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The newsletter on Human Papillomavirus

HPVWorld

HPV Screening in the way to cervical cancer elimination

Marc Arbyn with

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We know HPV is a family of viruses that can cause cancer
We have excellent HPV vaccines and HPV screening tests
We can envisage cervical cancer elimination

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nº 54 Interview with

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Marc Arbyn

What has been your area of expertise in relation to HPV screening?

Currently our main expertise comprises synthesizing the evidence related to prevention and treatment of HPV-related cancer by performing systematic reviews, meta-analyses and Cochrane reviews. Within our Unit of Cancer Epidemiology which is part of the Belgian Cancer Center (Scientific Direction of Public Health & Surveillance) of the Sciensano (previously Institute of Public Health) in Brussels, we have built up a core group of young scientists who have learnt the methodology of performing high-quality meta-analyses who are sharing their skills with other international teams. This work is done as contribution to the development of evidence-based practice guidelines. We also have developed new statistical methods and software for synthesizing data, such as the *metaprop* for procedure to pool proportions, diagnostic network meta-analysis and pretest-posttest probability plots. Pooling of survival data by digitizing Kaplan-Meier curves is an ongoing statistical project that was initiated at our unit. We also conducted age-cohort-period analyses of the incidence of and mortality from cervical cancer at European and world level.

What is the Cochrane collaboration and which contributions have they made to the HPV field?

The Cochrane Collaboration is an international, independent not-for-profit organization involving a network of researchers, health professionals, patients, carers and people interested in health. Its main objective is to evaluate interventions for prevention, treatment & rehabilitation by producing systematic reviews of primary research using established methods for summarising and reporting evidence. These reviews are published in the Cochrane Database of Systematic Reviews (http://www.cochranelibrary.com/cochrane-database-of-systematic-reviews/).

A few years ago, we received a grant from the Gynaecological Cancer Cochrane Review Collaboration to conduct a number of Cochrane reviews.

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EU Guidelines on Quality Assurance of Cervical Cancer Screening recommend primary HPV screening in all member states at an interval of at least 5 years and starting from the age of 30-35 years

Several new Cochrane reviews have been accomplished such as those on triage of women with minor cervical cytological abnormalities, the comparison of the accuracy of cytology and HPV tests in primary cervical cancer screening, safety and efficacy of HPV vaccines, and on obstetrical harm associated with treatment of cervical precancer.[1-4]

We observe today that systematic reviews of important clinical questions are too often repeated by national or regional health technology assessment agencies. This yields a multiplicity of reports of heterogeneous quality, sometimes with conflicting conclusions. We advocate international collaboration and coordination to avoid a waste and dilution of resources and maximising quality. The Cochrane collaboration has a world-wide focus and is accessible for all motivated and skilled experts. It can contribute in making future high quality reviews. We invite young scientists to contact the Cochrane website and to follow their courses. We are happy to observe that our unit in Brussels receives funding from the European Union and also from national organisations (France, the Netherlands, Germany, USA, Australia…) to perform reviews on HPV testing on self-samples, triage of HPV+ women and obstetrical complications following excision of cervical precancer.

Which are currently the guidelines of the EU in relation to HPV screening?

The 2nd edition of the EU Guidelines on Quality Assurance of Cervical Cancer Screening published in 2008 recommended HPV testing in triage of women with atypical cervical cytology and in surveillance after treatment of cervical precancer.[5,6] The supplements to these guidelines, published in 2015, recommend primary HPV screening in all member states at an interval of at least 5 years and starting from the age of 30-35 years.[7]

Which countries in Europe have clearly switched to HPV screening as an alternative to cytology-based screening?

An overview of countries that have switched or that are planning to switch to HPV-based screening is included in the 2016 Eurogin Roadmap.[8] The Netherlands and Sweden were the two first countries that introduced nationwide HPV-based screening in 2017. In Italy, HPV-based screening is running already in several regional programmes. Several other European countries have made decisions to introduce screening with validated HPV assays. We are proud to announce that also in our country, ministers of health decided (July 2018) to introduce screening with HPV testing only instead of cytology, after long discussions on screening with both cytology and HPV (co-testing). The introduction of new HPV-based screening policies in several countries is described in more detail in the papers of this HPV World issue.

What is your view on self-sampling for HPV testing in Europe?

From our reviews we concluded that HPV testing on vaginal self-samples using a valid PCR-based assay is as accurate as on a clinician-taken self-samples. Offering devices for self-sampling generally

is more effective to reach non- or under-screened women than sending mailed invitations to have a cervical sample taken by a clinician. More details from an updated meta-analysis can be found in Arbyn et al. (this issue page 14).

How many HPV tests are considered validated for screening programs?

Two high-risk (hr) HPV tests were validated for cervical cancer screening in randomised trials showing improved protection against cervical cancer: Hybrid Capture II test and the GP5/6+ PCR-EIA. Five more hrHPV DNA tests, fulfilling all the international minimal accuracy and reproducibility criteria, were included in a review of 2015 listing all the validated tests.[9] Three other tests fulfilled partially the criteria. An updated list, actualised in July 2018, adding three more test, is included in Arbyn and Hillemanns (this issue page 6).

Which triage methods seem most suitable for a screening program based on HPV testing?

Many options are available to triage HPV-positive women. We can distinguish the reflex-triage applied on the sample used for HPV-screening and 2nd time triage applied when reflex triage was negative. The most often recommended reflex-triage methods are cytology at cut-off ASC-US or LSIL combined or not with genotyping for HPV16/18. Cytology and/or hrHPV retesting are the most often recommend options for 2nd time triage for women with negative reflex (1st time) triage result. Many more alternative possibilities are being evaluated including mRNA testing, protein markers (p16/Ki67, E6/7), methylation and other markers. Triage of HPV+ women is currently one of priorities for ongoing meta-analytical work at our unity.

Which are the most visible changes in the organization of HPV based screening programs as opposed to cytology based programs?

The use a machine-based test detecting nucleic acids of the virus and restriction of cytology to triage of HPV-positive women will have a huge impact on laboratory practice. HPV testing will facilitate automation, scale increase, high-throughput and accompanying cost reductions. It is not surprising that at least a part of the cytopathology society is opposed against introduction of HPV screening. Therefore careful planning and respect-full communication with the concerned stakeholders is and will be crucial. HPV-based screening at longer intervals including adherence to triage guidelines will require a higher level organisation and good communication between women, screening organisations and health professionals. As already mentioned, HPV testing will enable strategies including use of self-samples.

What is the influence of receiving HPV vaccinated cohorts into the screening programs?

In vaccinated cohorts, we will observe a reduction in the incidence and prevalence of infection with HPV types included in the vaccines or genetically linked with the vaccine types. Also the burden of associated lesions will decrease yielding lower predictive values of all tests. The reduction of infection and lesions will be lower in women who were vaccinated at an older age than in those who were vaccinated before sexual exposure to HPV. How this shift will influence screening policies of vaccinated cohorts is discussed in this issue in the paper of Giorgi-Rossi et al. (this issue page 60). Vaccinated cohorts may need less frequent screening starting at an older age with more specific methods.

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What is your opinion on the recent declaration of cervical cancer as an eliminable disease?

This recent declaration will boost countries with already well organised screening and vaccination programmes to perform even better than before. Karen Canfell and colleagues, describe in this issue how Australia is going to tackle this challenge (this issue page 50). At the same time, it invites countries who did not (yet) develop fully organised preventive programmes or who still have to start – in particular developing countries – to do so. The availability of and access to new point-of-care HPV tests applicable in field conditions and the possibility to perform HPV testing on self-samples increase the possibilities to reach the generations of currently adult women already exposed to HPV infection. Integrating the implementation of these new screening tools with vaccination of young girls and young women should make cervical cancer a rare disease in many parts of the world. ■

Integrating the implementation of these new screening tools with vaccination of young girls and young women should make cervical cancer a rare disease in many parts of the world

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HPV assays validated for primary cervical cancer screening

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American, Australian and European guidelines recommend implementation of HPV-based cervical cancer screening. As described elsewhere in this issue of HPV World, several countries have recently introduced the HPV test for primary screening or are considering to switch from cytological to viral screening in the near future. The evidence supporting this paradigm shift is derived from randomised trials demonstrating a reduced incidence of cervical precancer and cancer among women with a negative HPV test compared to those with a negative cytology result. Two essays were used in the pivotal trials: Hybrid Capture II (HC2, Qiagen, Gaithersburg, MD, USA) and GP5+6+ PCR-EIA which both detect DNA of 13 or 14 high-risk (hr) HPV types. Based on international consensus, equivalency criteria have been accepted that other hrHPV DNA tests have to fulfil in order to accept them in cervical screening. These criteria include good intra- & inter-reproducibility and non-inferior accuracy to detect CIN2 or worse lesions compared to the two standard comparator tests.[1] In 2015, a systematic review of screening and validation studies was performed which yielded a list of assays fulfilling the international criteria.[2] The following commercially available hrHPV DNA assays were considered as fully validated (in alphabetic order): cobas 4800 HPV test (Roche Molecular System, Pleasanton, CF, USA); HPV-Risk assay (Self-Screen BV, Amsterdam, Netherlands); Onclarity HPV assay (BD Diagnostics, Sparks, MD, USA); PapilloCheck HPV-screening test (Greiner Bio-One, Frickenhausen, Germany), and RealTime hrHPV test (Abbott, Wiesbaden, Germany). Three hrHPV DNA tests were considered as partially validated: Cervista (Hologic, Bedford, MA, USA), LMNX genotyping kit HPV GP (Diassay B.V., Rijkswijk, Netherlands), the in-house RIATOL qPCR (Antwerp, Belgium). The first of these three partially validated tests showed in-consistent non-inferiority compared to HC2, and the latter two showed non-inferior accuracy but had incomplete reproducibility information.[1]

The international cross-sectional equivalency criteria for validation of hrHPV DNA assays usable for screening have received a high level of acceptance in the HPV community and among decision makers

Since the publication of the previous list,[2] more studies have been conducted in agreement with the VALGENT[3] or Meijer[1] validation protocols (Table 1). Four reports corroborated the validation status of the HPV-Risk assay,[4] the Onclarity HPV assay[5,6] and the PapilloCheck HPV-screening test.[7] Two new assays could be added to the

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Table 1

Relative sensitivity and specificity for CIN2+ of hrHPV DNA assays compared to the standard comparator tests (HC2 or GP5+/6+ PCR-EIA), evaluated after the publication of the prior systematic review of tests which fulfil international criteria for application in cervical cancer screening[2]

Evaluated Assay	Study	Comparator assay	Relative sensitivity (90% CI)	Relative specificity (90% CI)	Non inferiority p_{sens} ‡	Non inferiority p_{spec} ‡
Studies confirming previously validated hrHPV DNA assays						
BD Onclarity	Cuschieri, 2015[5]	GP5+/6+	1.02 (0.997-1.046)	0.99 (0.976-1.000)	0.009	0.155
	Ejegod, 2016[6]	HC2	0.98 (0.937-1.032)	1.00 (0.984-1.008)	0.025	0.017
PapilloCheck	Heard, 2016[7]	GP5+/6+	1.02 (0.985-1.066)	0.99 (0.976-1.007)	<0.001	0.097
HPV-Risk assay	Polman, 2018[4]	HC2	0.98 (0.924-1.015)	1.02 (1.008-1.030)	<0.001	<0.001
Studies on newly validated hrHPV DNA assays						
Anyplex II HPV HR	Hesselink, 2016[8]	GP5+/6+	1.00 (-)*	0.99 (0.984-1.006)	0.005	0.023
	Jung, 2016[9]	HC2	1.06 (0.991-1.128)	1.00 (0.982-1.016)	0.007	0.035
Xpert HPV	Cuschieri, 2016[10]	GP5+/6+	0.98 (0.941-1.030)	1.01 (0.999-1.014)	0.019	<0.001
Linear Array	Xu, 2018[11]	HC2	1.02 (0.987-1.057)	1.02 (1.007-1.027)	0.001	<0.001

** confidence interval not computable; ‡ p values for non-inferiority of the evaluated assay compared to the comparator assay, assessed in a matched study, where the aimed relative sensitivity >0.90 and relative specificity >0.98. CIN2+: cervical intraepithelial neoplasia of grade II or worse; HC2: Hybrid Capture II assay; GP5+/6+: GP5+/6+ polymerase chain reaction with enzyme-immunoassay identification of 14 high-risk HPV types.*

list of validated hrHPV DNA assays. The Anyplex II HPV HR (Seegene, Seoul, Korea), a full-genotyping assay identifying separately 14 hr types by RT PCR was evaluated in two studies.[8,9] Xpert HPV (Cepheid, Sunnyvale, USA), a cartridge-based point-of care test that distinguishes HPV16, HPV18/45 and the aggregation of 11 other hr-HPV types, was assessed in the Scottish VAL-

Table 2

List of items requiring adaptation in the future guideline for validation of cervical cancer screening tests

1	Longitudinal performance indicator: longitudinal sensitivity, specificity; cumulative risk after a negative or positive test with definition of acceptance benchmarks. Absolute vs relative performance indicators.
2	Statistical test for comparison of assays: non-inferiority test of matched proportions, McNemar statistics, confidence interval around relative accuracy parameters.
3	Acceptance of other comparator tests than HC2 and GP5+/6+ PCR.
4	Target lesion: CIN2+, CIN3+, cancer.
5	Duration of follow-up time: 3 or 5years or longer.
6	Source of data, study design: randomised trials, cohort studies, screening data-bases linked to pathology/cancer registries.
7	Criteria for validation of HPV tests on self-collected samples.
8	Specifications regarding storage/transport media.
9	Requirements for HPV genotyping tests (limited, for instance HPV16 & 18; extended, for instance 5 most carcinogenic types and groups of other hr types; full genotyping with separate identification of all genotypes; genotyping beyond the group of hrHPV types).
10	Assessment of sample adequacy (for instance amplifiability of human genes).
11	Viral load measurement (quantified or semi-quantified signal), flexibility of test cut-off.
12	Principles for grading the level of evidence for test validation.

GENT 2 framework.[10] Both assays showed similar accuracy for detection of CIN2+ compared to the standard comparator tests and demonstrated excellent reproducibility. The Linear Array HPV Genotyping Test (Roche Molecular Diagnostics, Branchburg, NJ, USA) enables type-specific identification of 37 HPV types. The aggregate of 13 hrHPV types identified with this test was found in VALGENT 3 to be as sensitive but more specific for CIN2+ compared to HC2.[11]

The international cross-sectional equivalency criteria for validation of hrHPV DNA assays usable for screening have received a high level of acceptance in the HPV community and among decision makers. However, at the Cape Town Workshop (31st International Conference of the Papillomavirus Society [IPV], 2017), the need was expressed to adapt the validation guidelines. A future version should define longitudinal criteria applicable for assays that target

other molecules than HPV DNA (RNA, proteins, methylation markers). Lack of such a criterion has divided the HPV community regarding validation of the APTIMA assay, which has demonstrated similar sensitivity and better specificity compared to HC2, but for which 5-year safety (similar five-year cumulative incidence of CIN3+ after negative APTIMA or HC2) still had to be demonstrated in a published peer-reviewed report. Table 2 contains the list of items that need further definition.

Intensive work is being done and a draft for the future validation guideline is planned to be presented for further debate at the next conference of the IPV society (Sidney, October 2018).

A future version of the guidelines should define longitudinal criteria applicable for assays that target other molecules than HPV DNA (RNA, proteins, methylation markers)

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Are HPV DNA or HPV E6/E7 mRNA assays the better solution for Cervical Cancer Screening?

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Currently, more than 200 commercial test methods are available for the detection of Human Papillomavirus (HPV) in cervical swab samples. These tests largely differ in the test principle, the detection of HPV DNA or RNA, as well as the targeted viral genome region.[1] While some test methods are limited to the detection of the so-called high-risk (hr) HPV types, which are classified by the World Health Organization (WHO) as carcinogenic to humans, there are also several test methods that additionally identify the two HPV types 16 and 18, as well as aggregate of other high-risk types, since the former two types have the highest risk potential for cervical cancer. In addition, there are numerous tests that are based on various methodologies and that allow more extended genotyping. Only one commercially available test allows detection of viral activity by targeting transcripts of the oncogenes E6 and E7 from all high-risk types: APTIMA HPV (Hologic, Bedford, MA, USA).

When comparing HPV DNA- and RNA-based detection methods, it is important to consider that the detection limit from which a test indicates a positive result, as defined by the manufacturer, is not primarily determined by the analytical sensitivity of the respective test. Rather, the detection limit should be determined in clinical trials in which an optimal ratio of clinical sensitivity to clinical specificity determines the cut-off, i.e. the threshold for a positive test result. Thus, all test methods that aim at maximum sensitivity are not suitable for use in early detection cervical cancer screening programs, because they would detect a large number of "latent infections", which are not clinically significant and would lead to unnecessary follow-up investigations for the women, individual uncertainty and unnecessary costs for health care systems.

To avoid the requirement for each new HPV test to prove its performance in large clinical trials, an international expert group established guidelines for new HPV testing methods used for cervical cancer screening.[2] These guidelines consider the HC2 (Digene Hybrid Capture 2 High-Risk HPV DNA Test (Qiagen)) or GP5+/6+ PCR as standard comparator tests. These two tests have demonstrated superior protection against future CIN3+ and cancer when used in primary screening than good-quality cytology.[3,4] The guidelines call for a non-inferior clinical sensitivity and clinical specificity, accepting the bench marks 0.90

All test methods that aim at maximum sensitivity are not suitable for use in early detection cervical cancer screening programs, because they would detect a large number of "latent infections", which are not clinically significant

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Table 1

Assay Evidence and Approval Level for six different HPV test

Assay	CE-IVD Approval	FDA Approval	Meijer Criteria	Peer-reviewed Evidence Level	3yr NPV Evidence	5+ yr NPV Evidence
HC2 (Qiagen)	✓	✓	✓	+++++	Publication 3,4	Publication 3,4
APTIMA (Hologic)	✓	✓	✓	+++	Publication 11	Publication 12
Cobas (Roche)	✓	✓	✓	+++	Publication 9	Publication 12
RealTime (Abbott)	✓	✗	✓	++	Publication 10	none
Onclarity (BD)	✓	✓	✓	+	none	none
Xpert (Cepheid)	✓	✗	✓	+	none	none

Note: Evidence from posters is not peer-reviewed and is considered as insufficient for clinical validation.

for relative sensitivity and 0.98, for relative specificity compared to the HC2 test or GP5+/6+ EIA-PCR. Furthermore, comparative studies should be performed using cervical specimens from a representative routine screening population of women who are at least 30 years old. In addition, the study cohort should contain at least 60 cases of precancerous lesions (Cervical Intraepithelial Neoplasia Grade 2, CIN2+) as well as a minimum of 800 smears of females with no severe lesions (≤CIN1). Moreover, the new test method is expected to achieve a high intra- and inter-laboratory reproducibility of at least 87%. The evaluation of a novel test after performing these studies should be carried out with a "non-inferiority" test.[2] Although these guidelines are undoubtedly helpful, they might no longer be sufficient to justify the use of an HPV test procedure in the cervical cancer screening programs coming ahead. New HPV tests introduced in screening will need to be monitored carefully to verify longitudinal performance in mass screening conditions and replaced or adjusted when required. Finally, novel test methodologies require acceptance by competent regulatory bodies involving experts and stakeholders, and be economically affordable.

In the United States, most of these criteria are specifically examined by the FDA during their approval process. In Europe, no comparable authorization procedure exists. However, validation protocols such as VALGENT[5] or Meijer[2] are widely accepted. The Directive 98/79/EC of the European Parliament and of the Council of 27 Oc-

tober 1998 on in-vitro diagnostic medical devices (CE marking) only requires proof of conformity of new methods for the detection of HPV, which is not comparable to the certification process of the FDA. However, even FDA-approved HPV tests do not necessarily meet all the requirements for application in cervical cancer screening programs as demonstrated by a study comparing an FDA approved DNA-test (Cervista, Hologic) that showed twice as high HPV positivity rate in cytologically normal women compared to HC2. By increasing the cut-off, this lack of clinical specificity could be remedied without loss of sensitivity.[6,7] However, in the US this change would require a new approval by the FDA.

Logically, perhaps a test that detects the activity of the viral oncogenes by detecting viral mRNA should be more specific than tests merely detecting HPV DNA, which might be present in the form of viral particles even outside of cells and therefore does not necessarily indicate disease or even HPV infection. In fact, published studies show a sensitivity of the RNA-based test comparable to the HC2 (ratio of 0.98 (CI 0.95–1.01), together with a significantly increased specificity (ratio 1.04 (CI 1.02–1.07) of the RNA test.5 This increased specificity will result in a considerable reduction (23%) of follow-up investigations due to a positive test result and therefore decrease costs for follow-up.[8]

Many countries have or are about to introduce HPV-based cervical cancer screening. For three DNA tests based on the detection of the whole genome or the genomic region coding for the main capsid protein and the RNA test for group detection of the E6/E7 mRNA of the high-risk HPV types, data from prospective studies have become available over at least three years suggesting comparable safety to the standard comparator test over this interval (Table 1).[9-11] All of these tests also allow the possibility to simultaneously or subsequently detect HPV16/18. This provides a possibility to triage the primary result to evaluate the individual risk for CIN2+ in HPV-positive women.

All commercially available HPV test methods in Europe must be CE-marked. However, the CE mark does not represent a certification for a test to be used in cervical cancer screening programs. For the large majority of available HPV tests, even no published data exist. The criteria required by Meijer et al.[2] concern only HPV DNA assays which are today met for only a few ones[5] (this issue page 6) of which four have received FDA approval for the US market. However, FDA approval is not relevant in Europe and even this approval does not guarantee the suitability for mass screening. Therefore, HPV tests used in primary screening in Europe should be reclassified to meet the requirements of Class C high-risk IVDs in accordance with the requirements of the International Medical Device Regulators Forum (IMDRF, http://www.imdrf.org/).

Longitudinal performance over a 5-year period is still required; which may be available in the near future. Once this level of evidence is reflected in the peer-reviewed literature, APTIMA might become a preferred assay for cervical cancer screening

In summary numerous studies from different populations (screening, referral) consistently demonstrated a similar cross-sectional sensitivity paired with higher clinical specificity when APTIMA was compared to other FDA approved HPV DNA tests, which reduces the burden of follow-up. Since APTIMA is not a DNA test, longitudinal performance over a 5-year period is still required; which may be available in the near future. Once this level of evidence is reflected in the peer-reviewed literature, APTIMA might become a preferred assay for cervical cancer screening. Just at the moment of publication of this HPV World paper non-inferior longitudinal (over 5-7 years) sensitivity of APTIMA compared to the FDA approved cobas 4800 was demonstrated in a Swedish biobank linkage study.[12] ■

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HPV testing on self-samples: the evidence of Today

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High-quality evidence derived from randomised trials is today available indicating that primary cervical-cancer screening using a high-risk (hr) HPV DNA test is more effective than cervical cytology to prevent future cervical precancer and cancer.[1,2] HPV testing is more sensitive for detection of cancer precursor lesions compared to cytology, allowing for a safe extension of screening intervals. Another advantage is that HPV testing can be performed on vaginal self-samples taken by the woman herself, whereas cytology on self-samples generally shows poor accuracy.[3,4]

In this short paper we address two questions: 1) Is HPV testing on a self-sample as accurate (i.e., sensitive and specific) as on a clinician-collected samples to detect underlying high-grade cervical intraepithelial neoplasia (CIN2+)? and 2) Can strategies providing kits for self-sampling be more effective to reach under-screened women than conventional invitational strategies?

To answer these questions, two meta-analyses[3,5] were updated including literature up-to November 2017. The review was conducted upon request of the U.S. Centers for Disease Control and Prevention, to assess the available evidence regarding possible application of HPV-screening on self-samples in the US.

Accuracy of hrHPV testing on vaginal self-samples compared to clinician-taken samples

A remarkable finding of the meta-analysis was that the absolute accuracy (in particular the specificity) for CIN2+ varied by clinical setting (primary screening or follow-up because of previous cervical abnormalities), whereas the relative accuracy of hrHPV testing on self-samples compared to clinician-samples was robust justifying pooling over multiple settings. Consistent differences were found by test platforms systems based on a principle of signal amplification (like Hybrid Capture or Cervista) versus target amplification by polymerase chain reaction (PCR). Signal-amplification assays were significantly less sensitive on self- vs clinician collected samples (relative sensitivity = 0.85, 95% CI 0.80-0.89) if HC2 or Cervista were used whereas the relative sensitivity did not differ significantly from unity when validated PCRs were applied (see Figures 1 and 2). Also the specificity of signal-amplification assays was 4% lower on self-samples (ratio=0.96, 95% CI 9.93-0.98) whereas the loss of specificity of PCR-based assays was only 2% on self-samples (ratio=0.98, 95% CI 9.97-0.99) compared to clinician-collected specimens. The meta-analysis

Consistent differences were found by test platforms systems based on a principle of signal amplification versus target amplification by polymerase chain reaction (PCR)

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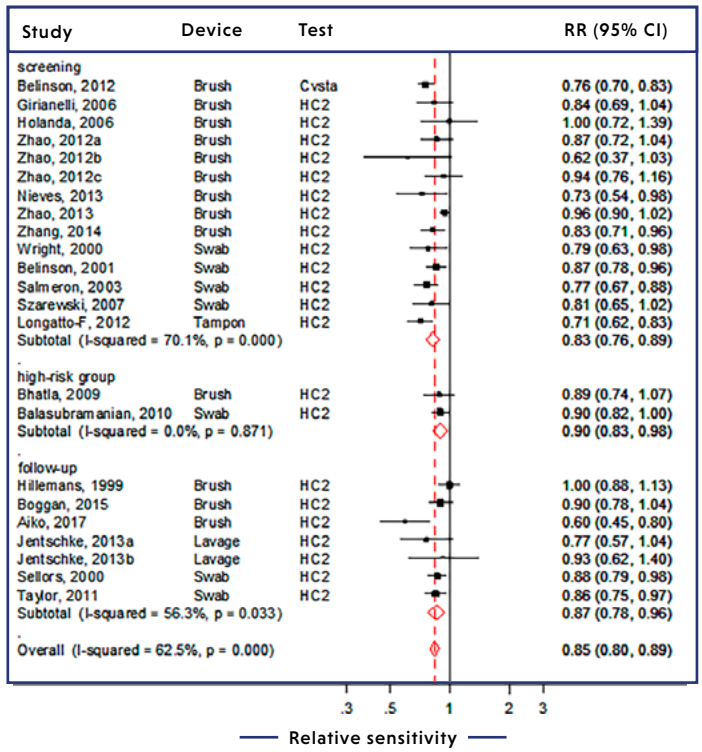
Figure 1

Relative sensitivity of hrHPV testing with HC2 or Cervista on self- versus clinician-taken samples



Text-legend: Black reference line (sensitivity of 1) reflects the sensitivity of clinician -taken specimens using signal amplification tests. Red line (sensitivity 0.85 reflects the lower sensitivity of self collected samples using signal amplification assays

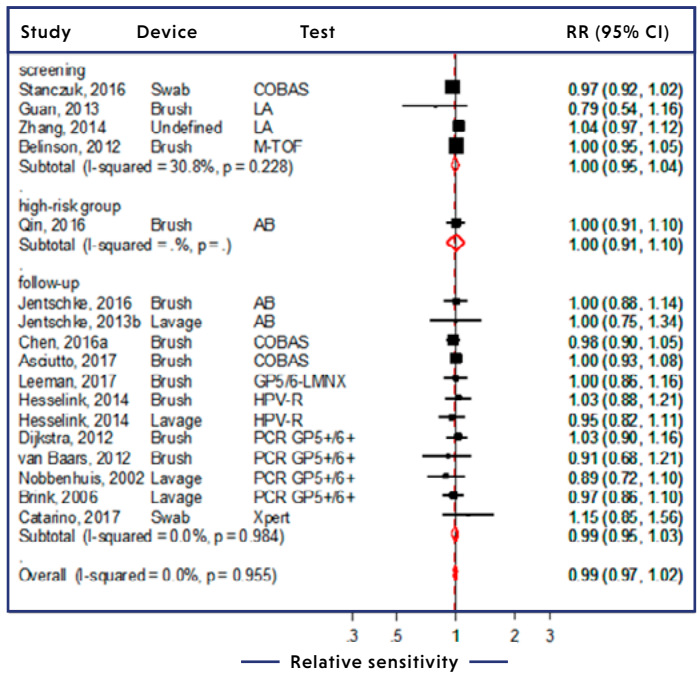
did not identify significant self-sample device or storage medium effects.

Response to the offer a self-sample kit compared to the invitation to have a cervical sample taken by a health worker

The second updated meta-analysis included 23 randomised trials targeting attendance to screening among under-screened women. The pooled results showed that 19% (range 6-34%) who received a self-sample kit at home returned it to the laboratory. By comparison, the controls receiving an invitation to have a cervical specimen taken by a clinician, showed a pooled response of 11% (range 2-26%). The pooled participation ratio of self-sampling to screening at the clinic was 1.78 (CI 1.29-2.45). Opt-in self-sample strategies were less effective than mail-to-all stra-

Figure 2

Relative sensitivity of hrHPV testing with clinically validated PCR-based assays on self- versus clinician-taken samples



Text-legend: Black and red line superimposed: reflect no differences in sensitivity between clinican taken or self taken specimens when PCR-based assays for HPV testing are used.

Pilot studies should be set up before regional/national roll-out of self-sampling strategies

tegies. Compliance to follow-up among women with hrHPV-positive self-samples was on average 80.0% (CI 65.6-91.4%) which was lower than for screen-positive women in the control arm, but the difference was not significant (relative risk of 0.91, 95% CI 0.80-1.05).

Conclusions

Under the condition of using validated PCR-based assays, hrHPV testing on self-samples is as accurate as on clinician-taken samples. Offering self-sampling kits generally is more effective in reaching under-screened women than sending invitations to be screened at a clinic. However, response rates are highly variable among settings and therefore pilots should be set up before regional/national roll-out of self-sampling strategies.

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Self-sampling to reach non-participating women

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Even the best organised, free of charge, national cervical cancer screening programs only attracts approx. 3 out of 4 invited women for screening. In Denmark, the 25% non-attending women accounts for almost half the cervical cancers diagnosed annually[1]. Reasons for non-attendance varies across the globe, yet universal motives include not liking/embarrassment in connection with the gynaecology examination, issues with access to doctor's appointments, or quite simply that women don't think they need screening for one reason or the other[2]. Self-sampling in the comfort of the woman's own home, in her own good time, and without risk of social, cultural or religious stigmatization offers an opportunity to target one of the largest single challenges of organised cervical cancer screening, the participation rate[1,3,4]. Here, we will summarize some of our experiences and considerations with self-sampling from the Copenhagen Self-sampling Initiative (CSi), inviting almost 24.000 screening non-attenders for self-sampling.[5,6]

Two main strategies have been evaluated: the Opt-out (also called "mail-to all") strategy where identified non-responders are mailed a self-sampling kit directly, or Opt-in where identified non-responders are invited to request a self-sampling kit

Opt-in or Opt-out: That's the question…

How to best recruit non-attenders to screening is the question, and several clinical trails have investigated self-sampling as alternative to clinical taken samples. Two main strategies have been evaluated, the Opt-out (also called "mail-to all") strategy where identified non-responders are mailed a self-sampling kit directly, or Opt-in where identified non-responders are invited to request a self-sampling kit. The former strategy has the advantage of presenting the self-sampling kit to all non-responders in the hope that more will accept and return a sample for analysis, but the disadvantage is a high loss of unused kits never returned for analysis. In other word, you may recruit more non-responders but it comes at a (costly) premium. The Opt-in strategy has the advantage of lower costs by only shipping the kits to women who after invitation actively request the kit. The disadvantage is that non-responders will have to go through the additional step of actively ordering the self-sampling kit which may lead to a lower participation.[2] Table 1 shows key features from a selection of HPV self-sampling studies.

In terms of participation, the studies vary widely. From 6.4% (Szarewski et al, UK, Opt-out) to 39% (Sanner et al, Sweden, Opt-in), reflecting the design of the self-sampling approach, the population targeted, when and where.[2] At

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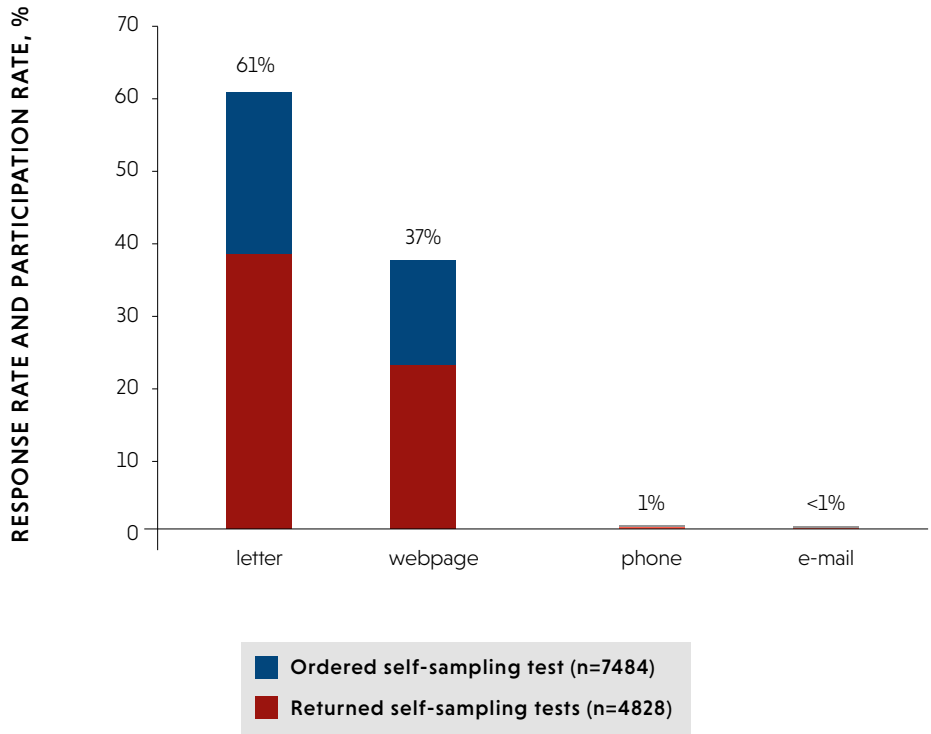
Table 1

Summary of studies assessing different invitation strategies for self-sampling

Invitation strategy	Country & Study design	Study size	Target age (years)	Participation Rate	Reference
Opt-in	Denmark Cross sectional	N=4874	27-64	**20%** by self-sampling+ **10%** by clinician taken samples after invitation	Lam J.U.H. et al., Int J Cancer 2017
	Sweden Cross sectional	N=369	35-50	**32.0%**	Stenvall H. et al., Acta Derm Venereol 2007
	Sweden Cross sectional	N=3000	30-58	**39.0%**	Sanner K. et al., Br J Cancer 2009
	Sweden RCT	N=800	30-62	**16.0%**	Broberg G. et al., Int J Cancer 2014
Opt-in & opt-out	Italy RCT	Opt-in: N=622 Opt-out: N=622	35-65	Opt-in : **8.7%** Opt-out: **19.6%**	Giorgi Rossi P. et al., Br J Cancer 2011
	Italy RCT	Opt-in: 4513 Opt-out: 4516	30-64	Opt-in: **10.5%** Opt-out: **19.6%**	Giorgi Rossi P. et al., Br J Cancer 2015
Opt-out	Netherlands RCT	N= 2546	30-50	**28.9%**	Bais A.G. et al., Int J Cancer 2007
	UK RCT	N=27,792	30-60	**26.6%**	Gök M. et al., BMJ 2010
	Finland RCT	N=8000	30-65	**39.0%**	Gyllensten U. et al., Br J Cancer 2011
	Sweden RCT	N=1500	NR	**6.4%**	Szarewski A. et al., Br J Cancer 2011
	UK RCT	N=2,397	30-60	**27.7%**	Virtanen A. et al., Cancer Epidemiol Biomarkers Prev 2011
	Finland RCT	N=2000	39-60	**34.0%**	Wikström I. et al., Br J Cancer 2011
	Netherlands RCT	N=26,145	26-63	**30.8%**	Gök M. et al., Int J Cancer 2012
	Sweden RCT	N=1000	32-65	**14.7%**	Darlin L. et al., J Clin Virol 2013
	France RCT	N=8,829	35-69	**18.4%**	Sancho-Garnier H. et al., Int J Cancer 2013
	UK RCT	N=3,000	25-65	**13.0%**	Cadman L. et al., J Med Screen 2014

Figure 1

Response and participation rate by letter, webpage, phone and email



current it is not possible to point to Opt-in or Opt-out as the universally superior option, and HPV self-sampling as a supplement to organised cervical screening should be designed and operationalized with respect to the screening program it is proposed to supplement.[7] In our setting, of 23,632 women invited, 20% returned the self-sample for analysis with 39% of those being long term unscreened (≥10 years unscreened).[5]

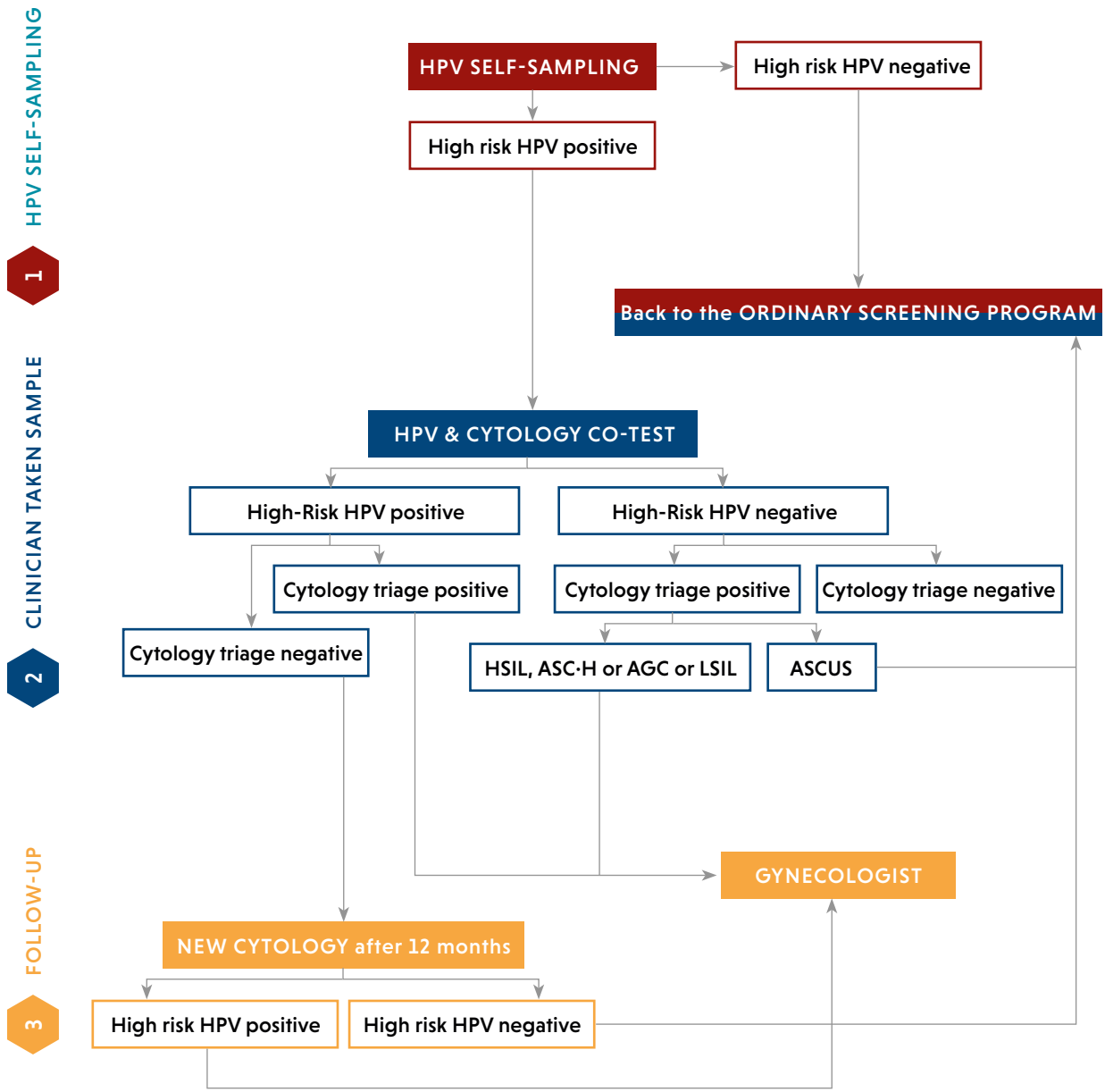
HPV self-sampling to screening non-attenders should not only be evaluated on the number of returned samples, but also include derived screening activity

The effect of HPV self-sampling on screening participation

Most often, studies on HPV self-sampling compare to a group of women offered clinician based sampling are offered clinician based sampling. We however, also focused on the screening participation by clinician taken samples after the non-attenders received the invitation for self-sampling, acknowledging that the total participation rate of a self-sampling initiative will consist of both. In our setting, an additional 10% of the non-attenders invited for self-sampling chose to have a clinician taken sample.[3] Overall, this resulted in 30% participation rate.

Figure 2

Proposed follow-up strategy for HPV positive women by self-sampling



The point is, that introducing HPV self-sampling as an alternative to screening non-attenders should be evaluated not only on the directly measurable effect in term of returned brushes for analysis. The derived "motivational effect" for screening participation may be substantial amongst non-attenders. Passive register follow-up in 2017 of the women invited for CSi showed that 2 year after the invitations for self-sampling, 18.2% of the invited women had a regular, clinician taken sample registered.[6] This is an increase from the 10% in the implementation period.[5] Without arguing this as a direct effect of the self-sampling invitations, at least it indicates that a large proportion of screening non-attenders are susceptible to accept screening. In retrospect, it may not be surprising that women presented with options for screening with screenings options actively choses between those options.

The power of communication

"The single biggest problem in communication is the illusion that it has taken place" wrote George Berhard Shaw. Communication strategies are pivotal to informing women about screening and why it is important to participate. One of the key design items we focused on in CSi was to provide relevant information and facilitate easy access to "Opt-in" by offering a web-based response platform. The special designed web-page system with App like features included a re-directing QR code on the invitation letter for smart phone, tablets or computer use knowing that 95-98% of all Danish women have access to a smart phone or similar devices. Moreover we focused on offering language options other than Danish on the web-platform, thereby attempting to bridge any linguistic divides. Looking at all responders, almost 40% used the electronic platform for opting in[5] (Figure 1), underlining that offering easy ways to accept the invitation is beneficiary for accruing participation. The effect of multi-language information is yet to be reported, but almost 30% of those accepting self-sampling were of non-danish origin[7], which is double up compared to the proportion of non-Danes in the general population.

HPV self-sampling is a viable supplement to recruit screening non-responders

From an operationalization point-of-view these are interesting points. Firstly, communication through web and app based platforms holds a huge potential to improve the user experience compared to letter based correspondence, but it also confers large cost savings on postage for the program. Secondly, language versions of invitation and web based contents require a small effort for a potentially great gain in participation. We are currently exploring these items in more detail in the coming three years, 2017-2019, as self-sampling is rolled out as a supplementary offer to screening non-attenders in our program.

Bringing HPV self-sampling into the organised screening program

HPV self-sampling to increase screening participation is becoming an essential supplement to organised screening. Yet, a number of key features still needs to be addressed to ensure optimal performance of self-sampling in organised screening programs. Firstly, how to follow-up HPV positive women by self-sampling? Here we propose a conservative strategy (Figure 2) referring HPV positive women for a clinician taken sample for cytology and HPV co-testing. Based upon this follow up sample, the woman can be referred in concordance with standard-of-care practice, national recommendations or guidelines, in effect shuttling her into the organised screening

program. Loss to follow up after self-sampling has been voiced as a concern, but in CSi, 87% (N=639) of the self-sampling positive women went for follow-up.[6] This resulted in an initial detection of 101 ≥CIN2 cases with more to come as follow up becomes more complete over time.[6] But does the follow-up necessarily have to be by regular, clinician taken sample? Or could a subset of women benefit from being referred directly for colposcopy saving them at least one gynaecological examination? This is still an open question that should be addressed weighting the balance between the absolute minimum required versus too many examinations, knowing that the examinations are often the barrier to screening.

Risk-based triage strategies using genotype information or methylation markers could potentially come in play, given that both types of analysis can be conducted directly on the original self-sample. Finally, routine self-sampling emphasises the need for HPV assay validation criteria on self-samples. However, no joint international recommendations or requirements have been established to this end.

In conclusion, HPV self-sampling is a viable supplement to recruit screening non-attenders. How and in which way HPV self-sampling will be part of organised screening programs must be defined locally, in order to get the best synergy effects with the regular screening program. By the end of the day, what matter is getting non-responders screened.

Disclosure of interests:
JB used to serve as a paid advisor to Roche and Genomica, and has received honoraria from Hologic/Gen-Probe, Roche, Qiagen, Genomica, and BD diagnostics for lectures. He is principal investigator on studies funded by BD diagnostics, and Qiagen Ltd. DE has no interests to declare. ■

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nº 59

The changing landscape of HPV in Scotland and the contribution of VALGENT 2: a framework for comparing and validating HPV assays

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Established international validation guidelines (2009) contain performance criteria against which HPV tests, destined for application in cervical screening contexts, can be adjudicated and said criteria have made an important contribution to the practice and culture of HPV assay validation.[1] However, the guidelines do not include a piece for validation of the genotyping element of assays although this aspect is likely to have increasing importance.

Scotland, has a national, organised cervical screening programme, associated with an uptake of around 70%. In line with the rest of the United Kingdom, women are screened every three years between the ages of 25-50 and every five years between the ages of 50-65.[2] HPV testing as a test of cure of treatment has been in place nationally since 2012 and a key, planned development relates to the implementation of HPV-primary screening (to replace Pap) in 2019. The proposed algorithm is that all High Risk (HR)-HPV positive women will be triaged to cytology. However, incumbent on the programme is the assessment and consideration of emerging HPV assays, including those which may have a typing component, to determine how these could add value and efficiency to screening protocols. To this end, participation in international endeavours such as VALGENT designed to determine the performance of clinical HPV tests is apposite.[3] Furthermore, the organised nature of screening in Scotland and the existence of a central IT system, the Scottish Cervical Call Recall System (SCCRs) which contains a woman's entire screening record (including information on all management, recall, laboratory results, clinical results and vaccination status) facilitates population-based research and service evaluations designed to further refine and evolve cervical screening.

Additionally, the use of genotyping assays has been essential in determining early measures of the impact of the prophylactic HPV vaccines. Scotland has delivered a national HPV vaccine programme since 2008 associated with high uptake (~90%) in the target group. Furthermore, as women were initially screened aged 20 until 2016 in Scotland it has been possible to perform longitudinal surveillance assessment of HPV prevalence in successive birth cohorts, including those offered the vaccine. Notably, HPV16 and 18 prevalence reduced from 30.0% (95% Confidence Interval-CI: 26.9, 33.1%) in females born in 1988, who represented an unvaccinated baseline cohort, to 4.5% (95% CI: 3.5, 5.7%) in females born in 1995 cohort of whom around 90% were vaccinated. The 4.5% prevalence in the vaccinated 1995 cohort was observed using a genotyping assay with a high

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K Cuschieri, R Bhatia (2018). The changing landscape of HPV in Scotland and the contribution of VALGENT 2: a framework for comparing and

One of the objectives of VALGENT is to gather data on type specific performance of assays to inform the creation of minimal requirements/characteristics of genotyping tests that can be applied to cervical screening

analytical sensitivity - the Optiplex HPV Genotyping Test, (Diamex, Heidelberg, Germany) and in fact, when a clinically validated assay (the RT HPV Test, Abbott Molecular, US) with genotyping capability was applied to samples from the 1995 cohort, HPV16/18 prevalence was 0.5%. These findings emphasise the influence that assay choice can exert on observed prevalence and underline the differences between assays calibrated to detect cervical intraepithelial neoplasia grade 2 or worse (CIN2+) rather than a low amount of virus within a sample. In keeping with the theme of clinically relevant genotyping; one of the objectives of VALGENT is to gather data on type specific performance of assays to inform the creation of minimal requirements/characteristics of genotyping tests that can be applied to cervical screening.

As described elsewhere in this HPV World issue, the VALGENT endeavour is iterative; essentially, a "host/hub" site collates cervical samples, of which several aliquots are made and distributed to collaborating laboratories.[3] From iteration 2 onwards the emphasis has been geared towards assays which would be suitable for service laboratories and screening/clinical applications, whereas VALGENT 1 also included assays with high analytical sensitivity designed for epidemiological use. In the context of VALGENT 2, which used samples from the Scottish Cervical Screening Population, one limited (Xpert HPV), one extended (Onclarity) and two full genotyping assays (PapilloCheck and LMNX Genotyping Kit GP HR) were evaluated and pathology information obtained, with due process of governance, using the national IT system: SCCRs (described above). Analysis focused on the 14 HR-HPV types in common to all assays and clinical performance of these tests in terms of relative sensitivity and specificity for CIN2+ compared to the GP5+/6+ PCR-EIA is summarised in Table 1. VALGENT 2 also differed from VALGENT 1 in that all samples were collected in PreservCyt rather than SurePath. The HPV results obtained as a consequence of VALGENT 2 did not affect patient management nor was HPV primary screening in place at the time of sample collection so the preface/work-up which allowed case and control definition was driven entirely by cytology. Again, this differed from VALGENT 1 where HPV status at primary screen was known. Having the different biospecimen types reflected/represented in the VALGENT iterations, in addition to the different work-up strategies to identify disease is of value as it represents the heterogeneity of practice in the "field".

Ultimately VALGENT will generate a matrix of type-specific positivity according to assay, stratified by underlying pathology. Such a matrix will support the determination of what is clinically relevant HPV typing information

With respect to the genotyping element of VALGENT 2, type specific concordance between assays was also determined. One of the comparisons

Table 1

Relative sensitivity and specificity of the HPV tests evaluated in VALGENT 2

	Relative Sensitivity (95% CI)	Relative Specificity (95% CI)	Reference
GP5+/6+-LMNX	1.02 (0.97-1.08)	1.00 (0.98-1.03)	Geraets et al. 2014[4]
Onclarity	1.02 (0.97-1.08)	0.99 (0.96-1.02)	Cuschieri et al. 2015[5]
Xpert HPV	1.00 (0.94-1.06)	1.00 (0.97-1.03)	Cuschieri et al. 2016[6]
PapilloCheck High-risk Test	1.02 (0.97-1.08)	0.99 (0.97-1.02)	Heard et al. 2016[7]

CI: Confidence Interval. The comparator assay being GP5+/6+ PCR-EIA. The sensitivity values presented are based on the detection of cervical intraepithelial neoplasia 2 or worse (CIN2+) and the specificity values are based on "disease free" samples from women who had two consecutive negative Pap screens. An additional outcome for the specificity calculation which included women with confirmed CIN1 or less was also computed. For further details, please refer to the original publications.

drawn from VALGENT 2 focused on LMNX Genotyping Kit GP HR vs Onclarity and is presented in Table 2a and 2b. Kappa values when computed for the types which are individually resolved by both assays (16,18,31,45,51,52) were excellent to good (over 0.7) although differences in agreement were observed according to whether an infection was present as a single or with other types. Ultimately VALGENT will generate a matrix of type-specific positivity according to assay, stratified by underlying pathology. Such a matrix will support the determination of what is clinically relevant HPV typing information. Furthermore, by depicting the level/extent of assay-driven differences in type specific concordance, such a matrix will inform on HPV prevalence comparisons where different HPV genotyping assays have been applied.[3]

As the VALGENT projects move into their fourth iteration (see Poljak & Ostrebenk and Bonde et al in this issue) and involve an even greater number of assays a sizeable data set will accrue which will provide insights to the community. Given the pace of change/implementation relating to HPV testing, internationally such a data set is timely. ■

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Table 2a

Concordance of GP5+/6+ LMNX and Onclarity in the total population for types individually resolved by both assays, irrespective of whether a type was present as a single or a multiple infection

HPV Type	Negative by both assays	Positive by both assays	Positive by Onclarity only	Positive by GP5+/6+ LMNX only	Kappa Value (95% CI)
16	1,180	107	5	4	0.956 (0.927-0.985)
18	1,255	36	1	4	0.933 (0.875-0.992)
31	1,235	54	7	0	0.936 (0.889-0.983)
45	1,264	27	2	3	0.913 (0.838-0.989)
51	1,252	34	6	4	0.868 (0.787-0.949)
52	1,237	38	21	0	0.775 (0.683-0.868)

CI: Confidence Interval.

Table 2b

Concordance of GP5+/6+ LMNX and Onclarity in the total population for types individually resolved by both assays, based on single infections only

HPV Type	Negative by both assays	Positive by both assays	Positive by Onclarity only	Positive by GP5+/6+ LMNX only	Kappa Value (95% CI)
16	1,163	75	5	4	0.940 (0.900-0.979)
18	1,216	26	1	4	0.910 (0.832-0.988)
31	1280	33	6	0	0.914 (0.846-0.982)
45	1,232	11	2	2	0.845 (0.694-0.995)
51	1,214	24	5	4	0.838 (0.734-0.942)
52	1,205	24	18	0	0.720 (0.597-0.844)

CI: Confidence Interval.

Text-legend: The kappa value measures (expressed as a %) the degree of concordance of two measurements, while adjusting for the underlying effect of the hazard. Kappa values >80% are considered as representing a very good agreement.

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Slovenian HPV Prevalence Study and VALGENT 3 framework

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In comparison to cytology, HPV-based primary cervical cancer screening provides greater protection against invasive cervical cancer, has better sensitivity for detection of underlying cervical intraepithelial neoplasia grade 2 or worse (CIN2+), and allows prolonged intervals between screening rounds. Despite recommendations that only clinically validated HPV tests with optimal balance between clinical sensitivity and clinical specificity should be used, several clinically non-validated HPV tests are used worldwide in daily practice. At least 246 commercial HPV tests and 214 test variants were available on the global market in October 2017, however only a small subset of commercial HPV tests has a documented clinical performance and performance evaluations were frequently not performed in line with agreed standards in the HPV community. To assess suitability and facilitate the acceptance of novel high-risk HPV tests for primary cervical cancer screening, carefully designed cohorts must be used to obtain conclusive evidence.

Slovenian HPV Prevalence Study

In 2009, a cross-sectional study was conducted in Slovenia to provide baseline data for pre-vaccination HPV prevalence.[1-3] Within 16 outpatient gynecology services with a nationwide coverage, a representative cohort of women attending the routine national cervical cancer screening program was established. Between December 2009 and August 2010, we prospectively enrolled 4,432 women aged 20-64 years. During the gynecological examination, two samples were obtained from each woman – one sample for routine cervical cytology testing and one sample for HPV DNA testing. The sample for HPV DNA testing was collected in PreservCyt ThinPrep solution (Hologic, Marlborough, MA) and transported to the laboratory within a week of collection. Upon the arrival to the laboratory, the samples were divided into several aliquots. The aliquots that were not immediately used for HPV testing were stored at –70°C. All cervical smear specimens obtained for cytological examination were processed under routine screening conditions. Women were referred to colposcopy at cytology threshold of atypical squamous cells-cannot exclude high-grade lesion (ASC-H) or worse in accordance with the Slovenian Cervical Cancer Screening Guidelines. In addition, all HPV16 and/or HPV18 positive women were invited for colposcopy regardless of their cytology result. Colposcopically directed punch biopsies obtained from the suspicious areas were histopathologically assessed by certified pathologists, who were blinded to the HPV status.

To provide longitudinal data, second screening round was commenced in December 2012 using similar approach as in the first screening round.[4]

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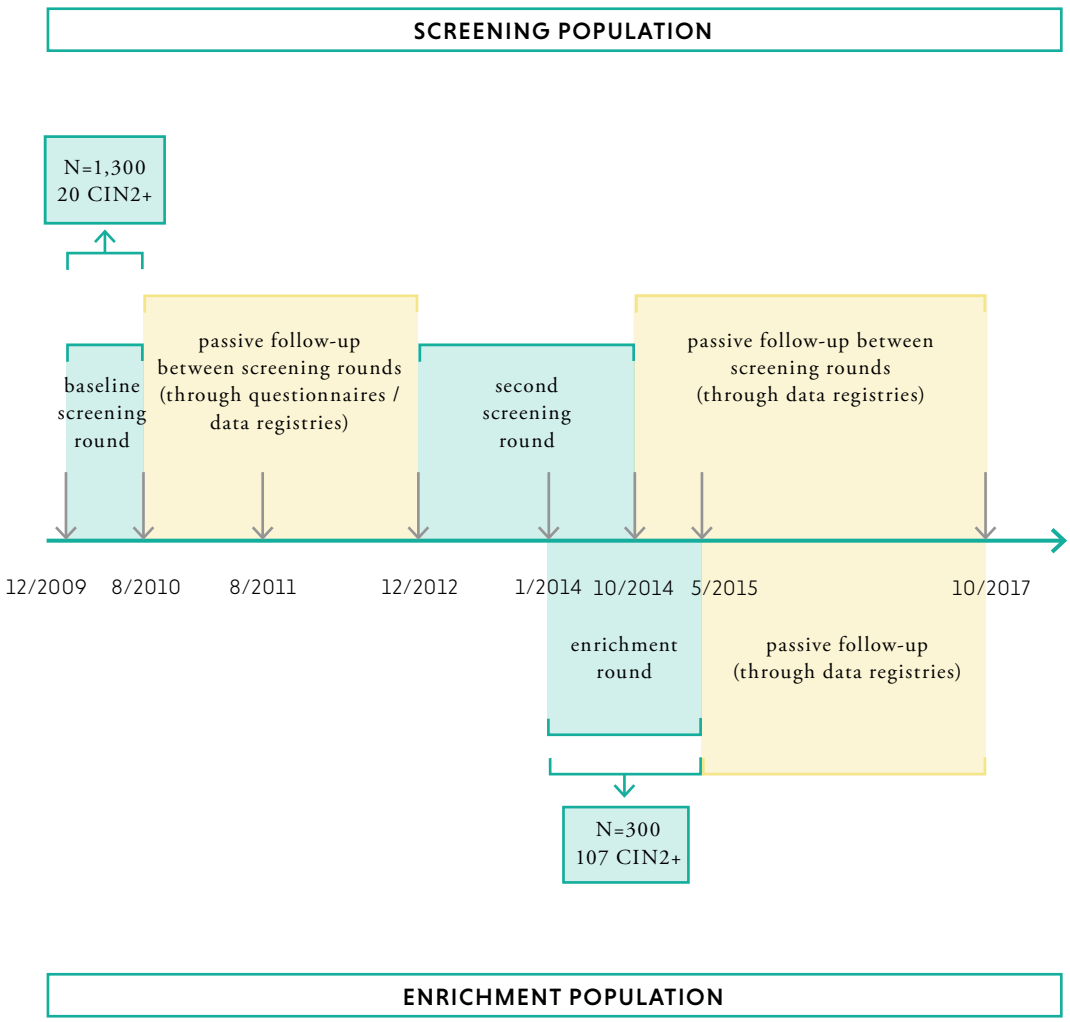
Figure 1

Overview of the 1,600 specimens used for the VALGENT 3



Between December 2009 and August 2010, a total of 1,300 sequential cervical samples were selected from women aged 25-64 years participating in the Slovenian national cervical cancer screening program (screening population). In addition, 300 cervical samples were obtained from women with abnormal cytology from January 2014 to May 2015 (enrichment population).

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Table 1

HPV tests included in the VALGENT 3

Aliquot	HPV test evaluated	Participating laboratory
1	Hybrid Capture 2 (HC2) HPV DNA Test (Qiagen, Gaithersburg, MD)	Ljubljana (Slovenia)
2	RealTime High Risk HPV test (Abbott Molecular, Des Plaines, IL)	Ljubljana (Slovenia)
3	Linear Array HPV Genotyping Test (Roche Molecular Systems, Alameda, CA)	Ljubljana (Slovenia)
4	EUROArray HPV (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany)	Edinburgh (Scotland)
5	INNO-LiPA HPV Genotyping Extra II (Fujirebio Europe, Ghent, Belgium)	Ghent (Belgium)
6	GP5+/6+ PCR with EIA & LNX (Voorburg, The Netherlands)	Voorburg (The Netherlands)
7	Anyplex II HPV HR Detection (Seegene, Seoul, South Korea)	Ljubljana (Slovenia)
8	Anyplex II HPV28 Detection (Seegene, Seoul, South Korea)	Ljubljana (Slovenia)
9	HPV-Risk assay (Self-Screen BV, Amsterdam, The Netherlands)	Amsterdam (The Netherlands)
10	In-house qPCR (E6/E7 specific) (Antwerp, Belgium)	Antwerp (Belgium)
12	Cobas 4800 HPV Test (Roche Molecular Systems, Alameda, CA)	Hannover (Germany)
13	14 High-risk HPV with 16/18 Genotyping Real-time PCR Kit (Hybribio, Beijing, China)	Ljubljana (Slovenia)
14	21 HPV GenoArray Diagnostic Kit (Hybribio, Beijing, China)	Ljubljana (Slovenia)

The Slovenian HPV Prevalence Study represents a valuable collection of clinical samples that can be used for ongoing and future validation studies of HPV tests designed to be used in primary HPV screening setting

To collect data for possible follow-up visits that occurred between two screening rounds two questionnaires (patient-based and physician-based) were used, as well as data from national screening registry for non-responders.

In addition to cervical samples, 3,195 and 2,041 serum samples were collected from women enrolled in the first and second screening round, respectively, in order to assess the cumulative exposure to HPV in this cohort of women, to evaluate the correlation between persistence and/or clearance of HPV infection and to estimate the protective effect of naturally acquired serum antibodies against incident HPV infections.[5-7]

The Slovenian HPV Prevalence Study is the largest screening cohort in this part of Europe to date, with reliable longitudinal data and thus represents valuable collection of clinical samples that can be used for ongoing and future validation studies of HPV tests designed to be used in primary HPV screening setting.

VALGENT is a powerful tool for providing comprehensive evidence of the performance of HPV tests used in primary HPV screening setting

VALGENT 3

The VALGENT (VALidation of HPV GENotyping Tests) framework is an international collaboration designed to promote clinical validation and to assess the comparative performance of HPV tests with limited, extended or full genotyping ability.[8] The study protocol is comprised of continuous samples obtained from women participating in a screening program, enriched with samples obtained from women with cytopathological abnormalities.

From sample collection of Slovenian HPV Prevalence Study, a total of 1,300 sequential cervical samples (screening population) were included in the VALGENT 3 framework (Figure 1). In addition, enrichment population consisted of 100 women with atypical squamous cervical cells of undetermined significance (ASC-US), 100 women with low-grade squamous intraepithelial lesion (LSIL) and 100 women with high-grade squamous intraepithelial lesion (HSIL) (Figure 1). The average age of women in the total study population (screening and enrichment population) was 39 years (range, 20-77), with 18.4% of the population below 30 years old.

VALGENT is a powerful tool for providing comprehensive evidence of the performance of HPV tests used in primary HPV screening setting. Using internationally recognized uniform criteria, validation is based on comparison of the novel HPV tests to the standard comparator (e.g., Hybrid Capture 2 (Qiagen Gaithersburg,

MD, USA) and/or GP5+/6+ PCR). AML laboratory in Antwerp, Belgium, provided samples for VALGENT 1 and Scottish HPV Reference Laboratory in Edinburgh, Scotland, provided samples for VALGENT 2. VALGENT 4 is still ongoing and the samples are being collated in Copenhagen, Denmark.

HPV tests included in the VALGENT 3 are summarized in Table 1. Majority of the tests validated within the VALGENT 3 framework, fulfill the Meijer's criteria for use in clinical practice. Based on the samples enrolled in the Slovenian HPV Prevalence Study, VALGENT 3 framework will provide important head-to-head data on clinical accuracy of the majority of the most important HPV tests currently available on the market. ■

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The VALGENT 4: Robust analytical and clinical validation of 11 HPV assays with genotyping on cervical samples collected in SurePath medium

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From a technology point of view, HPV assays are undergoing a rapid evolution these years as focus shifts towards large scale implementation of primary HPV screening in a number of countries. The first generation of clinical HPV assays were developed solely to detect oncogenic HPV genotypes using DNA PCR or hybridization techniques and these assays mainly reported the test outcome as either HPV positive or negative, with no individual HPV genotype information. Newer generations of commercially available HPV assays depend upon a wide variety of DNA and RNA detection techniques and allow for a more detailed reporting of HPV genotypes.

Current HPV genotyping assays can be divided into three categories: 1) Assays with ***limited genotyping*** that report separate identification of HPV16 and HPV18 or HPV18/HPV45, combined with pooled detection of the remainder of the carcinogenic types, 2) Assays with ***extended genotyping*** that report separate identification of ≥5 genotypes combined with one or more bulk detections of the remainder, and 3) Assays with ***full genotyping***, reporting individual identification of all carcinogenic HPV genotypes. Of these, we have no doubt that the trend is towards higher data-resolution to ensure optimal screening and referral algorithms. After all, not all oncogenic genotypes are equal when it comes to absolute risk of disease.

The international validation criterion for HPV assays for screening use is described elsewhere.[1] However, in the context of genotyping assays, qualifying by the criterion is based upon the clinical accuracy for detection of cervical precancer lesions by aggregating all type-specific information into high-risk HPV positive or negative. This limitation requires considerations as novel assays trend towards allowing extended or full genotyping. The VALGENT framework enables comparison and validation of HPV genotyping assays using a relevant sample population with sufficient disease to confirm clinical performance using a validated comparator assay like the international guidelines.[2] However, VALGENT includes the possibility to absorb the dimension of HPV-type specificity.

The VALGENT framework enables comparison and validation of HPV genotyping assays using a relevant sample population with sufficient disease to confirm clinical performance using a validated comparator assay like the international guideline

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Table 1

HPV genotyping assays evaluated, concurrent material required and scientific partners under the VALGENT 4 study protocol

VALGENT 4 included Assays	Aliquot	Amplicon length	Scientific partner
BD Onclarity HPV Assay	Original Material	79-137 bp.	Copenhagen University Hospital, Pathology Laboratory, Hvidovre, Denmark
Genomica CLART HPV4 assay	DNA	465 bp.	
Agena HPV MassArray assay	DNA	90-122 bp.	
Roche cobas 4800 HPV Test	Original Material	~200 bp.	Norwegian HPV Reference Lab, Akerhus University Hospital Norway
Fujirebio INNO-LiPA Genotyping Extra II test	DNA	65 bp.	Ghent University, Ghent, Belgium
SeeGene Anyplex HPV28 detection test Seegene Anyplex II HPV test	DNA or Original Material	~150 bp.	Infection and Cancer Laboratory. Cancer Epidemiology Research Program, Barcelona, Spain
Self-screen HPV-Risk assay	DNA	~150 bp.	VU University Medical Center, Amsterdam, The Netherlands
Genefirst HPV-MPA Genotyping Test	DNA	150 bp.	Scottish HPV Reference Lab, Royal Infirmary of Edinburgh Scotland
Liferiver Harmony test Liferiver Venus test Genefirst HPV-MPA Genotyping Test	DNA	100-200 bp. 100-200 bp.	
Comparator assays			
GP5+/6+ EIA Luminex	DNA		DDL Diagnostic Laboratory, Rijswijk, The Netherlands
GP5+/6+ PCR EIA kit HPV GP HR	DNA		International HPV Reference Center, Karolinska University Hospital, Stockholm, Sweden

Compared to the previous VALGENT installments, the VALGENT 4 protocol includes several novelties. Firstly, the VALGENT 4 panel was generated using fresh BD SurePath™ (Becton, Dickinson and Company) collected screening samples from the Danish cervical cancer screening program which services a well-screened population with a high background risk of cervical cancer. The panel exclusively includes samples from women ≥30 years of age which is in line with the majority of HPV primary screening guidelines. In total, eleven different HPV genotyping assays from 8 different manufacturers were evaluated in the VALGENT 4 study (Table 1), using GP5+/6+ PCR-EIA with genotyping as comparator in line with previous VALGENT installments (See Table 1). Only DNA assays were evaluated.

Eleven different HPV genotyping assays from 8 different manufacturers were evaluated in the VALGENT 4 study using GP5+/6+ PCR-EIA with genotyping as comparator

A key element in VALGENT 4 is that all assays requiring a DNA input were evaluated on DNA extraction of the panel samples provided by the parent Copenhagen laboratory. By this, any variability introduced by various 3rd party DNA extraction platforms on HPV assay performance is sought eliminated. Of the 11 assays evaluated in VALGENT 4, only BD Onclarity, Seegene Anyplex HPV28/HPV II HR and Roche Cobas HPV assays required original SurePath LBC material as input material for analysis, mainly as these assays run on full IVD work flows including integrated DNA extraction. Moreover, quality assurance was redefined and all included panel samples where characterized using a novel quality measuring assay, the ExomeQC panel (AGENA Bioscience, Germany). In short, this quality assurance assay measures the analytically available DNA as well as the relative level of DNA fragmentation in the individual sample. It also allows us to compare assay performance between the 11 included HPV assays with respect to robustness to variation in sample quality.

Data compilation by VALGENT 4 partner laboratories is currently being completed, and statistical work, (performed at the Sciensano Institute in Brussels) is in progress. Clinical validation of HPV assays for use in screening has primarily been undertaken on ThinPrep collected samples,[2-9] with only one assay to date, the BD Onclarity, being validated on both ThinPrep[5] *and* SurePath collected samples.[10] Compared to previous VALGENT installments a few assays are repeaters, from VALGENT 2 its BD Onclarity, and from VALGENT 3 it is cobas4800, INNO-Lipa, Anyplex HR and HPV28, as well as Self-Screens Risk HPV assay. It will be interesting to see how the many HPV genotyping assays included in VALGENT 4 perform on SurePath collected screening samples in this large-scale performance comparison. Not only from the clinical performance perspective but also with respect to assay concordance at the individual genotype level, and how the individual assay's genotype detection correlates to the clinical endpoints. ■

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The BD Onclarity HPV assay: Key data pointing to clinical validity

In an era of rapid evolution to HPV primary screening, data on assay performance matters. The BD Onclarity™ HPV Assay (Onclarity) is a Real-Time PCR assay that utilizes gene-specific E6 and E7 targets for 14 types, enabling integrated extended high-risk HPV genotyping with individual genotyping of HPV 16, 18, 31, 45, 51 and 52 and reporting the remaining HPV types in three distinct groups (33/58, 56/59/66, and 35/39/68). The assay also detects the human beta-globin gene, which acts as a sample adequacy control. Onclarity is run on the fully integrated BD Viper™ LT System (Viper LT) which can process both BD SurePath™ (SurePath) and Hologic PreservCyt® (PreservCyt) liquid-based cytology (LBC) specimens.

The clinical validity of Onclarity, and equivalence to other HPV assays has been established. A systematic review and meta-analysis listed Onclarity among hrHPV assays that fulfill international criteria for use in primary screening.[1] The VALGENT 2 study demonstrated non-inferior sensitivity and specificity of Onclarity for CIN2+ compared to GP5+/6+ PCR using ThinPrep samples from the Scottish cervical cancer screening program.[2]

Furthermore, the Predictors 2, 3, and 4 studies established that Onclarity sensitivity for ≥CIN2/3 was not significantly different from HC2 or Roche cobas® HPV (Cobas), out of PreservCyt[3,4,5] and not significantly different from HC2 out of SurePath.[6] Specificity for ≥CIN2 for Onclarity and Cobas were equivalent,[3,6] but HC2 was significantly less specific in one study[4] and not significantly different in another study.[6]

Risk discrimination by extended genotyping results from the Clinical Evaluation of the BD Onclarity HPV Assay on the BD Viper LT System with Cervical Specimens Clinical Trial (Onclarity Clinical Trial)[7] for the subset of women ≥25 are presented in Table 1. Similar risk discrimination by Onclarity extended genotyping results from studies conducted with Kaiser Permanente Northern California (KPNC) subjects by the National Cancer Institute (NCI), for the subsets of 1903 women with ASC-US tested with Onclarity and for 8664 subjects with positive HPV are presented in Table 2.[9,10]

The KPNC/NCI authors stratified extended genotype results into 5 tiers (HPV 16, else 18/45, else 31/33/58/52, else 51/35/39/56/59/66/68, else HPV-negative). The LBC results were stratified into 3 tiers (high-grade (HSIL, ASC-H, AGC), LSIL/ASC-US, NILM). For the resultant 15 combinations of hrHPV genotype and cytology, the 3-year CIN3+ risks ranged 1000-fold from 60.6%

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Table 1

Baseline risk of ≥CIN3 (p16-assisted H&E, adjudicated) by HPV genotype, multivariate Bayesian method, expressed as % (95% confidence intervals), from the Onclarity Clinical Trial.[7,8] Women aged 25 or above at screening time.

Genotype	NILM ≥25	ASC-US ≥25	LSIL ≥25	All subjects ≥25
16	9.2 (6.4, 12.6)	20.4 (13.1, 29.0)	16.3 (9.7, 25.3)	19.9 (16.3, 23.7)
31	7.7 (4.7, 11.9)	8.9 (1.9, 18.8)	8.2 (1.9, 17.8)	10.0 (7.0, 13.2)
18	3.8 (0.9, 8.3)	6.8 (1.0, 17.5)	7.1 (1.3, 18.3)	6.4 (3.1, 10.2)
33/58	3.2 (1.2, 6.1)	3.9 (0.1, 3.7)	4.6 (0.2, 12.5)	4.8 (2.5, 7.6)
52	2.0 (0.6, 4.0)	7.0 (1.9, 14.2)	3.2 (0.1, 10.8)	3.4 (1.7, 5.6)
45	1.5 (0.2, 4.1)	3.9 (0.1, 13.8)	6.0 (0.2, 20.1)	2.4 (0.7, 4.8)
51	1.3 (0.0, 4.0)	3.7 (0.1, 12.5)	2.6 (0.1, 9.0)	1.6 (0.1, 4.3)
35/39/68	1.0 (0.3, 2.4)	4.2 (1.0, 9.6)	1.5 (0.0, 5.3)	1.7 (0.8, 2.8)
56/59/66	0.8 (0.1, 2.6)	1.4 (0.0, 4.8)	1.1 (0.0, 3.9)	0.5 (0.1, 1.3)

text-legend of the underlined box: Women with HPV 16 in their cervical specimen and cytology of LSIL showed a probability of CIN3+ of 16.3% in a three-year follow up time

(HPV 16 and HSIL) to 0.06% (hrHPV--negative and NILM). Onclarity combined with cytology predicts risks of cervical precancer/cancer in refined strata varying from extremely high to extremely low risk.[10] The Onclarity assay design also overcomes the issue of pooled masking of the true underlying genotype-specific risks for CIN3+ disease posed by non HPV-16/18 types (Figure 1).

Other recent studies for risk stratification included a proof-of-principle study that demonstrated that a totally automated algorithm using features from the BD FocalPoint™ Slide Profiler system performing computer-interpreted cytology and matched ≥ASC-US by human-interpreted Bethesda system cytology demonstrated excellent risk based triage when genotyping was added to

Onclarity combined with cytology predicts risks of cervical precancer/cancer in refined strata varying from extremely high to extremely low risk

Table 2

3-year risk of ≥CIN3 by HPV genotype, univariate hierarchical method, expressed as % (95% confidence intervals) for the Onclarity HPV Assay, from NCI studies at KPNC[9,10]

Genotype	ASCUS≥21	All subjects (median age 39.0)
16	16.0 (14.1, 18.0)	21.9 (20.1, 23.7)
31	7.0 (5.3, 9.4)	8.1 (6.6, 9.5)
18	7.4 (5.1, 10.7)	11.5 (9.2, 13.5)
33/58	7.1 (5.1, 9.6)	8.6 (6.9, 10.3)
52	4.3 (3.2, 5.9)	5.6 (4.4, 6.7)
45	3.9 (1.9, 7.7)	5.4 (3.8, 7.0)
51	2.7 (1.7, 4.5)	2.9 (1.9, 3.9)
35/39/68	1.6 (1.0, 2.3)	2.0 (1.5, 2.5)
56/59/66	1.3 (0.7, 2.3)	1.5 (1.0, 1.9)

the triage strategy.[11] Such studies demonstrate the feasibility of automation of triage especially in areas lacking in cytology expertise.

The Onclarity HPV assay is clinically validated and as of mid-February 2018, US FDA approved for use in ASCUS triage, HPV primary screening and co-testing

In summary, the Onclarity HPV assay is clinically validated and as of mid-February 2018, US FDA approved for use in ASCUS triage, HPV primary screening and co-testing. The ability to collect a single sample that generates both a cytology result and an HPV result with genotyping as well as potentially end to end automated processing that can be applied to virtually all algorithms for cervical cancer screening is certainly an attractive package from both the laboratory as well as clinical standpoint. ■

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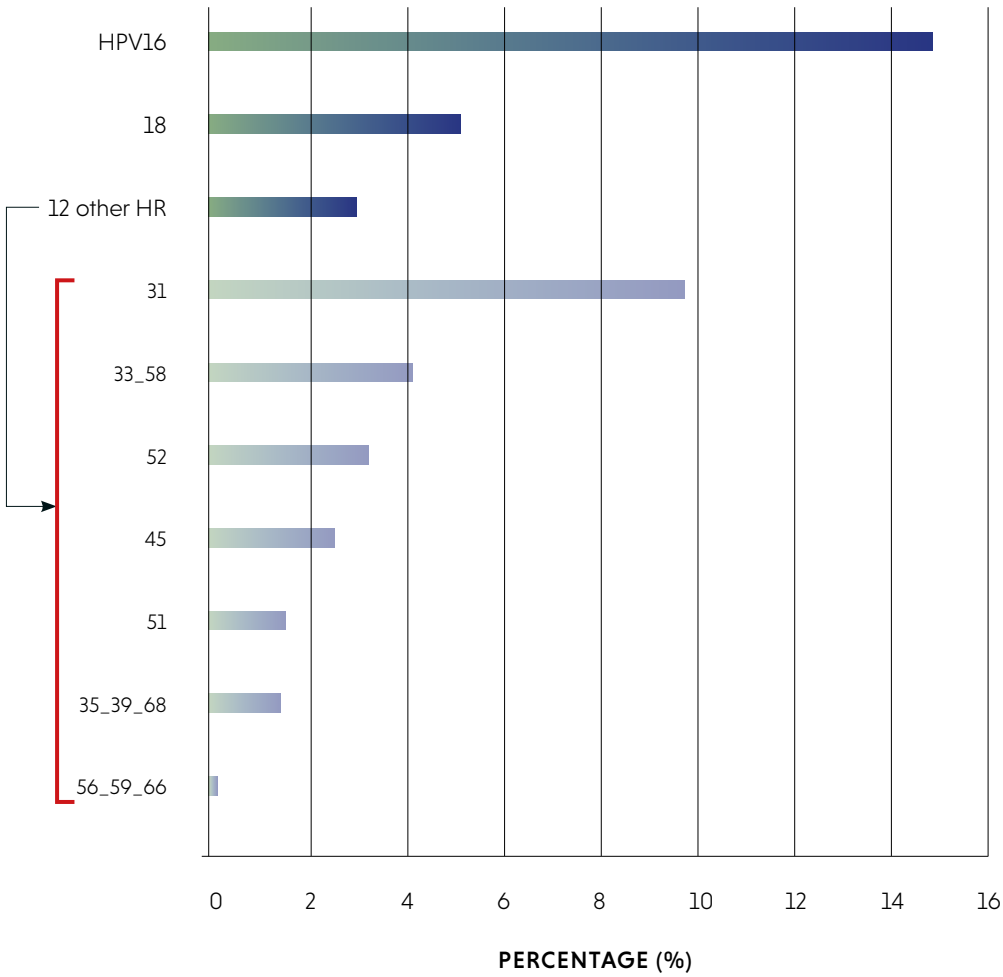
Figure 1

Absolute Risk CIN3+



- Pooled results from 12 "Other high risk types" masks the differences in prognosis between HPV 31 (absolute risk close to 10%) and HPV 51 (absolute risk below 2%)

Onclarity US Premarket Approval Baseline Study illustrating how pooling of non-HPV16/18 types ("12-other") masks the true underlying risks of CIN3+ disease. Bar chart shows absolute risk of CIN3+ as determined by the Onclarity assay compared to risk estimate calculated from the 12-other pool. Pooling masks the underlying risks posed by HPV 31 and 33_58. (Data source: 33,858 enrolled subjects; > 6000 with colposcopic biopsies; adjudicated H&E histology + p16; Age = 25+).7,8

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Quote this article as:

MH Stoler, TC Wright (2018). The BD Onclarity HPV assay: Key data pointing to clinical validity. **www.HPVWorld.com**, *62*

Primary HPV screening in the US with the Cobas® assay

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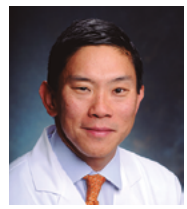

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Large-scale cervical cancer screening by cytology became common practice in the United States (US) in the 1960s. Since that time, best screening practices have been refined as studies have shed light on the optimal ages to start and cease screening, screening intervals, and the use of concomitant high-risk human papillomavirus (hrHPV) testing. Originally proposed by Wright et al in 2004, concomitant hrHPV testing, also known as co-testing, has become the preferred cervical cancer screening strategy in women >30 years of age.[1] Furthermore, in 2012, the American Society for Colposcopy and Cervical Pathology management guidelines were revised, recognizing the utility of co-testing as a surveillance or follow up test aftertreatment of cervical precancer. Most recently, hrHPV testing alone has been proposed as a mode of primary screening.[2]

Of the four HPV assays used for co-testing and triage of equivocal cytology (e.g., atypical squamous cells of undetermined significance) approved by the US Food and Drug Administration (FDA) agency, two have been approved for primary HPV screening. One of them, the Roche Cobas® 4800 HPV Test, is the first test approved by the FDA for primary cervical cancer screening without cytology. Using real-time polymerase chain reactions for amplification of the L1 gene of the HPV genome, the Cobas® 4800 system is able to distinguish HPV type 16 and 18 from the other twelve hrHPV types.

A number of large randomized trials and cohort studies have assessed primary hrHPV tests as a primary screening strategy and corroborate the enhanced sensitivity of primary HPV screening over cytology alone.[3,4] Gage et al analyzed a cohort of 1 million women in Northern California. The study concluded that the 3-year cumulative incidence rate (CIR) of cervical precancerous abnormalities (CIN3+) was lower after a negative hrHPV testing compared to after a negative cytology result (0.07% vs. 0.19%).[2] The ATHENA trial, a US prospective registration trial that utilized the Roche Cobas® 4800 system, also demonstrated the superior protection of primary hrHPV screening vs. cytology alone (3-year CIR of CIN3+ 0.3% vs. 0.8%).[2,5]

It should be noted that a certain percentage of invasive cervical cancers are hrHPV negative.[2] Furthermore, although a growing body of evidence validates the improved sensitivity of primary hrHPV screening, the practice is not without criticism in the US. Initial primary hrHPV screening may lead to unnecessary follow-up tests and biopsies. In women between 25 and 29 years of age, the ATHENA trial noted a baseline hrHPV rate and abnormal cytology rate (≥ ASCUS) of 21.1% and 9.8%, respectively.[5] Using the FDA-approved primary HPV screening algorithm, which includes genotyping for HPV 16 and 18 and reflex cytology for other hrHPV

The ATHENA trial that utilized the Roche Cobas® 4800 system, demonstrated the superior protection of primary hrHPV screening vs. cytology alone (3-year Cumulative Incidence Rate of CIN3+ 0.3% vs. 0.8%)

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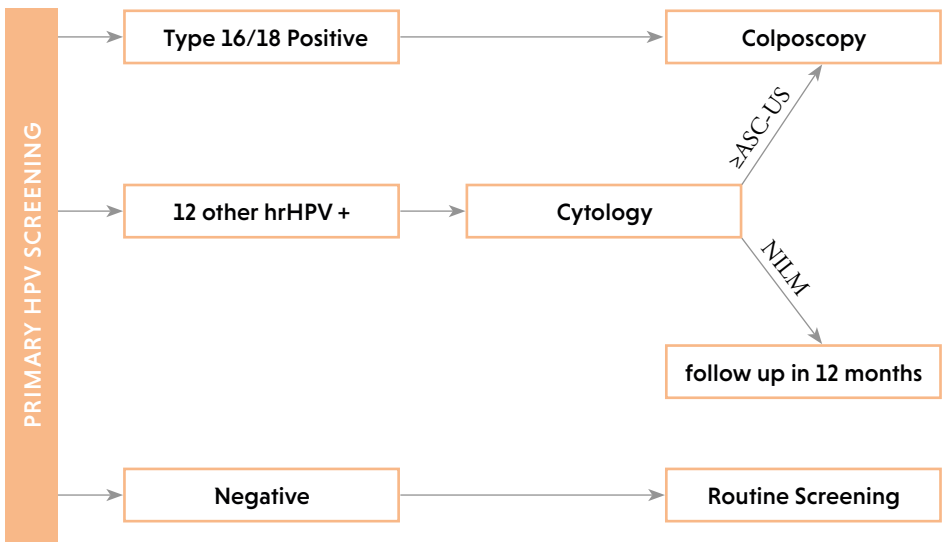
Figure 1

Recommended primary HPV screening algorithm[2]



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

types as defined in Figure 1, the trial detected almost one third of all CIN2+ cases in this age group. But using the algorithm also doubled the number of colposcopies compared with screening starting at age 30.[2] Most importantly, more studies will be required to determine if this early detection of CIN2+ will translate to decreased cervical cancer morbidity and mortality. ■

Although a growing body of evidence validates the improved sensitivity of primary hrHPV screening, the practice may lead to unnecessary follow-up tests and biopsies

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nº 64

Xpert HPV: Basis and key results in best trials

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Nearly 100% of cervical cancers are causally -related to infection with high-risk types of HPV, with 70% of cases attributed to infection with HPV16 and 18.[1] Papanicolaou cytology was introduced as a screening test to detect precancerous lesions of the cervix in the 1940s. In those countries that achieved high coverage of the target population linked to treatment of abnormalities and follow-up to detect recurrences, there was a dramatic reduction in cervical cancer incidence and mortality. Cytology-based screening programmes have not been replicated in developing countries, where 88% of cases occur due to poor or absent screening programmes and lack of access to appropriate treatment. The relatively sophisticated infrastructure required by cytology, colposcopy and histological sampling have proved to be too costly and too complex to initiate or sustain in the low and middle income settings.

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The concept of Screen and Treat was introduced into clinical research trials in the past 15 years, comparing a variety of HPV molecular tests (often Hybrid Capture 2 (HC2) or careHPV (Qiagen, Germantown, MD)), to Visual Inspection with Acetic Acid (VIA). HPV DNA testing for high-risk types has shown a consistently higher sensitivity but a lower specificity compared to cytology. Recently a new type of HPV DNA test has become commercially available. The test produced by Cepheid (Sunnyvale, CA) and known as Xpert HPV is based on the same platform as GeneXpert for detection of Tuberculosis and Rifampicin resistance (MTB/RIF), was clinically validated for cervical screening according to the VALGENT protocol[2] and is on the World Health Organisation (WHO) pre-approval list.[3] The Xpert HPV assay, is a real-time polymerase chain reaction (PCR) assay for the detection of 14 high-risk types of HPV through five separate channels (HPV16, HPV18 and 45, HPV31, 33, 39, 52 and 58, HPV51 and 59 and HPV39, 56, 66 and 68). The assay is formulated in a single use cartridge, provides a result within one hour, can be performed by non-laboratory trained health-care workers and requires minimal hands on time. The test requires 1 ml of cervical specimen collected in PreservCyt (Thinprep: Hologic, Bedford MA).

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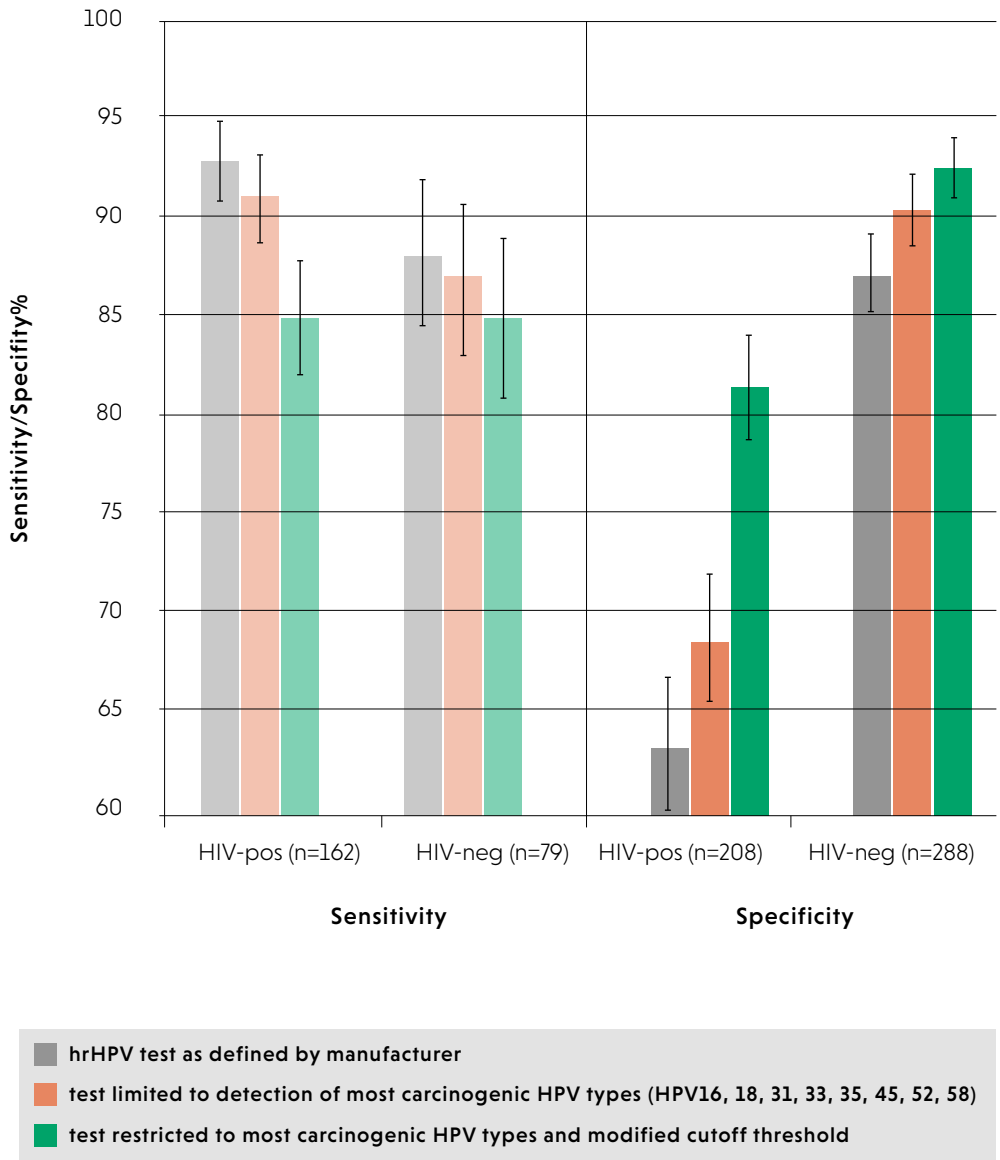
Figure 1

Sensitivity (left) and specificity (right) of Xpert™ HPV as defined by the manufacturer, restricting to specific HPV types, and optimizing cutoff thresholds for specific HPV channels, to detect CIN2+ in HIV+ and HIV- women



Einstein et al.[4] compared Xpert HPV testing to Cobas HPV test (Roche Molecular Systems, Pleasanton, CA) and HC2 to histological outcomes. The sensitivity of Xpert HPV for CIN2+ was equal to that of the Cobas HPV test (90.8% versus 90.8%) and greater than that of HC2 (90.8% versus 81.6%). Xpert HPV had a higher specificity than the Cobas HPV test (42.6% versus 39.6%, p = 0.02) but a lower specificity than HC2 (42.6% versus 47.7%, p = <0.001).

In the same study, Castle et al.[5] performed two Pap smears on 658 women for Xpert HPV testing and compared test results with HC2 and the Cobas HPV test. They showed that the kappa values of the two Xpert HPV results and the Cobas HPV results were 0.85 and 0.83 and the HC2 results were 0.72 and 0.74. Our group recruited 1120 women aged 30–65 years (of which roughly half were HIV-positive) from a colposcopy clinic and unscreened women from the general population in Cape Town, South Africa. All women underwent colposcopy and histological sampling in addition to on site Xpert HPV testing. We then calculated the sensitivity of Xpert HPV to detect histologically-confirmed CIN2+ and the specificity of no disease in the screening population using three approaches to classify "screen-positive" from the output of the test. Firstly, we calculated sensitivity and specificity when the Xpert HPV test was run as per the manufacturer's instructions i.e. using the pre-defined cut-offs on all of the five channels to define screen-positive (Figure 1, grey bars). Sensitivity to detect CIN2+ was excellent for HIV-negative (88.3%) and HIV-positive (93%) women. However, specificity while reasonable in HIV-negative women (87.3%) was low in HIV-positive women (63.6%). Secondly, we evaluated the effects of defining screen-positive based only on the three channels detecting HPV16, 18, 45, 31, 33, 39, 52 and 58. Using this approach, sensitivity to detect CIN2+ remained excellent for both HIV-negative and positive women and specificity improved to 90.5% for HIV-negative women and to 68.9% for HIV-positive women (Figure 1, salmon bars). Thirdly, we evaluated the effects of altering the Cycle threshold cut-off levels for the channels detecting HPV types 16, 18, 45, 31,33, 35, 52 and 58. We developed a logistic regression model and selected the cut-offs that could attain 85% sensitivity. In this scenario, specificity improved further for HIV-negative women attaining 92.6% and for HIV-positive women attained 81.6% (Figure 1, green bars).

The Xpert HPV test is sensitive, specific and reliable and may easily be adapted to a point of care test, making screening and treatment of women possible in one visit

The Xpert HPV test is sensitive, specific and reliable and may easily be adapted to a point of care test, making screening and treatment of women possible in one visit. The algorithm developed by our group[6,7] ensures that sensitivity is maintained with a marked improvement in specificity, particularly in HIV-positive women. This study has now moved into an implementation phase and women who meet the criteria for treatment as postulated by the algorithm, are being treated at the same visit using thermocoagulation in a nurse-driven process. In parallel, we are evaluating the impact of introducing Screen and Treat services on the health system and health care providers at primary/district levels of care. ■

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nº 65

Australia on-track to be the first country to achieve cervical cancer elimination

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Recently, the International Papillomavirus Society (IPVS) issued a 'call to action' to health authorities to achieve elimination of cervical cancer as a public health problem.[1] In principle, cervical cancer rates can eventually be reduced to near-zero given the highly effective primary prevention (HPV vaccination) and secondary prevention (cervical screening) interventions now available.[2,3] But even if these interventions can be deployed very rapidly at a global level, the timeline to elimination is uncertain.

The issue of timing is complex, because HPV vaccination is most effective in younger cohorts prior to HPV exposure. But cervical cancer occurs in mid-adult and older women, and it will thus take several decades for the full impact of vaccination to be realised. Cervical screening has a much nearer-term impact on cervical cancer, but the extent of coverage and level of organisation for screening varies widely between countries. Furthermore, a large body of evidence now demonstrates that primary HPV screening is more effective than cytology in protecting against invasive cervical cancer; so countries introducing primary HPV should be able to accelerate the reductions.[3,4] So how will all these factors combine to influence the timing of cervical cancer elimination?

It is useful to look at a specific example. Australia is poised to be the first country to approach cervical cancer elimination, since it has now fully implemented all these major prevention interventions. Australia was the first country to introduce a national publicly-funded HPV vaccination program in 2007, with a wide catch-up age range from 12 to 26 years. In 2013, Australia introduced vaccination for adolescent males, and in 2018 the next generation nonavalent vaccine was introduced, which protects against seven carcinogenic types which are associated with ~90% of cervical cancers. Multiple studies have documented the impact on health outcomes: the prevalence of vaccine-included type-specific infections in young women aged 25-35 years has now drop-ped by a factor of 10 (even in unvaccinated females, due to herd immunity),[5] the prevalence of anogenital warts has dropped substantially in both females and heterosexual males,[6] cervical precancerous abnormalities (CIN2/3) have now dropped by 41% nationally in women aged 20-24 years,[7] and the rate of excisional treatment has now also fallen in young women.[8]

Australia has also had a comprehensive organised screening program since 1991, which by 2010 had already halved cervical cancer incidence rates in women aged 25+ years.[9] Prompted by the impact of vaccination and established evidence on primary HPV-based screening, on December 1st, 2017, Australia transitioned to 5-yearly screening with validated HPV assays, which is expected to reduce cervical cancer incidence and mortality rates by at least a further 20% (Table 1).[10] First out-

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Table 1

Projected long term impact of switching to primary HPV screening on health outcomes, costs and health resources utilisation[10]

	CYTOLOGY SCREENING		HPV: FINAL GUIDELINES*	
	If HPV vaccination had not been introduced	Cohort offered vaccination at age 12 year	If HPV vaccination had not been introduced (reduction compared to cytology screening program)	Cohort offered vaccination at 12 years (reduction compared to cytology screening program)
Cervical cancer incidence †	6·92	2·87	4·73 (-31%)	2·17 (-24%)
Cervical cancer mortality †	1·80	0·74	1·15 (-36%)	0·53 (-29%)
Cervical cancer cases (n) ‡	850	353	584 (-265;-31%)	267 (-85;-24%)
Cervical cancer deaths (n) ‡	227	94	145 (-82;-36%)	66 (-28;-29%)
Colposcopies (n) ‡	85795	60995	116889 (31094; 36%)	56479 (-4516;-7%)
Treatments (n) ‡	22661	13899	23963 (1302;6%)	13240 (-659;-5%)
Annual cost‡ of screening programme (AUS$)	$223 million	$192 million	$182 million ($41 million; -19%)	$142 million ($50 million; -26%)

*"Cytology screening" is the prior cytology-based program (2-yearly cytology from ages 18-20 to 69 years). "HPV: Final Guidelines" are final estimates for the HPV-based screening program (5-yearly HPV screening ages 25-74 years) after considering detailed clinical management guidelines for colposcopy referral and post-colposcopy management in new program. †Age-standardised rate (0–84 years), standardised using the 2001 Australian standard population and represented per 100 000 women. ‡Using the female Australian standard population as predicted for 2017.

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comes from a major trial of screening in unvaccinated populations, Compass, have demonstrated that the increased detection of CIN2+ with HPV compared to cytology screening (well documented in unvaccinated populations) is seen even in Australia's population, with its high vaccine uptake.[11]

Recently, we modelled the impact of these multiple interventions on cervical precancerous abnormalities, invasive cervical cancer and mortality, out to 2035 (Figure 1).[12] Because of the increased sensitivity of HPV testing, it is initially expected to result in an apparent transitional increase in cancer rates due to earlier detection. In the intermediate term, cervical cancer rates are expected to halve (again) by 2035, and mortality rates should remain stable until about 2020, but then decline by 45% by 2035. These findings indicated that both HPV vaccination and primary HPV screening represent significant and timely steps in Australia's journey towards elimination of cervical cancer.

In the intermediate term, cervical cancer rates are expected to halve (again) by 2035, and mortality rates should remain stable until about 2020, but then decline by 45% by 2035

Thus, the experience in Australia demonstrates that there is potential to drastically reduce the incidence of one of the world's major cancers in women. However, the large majority of the global cervical cancer burden is in low and middle income countries where access to screening is very limited or non-existent.[13] The key will be effective action to fund, implement and monitor widespread HPV vaccination and cervical screening initiatives in these countries, which have the greatest need.

The key will be effective action to fund, implement and monitor widespread HPV vaccination and cervical screening initiatives

Acknowledgement: This work was funded via a number of National Medical Research Council (NHMRC) Australia grants and the authors are part of C4, The NHMRC Centre of Research Excellence in Cervical Cancer Control. ■

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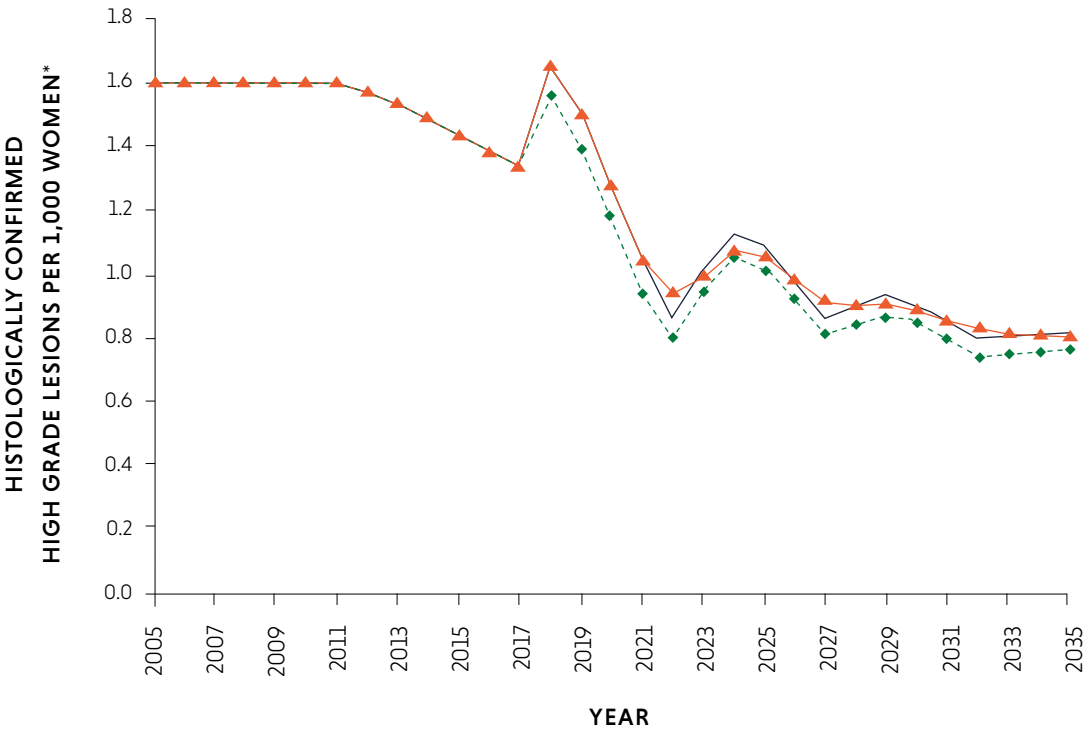
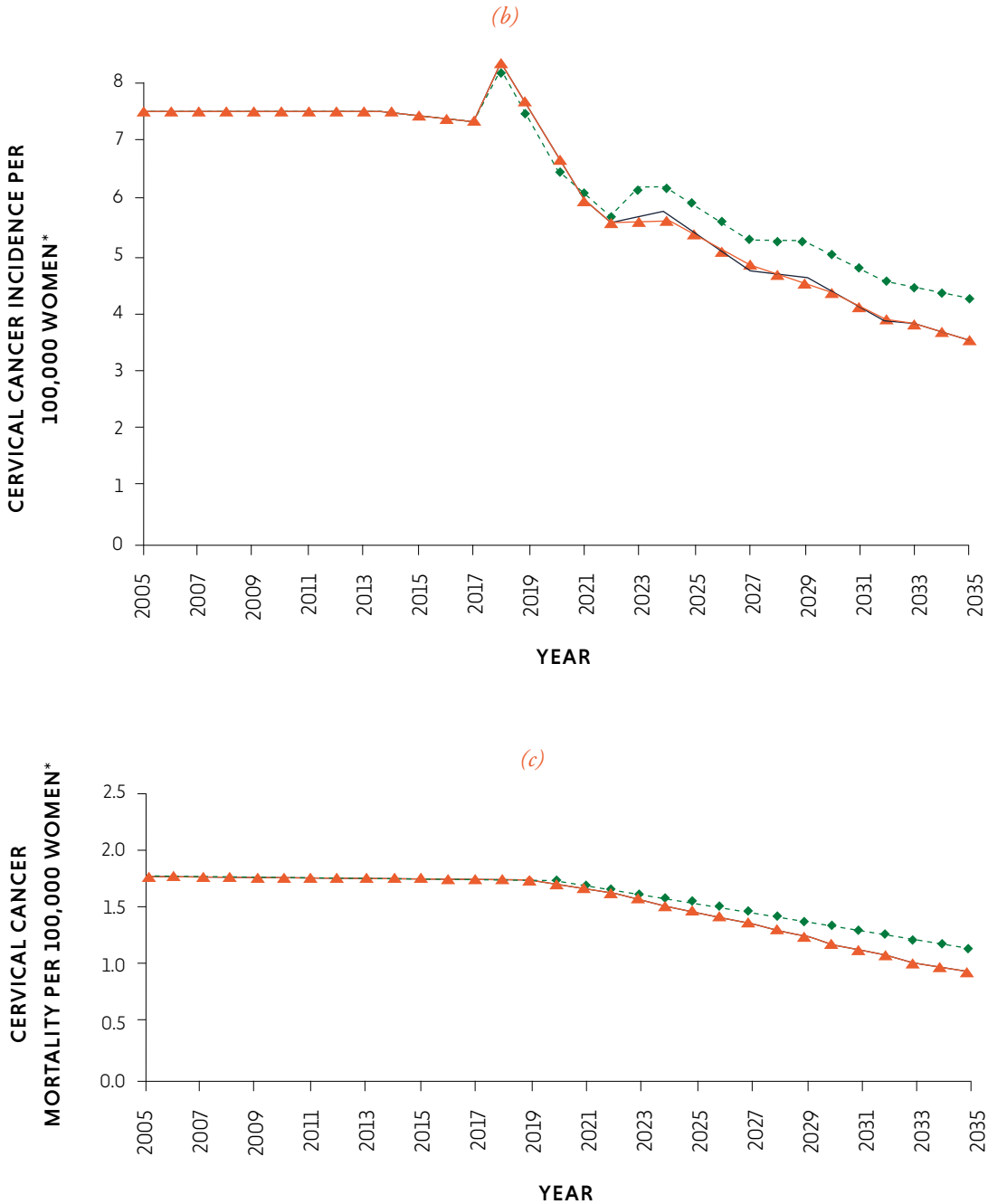
Figure 1

Combined effect of HPV vaccination and HPV screening on detected CIN2/3 (a), cervical cancer incidence (b) and cervical cancer mortality (c) in Australia to 2035[12]

(a)



* Age-standardised rate (0–84 years), standardised using the 2001 Australian population



Text legend: "Sensitivity Analysis" explores the impact on disease reduction (i.e. cervical cancer incidence) in a changing range of values of the key variables influencing the prediction (i.e. the variation in disease incidence reduction in the presence of low, intermediate or high vaccination coverage rates). For the three outcomes displayed in the figure the lower bounds of two critical screening variables, namely screening coverage and test sensitivity are shown.

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Integrated HPV-based cervical cancer screening and HPV vaccination is the only way forward for Central and Eastern European countries

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In the next few decades, several million women living in the Central and Eastern Europe (CEE) will be at high risk of developing cervical cancer. At present, cervical cancer incidence and mortality are higher in CEE countries than elsewhere in Europe and are rising in certain countries, partly due to an absence of screening interventions that are, at best, opportunistic with relatively low coverage and quality.[1-4] In CEE approximately 40,000 women develop cervical cancer and 20,000 die from the disease yearly and cumulative risk for getting the disease in Eastern Europe is 4 to 5 times higher than in Western and Nordic Europe.[3-5]

In CEE approximately 40,000 women develop cervical cancer and 20,000 die from the disease yearly and cumulative risk for getting the disease in Eastern Europe is 4 to 5 times higher than in Western and Nordic Europe

The process of post-socialistic transition, taking place at a different pace in each CEE country, has significantly affected all health-associated issues and the political attitude towards health problems, including cervical cancer prevention.[5-8] Emerging problems in CEE region are related to the high proportion of female smokers and the dramatic increase of human immunodeficiency virus infection incidence in some CEE countries in the recent years. Consequently, in several CEE countries at least one woman in 50 will develop cervical cancer before the age of 75 years. The high burden of cervical cancer in CEE can be explained by a historical lack of effective screening and changed sexual behavior and, subsequently, increased exposure to human papillomavirus (HPV) infection among women born after 1930. In addition, in the CEE there is a paucity of detailed data impeding the evaluation of variations in the incidence of and mortality from other HPV- related cancers and disease and several gaps in knowledge also exist concerning HPV prevalence and type distribution in the general population and among women with cervical pre-cancer lesions.[2-6]

Although the introduction of prophylactic vaccination against HPV in CEE would substantially reduce the number of future cases of cervical cancer, the full effect, in terms of a reduction in all-ages cervical cancer incidence, will not be detectable for more than 30 years. Hence, the implementation of high-quality screening activities should still play a major role in the prevention of cervical cancer and bridge the gap until the longer-term effects of HPV vaccination programs are seen.

In the part of Europe where high-quality, cytology-based cervical cancer screening programs were implemented several decades ago (e.g. in Northern Europe), the intervention has countered the

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A particularly positive example comes from Slovenia, where, in a relatively short time and with affordable investment, the country moved from an opportunistic to an organized national screening program; the result was a dramatic drop in cervical cancer incidence rates, from 15 to 6 cases per 100,000 during 2003-2015

increased exposure to HPV infection, and incidence of cervical cancer has uniformly decreased, making cervical cancer a relatively rare disease.[1] In contrast, great majority of CEE countries failed to establish organized, high-quality or high-coverage cervical cancer screening programs. Until recently, across whole CEE region cytology-based screening was mainly opportunistic, with low coverage and low quality control on cytology.[6,7] Some efforts, however, have been made, especially after the release of the European Union guidelines on screening in 2008. Baltic countries and some of the Central European countries established organized cytology-based screening programs in the last decade that partly function, although low coverage, absence of quality assurance, and opportunistic screening outside the main program are major obstacles.[6,7] A particularly positive example comes from Slovenia, where, in a relatively short time and with affordable investment, the country moved from an opportunistic to an organized national screening program; the result was a dramatic drop in cervical cancer incidence rates, from 15 to six cases per 100,000 during 2003–2015.[6] However, none of the CEE countries

The resolution of the problem of cervical cancer in CEE region is not anymore a matter of further scientific research, but rather the implementation of public health care programs

Immediate action is necessary, including the establishment of continuous, concerted and stepwise programs of cervical cancer prevention and programs for changing perceptions and attitudes in public, medical profession, and government

seem to have planned for the use of HPV-based screening which, compared with cytology, provides better and more durable negative predictive value against high-grade cervical disease and cancer, requires a simpler logistic and health-care infrastructure, is more reproducible, and is likely to be more cost effective. The use of HPV screen-ing is recommended by WHO guidelines for countries without an already functioning effective, high-coverage cytology-based program,[9] and for all member states of the European Union.[10]

Recent model study using six countries in CEE region (Estonia, Lithuania, Latvia, Belarus, Bulgaria, and Russia) projected the number of women that could be spared from cervical cancer over the next 25 years in the region upon swift introduction in 2017 of effective cervical cancer screening programs.[11] Under the assumption that screening-related gains could be as favorable as those shown in the long-term trends in cervical cancer incidence in Denmark, we estimate that 180,000 new cases of cervical cancer could be prevented from 2017 to 2040 in the six studied countries only.[11] The scale of the rapid increase in risk in recent generations of women, most of whom are outside the target age range of the HPV vaccine, and the clear evidence of a prevention effect can and must strengthen the resolve to immediately launch effective screening programs in CEE countries. A lack of action will result in dramatic increase of the number of women diagnosed with cervical cancer. The use of HPV-based screening program in combination with a prompt introduction of HPV vaccination, could drastically reduce the burden of cervical cancer.[11]

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At present, cervical cancer screening as well as HPV vaccination are restricted or ineffective in CEE countries. The resolution of the problem of cervical cancer in CEE region is not anymore a matter of further scientific research, but rather the implementation of public health care programs.[2,10] HPV vaccination is the best strategy for preventing cervical cancer in CEE countries in the long term, yet strengthening screening activities is a key intervention to prevent a future increase in cervical cancer diagnoses in the next two or three generations of women.[11] Protocols combining HPV vaccination of adolescents with a few rounds of organized HPV-based screening have been proposed as a viable option in high-risk populations such as the CEE countries.[12] In the absence of action, the cervical cancer risk in women living in CEE might reach levels similar to those seen in some sub-Saharan African countries today and in countries of Northern Europe half a century ago.[11] Additionally, all CEE countries should enhance advocacy, communication, and social mobilization strategies to increase awareness of the burden of HPV-related diseases and adequacy of joint primary and secondary prevention strategies, especially its synergistic effect.[10] The involvement of stakeholders at all levels is necessary, including medical professionals, decision makers, non-government organizations, press, women's groups, etc., aiming to enhance the political will, the economic resources and the administrative infrastructure to control cervical cancer. [4,8,10] Immediate action is necessary, including the establishment of continuous, concerted and stepwise programs of cervical cancer prevention and programs for changing perceptions and attitudes in public, medical profession, and government. We are all fully aware that the implementation of functioning organized cervical screening programs with accessible and effective treatment of precancerous lesions, coupled with universal HPV vaccination, is the challenging future for the majority of the CEE countries. But, this is certainly the only way forward. ■

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Italian Consensus Conference on Cervical Cancer Screening in HPV Vaccinated Women: Recommendations

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In Italy, HPV vaccination is actively offered free of charge to 12-year-old girls since 2007–2008 (depending on the region). In addition, some Italian regions have extended active offer to older female age-groups, including also girls in their 15th year of age.

In the near future, these cohorts of women will be reaching the age for screening (25 years). This happens while screening is moving from being cytology-based to HPV-based. This situation represents a challenge but also an opportunity for unprecedented reorganisation of CC prevention.[1]

In November 2015, the National Screening Monitoring Centre Directive and the Italian Group for Cervical Screening (GISCi) Coordination Committee in collaboration with different scientific professional societies for gynaecology, colposcopy, histo- and cytopathology, virology and virology organised a Consensus Conference aimed at the collection of available evidence required to define the best screening policy for girls vaccinated against HPV. The Consensus Conference identified and defined the central and local actions to be implemented in order to optimize the integration of primary prevention programs with secondary prevention programs, as well as research activities connected with the knowledge needed for change. Further, for each question, a Jury made recommendations and expressed an answer, which could be: (I) consensus for the recommendation; (II) consensus for the recommendation but need for reformulation, providing relevant indications; (III) no consensus for the recommendation.

Screening is moving from being cytology-based to HPV-based. This situation represents a challenge but also an opportunity for unprecedented reorganisation of CC prevention

The Italian integral report is published on the internet,[2,3] and has been officially presented to decision makers: the Ministry of Health and the State-Regions Conference. An English summary has been also published.[4] Here we present the recommendations as answers to four main policy questions handled by the group.

Question 1: Do the protocols for screening programs need to be changed upon the arrival of the cohorts of vaccinated women? If so, which policy appears to be the most effectively and operatively manageable, a tailored or a one-size-fits-all strategy?

Recommendation: First, the Jury stresses the fact that screening activity must continue and be

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performed within organized screening programs also for vaccinated women.

Second, the Jury considers changing the screening program protocols upon the arrival of the vaccinated cohorts as appropriate. The Jury recommends that tailored protocols, according to vaccination status, are gradually extended to all Italian Regions, in parallel with the implementation and validation (for quality and completeness) of IT systems.

Tailored screening could at some point be replaced by one size fits all screening protocols, when the vaccination coverage has reached levels such that infections from HPV16/18 (included in the vaccines currently used) can be considered practically negligible. This, according to the Jury, could be well below 95%.

Question 2: At what age should screening start? Which test should be used? How often should it be done?

Recommendation: For girls vaccinated in their 12th year, the Jury accepts the proposal to move the starting age for screening from 25 to 30 years, using HPV test as primary screening test. For non-vaccinated women, the current protocol must be continued, with cytological screening in the frame of 25-29 age and HPV test with cytology triage from age 30 to 64.

The Jury recognizes the lack of evidence on the optimal interval between screening rounds in vaccinated women, while acknowledging the strong rationale for an interval longer than 5 years, the interval currently recommended in the female population in general. Furthermore, the Jury adheres with full consent to the proposal to promptly start studies on this issue.

Question 3: Should the strategy be different for the cohorts vaccinated in their 15th year (or later) with respect to those in their 12th year?

Recommendation: The Jury is favorable to the recommendation not to change current screening protocols with primary Pap test for women vaccinated in their 15th year or later. Indeed, the estimated median age of sexual debut in Italy is 17 years old. Hence, it can be assumed that less than half of the girls vaccinated in their 16th year and more than half of those vaccinated subsequently have already had sexual intercourse and therefore may not be HPV-naive at vaccination (Table 1).

Question 4: Which actions need to be scheduled from now and up to 2021 in order to acquire missing evidence and to make the integration of primary and secondary prevention practically possible?

Recommendation: The Jury underlines the need to implement a link between vaccination records (indicating the number of doses, vaccination date of each dose and type of vaccine administered) and screening registers, and recommends the construction of archives at a regional and national level reciprocally connected.

The Jury also considers that a substantial effort should be dedicated to training healthcare operators, so that they can provide to the general popu-

The Consensus Conference identified and defined the central and local actions to be implemented in order to optimize the integration of primary prevention programs with secondary prevention programs

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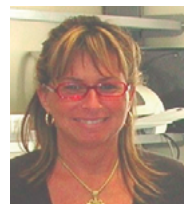

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Table 1

Recommendations for cervical cancer screening in vaccinated women

Vaccination at 12th year of age (primary target of the organized vaccination program)	**Background:** Presumably HPV-naïve at the time of vaccination and thus with an expected high vaccine efficacy. **Strategy:** A tailored strategy (according to vaccination status), followed by one-size-fits-all strategy when the high vaccination coverage minimize the burden of HPV16/18 infections. Primary screening using HPV-test (with cytological triage in HPV-positive), with a screening starting age delayed to 30 years. **Screening interval:** Strong rationale for an extension of interval over 5 years. Need to be evaluated in clinical studies.
Vaccination at ≤15th year of age	**Background:** A relevant number of these girls may have already been infected at the time of vaccination, and thus the level of protection of HPV vaccines is not well identified at this time. **Strategy:** Current protocol must be continued (cytological screening in the frame of 25-29 age and HPV test, with cytology as triage, from age 30 to 64). **Screening interval:** To be decided after the CIN3+ detection rate at the 2nd round in HPV-negative women.

lation useful and scientifically correct information on the changes to screening practices, their efficacy, the type of test used and the starting age.

Moreover, the Jury also underlines the need to promote conducting studies:

1. to monitor the activities of both programs and to provide appropriate epidemiologic surveillance. As vaccination implementation increases and more cohorts will be involved, the evidence of protection will also increase and therefore we will have more robust post-vaccination data.
2. to identify conservative protocols using HPV test also in women between 25 and 29 years.
3. to conduct surveys aimed at identifying tools/methods and appropriate ways to communicate the change of screening to women and clinicians.
4. to assess whether the nonavalent HPV vaccine may change the fundamental elements of the decision-making tree presented in this document. Indeed, in the future predicted scenario of higher prevention with the new vaccine of cervical pre-cancer lesions (which are the target of screening program) harms of screening may outweigh its benefits. ■

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Primary hrHPV population screening for cervical cancer in the Netherlands

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In 2017, the Dutch cervical cancer screening programme started the implementation of primary hrHPV screening with cytology triage at the general practitioner (GP) office. All hrHPV-positive women with cytological abnormalities (≥ ASC-US) are referred to the gynaecologist, instead of referring only women with HSIL or worse in the previous cytology-based screening programme. If there are no abnormal cells, the woman is advised to have a second cytology test in six months as part of the screening programme.

Liquid-based cervical cytology specimens are taken at the GP office but women who do not respond to the initial invitation can order a self-sampling device. In case of a hrHPV-positive self-sample, a GP-visit is needed to collect a cervical cytology specimen, because cytology is not possible on self-samples. Figure 1 presents a schematic overview of the screening programme.

Invitation scheme

Women aged 30, 35, 40, 50 or 60 years old receive an invitation for the population screening. At the age of 45 and 55 years, only women who had a hrHPV test positive or not performed at ages 40 or 50 years, respectively, receive an invitation. Women who had a hrHPV-positive test at the age of 60 years, receive a final invitation at the age of 65.

Women who do not respond to the initial invitation can order a self-sampling device

Tests performed

All laboratories use the cobas® 4800 HPV test (Roche Diagnostics, Alameda CA, USA) to test the clinical and self-samples. The cobas® 4800 HPV test is a CE/IVD certified kit for use in combination with the cobas® 4800 nucleic acid extraction PCR setup, real-time amplification and software system. As part of the assay procedure, each sample is tested for the presence of human cells by amplification of the human beta-globin gene.

The Evalyn® Brush (Rovers Medical Devices, Oss, the Netherlands) is used for self-sampling whereas ThinPrep® (Hologic, Bedford, MA, USA) is used as transport medium for cervical cytology specimens.

Quality control in general

The primary hrHPV screening programme implementation reduced the number of laboratories from 40 to 5. Two national reference officers, one for HPV and one for cytology, chair the national quality platform with representatives of the five laboratories. This platform exchanges experiences and methods used to enhance a uniform practical approach of the screening. The five laboratories are accredited by the Dutch CCKL (Coordination Commission to promote the Quality Control of Laboratory Research) or ISO 15189 and back up for each other if needed.

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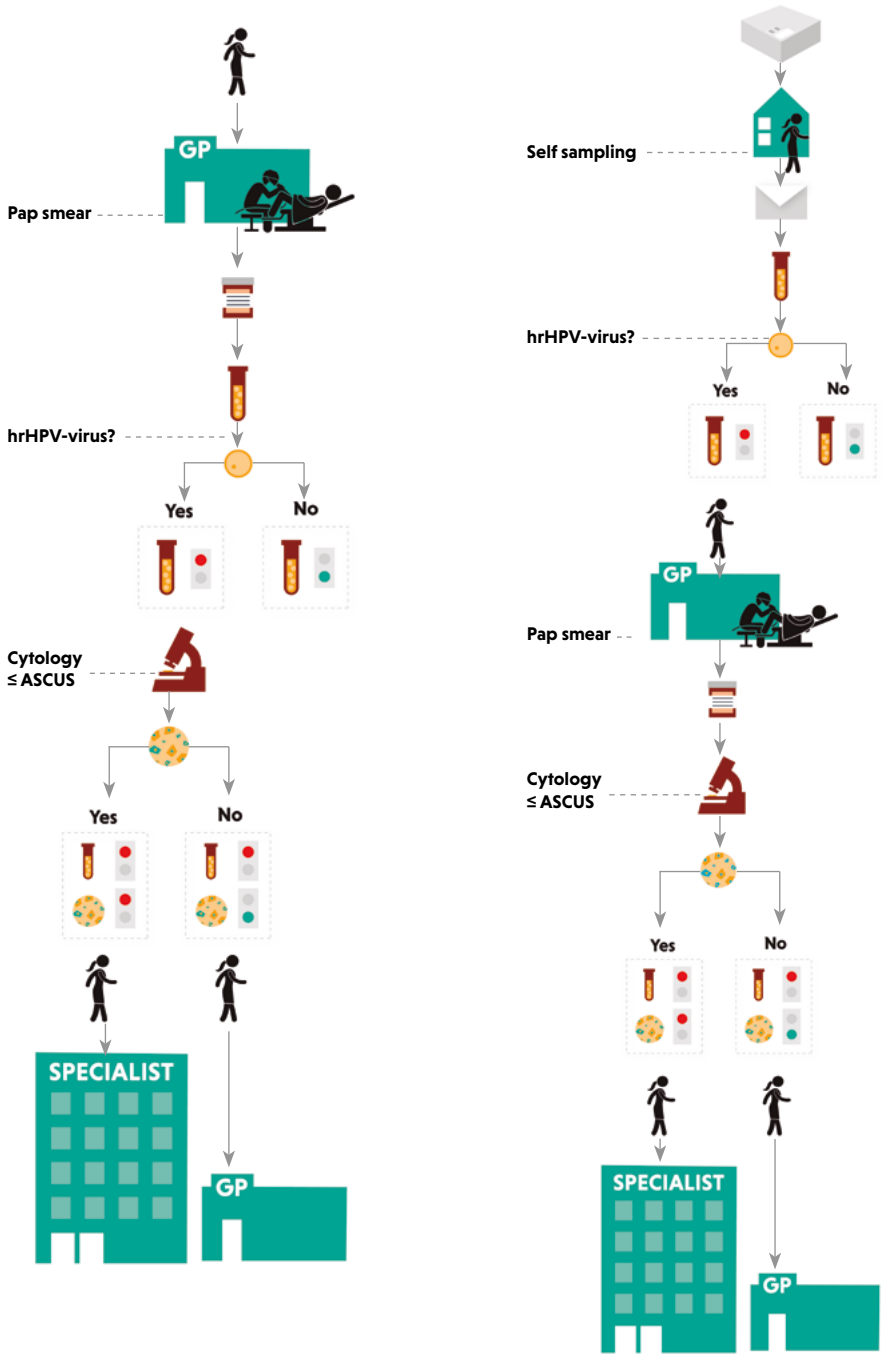
Table 1

Schematic overview of Cervical Cancer Population Screening – cervical cytology specimen (left) and self-sampling device (right)[1]



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There is a quality control programme on HPV and cytology on a structural basis

As part of the preparations towards the renewed programme, the suppliers of the HPV test and thin-layer cytology trained the employees of the laboratories. Additionally, cytologists and pathologists analysed two learning sets of samples to get used to a higher percentage of cytological abnormalities. Furthermore, there is a quality control programme on HPV and cytology on a structural basis.

Quality control of HPV and cytology testing
Besides proficiency panels of hrHPV samples and cytological samples, the quality control programme includes monitoring of the analytical performance of the hrHPV-test:

1. A verification and release programme for acceptance testing of equipment upon installation, repair or major maintenance activity. This programme is also used to test and release (new lots) of critical reagents.

2. A run control programme with a manufacturer-independent control sample in each HPV run. In addition, standardized procedures and protocols are written by the quality platform and used by all laboratories.

The results of the first year of primary hrHPV screening will be published on the English website of the National Institute for Public Health and the Environment as soon as available.[2] ■

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Core elements of the new HPV-based cervical cancer screening programme in Italy

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Italian recommendations on HPV testing were based on a Health Technology assessment report published in 2012.[1] It included a section on efficacy and undesired effects, based on a systematic review of literature, which represented a preliminary version of the chapter on HPV testing for primary screening developed for the Supplements to the "European Guidelines on cervical cancer screening" (published in their final version in 2015)[2] and sections about costs, organisational problems and social, ethical and legal issues mainly based on the experience of Italian pilot projects. Crucial recommendations were:

10 years is an upper bound for re-testing after a negative HPV test

Interval between negative screens of at least 5 years. This was based on data about the risk of CIN2/3 after a negative HPV[3,4] test and were strongly reinforced by the pooled analysis of European RCTs,[5] that showed the 5.5-year invasive cervical cancer risk after a negative HPV test to be about half the 3.5-year risk after normal cytology. Data from the POBASCAM study follow-up[6] show that the 10-year CIN2+ risk after a negative HPV is similar to the 5-year risk after normal cytology, suggesting that 10 years is an upper bound for re-testing after a negative HPV test.

Start of HPV-based screening not before age 30-35. This recommendation was based on the finding that in NTCC the cumulative detection of CIN2/3 up to including the second screening round, was much higher in the experimental than in the conventional arm, suggesting overdiagnosis of spontaneously regressive lesions.[7] As the treatment of high-grade CIN is associated with complications in pregnancy,[8] overtreatment should be avoided especially in younger women. However, the NTCC results could also be explained by very large gain in lead time with HPV at younger age while the POBASCAM trial[9] showed no evidence of overdiagnosis from age 30 (the youngest invited group). Finally the pooled analysis of EU trials[5] showed that the largest gain in protection from invasive cervical cancer by HPV was at age 30-39. Thus starting at age 30 is plausibly the best compromise.

Stand-alone HPV as primary test. Cytology should be only used as triage test in HPV+ women. This recommendation was based on the minimal increase of sensitivity observed with co-testing vs. stand-alone HPV[4] and on a similar reduction of CIN2+ in the experimental arm at round 2 ob-

The pooled analysis of EU trials showed that the largest gain in protection from invasive cervical cancer by HPV -screening was at ages 30-39. Thus starting at age 30 is plausibly the best compromise

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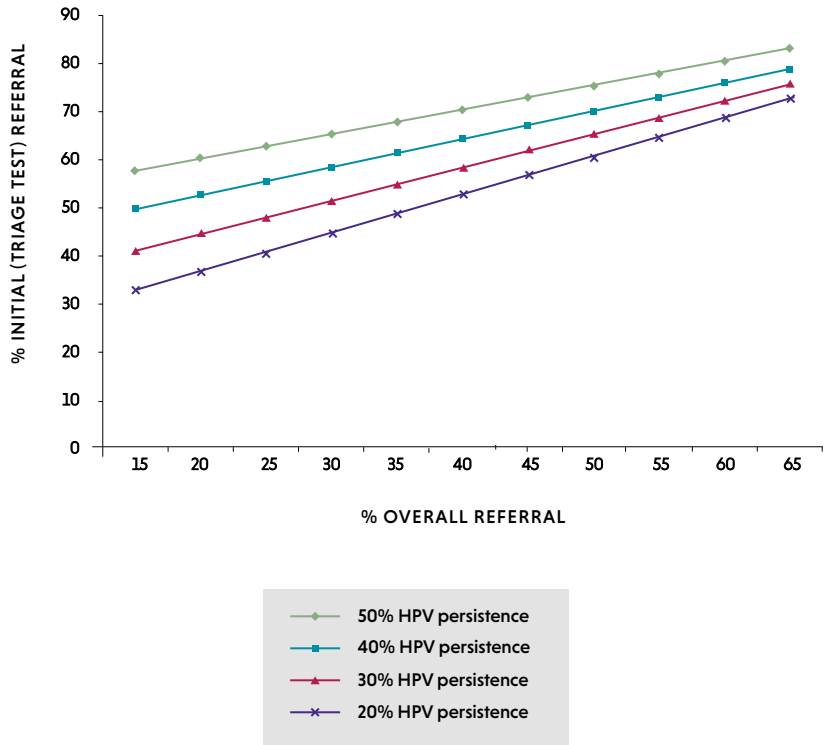
Figure 1

Triage protocol based on an immediate triage test, repeat HPV in triage-negatives after some time and referral of those who persist positive. Overall referral to colposcopy as a function of initial referral and of HPV persistence



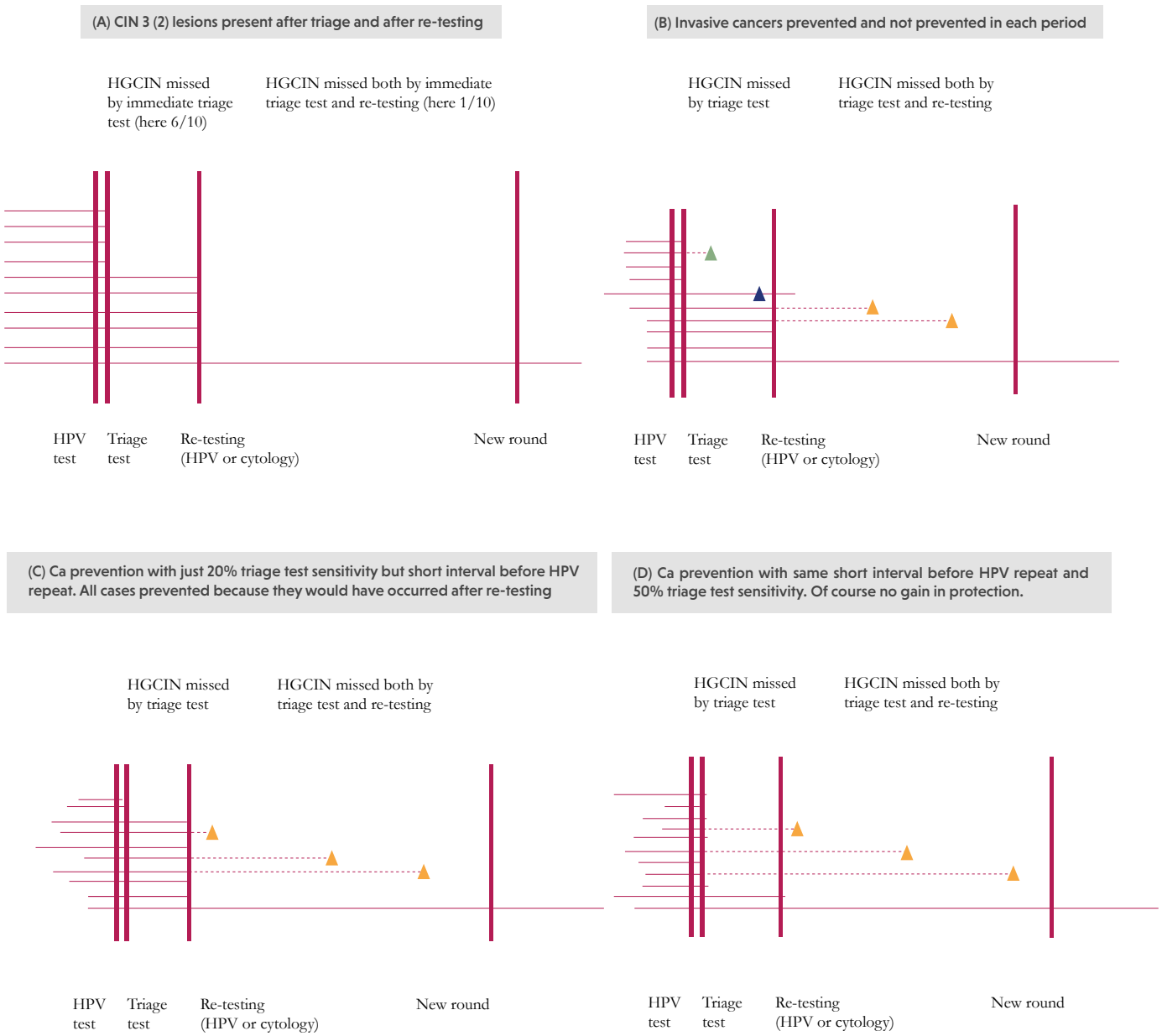
Overall persistence is computed as O = I + (100-I) x P where O is overall referral, I is initial referral and P is HPV persistence. Of course the overall referral with 50% initial referral and 20% HPV persistence is the same as with 20% initial referral and 50% HPV persistence.

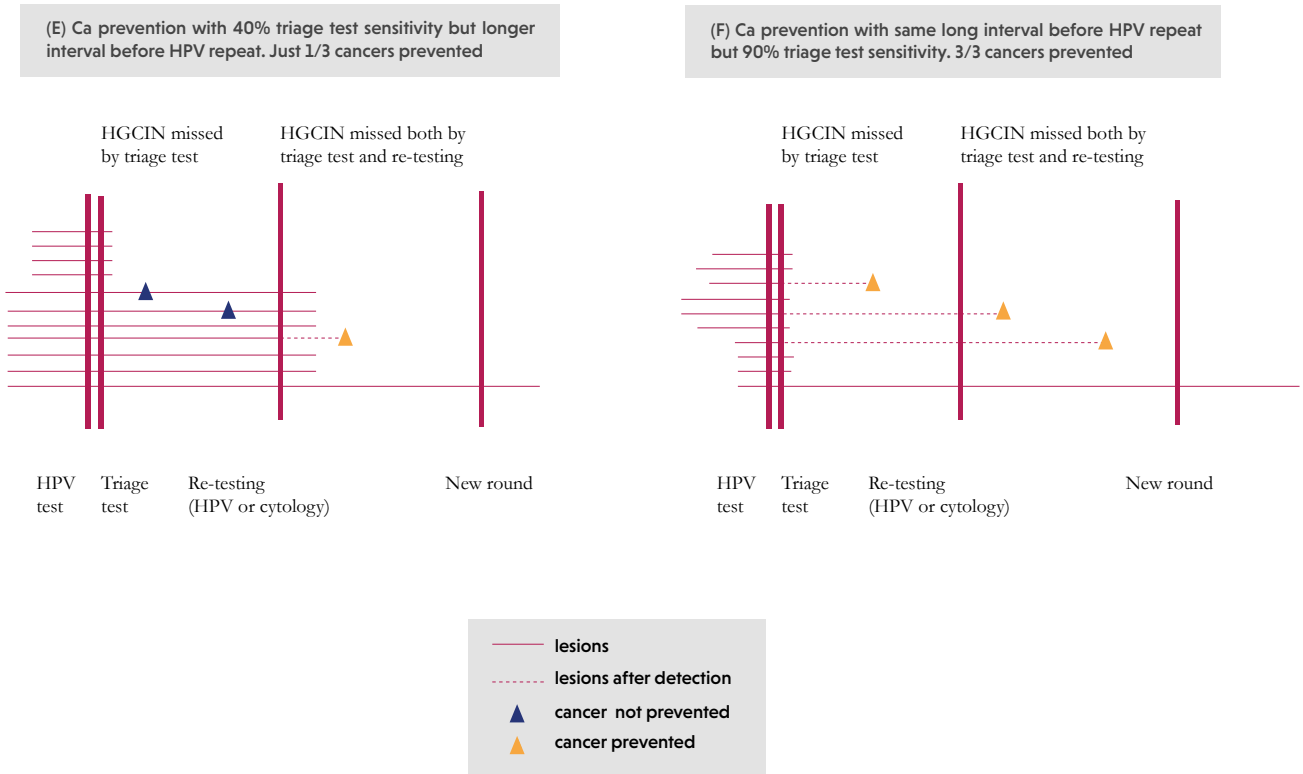
served after stand-alone HPV and after co-testing.[7] Conversely costs and undesired effects are much higher with co-testing.

Triage of HPV positive women (no direct referral). The recommendation was based on the increase in referral to colposcopy and decrease in positive predictive value observed in NTCC,[6] where direct referral was applied. The pooled analysis of RCTs[5] showed no evidence of heterogeneity between RCTs for the relative efficacy of HPV vs. cytology-based screening while there was a clear

Figure 2

Triage protocol based on an immediate triage test and repeat HPV in triage- negatives after some time. Effect of triage test sensitivity and interval before re-testing on cancer pevention





(A) Lesions present at baseline will still be present at HPV re-testing if missed by the triage test and at the new screening round if missed by both triage test and repeat HPV. (B) HGCIN missed only by immediate triage test are relevant only for cancer risk before re-testing. (C) If repeat is at short interval, then cancer risk will be low even with low sensitivity of the triage test. Remember time needed for progression to invasion is very long (1/3 of CIN3 in 30 years). Only HGCIN present from long time will progress before repeat. (D) With short interval before re-testing increases in sensitivity of triage test entail minimal decrease in cancer risk (E) With longer interval and same sensitivity of immediate triage test more HGCIN will progress to invasive cancer. (F) For repeat at long interval, high sensitivity of initial triage test is needed to avoid cancer.

heterogeneity for the biopsy rate (double with HPV vs. cytology in NTCC, similar in the other trials). The recommended protocol entails reflex cytology testing of HPV+ women, with referral to colposcopy of women with ASC-US+. The remaining are invited for repeat HPV testing after 12 months and referred to colposcopy if still positive.

This scheme was substantially shared by all RCTs. Further Italian data showed,[10] with this approach, a limited variability between local programs in the overall referral to colposcopy despite high variability in immediate referral (due to subjective interpretation of cytology). Anyway, because about halve of HPV infections clear in 1 year, then >50% of

HPV+ women will be referred to colposcopy if referral is based on HPV persistence after 1 year, decreasing with increasing interval before HPV repeat (Figure 1). The risk of invasive cancer up to HPV repeat (afterwards it is plausibly very low because of the high sensitivity of HPV) depends on the frequency, in the screened population, of pre-cancers progressing to cancer before it and on the cross-sectional sensitivity of the triage test or combination of tests (Figure 2). Prolonged intervals will plausibly be safer from the second screening round with HPV, when long-lasting lesions repeatedly missed by cytology will have been removed.[11] ■

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Experiences with the use of HPV testing in cervical cancer screening in Sweden

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We summarize some of the key findings of our implementation trials of HPV screening and use of population-based data for evaluating effectiveness and as a basis for incremental optimization of the organized program.

In the 1990s there was a widespread debate over the HPV screening concept. The major arguments against that were circulating concerned too high prevalences and life-long labelling of HPV positive women. The finding that HPV positivity cleared after effective treatment of cervical intraepithelial neoplasia (CIN) was therefore not only important in defining a new indication for HPV testing, but it also contributed to the understanding that HPV infection is not life-long.[1] Also, we realized that the high proportion of positives could be easily avoided by i) ensuring that internationally standardized and reliable HPV testing without contamination was used,[2] ii) avoiding HPV testing in the youngest age groups where the infection is common, and iii) screening for HPV persistence, as this (in contrast to transient HPV presence) is a central risk factor for cervical cancer.[3]

To enable better transition to real-life use, we use randomized health services studies (RHS), where the entire study is run within the usual healthcare system with both policies being compared financed within the system

To ensure generalizability, we launched a nationwide randomized clinical trial (RCT-Swedescreen) of primary HPV testing nested in the organized cervical cancer screening program in 1997. There was an increased detection of CIN2 and CIN3 that was followed by a decrease in the next screening round.[4] Analysis of the trial database indicated that primary HPV screening with reflex cytology only of the HPV positives would be the most cost-effective strategy.[5] A registry-based follow-up up to 14 years after enrollment identified subsequent screenings on 97.5% of the enrolled women and concluded that the extra CIN2 and CIN3 lesions detected by HPV screening did not represent overdiagnosis of regressive lesions, but earlier diagnosis of lesions that would have been detected later by cytology screening.[6]

Moving from research trials to clinical use can be problematic because of issues regarding e.g. generalizability, logistics, financing, and population acceptability. To enable better transition to real-life use, we use randomized health services studies (RHS), where the entire study is run within the usual healthcare system (with both policies being compared financed within the system).[7] When implementing HPV testing of women with low-grade cytological findings, we randomized outpatient clinics to either HPV triage and colposcopy of those that were positive or colposcopy of all low-grade cytological findings without HPV triage which was routine

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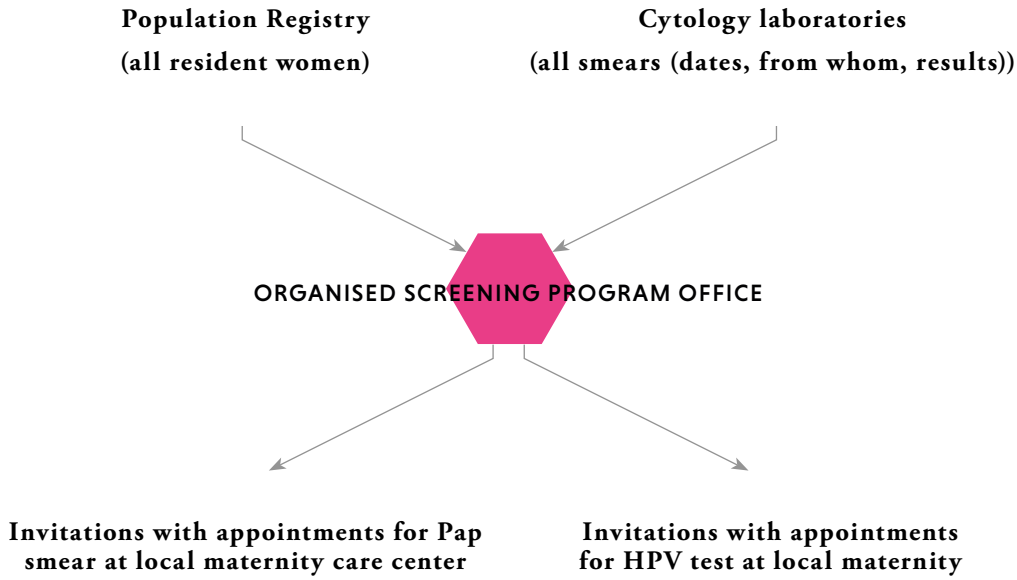
Figure 1

Algorithm of the randomized health service study (RHS) within organized screening



The computer at the organized screening office receives files detailing the population of resident women and files detailing which women have taken smears and when from the laboratories. Women eligible for organized screening are then invited by letter to an appointment at a screening station (maternity care center) close to the residence of the woman. In the Swedish RHS, the women received, at random, either an invitation to HPV screening or to cytology screening.

at the time.[8] For primary HPV screening, resident women were randomized to either receive an invitation to cytology screening or an invitation to HPV screening (Figure 1). The organized program first determines the target population with an extract of the population registry and subtracts the women who already have a cervical sample taken within the recommended interval. This reduces overscreening of already tested women and results in that non-attending women will remain in the target population and receive a new invitation the next year (Figure 2; never-attending women will receive 47 invitations per life-time). Population attendance according to recommendation is 83% and 10-year attendance is 96%. Organized sending of self-sampling kits to the 4% of the population that is long-term non-attenders can result in a further increase of population coverage, to 97%.

In 2012, we started an RHS of primary HPV screening vs. cytology screening that enrolled >400,000 women. The trial initially targeted only older women[9] but was subsequently enlarged to encompass all resident women aged 30-64. As of 2017, the yield of CIN2 or worse

(CIN2+) has been almost identical in both arms. Later referral of women with HPV persistence is expected to increase the sensitivity.

Initially, HPV+/Cytology- women were referred for repeat HPV testing after 1 year, but data found no increased cancer risk for cytology- women within 3 years; thus, those women will have a repeat test 3 year later and will be referred only if HPV is positive in 2 consecutive screening rounds. Long-term follow-up of the original RCT found that all women with two consecutive HPV positive samples either developed treatable CIN2+ or became HPV negative and had no subsequent risk. After 7 years there were no longer any women with HPV persistence that did not have CIN2+.[10] The current policy is that women with HPV persistence should be referred to colposcopy after 3 years persistence and if no CIN2+ at 3 years, referred again after 6 years of persistence.

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In 2015, the Swedish National Board of Health and Welfare mandated HPV screening in the ages 30-64 (with repeat annual invitations up to age 70 for non-attending women). In 31st December 2016, the RHS of primary HPV screening was stopped and all resident women offered HPV screening according to the national guidelines. At the time of writing, at least six regions in Sweden have started implementation of primary HPV screening and all the remaining regions report that they are preparing the launch. Thus, our country has finally, after some 25 years of continued and large-scale research, made good use of the opportunities that HPV testing offers. The use of RHS within the organized program was crucial to enable incremental optimization of programs through controlled implementation/evaluation of new policies. ■

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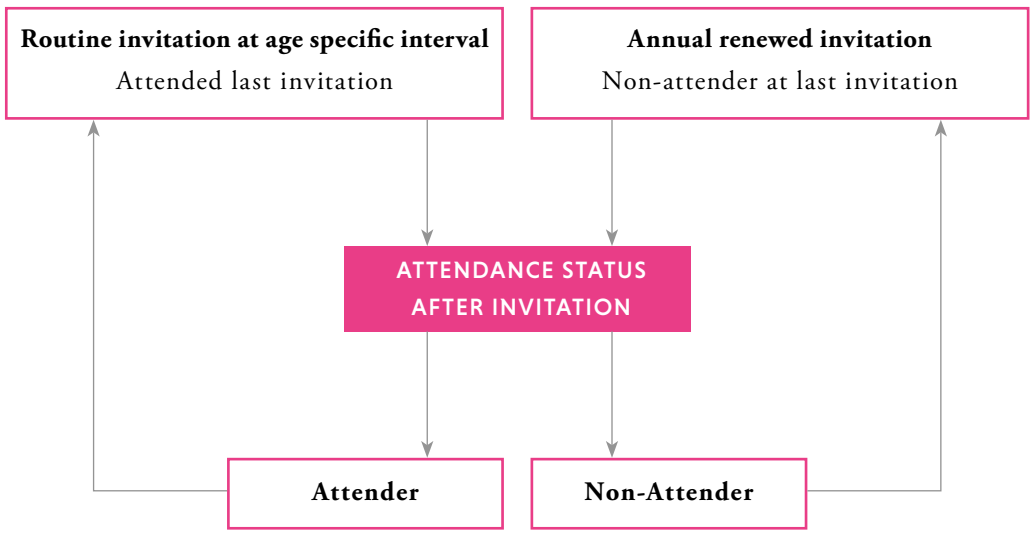
J Dillner, M Elfström (2018). Experiences with the use of HPV testing in cervical cancer screening in Sweden. **www.HPVWorld.com**, *70*

Figure 2

Invitations scheme



Invitation from the organized program are either sent based on the recommend ed age-specific screening intervals to women who have been screened. All women who have not been screened are sent an annual invitation.

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nº 71

HPV-based cervical cancer screening and management of abnormal screening results in the US

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The evidence supporting HPV screening

The evidence supporting switching from cytology screening to HPV screening is very uniform and clear. Multiple large randomized trials have demonstrated that HPV testing is more sensitive at detecting precancer compared to cervical cytology, leading to longer term protection against cancer.[1] In a pooled analysis of randomized trials, HPV testing provided greater protection against invasive cervical cancer compared to cytology.[2] European RCTs as well as a large observational study from Kaiser Permanente demonstrated that the benefit of adding cytology to HPV testing is very low at the cost of performing cytology in the entire population, substantially reducing cost-effectiveness compared to primary HPV screening alone. The mistaken notion that HPV alone screening may miss a substantial subset of treatable cervical cancers has been debunked.[3] Despite the strong evidence supporting HPV-based cervical cancer screening, its implementation is slow and varies strongly between healthcare settings. Here, we summarize the current status and future directions of HPV-based screening and management in the US, and we describe specifics of the US system and their influence on implementation.

Current primary screening options in the US

Cervical cancer screening is currently undergoing a major transition in the US. The US was the first country to approve and recommend HPV tests for use in cervical screening, initially for triage of ASCUS cytology in 2003, and later for use as an adjunct to cytology in primary screening. Recently, the first HPV-alone screening strategy was approved.[4] However, new approaches did not supplant existing strategies; instead, they became additional options. Three options are now available (Pap cytology, HPV testing, cotesting with both cytology and HPV), which differ by starting age, screening interval, and management options (Table 1). This abundance of choices is challenging for providers and has led to a lot of confusion, frequently resulting in poor implementation of specific strategies, with a strong tendency to screen much more frequently than needed.

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Table 1

Screening options in the US

Primary Screening	Cytology	HPV alone		HPV-cytology cotesting
Screening interval	3-year	3-year	5-year	5-year
Screening age	21-65	25-65	30-65	30-65
Triage	HPV	HPV16/18 and cytology	HPV16/18 and cytology	HPV16/18 or HPV-positive ASC-US or worse to colposcopy. 1-year repeat co-testing for HPV+/NILM
Regulatory approval	FDA approval of liquid based cytology, conventional Pap not regulated	FDA approval of two HPV tests (Cobas and Onclarity)	No	FDA approval for several HPV tests (HC2, Cervista, Cobas, Aptima, Onclarity)
Guidelines recommendation	2012 USPSTF 2012 ACS/ASCCP 2018 USPSTF	2015 ASCCP/ACS interim guidance	2018 USPSTF	2012/2017 USPSTF 2012 ACS/ASCCP

Regulatory approval vs. guidelines recommendations

It is important to differentiate regulatory approval of specific tests from organizational guidelines that recommend screening strategies. Regulatory approval through the Food and Drug Administration (FDA) focuses on safety and clinical efficacy of a specific test based on large, typically industry-sponsored Premarket Approval (PMA) trials. The result of a successful PMA process is regulatory approval of the new test for the pre-specified indication that was evaluated in the trial. Regulatory approval is binding and can be enforced.

In contrast, guidelines recommend a screening strategy, not specific assays. Multiple organizations develop guidelines for cervical cancer screening. In 2012, several professional societies led by the American Cancer Society developed screening guidelines that recommended HPV-cytology co-testing at 5-year screening intervals as the preferred screening option with cytology at 3-year intervals as an alternative. The independent US Preventive Services Task Force (USPSTF) came out with the same recommendations, for the first time providing a unified set of recommendations for providers. The USPSTF guidelines recently underwent a revision, and the previously announced draft recommendations proposed to switch to HPV alone at 5-year in-

tervals while keeping the recommendation for 3-year cytology. The guidelines attempt to arrive at a cost-effective public health recommendation. Many clinical stakeholders have voiced strong concerns regarding the draft USPSTF recommendation for HPV alone testing with 5-year screening intervals, citing the long interval and slightly reduced protection of this approach compared with the old standard of yearly Pap cytology. The final USPSTF guidelines now still include the option for co-testing.

It is important to evaluate HPV screening and triage strategies jointly, since they are conditional on each other in producing an optimally accurate answer of who needs treatment of precancer to prevent cervical cancer

Importantly, there is no organized screening program in the US, and in most settings, screening recommendations are not binding and are not enforced except in some cases via insurance reimbursement policies. Many providers still screen women annually with cytology or even with HPV-cytology co-testing. Currently, there is little disincentive by reimbursement systems outside of government health services (Medicare and Medicaid) that addresses too-frequent screening. The separation of regulatory approval from guidelines recommendations can create challenges for manufacturers of screening tests and regulatory authorities when transformative changes of practice are considered: Guidelines typically restrict to approve tests for specific indications, and regulatory trials typically focus on applications that are recommended by guidelines. This impasse is eventually solvable by introduction of new data from large independent clinical studies, which, in the case of cervical screening, are now underway.

Screening age and triage

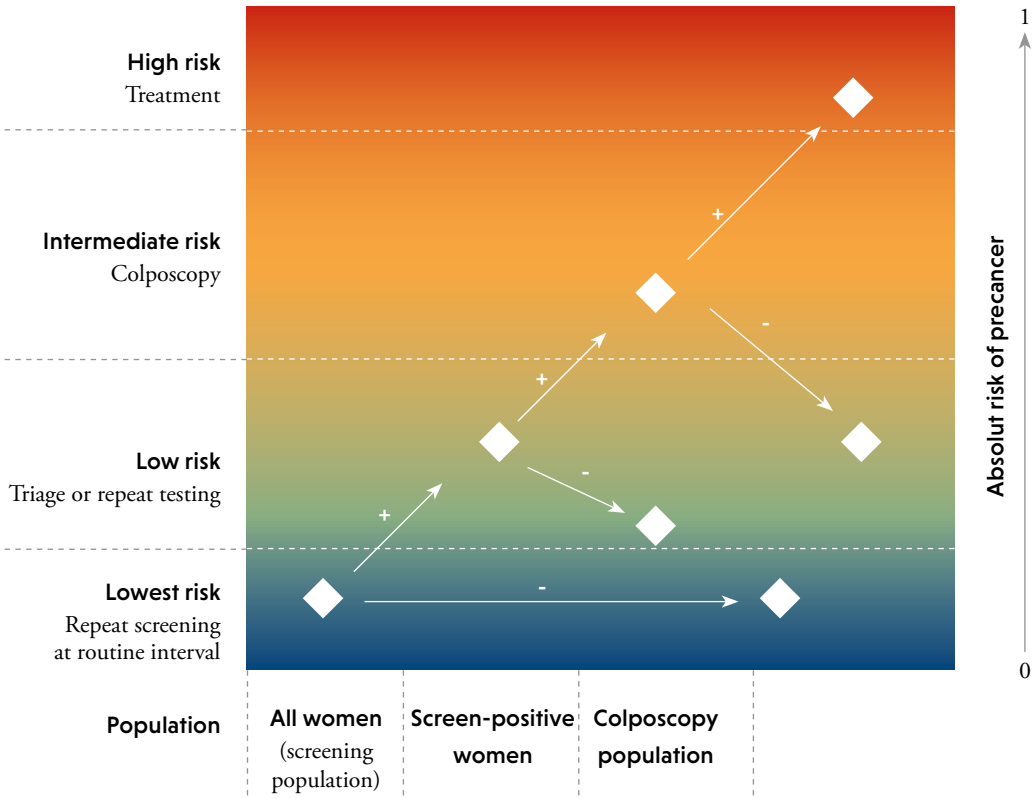
The primary screening approaches have different starting ages: Primary cytology is recommended from age 21 to 65, while use of HPV testing tends to start at older ages (at 25) because of the high prevalence of transient HPV infections in women age 20-24. The starting age for co-testing is 30, while FDA approval and interim guidance for primary HPV screening alone permits a starting age of 25 (Table 1). Importantly, HPV-based screening requires triage tests to decide who among the HPV-positive women needs to go to colposcopically-directed biopsy to decide whether precancer is present that requires treatment.[5] A specific triage test can counterbalance a higher test positivity of the primary screening test, thereby allowing for an earlier starting age of HPV screening. Currently, cytology and HPV16/18 genotyping are the only approved triage strategies. Novel triage tests now under development will need to undergo regulatory evaluation similar to the trials that were conducted for primary screening tests. Regulatory groups are reluctant to evaluate combinations of tests; however, it is important to evaluate HPV screening and triage strategies jointly, since they are conditional on each other in producing an optimally accurate answer of who needs treatment of precancer to prevent cervical cancer.

Towards risk-based screening and management

Given the number of existing screening and triage options in the US, and several additional options undergoing regulatory approval trials, we do not expect a single screening strategy in the US in the future. To address this, there is an ongoing effort in collaboration between multiple professional societies led by the ASCCP and

Figure 1

Risk based screening and management



While there is a continuous risk range from 0 to 1 for having cervical precancers, there are only four risk areas with different clinical management. At the lowest risk, women return to primary screening at 3- or 5-year intervals. At low risk, they need additional triage or repeat testing after 1 year. At intermediate risk, women need to be evaluated at colposcopy. At the highest risk, immediate treatment is warranted. At each step, additional tests can put women into a higher or lower risk category, depending on the test result.

The goal is to develop risk-based clinical action thresholds that are independent of specific screening and triage tests

NCI to develop risk-based cervical cancer screening and management guidelines (Figure 1).[6,7] The goal is to develop risk-based clinical action thresholds that are independent of specific screening and triage tests. Current and future assays can be benchmarked against these thresholds, and permit us to update recommendations more easily when new tests become available. Implementation of these risk-based guidelines will be supported by applications that run on mobile devices or in electronic medical record systems to provide immediate risk-based recommendations using information from laboratory tests, previous screening results, and results from the clinical evaluation.

Summary and conclusions

Cervical cancer screening and management is undergoing a transition phase in the US. Within the next two years, new screening and management guidelines will be announced by various societies. The goal is to unify the messages about cervical cancer screening as much as possible, and to improve adoption and implementation of new screening approaches, particularly when these affect providers through extended screening intervals and switch from one technology to another (e.g. cytology to HPV testing).[8] ■

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