in screening, also called co-testing
(footnote continued)

safely be introduced as the standalone primary screening method to maximize efficiency associated with extended screening intervals. In the same KPNC analysis of >1 million women screened in routine clinical practice using cytology and HPV testing, not only was CIN3+ risk much lower in the HPV negative group compared with cytology negative group, but 5-year risk was almost equal in the co-test negative group compared with HPV negative group (0.11% versus 0.14%, respectively) (111). Although we appreciate that these risks are not identical, both are lower than the risk of CIN3+ associated with cytology screening every three years (111) – the current recommended screening strategy in Canada (64). Furthermore, we do not expect that the very small decrease in cancer risk associated with co-testing (versus HPV testing alone) would be considered affordable in most resource constrained nations, such as Canada, with important competing health priorities (114). In a recent study comparing the performance of co-testing versus HPV testing alone in multiple clinic practices (143), Blatt and colleagues report that standalone HPV testing is less sensitive and may lead to higher number of missed cervical cancer cases, compared with co-testing. However, the issue is that they restricted follow-up to one year, which likely led to misattribution of disease detected by HPV testing and missed by Pap testing. According to current US guidelines, women that are HPV positive but Pap test negative should return within one year for rescreening; however, some return visits past this one year anniversary are were not included in this analysis, thus we should expect additional cases to be detected in the Pap test positive group since these women are referred immediately for colposcopy (144).

Castle also highlights the importance of considering the costs and benefits of co-testing versus HPV testing alone over a woman's "screening lifetime", e.g., over at 30-year period, standalone HPV testing every three years would result in four additional HPV tests but six less Pap tests, and possibly offers greater safety than co-testing every five years (111, 144). If countries decide to introduce HPV testing alone as the primary cervical screening approach, there are alternative algorithms that have been proposed and evaluated in large population screening trials and demonstration projects. The two algorithms that we focus on in this report are: 1) HPV testing followed by cytology triage and 2) HPV testing followed by HPV16/18 genotyping + cytology triage.

Considering the high specificity, as well as the comfort and confidence that clinicians/patients now place in performing or receiving a Pap exam, cytology should continue to play a role in screening, via triaging HPV positive women for colposcopy. Based on current evidence evaluating different triage strategies and tests that have received regulatory approval, at this

(recommended in the US since HC2 was approved in co-testing in 2003). In Canada, only ASC-US triage has become a common use of HPV testing. Co-testing has not been used in Canada except as an option in private sector.

point there are only two triage approaches that may be implemented – one that includes only cytology and one that incorporates HPV genotyping (along with cytology). These algorithms are presented in Figure 3a and 3b, respectively. The only difference between these two strategies is that some women may be referred directly to colposcopy if positive for specific HPV types (either HPV 16 or 18); whereas *all other women* (positive for other HR-HPV types) in the genotyping approach, and *all women* (positive for any HR-HPV type) in the cytology triage approach would be referred to colposcopy if cytology results are abnormal (≥ ASC-US). For women who test HPV positive but have no evidence of disease (based on cytology or colposcopy), repeat HPV and cytology testing should be performed within 12 months. Re-screening following a negative primary HR-HPV screen should occur no sooner than every three years (e.g., every 3- or 5-years) depending on the risk tolerance in a given setting, recognizing that no screening program will prevent all cervical cancer. In Canada, risk tolerance based on current practice of repeat cytology every three years is equivalent to HPV primary screening not less than every five years (64, 111).

HPV/Pap triage approach

The strategy of HPV/Pap triage (145) takes advantage of the desirable properties of both tests, i.e., the high sensitivity of HPV testing and the high specificity of cytology. The concerns associated with maintaining cytology as the primary screening test in the post-vaccine era do not apply in this triage scenario; however, it is possible that smears evaluated by cytotechnologists known to have originated from HPV-positive women (unlike the current situation where cytotechnologists are generally unaware of the specimen HPV status) may be scrutinized more closely given the higher likelihood that a cervical abnormality is present. Originally, it was suggested that this 'artificially enriched' HPV positive population, with higher lesion prevalence and fewer cases of inflammation or reactive atypia (i.e., greater signal-to-noise ratio) would lead to improved diagnostic accuracy (93, 145). However, in a recent Canadian study designed specifically to evaluate this question, investigators found that samples reread by cytotechnologists after revealing the patients' positive HPV status led to slightly worse diagnostic performance (somewhat greater number of false-positive results and lower specificity), perhaps due to increased awareness of possible abnormalities (146). The HPV/Pap triage strategy is now being evaluated in the population based British Columbia HPV FOCAL trial (118), and was also recently evaluated in a community based demonstration project (VASCAR study) in Montreal (119). Results from both of these studies will provide critical information in guiding the development of screening recommendations focusing on this approach.

The HPV FOCAL study is the first North American RCT to compare HPV testing (HC2 with reflex Pap triage using LBC) versus Pap testing (with reflex HPV testing in triage of ASC-US cases) in cervical cancer screening; with a screening interval of four years in the intervention HPV testing arm (two years in the safety check arm) and two years in the control arm (147). As of

January/2011, 18,648 females aged 25-65 years had been randomized to receive either HPV testing with the HC2 test (n=12,494; including both intervention/safety check arms) or LBC with ThinPrep® (n=6,154) as the primary screening test (118). Interim results from round one of this screening trial suggest the HPV/Pap triage approach leads to greater overall detection rates of CIN2+ and CIN3+ (16.1 and 8.0 per 1000 tested in the HPV arm compared with 11.0 and 5.0 per 1000 tested in the control arm, respectively) but also a greater number of colposcopy referrals (57.2 versus 33.2 per 1000 tests in the HPV and control arm, respectively) (118). Recognizing that increased colposcopy referrals and their associated diagnostic and treatment procedures (considered a surrogate for harms from screening) is important, Coldman and colleagues (148) recently estimated the impact of implementing HPV primary screening (with Pap triage) on referral for colposcopy in the British Columbia screening program. Investigators utilized HPV FOCAL trial age-specific/screening-specific results (weighted by screening program distribution) and found that although HPV testing may initially increase rates of referral (compared with adoption of LBC primary screening), cumulative rates over the long-term would be similar, except among younger females aged 25-29 (for this group it would remain higher) and that adoption of either approach (primary HPV or LBC screening) would increase colposcopy referrals in the province, driven by more conservative management of abnormalities in the trial protocol compared to current practice (148). In the FOCAL trial, the lower colposcopy rate over time may be attributed to the lower incident HPV infection rate compared with the cross-sectional baseline HPV prevalence, which was approximately 50% higher.

The VASCAR study is the first community based demonstration project in North America to evaluate primary HPV DNA testing (using HC2) with conventional cytology (≥ASC-US) for triage to colposcopy (119). Beyond the collection of important information surrounding the performance of this approach compared with traditional screening practices, this project provides us with insight into the potential obstacles that must be overcome to ensure successful introduction of primary screening at the provincial/national level. 28,939 women were considered for inclusion in the study and after exclusion criteria were applied, screening results from 26,193 women aged 30-65 years were compared with the historic control era, i.e., cytology screening in the 3 years before VASCAR. Improvements were observed in the detection of high-grade precancerous lesions (6.58 versus 2.37 per 1000 women), as well as in the detection rate of these high-grade lesions among women referred for colposcopy (340.00 versus 163.02 per 1000 colposcopies) and lower median time from a positive Pap triage result to colposcopy (3.14 months in VASCAR versus 10.98 months in the historic period), with a slight rise in rate of colposcopy referrals in this primary HPV screened population (19.36 versus 14.54 per 1000 women) (119). Investigators attributed the improvement in time to colposcopy to the reduced workload of Pap smears being read by cytotechnologists (93% reduction), and the heightened sense of urgency felt by providers to refer a patient with an abnormal Pap test and presence of HR-HPV type(s) for colposcopy.

VASCAR provided also an important lesson in routine implementation of HPV testing. LBC is not currently publicly funded in Quebec, which prompted the need for conventional Pap tests to be used in triaging HPV-positive women. However, initial ethical approval of the study required that once a Pap smear is prepared it must be read and a result must be provided. LBC use would have obviated this legal concern because the cell suspension that serves for both HPV testing and Pap triage does not imply an accession number for the patient. The suspension can be safely stored and a smear prepared for reading only after the HPV test is completed and the result is positive. Therefore, this obstacle forced a second visit for a woman who was HPV positive. Expectedly, given the delays in having notifications sent out and scheduling new appointments for Pap tests, less than half of HR-HPV positive patients (first round screening) had been triaged with Pap cytology at the time of the VASCAR report (119).

This experience should serve as an important lesson for the introduction of primary HPV testing in settings that currently administer conventional Pap cytology screening. By switching to liquid based cytologic samples, efficiency could be improved because the screening process (i.e., all medical acts pre-colposcopy) could be reduced to single visit. The other important lesson to be learned from this demonstration project is that in the initial rollout of HPV primary screening, there may be a learning curve for some healthcare workers who violate the new protocol. For example, in the VASCAR study, 3,414 protocol violations were reported (11.7%), most of which occurred in the first year. A Pap smear being conducted at the initial screening visit (rather than the recommended HPV test) was the most common protocol violation (9.3%); however, among 11 individuals (0.04%) a repeat Pap smear was taken from women who had been referred for colposcopy following positive cytologic triage.

HPV testing followed by HPV16/18 genotyping + cytology triage

In 2015, the Roche cobas® assay became the first standalone HPV test approved by the FDA for cervical cancer screening. This assay separately tests for HPVs 16 and 18 (the two highest risk genotypes) and provides a combined result for 12 other HR-HPV types. The United States ATHENA study (112, 149) (n=41,955) was designed to evaluate the genotyping screening strategy compatible with the cobas® test among females ≥25 years (Figure 3b), along with two other strategies: cytology with reflex HPV testing for management of ASC-US, and a hybrid strategy that incorporates HPV/cytology co-testing for women ≥30 years, or cytology alone for women 25-29 years. The 3-year end-of-study results from this trial, which contributed to the FDA's recent approval decision, are summarized below.

Among females in this trial who at baseline were classified as cytology negative, HPV negative, or cytology and HPV negative, the 3-year cumulative incidence rate (CIR) of CIN3+ was 0.8%, 0.3%, and 0.3%, respectively. Similarly, the overall sensitivity/specificity for CIN3+ associated

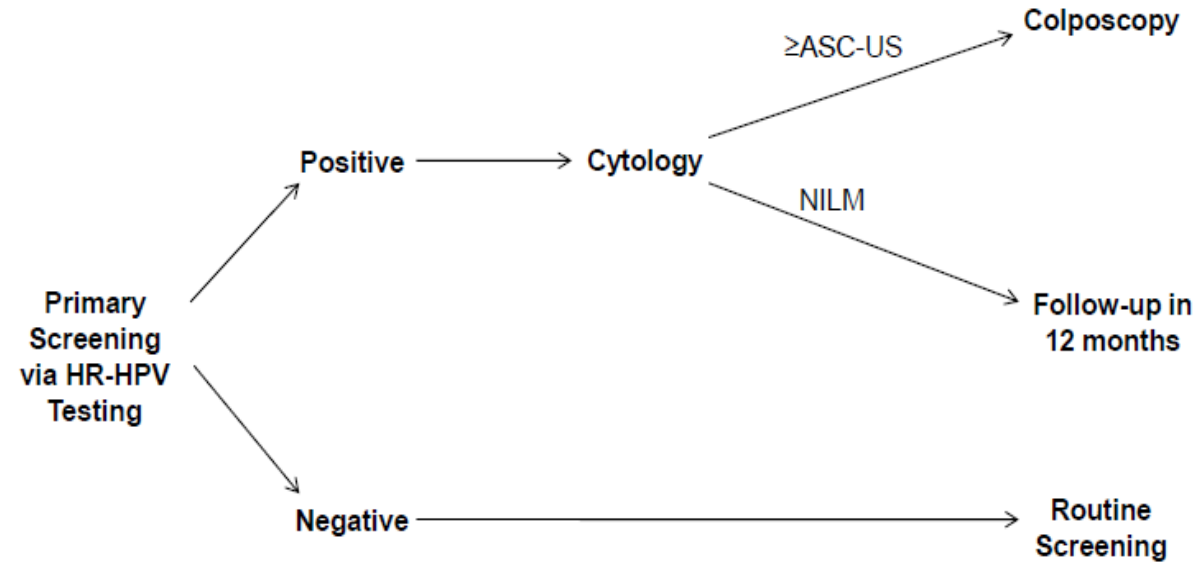
with each of these respective screening strategies (cytology, HPV primary, and hybrid strategy) was 47.8%/97.1%, 76.1%/93.5%, and 61.7%/94.6%, respectively. Compared with cytology and the hybrid testing strategies, primary HPV testing led to a 64.2% and 22.5% increase in the total number of CIN3+ cases detected, but a greater number of colposcopies over the 3-year study period. The potential benefits/harms associated with increased detection of high-grade precancerous lesions/colposcopy referrals was consistent across age groups, but was most pronounced among females <30 years of age. Despite the greater total number of colposcopies associated with this strategy, it led to fewer screening tests and a similar number of colposcopies per case detected, compared with the hybrid (co-testing) strategy (112).

The higher detection of CIN3+ cases by the primary HPV strategy in this trial reflects the possible benefit of HPV genotyping (i.e., testing specifically for HPVs16/18) in the triage of HPV positive women for colposcopy. In a recent study of HPV genotyping (nested within a large cohort of HPV positive women ≥30 years being followed at KPNC), Schiffman and colleagues examined the individual HPV types that provided the most useful risk stratification in the management of HPV positive/cytology negative results (150). Three year cumulative risk of CIN3+ associated with HPV16 and HPV18 infection status among these cytology negative women was 10.63% and 5.89%, respectively. These risk estimates were higher (for HPV16) and approximately the same (for HPV18) as the risk of CIN3+ among women in the same population who were HPV positive (with ASC-US cytology), i.e., the threshold for immediate colposcopy referral according to current US and Canadian screening guidelines. In a separate analysis focusing on the same KPNC cohort study population involving testing of >17,000 specimens using the cobas® HPV test (all from initially HC2 positive women), investigators reported excellent agreement between genotyping results from this test and the well-established LINEAR ARRAY HPV Genotyping Test (Roche Molecular Systems) (kappa=0.86) (151). The 3-year CIR of CIN3+ associated with the three cobas® channels (HPV16, else HPV18, else other HR-HPV) in this KPNC study was 18.5%, 7.8%, and 4.3%, respectively (slightly lower compared with reported estimates from the ATHENA trial, which were 25.2%, 11.0% and 5.4%, respectively) (112). Despite the relatively low risk of CIN3+ among HPV18 positive women in the KPNC analysis, the higher risk of invasive cancer and *in situ* adenocarcinoma associated with HPV18 (arising from glandular lesions that are more difficult to detect by cytologic screening) is another reason to support genotyping for this type, in addition to HPV16.

The possible role of HPV genotyping (HPV16 or HPV16/18) in triaging women for colposcopy is an approach that has been under consideration for some time (152, 153). When considering the benefit of detecting a higher number of cervical precancerous lesions associated with this strategy, it is also important to consider the harms that may be associated with greater number of colposcopies and their associated procedures; particularly among younger women (<30 years) that have a high prevalence of CIN2 lesions, which are likely to regress (63, 65-73). There

is currently no obvious winning strategy when it comes to selecting the best triage approach. We expect the decision will be based on local experience (including demonstration projects such as VASCAR) and successful implementation of primary HPV screening (incorporating either cytology, genotyping + cytology, or perhaps some other novel strategy) in similar settings.

a)



b)

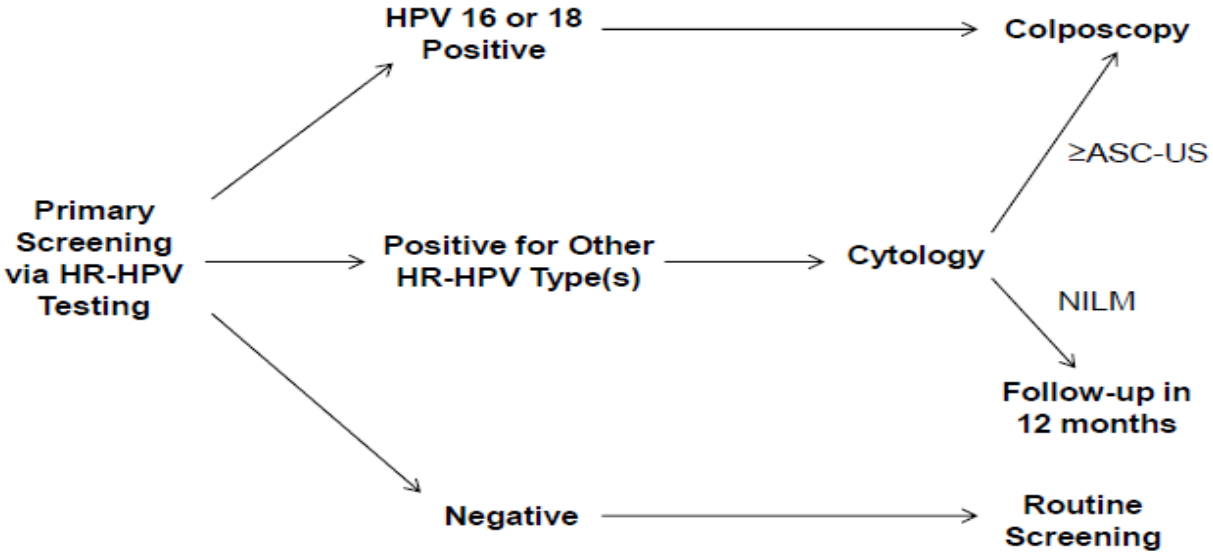


Figure 3. HPV primary screening algorithms incorporating triage with a) cytology only, and b) HPV16/18 genotyping + cytology. An important concern related to both algorithms is management of HPV positive women as a lifelong programmatic issue, including what to do after colposcopy fails to find precancer.

ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; NILM, negative for intraepithelial lesion or malignancy.

Current Cervical Cancer Screening Recommendations and Existing Knowledge Gaps Surrounding the Implementation of HPV primary screening

The Canadian Task Force on Preventive Health Care (CTFPHC) currently makes no recommendation concerning the use of HPV testing in primary cervical cancer screening (either as a standalone test or as part of co-testing with cytology) (64). It recommends cytology screening every three years, beginning at age 25. This is in contrast to the US consensus guidelines, which recommends HPV/cytology co-testing (as the preferred screening approach) every five years for women ≥30 years of age (with HR-HPV positive/cytology negative women returning after 12 months for repeat co-testing, or direct referral for colposcopy if positive for HPV types 16 or 18); or cytology screening every three years for females aged 21-29 years (75). However, the CTFPHC recommendations have not been universally accepted. For example, the Ontario Cervical Screening Guideline Working Group now recommends standalone primary HPV testing every five years for women aged 30 to 65 years, with Pap cytology triage (consistent with the screening algorithm presented in Figure 3a) (74).

Despite convincing evidence that HPV testing should be introduced in primary cervical cancer screening as the standalone test, there remains uncertainty surrounding certain parameters, including age to initiate/discontinue screening, length of screening interval, as well as the best approach to triage HPV positive women for colposcopy. The CTFPHC recommendation that cervical cancer screening be postponed until age 25 was intended to avoid overtreatment and other associated harms among younger women with high prevalence of cervical abnormalities that are unlikely to progress to cervical cancer (63, 65-72). Similarly, due to the high prevalence of HR-HPV infections among women 25-29 years of age, HPV testing of women <30 years is not recommended in any of the current US or Canadian guidelines based on the greater harms associated with higher rates of colposcopy referral and treatment (64, 74, 75). Although the cobas® test has been approved for use among women ≥25 years of age by the FDA, it is still uncertain whether identification and treatment of high-grade precancerous lesions in females 25-29 years of age would translate into a meaningful reduction in the incidence of cervical cancer, i.e., detection of most disease found in this age group may be safely deferred until age 30. Furthermore, as we alluded to previously, once the prevalence of vaccine targeted types (particularly HPV16) declines in the population, it may be decided that cervical cancer screening can safely be delayed based on the known natural history, i.e., slower progression rate from infection with non-vaccine targeted HR-HPV types to CIN3+ (131).

The Ontario Cervical Screening Guideline Working Group recommends cessation of screening at age 65 but acknowledges that the quality of evidence to support this is low (74). Arguments favouring 65 as the age to stop screening include the following: 1) most cases of cervical cancer have the onset with HPV infection acquired in late adolescence and early adulthood, 2) with primary HPV testing there is greater reassurance and more safety in ascertaining when a

women is lesion free, 3) HPV testing allows a longer window of detection opportunity before CIN3 develops, 4) there is no reason to suspect that natural history of HPV infection is different at age 60 compared with earlier ages, and 5) there is the societal (ethical) obligation to re-target resources towards other important competing healthcare priorities, especially considering the increasing proportion of the population becoming >65 years of age (154). Although suggested to be uncommon (75), some cases of cervical cancer among women >65 years of age (preventable by screening) would occur if screening is discontinued above this age, and therefore, this final argument (concerning societal or ethical obligation to prevent cancer) could also be used by opponents in favour of screening beyond this age limit. However, past studies estimating the annual incidence of cervical cancer have not accounted for hysterectomy – a procedure that is very common among women over age 65 – leading to corrected cervical cancer estimates in this >65 age group that are >80% higher than previously reported (155). Furthermore, greater HPV risk in the more recent birth cohorts now exiting screening (i.e., with sexual debut after the sexual revolution) may result in higher future rates of cervical cancer in this age group as well (156).

The high sensitivity of HPV testing in combination with the known natural history of cervical cancer (initiated with HR-HPV acquisition), safely permits the extension of cervical cancer screening intervals. But recently it was suggested that the benchmark for acceptable risk introduced in the 2012 guidelines (recommending cytology every three years) relative to the American Cancer Society 2002 guidelines (recommending conventional cytology annually) may be too high (75, 77, 157). Although the risk of CIN3 associated with HPV co-testing every five years is lower than the risk associated with cytology screening every three years (106, 110-113), Kinney and colleagues (77) make the case that extending the interval for co-testing (or HPV primary screening) to five years (from three years) will lead to an important increase in the lifetime risk of developing and dying from cervical cancer, i.e. an absolute increase of 0.27% and 0.06%, respectively. Ultimately, the decision of what interval length to invite women back for screening will depend on risk tolerance in a particular setting, including available resources.

The advantages/disadvantages of incorporating HPV genotyping in triaging HPV positive women for colposcopy have previously been discussed. However, in addition to cytology and genotyping, there are other molecular tests that have been developed and are currently being evaluated for use in cervical screening; including methylation (and consequent silencing) of host and viral genes (158-162), and cytologic methods that attempt to identify proliferating cells (e.g., $p16^{INK4a}$/Ki-67 staining) (163-166). Both of these approaches are more specific than HPV testing for detection of high-grade cervical precancer and are therefore most likely to be used in the triage of HPV positive women and those with ASC-US/LSIL for colposcopy. p16/Ki-67 dual staining may be accomplishing using the CINtec PLUS Cytology Kit (Roche mtm Laboratories AG, Mannheim, Germany), which is highly reproducible and has been shown to be

more sensitive than cytology and more specific than HPV testing for detection of CIN3+ (165-170). Unfortunately, p16/Ki-67 dual stain cytology (like other cell based assays) is not compatible with self-collected specimens. Therefore, women who self-collect a specimen for HPV testing that are positive would then be required to attend a clinic for collection of an additional specimen for triage testing and colposcopy. In a recent Dutch trial conducted among women who did not attend regular cervical screening but provided a self-collected sample for HPV testing, methylation testing of two genes (MAL and miR-124-2) performed on these self-collected cervicovaginal specimens was compared with physician-collected cytology and displayed similar (non-inferior) clinical performance for detection of CIN2+, among all HR-HPV positive specimens that were evaluated (161). Currently, no commercial methylation testing kit exists; however, considering the potential utility of methylation testing as a triage test (particularly for self-sampling), we expect many companies would be interested in developing one or more of these tests, or are in the process. But before this approach may be considered for clinical use, additional studies assessing its reliability and performance should be conducted, including direct comparisons of alternative triage tests (molecular technologies) for standard endpoints (e.g., specificity for detection of CIN3+), and in different settings.

The rapid pace of technological changes and new discoveries ensures that uncertainty will almost always exist in cervical cancer screening. However, selection of the best test/triage strategy for HPV positive women, or the most appropriate age groups and interval to screen does not change that fact that HPV testing is superior to Pap cytology. Although we should appreciate the importance of these decisions, uncertainty surrounding key issues exists in all screening programs, including those that have been in existence for many years.

Implementation of HPV Primary Screening throughout the World

Despite our expectation that HPV vaccination will eventually lead to declines in the incidence of cervical cancer and precancerous lesions, screening should, at least in the near future, continue to be a part of any comprehensive cervical cancer prevention program. In 1949, Canada was among the first countries to implement Pap cytology screening, and recently, has played a prominent role in evaluating its performance in comparison to HPV testing in cervical cancer screening. Due to the improved performance and efficiency offered by HPV testing, many countries have already moved towards introducing HPV testing as the standalone primary test. Although it is difficult to gather worldwide information on activities underway in every country, in 2012, Castle and colleagues (171) presented selected experiences of countries in North America, Europe, Asia and Africa in planning and implementation of HPV testing.

Recognizing that cytology screening has not been effective in reducing the cervical cancer burden, particularly among low-income women, Mexico recently became the first country to introduce standalone primary HPV testing into their population cervical cancer screening

program. The program has been implemented in all 32 states and targets women 35 to 65 years of age. HPV positive women are currently triaged using cytology and asked to return in 12 months if cytology is negative for repeat HPV testing, or asked to return in five years if their HPV test is negative. To date, over six million women in Mexico have been screened for HPV, and recent studies evaluating the performance of HPV self-sampling in this country suggest that improved screening coverage (achieved by home-based collection) comes without a great loss in overall performance (128, 129).

Like Mexico, Turkey is another middle-income country that lacks the resources and infrastructure to introduce an effective cytology cervical cancer screening program. However, recognizing the efficiency/performance advantages that HPV primary screening offers, and following extensive national/international consultations and pilot studies to assess feasibility, Turkey also recently introduced HPV primary screening. Its goal in the next five years is to screen 13.5 million women, applying an algorithm that is similar to Mexico's but incorporates genotyping for HPVs 16 and 18, i.e., women who are cytology negative but positive for either of these high-risk genotypes will immediately be referred for colposcopy. Primary HPV testing is being conducted using HC2, and tremendous implementation support is being provided by the manufacturer (QIAGEN), which has been asked to assist by providing complete sample collection sets, training of practitioners for sample collection, and to oversee the entire laboratory operation, including testing received samples, reporting, quality control, documentation processes, information technology, and employment of all testing staff including pathologists and microbiologists. All HPV testing is being consolidated and centralized into two major laboratories, which should improve and maintain the quality of testing, standardized processing of specimens, and reduce costs.

Italy also recently moved forward with HPV primary screening. Unlike Mexico and Turkey, Italy already has an established and successful cytology cervical cancer screening program in place; however, based on results from a health technology assessment (HTA) submitted to the ministry of health, it was acknowledged that early adoption of standalone HPV primary screening would be advantageous. Components of their HTA included results from the local New Technologies for Cervical Cancer (NTCC) screening trial (97), recent pilot studies evaluating the feasibility of this approach in regions of northern Italy (~80,000 women enrolled/year) and Abruzzo (~40,000 women enrolled/year), and an economic evaluation. To date, HPV primary screening programs have been introduced in nine Italian provinces, with >175,000 women tested each year. Other European countries that we are aware of, which are planning or expected to introduce HPV primary screening shortly include the Netherlands (introduction in 2016), Sweden (planning for 2017; with aim to implement individualized risk prediction algorithm into its program), and Scotland (planning for 2018/19; pending government approval of the business case that had been submitted). A commonality among countries that have been

early adopters of HPV primary screening is that local champions (including prominent researchers, clinicians and other key opinion leaders) have played key roles in convincing their respective ministries of health to move forward with planning activities and the commitment to introduce this approach.

Recognizing that cytology screening is no longer the only suitable test for primary cervical screening, a series of supplements describing the potential for HPV primary screening to improve cervical cancer prevention efforts will soon be published by the European Commission. These new European screening guidelines were also recently summarized in a separate article (172). Briefly, the new guidelines now recommend primary testing for oncogenic HPV types in organized, population-based programs, and avoidance of HPV/cytology co-testing, i.e., only one test should be used at any given age in cervical cancer screening. It is also recommended that routine HPV primary screening not begin until age 30, discontinued at the same upper age limit for cytology screening (e.g., age 60 or 65), and occur at regular 5 to 10 year intervals (depending on age and prior screening history). Cytology testing of HPV positive women (preferably using the same specimen collected at the initial HPV testing visit) was an approach suggested for triaging women to colposcopy.

A tremendous amount of evidence exists to support the introduction of HPV testing as the standalone primary screening test, so the focus in Canada should now be on practical steps to support its implementation, which requires the cooperation of governments, clinical leaders, cancer agencies, and other medical associations. In November/2014, the Pan-Canadian Cervical Screening Network (PCCSN) assembled a workshop, inviting cervical screening experts to discuss implications of implementing population-based HPV testing and to identify and address the needs of providers/practitioners, program participants, laboratories, and screening programs in HPV based cervical cancer screening. A report summarizing results from this workshop has been produced, outlining the needs of the respective stakeholder groups, and associated recommendations for action. One of the key messages from this report, which is expected to become publically available shortly, is how important it is to provide education to practitioners and screening participants on the natural history of HPV/cervical cancer (including the high incidence and clearance rate of HPV), while keeping the focus on cancer prevention rather than HPV (similar to this situation with vaccination). Both groups should also appreciate that the main reason for transitioning to HPV primary screening (despite the opportunity for an extended screening interval and cost-savings) is to improve the performance of screening. There is also expected to be a tremendous practitioner and patient learning curve associated with introduction of HPV testing, which was demonstrated in the VASCAR study by the initial high number of protocol violations.

Experience from Canadian pilot studies and demonstration projects (such as the HPV FOCAL and VASCAR studies), successful transitioning from cytology in other countries (e.g., Italy), and convincing data from cost-effectiveness studies (similar to recent analyses carried out for Quebec, Mexico and other European countries) (120-124) will be important components of HTAs and building a strong business case to support the switch to HPV primary screening. Provinces should also consider the opportunity for group pricing if multiple jurisdictions decide a move towards LBC and HPV testing. In light of the VASCAR study experience on the need for reading and reporting results on all conventional cytology smears regardless of HPV test results, provinces that have similar legal imperatives and have not yet implemented LBC should do so as a concurrent step with the introduction of HPV testing. As discussed, this would permit reflex cytology testing and avoiding costly and harmful delays in referring patients for colposcopy. Introduction of HPV primary screening will also require the establishment of organized programs incorporating an information system with a protocol for identification, invitation, screening, follow-up and monitoring of participants. As different professional societies in Canada move towards recommending HPV primary screening, they should strive for consensus in their guidelines. This would prevent clinicians from “cherry picking” recommendations they decide to follow (117).

Switching from Pap cytology to HPV primary screening with extended intervals is expected to be a difficult transition for some patients and providers. In a recent Canadian study (nested within the HPV FOCAL trial; n=981) that was designed to assess the potential impact of HPV testing on women’s intentions to be screened, 84% of women responded that they intended to attend HPV-based cervical cancer screening; however, this number dropped to 54% when the screening interval was extended, and dropped even further (to 51%) when the starting age for screening was delayed to 25 years (173). In a similar study evaluating the opinion of BC-registered colposcopists, the majority (53%) in 2011 believed that an interval length of four years between HPV tests is too long (174). Acknowledging the importance of proper education in the acceptance of HPV vaccination (another novel intervention aimed at cervical cancer prevention) (59), education of practitioners about HPV primary screening so that they can properly educate their patients will be critical to ensuring that compliance with cervical cancer screening remains high.

Recommendation of steps to be taken

The above concerns should be viewed as implementation items that require collective thinking among provinces and professional societies. None of the sources of variations discussed above are key obstacles to the implementation of HPV testing as primary technology in cervical cancer screening in Canada. Policymakers should not postpone decisions assuming that eventually RCTs will answer questions related to age to start screening, intervals, and age to stop screening. In addition to being ethically intractable, such questions cannot be answered by

RCTs because of the prohibitive cost in terms of sample size and long trial duration to yield clinically meaningful answers. Choice of HPV testing technology is also of secondary importance. All clinically validated and approved HPV tests perform with comparable sensitivity and specificity and follow internationally-accepted benchmarks of performance (175). All HPV assays approved in Canada and in the US are backed up by extensive clinical data that document their acceptable performance. Given their similarity, head-to-head comparisons of such assays via RCTs would be enormously costly and not likely to be funded by the public sector.

The adoption of LBC in some Canadian jurisdictions should serve as an example of pragmatic implementation. One cytology technology (conventional Pap tests) was replaced by another (LBC). Given their equivalence in performance, the decision to incorporate LBC was taken in light of cost-effectiveness analysis, practicality to laboratories and cytotechnologists and ability to serve as a platform for molecular testing. The same mindset of professional pragmatism should exist for adopting HPV testing in cervical cancer screening.

In summary, despite the sufficiency of the science on this subject, molecular HPV testing has not become a frontline strategy in cervical cancer screening in Canada. Of particular concern is the fact that the first cohorts of vaccinated women are reaching screening age. Pap cytology will be an inadequate mainstay of cervical cancer screening for the near future, a situation that will be further aggravated after the provinces switch to the nonavalent HPV vaccine. At present, most of the hesitation comes from the mistaken perception that cervical cancer screening must first be properly organized before technological changes can be made. In fact, the opportunity for changing the core technology is a major incentive for implementing organized screening in Canada. Another reason for hesitation is the decades-long reliance that cervical cancer control has had on cytopathology, as its core professional discipline. Adoption of HPV testing for ASCUS triage, albeit beneficial, now serves as a distraction in professional education and in taking the focus out of the value of HPV testing as the ideal anchor technology in cervical cancer screening.

Disclosure of potential conflicts of interest

F.C. receives financial support to perform research projects as well as for oral presentations on HPV from Merck Sharp and Dome, and Roche Molecular Systems. A.F. served as a member of the pathology panels for screening and vaccine clinical trials conducted by BD, Cepheid, Ventana/Roche, Roche and Merck. E.L.F. has occasionally served as a consultant to Merck, GSK, Roche, and Gen-Probe, and his institution received unrestricted grants from Merck. M.H.M served as a site principal investigator for the Merck nonavalent HPV vaccine trial and has received unrestricted research grants from Qiagen and Merck. G.O. has not personally received funds but has received support to conduct adjunct studies evaluating the accuracy of different

HPV assays, as part of the HPV FOCAL study. S.R. received honoraria, and research grants from Hologic and Roche diagnostics. No potential conflicts of interest were disclosed by the other authors.

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