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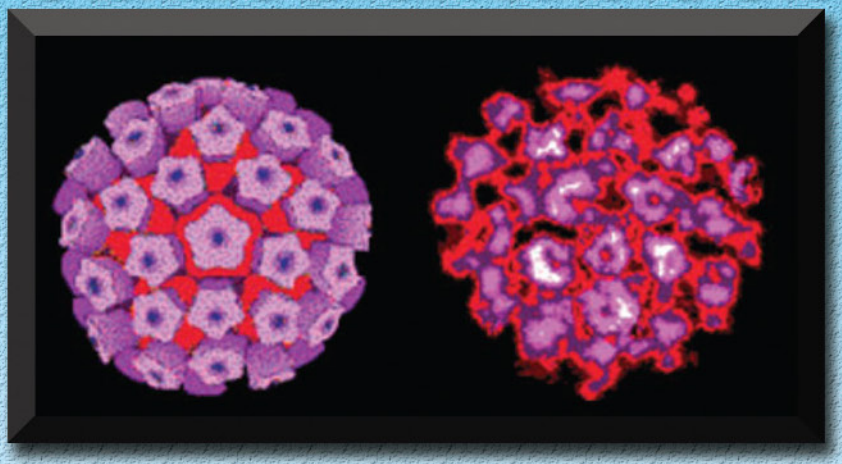
HPV DNA-BASED SCREENING TEST FOR CERVICAL CANCER



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MINISTRY OF HEALTH MALAYSIA

Health Technology Assessment Report

HPV DNA-BASED SCREENING TEST FOR CERVICAL CANCER

DISCLAIMER

This Health Technology Assessment has been developed from analysis, interpretation and synthesis of scientific research and/or technology assessment conducted by other organisations. It also incorporates, where available, Malaysian data, and information provided by experts to the Ministry of Health Malaysia. While effort has been made to do so, this document may not fully reflect all scientific research available. Additionally, other relevant scientific findings may have been reported since completion of the review.

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EXECUTIVE SUMMARY

Background

Cancer of the uterine cervix is a leading cause of mortality and morbidity among women worldwide. Current estimates indicate that every year 529,828 women are diagnosed with cervical cancer and 275,128 die from the disease. In 2006, cervical cancer was reported to be the third most common cancer among Malaysian women. The overall age-standardized incident rate (ASR) of cervical cancer in Malaysia was 12.2% per 100,000 populations. Cervical cancer incidence rate increased with age after 30 years and has its peak at ages 60-69 years. Research worldwide has clearly shown that virtually all cervical cancer is caused by human papilloma virus (HPV) infection. The virus is transmitted to the cervix and vaginal tissues primarily by sexual intercourse.

The varying carcinogenicity of these HPV type is partly related to the expression of two oncogenes E6 and E7. The International Agency for Research on Cancer (IARC) has classified 12 HPV types as high risk: type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59. Of these, HPV-16 and 18 are most carcinogenic and contribute to over 70% of all cervical cancer cases, between 41%-67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions worldwide.

Screening programs for cervical cancer have been instituted in many countries and are responsible for a substantial fall in the incidence and mortality rates attributed to cervical cancer. Primary cervical cancer screening by cytological examination of cervical cells with a Pap test is considered the most successful cancer screening programme to date. Despite its success, cytology has limitations especially technical limitations regarding sampling and laboratory errors in screening and interpretation. Therefore in recent years, there has been interest for developing new tests with adequate sensitivity and specificity for detecting clinically significant cervical cancer precursors. One such method is Human Papillomavirus Testing via viral DNA detection which is based on the knowledge that infection with HPV is at high risk for development of cervical cancer.

Detection of high risk HPV DNA is considered to be potentially useful in three clinical applications: triage of borderline abnormalities, primary screening in selected age groups, and follow-up of treatment for precancerous or neoplastic lesions.

Technical features

DNA testing for HPV has gained widespread acceptance as an additional cervical cancer screening tool and as follow-up to abnormal changes detected with a Pap smear. There are now several such DNA HPV tests, some of which have been approved for marketing by the FDA, that can detect either the majority of the high-risk types of HPV or specific subtypes, such as HPV-16 and HPV-18.

Policy Question

Should HPV DNA-based test be used in the cervical cancer programme as a primary screening test for cervical cancer in Malaysian women?

Objectives

a) To assess the effectiveness/efficacy, cost effectiveness, social, organizational and legal implications of using HPV DNA-based test as a primary screening test for cervical cancer.

b) To assess the effectiveness of using HPV DNA-based test for triage in primary cervical cancer screening.

c) To assess the role of HPV DNA based test in clinical management and as follow –up to detect treatment failure.

Methods

Literature search was done by two Information Specialists who searched for published articles pertaining to use of HPV DNA-based screening test for cervical cancer. The following electronic databases were searched: MEDLINE via OVID, PubMed, and EBM reviews – Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, HTA Databases, EBM reviews – NHS Economic Evaluation Database, EBM Full Text –Cochrane DSR and DARE. The search terms as in Appendix 3 were used either singly or in combination. The search was limited to publication year from 2000-2010. Additional articles were identified by reviewing the bibliographies of retrieved articles and hand searching of journals. All relevant literature was appraised using the Critical Appraisal Skills Programme (CASP) and evidence was graded based on guidelines from U.S./Canadian Preventive Services Task Force.

Results and conclusion

Effectiveness of HPV DNA Based Screening Test for Cervical Cancer. There was good level of evidence to show that HPV DNA-Based testing may be able to decrease the incidence and mortality rates related to invasive cervical cancer. There was good level of evidence to suggest that HPV DNA-Based Screening Test for Cervical Cancer has moderate accuracy if used alone but much higher sensitivity if used in combination with Pap smear. The sensitivity of HPV testing for cervical intraepithelial neoplasia of grade 2 or 3 was 94.6% (95% CI, 84.2 % to 100%), compared to Pap testing which had a sensitivity of 55.4% (95% CI, 33.6 % to 77.2%). The sensitivity of both tests used together was 100%, and the specificity was 92.5%. Compared to the other tests, The Hybrid Capture 2 assay showed a sensitivity for CIN2+ of 62% (95% CI, 56%–68%) and a specificity of 94% (95% CI, 92%–95%) whereas VIA and VIAM had a sensitivity of 79%, specificity of 85%, while VILI had a sensitivity of about 89%, specificity 85%. Visual inspection is an alternative low-technology screening tests usually done in low resource settings with potential difficulties in implementing cervical cytology-based screening. However a clear understanding of the anatomy, physiology and pathology of the cervix is absolutely essential to understand the basis and to interpret the outcome of screening using VIA, VILI and VIAM.

There was moderate level of evidence to show that HPV-triage using the Hybrid Capture 2 assay was more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. Among women aged 35 or older primary HPV DNA-Based screening with cytology triage is also more specific than conventional screening such as Pap smear which decreases referrals and follow-up tests. False negatives would be reduced, double negative patients could be safely screened at longer intervals (reducing costs) and patients as being at high risk but not having identifiable cervical cancer could be monitored closely. Compared with cytology, primary screening with HPV DNA-Based test followed by cytological triage and repeat HPV DNA-Based test of HPV DNA– Based positive women with normal cytology increased the sensitivity for CN3+ detection by 30% (95% CI = 9% to 54%), and resulted in a mere 12% increase in the number of screening tests.

There was good to fair level of evidence to suggest that after treatment of cervical lesions, HPV DNA-Based test easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. Treatment failure expressed in terms of residual or recurrent CIN, occurred on average in 10.2% (95% CI: 6.7% –13.8%) of treated cases. The sensitivity of HPV DNA-Based test detection in predicting treatment failure ranged from 67% to 100% and was on average 94.4% (95% CI: 90.9% – 97.9%). There was also good to fair level of evidence on the role of HPV DNA-Based test in post-treatment follow-up of patients after therapeutic excision of the cervix due to positive screening tests. A negative HPV DNA-Based test in the post-treatment period excluded not only the recurring CIN but also the development of persisting cytological atypia (negative predictive value (NPV): 100%). A negative HPV DNA-Based test eliminates the risk of recurrent disease after treatment for CIN. In a positive HPV DNA-Based test, this may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity.

There was fair level of evidence with the assumption that the countries (South Africa, Thailand and Peru) in the studies mentioned represent almost the same resource setting as Malaysia suggesting cost effective options in Malaysia. The studies showed that the ICER was lower than the GDP per capita in Malaysia which was noted to be about USD 5151 in 2008 (According to the International Commission on Macroeconomics and Health guidelines, interventions with an ICER between one and three times gross domestic product (GDP) per capita are considered cost effective). The four strategies derived from four different studies were:

- Using HPV DNA testing every five years as a screening strategy in Colombia (Gamboa A et al). The ICER was USD$44 in Colombia.
- A single life time screening with HPV DNA testing coupled with immediate cryotherapy once with positive results of HPV (Goldie S J et al). The ICER was less than $62 in South Africa.
- With at least 1 visit screening with HPV DNA once in a lifetime was the most cost effective strategies (Goldie S J et al). The ICER was USD$467 for South Africa, USD $170 for Thailand and USD $152 for Peru.
- Conventional cytology followed by HPV triage for equivocal cytology was the most cost effective strategy (Vijayaraghavan A et al). The ICER was USD$409 for South Africa

Beyond the cost issues that arise out of unnecessary testing, another point to consider is the physical discomfort and anxiety that a woman suffers in anticipation of an often unnecessary, invasive procedure. HPV testing may have an adverse psychosocial impact on women who test HPV positive when it is used as a primary screening test alongside conventional cytology. Consideration of the psychosocial consequences of HPV testing is important.

Health care planners who are considering implementing any type of cervical cancer screening must develop clinical protocols that are responsive to the natural history of cervical disease, the diagnostic characteristics of the screening technology, disease prevalence in the target population, and the Malaysian needs and concerns.

As recommended by the Cytopathology Education and Technology Consortium, and endorsed by American Society for Clinical Pathology (ASCP), the American Cancer Society, and several other professional medical societies, HPV DNA-Based testing should be used only for high-risk HPV types and co-testing with the Pap test in women over 30 years of age provides predictive safety for at least three years in women who are negative on both tests.

Recommendation

Based on the above review, HPV DNA-based testing may be incorporated in the cervical screening program. HPV DNA-based testing may be done every five years as a primary screening strategy or combined with Pap test in women over 30 years of age for an interval / frequency of at least three to five years in women who are negative on both tests in the annual screening. Although HPV DNA-based test is expensive (about RM 91.50- RM183 while Pap smear costs about RM 14.16 per test), it has higher sensitivity than Pap smear. For the primary screening strategy, it is suggested that HPV DNA-based testing may be done every five years since the test is expensive for the moment.

Alternatively a single life time screening using HPV DNA-based test was one of the most cost effective strategies carried out in South Africa, Thailand and Peru which Malaysia may emulate. However, local economic evaluation and research should be conducted with due consideration for our Malaysian healthcare systems as well as local costing that will further provide more evidence to support the above strategies.

HPV DNA-based test can be used to triage patients for atypical squamous cells of undetermined significance (ASCUS) in women aged 35 or older, whereby these women will undergo HPV DNA-based testing after conventional cytology. This strategy is recommended since it has been shown that this strategy is less expensive and more effective with higher specificity than screening using repeated cytology alone.

HPV DNA-based testing may be recommended as a follow up screening for post treatment cases since HPV DNA-based test easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. A negative HPV DNA-based test in the post-treatment period eliminates the risk of recurrent disease after treatment for CIN while a positive HPV DNA-based test, may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity.

A standard guideline needs to be developed for cervical cancer screening and management of abnormal findings if HPV DNA-based testing is adopted as a screening test for cervical cancer screening in Malaysia. Organisational issues such as training, manpower, good referral system, and funding need to be addressed at all levels.

TABLE OF CONTENTS

ABBREVIATIONS

AGC	Atypical glandular cell
AEC	Atypical endocervical cell
AIS	Endocervical adenocarcinoma in situ
ASCUS	Atypical squamous cells of undetermined significance
AGUS	Atypical glandular cells of undetermined significance
AUC	Area under receiver operating characteristic curve
CE	Cost Effectiveness
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
DVI	Direct Visual Inspection
FP	False-positive
GDP	Gross Domestic Product
HC2	Hybrid Capture 2
HCA	Hybrid Capture Assay
HPV DNA	Human Papillomavirus Deoxyribonucleic acid
HD-C	HPV DNA chip
HR-HPV	High Risk Human Papillomavirus
HGSIL/HSIL	High Grade Squamous Intraepithelial Lesions
HTA	Health Technology Assessment
hpVIR	Real-time PCR (hpVIR)
ICER	Incremental Cost Effectiveness Ratio
LBC	Liquid based cytology
LGSIL/LSIL	Low grade squamous intraepithelial lesion
LA	Linear Array
NOS	Not otherwise specified
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
PCR – B	PCR in biopsies
PCR- S	PCR in Swabs
PPV	Positive Predictive Value
QALY	Quality Adjusted Life Year
RR	Risk Ratio, Relative risk
ROC	Receiver Operating Characteristic
SIL	Squamous Intraepithelial Lesions
SCC	Squamous Cell Carcinoma
VIA	Visual Inspection Acetic Acid
VIAM	Visual Inspection Acetic with magnification
VILI	Visual Inspection with Lugol's Iodine
WNL	Within Normal Limit
YLS	Years Life Saved

1 BACKGROUND

Cancer of the uterine cervix is one of the leading causes of mortality and morbidity among women worldwide. Current estimates indicate that every year 529,828 women are diagnosed with cervical cancer and 275,128 die from the disease. Using crude incidence rates, cervical cancer ranks as the third most frequent cancer in women in the world, and the second most frequent cancer among women between 15 and 44 years of age. After age-standardization, cervical cancer ranks as the second most frequent cancer in women in the World.[1] In 2006, cervical cancer was reported to be the third most common cancer among Malaysian women. The overall age-standardized incident rate (ASR) of cervical cancer in Malaysia was 12.2% per 100,000 populations. Cervical cancer incidence rate increased with age after 30 years and has its peak at ages 60-69 years. Chinese women were found to have the highest incidence for cervical cancer followed by Indian and Malay.[2]

Research worldwide has clearly shown that virtually all cervical cancer is caused by human papilloma virus (HPV) infection. HPV is a small, non-enveloped, double stranded circular deoxyribonucleic (DNA) virus, classified in the genus *papillomavirus* of the *Papoviridae* family of viruses. The virus is transmitted to the cervix and vaginal tissues primarily by sexual intercourse. HPV can infect and persist in vulvar, vaginal, and cervical tissue throughout a lifetime. To promote cervical cancer abnormalities, the virus must become integrated into the host genomic DNA. With viral integration, the oncogenic effect of the E6 and E7 proteins is enhanced and cellular changes characteristic of high-grade dysplasia and ultimately cancer is observed.[3,4]

More than 100 types of HPV have been identified, and approximately 50 types infect the epithelial membranes of the anogenital tract. The HPV strains are divided into two groups of either high risk or low risk based on their oncogenic potential and ability to induce tumours. The varying carcinogenicity of these HPV types is partly related to the expression of two oncogenes E6 and E7. The International Agency for Research on Cancer (IARC) has classified 12 HPV types as high risk: type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59. Of these, HPV-16 and 18 are most carcinogenic and contribute to over 70% of all cervical cancer cases, between 41%-67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions worldwide.[1,5]

Screening programs for cervical cancer have been instituted in many countries and are responsible for a substantial fall in the incidence and mortality rates attributed to cervical cancer. Primary cervical cancer screening by cytological examination of cervical cells with a Pap test is considered the most successful cancer screening programme to date.[6,7] Despite its success, cytology has its limitations especially technical limitations regarding sampling and laboratory errors in screening and interpretation. Therefore in recent years, there has been interest for developing new tests with adequate sensitivity and specificity for detecting clinically significant cervical cancer precursors. One such method is Human Papillomavirus Testing via viral DNA detection which is based on the knowledge that infection with HPV is essential for development of cervical cancer.

Detection of high risk HPV DNA is considered to be potentially useful in three clinical applications: triage of borderline abnormalities, primary screening in selected age groups, and follow-up of treatment for precancerous or neoplastic lesions.[8] The two major methods for detection of carcinogenic or high risk HPV DNA are hybridization with signal amplification and genomic amplification using polymerase chain reaction. Current technology using signal amplification approved by FDA is Hybrid Capture 2.

2. TECHNICAL FEATURES

The conventional Pap test has been the mainstay of cervical cancer screening since its inception in the 1950s. Screening protocols remained unchanged for the first four of the last five decades. Standardization of cervical cytology and reporting terminology was accomplished in 1988 with the implementation of the Bethesda system. Using the revised Bethesda cytology reporting system (2001), clinicians can better triage patients with abnormal cervical cytology based on less ambiguous terminology.[9-10]

Several other cytological classification systems are used worldwide and were utilised in the studies included in this report. For histological classifications the term cervical intraepithelial neoplasia is used, with CIN1 to CIN3 representing progressively worse outcomes. To aid in the interpretation of the patient inclusion criteria and comparisons with HPV detection utilised in the studies included in this report, the relationship between the different nomenclatures is presented in Table 1 below[11].

Table 1 Classification of cervical cytology (adapted from Broadstock 1999 and Cancer Research UK website1)

BETHESDA SYSTEM	RICHART	WORLD HEALTH ORGANISATION	UK CLASSIFICATION SYSTEM
Atypical squamous cells of undetermined significance (ASC-US)		Atypia	Borderline dyskaryosis
ASC, cannot exclude highgrade Squamous intraepithelial lesion (ASC-H)			
Low-grade squamous intraepithelial lesion (LSIL) – encompasses CIN1 and lowgrade changes due to HPV infection	Cervical intraepithelial neoplasia 1 (CIN1)	Mild dysplasia	Mild dyskaryosis
High-grade squamous intraepithelial lesion (HSIL) – encompasses both CIN2 and CIN3	Cervical intraepithelial neoplasia 2 (CIN2)	Moderate dysplasia	Moderate dyskaryosis
	Cervical intraepithelial neoplasia 3 (CIN3)	Severe dysplasia	Severe dyskaryosis or worse
Atypical glandular lesions Atypical endocervical lesions Adenocarcinoma in situ		Carcinoma in situ (CIS)	

Traditionally, genital HPV infection has been detected as abnormal cell changes on a Pap smear, a test used primarily to detect cancer of the cervix (the lower part of the uterus or womb) or conditions that may lead to cancer. During a Pap smear, the “normalness” of cervical cells is evaluated under a microscope. “Low-grade” changes to the cells on a Pap smear may indicate an HPV infection, but there is no clear distinction between high- and low-risk types.

DNA testing for HPV has gained widespread acceptance as an additional cervical cancer screening tool and as follow-up to abnormal changes detected with a Pap smear. There are now several such DNA HPV tests, some of which have been approved for marketing by the FDA, that can detect either the majority of the high-risk types of HPV or specific subtypes, such as HPV-16 and HPV-18.

2.1 Hybrid Capture 2

Digene Corporation, USA developed two products with hybrid capture technology: the first generation Hybrid Capture Tube (HCT) and more recent Hybrid Capture 2 (HC2). HCT was approved by US FDA in 1999 as an adjunctive test to cytology for the triage of women with equivocal cytology results. In 2003, FDA approved the second generation HC2 which detects additional four high risk viral types compared to HCT. HC2 is available in a 96-well microplate format with in-built positive and negative controls. It is an in-solution, hybridization test able to detect 13 high-risk types of HPV DNA (16, 18, 31,33, 35,39, 45, 51, 52, 56, 58, 59 and 68) and five low risk types (6, 11, 42, 43 and 44) using two different ribonucleic acid (RNA) probes, probe B (high-risk types) and probe A (low-risk types) in two separate reactions.[12]

To perform the HC2 assay, cervical samples are combined with an extraction buffer to release and denature the target HPV DNA. The released target DNA then combines with specific RNA probes to create RNA-DNA hybrids, which are captured onto a solid phase by an antibody specific for the hybrids. These captured RNA-DNA hybrids are then tagged with antibody reagents linked to alkaline phosphatase. A chemiluminescent substrate then produces light that is measured on a luminometer in a relative light units (RLU). The amount of light generated is proportional to the amount of target DNA in the original specimen. The recommended cut-off value for a positive test is 1 RLU which is equivalent to 1 pg HPV DNA/ml sampling buffer, corresponding to 5900 genomes per test well. The result does not provide information on specific types of HPV detected, instead gives a positive result when the DNA of any one of the types is present above a certain threshold.[12-13]

2.2 Polymerase Chain Reaction (PCR)-BASED ASSAY

PCR technology requires a cyclic, three step reaction. The assay is designed to selectively amplify the viral genome by a series of polymerization steps, which result in an exponential and reproducible increase in the nucleic acid sequences present in the biological specimen. The methodology relies on the amplification of selected portions of the gene of interest whose boundaries are defined by oligonucleotides that hybridize or anneal to their complementary sequences on the target strand that has been previously denatured. At defined temperatures, such oligonucleotides are extended by a thermoresistant DNA polymerase leading to formation of two new double stranded DNA molecules (called amplicon) using each of the original target DNA single strands as templates. By repeating the cycle of denaturation, annealing and extension, each newly synthesized double-stranded DNA molecule serve as a template for the next cycle, and the number of molecules increase exponentially. Analysis of the amplified products may be performed by gel electrophoresis, dot blot, restriction fragment length polymorphism analysis or sequencing.[12,14]

The sensitivity and specificity of PCR-based methods vary depending on the primer sets, the size of PCR product, reaction conditions, performance of the DNA polymerase used in the reaction, the spectrum of HPV DNA amplified, and the ability to detect multiple types. Most PCR based assays use consensus primers including MY09/11, PGMY09/11, GP5+/6+ and SPF1/2 which are directed to L1 gene, a highly conserved region of the HPV genome.[14]

2.3 Cytology

a) Conventional – Papanicolaou (Pap) test

The Pap smear is currently primary method for detection of dysplasias due to HPV. The current reporting system for Pap smear is the updated Bethesda System 2001. With a conventional Pap test, a cervical cell sample is smeared on a glass slide, stained with a special dye and viewed under the microscope by cytotechnologist or pathologist. A portion of the patient's cell sample is lost when the sampling device is discarded (abnormal cells may be present but may not have been put on the slide) and material such as blood and mucus may get on the slide and impede diagnosis.[13]

b) Monolayer – Liquid based cytology

Cervical samples are collected using a special cytobrush. The tip of the brush, which contains the sample, is removed and suspended or fixed in a cell-preserving solution for automated slide preparation at the laboratory. The liquid is spun and passed through a microfilter and then mechanically transferred to the slide as a monolayer. The slide is processed and interpreted as in conventional Pap smear. The fixed specimens are processed using either the ThinPrep 2000 test which was approved by the FDA in 1996 or the AutoCyte Prep system approved in 1999. The filtering process removes excess blood, mucous and inflammatory cells thus improving the quality of slide and increase the detection of cervical abnormalities.[15,16]

2.4 Visual inspection

Naked-eye visual inspection of the uterine cervix, after application of 5% acetic acid (VIA) and/or of Lugol's iodine (VILI), provides simple tests for the early detection of cervical precancerous lesions and early invasive cancer. VILI is similar to the Schiller's iodine test, which was used for early detection of cervical neoplasia in the third and fourth decades of the 20th century, but discontinued after the advent of cervical cytology testing. [17] The potential difficulties in implementing cervical cytology-based screening in low resource settings have prompted the investigation of the accuracy of alternative low-technology tests such as VIA and VILI in the early detection of cervical neoplasia. The results of VIA and VILI are immediately available and do not require any laboratory support. VILI is simple to administer, and a range of types of health care providers can perform the procedure with appropriate training. Squamous epithelium contains glycogen, whereas precancerous lesions and invasive cancer contain little or no glycogen. Application of iodine results in brown or black color staining in areas containing glycogen. In areas lacking glycogen, iodine is not absorbed and such areas remain colorless or turn yellow.

Visual inspection with acetic acid (VIA) can be done with the naked eye (also called cervicoscopy or direct visual inspection, [DVI]), or with low magnification (also called gynoscopy, aided VI, or VIAM). Visual inspection with magnification (VIAM) is the visualization of cervix under low magnification after application of acetic acid. Several devices had been used by different investigators. These are hand-held devices with built-in source to view cervix in community settings - a special lightweight monocular telescope called gynoscope (PATH 2000) and a magnivisualizer. [18]

3 POLICY QUESTION

Should HPV DNA-based test be used in the cervical cancer programme as a primary screening test for cervical cancer in Malaysian women?

4. OBJECTIVE

a) To assess the effectiveness/efficacy, cost effectiveness, social, organizational and legal implications of using HPV DNA-based test as a primary screening test for cervical cancer.

b) To assess the effectiveness of using HPV DNA-based test for triage in primary cervical cancer screening.

c) To assess the role of HPV DNA based test in clinical management and as follow –up to detect treatment failure.

5. METHODOLOGY

5.1 Literature Search Strategy

Literature search was done by two Information Specialists who searched for published articles pertaining to use of HPV DNA-based screening test for cervical cancer. The following electronic databases were searched: MEDLINE via OVID, PubMed, EBM reviews – Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, HTA Databases, EBM reviews – NHS Economic Evaluation Database, EBM Full Text –Cochrane DSR and DARE. The search terms as in Appendix 3 were used either singly or in combination. The search was limited to publication year from 2000-2010. Additional articles were identified by reviewing the bibliographies of retrieved articles and hand searching of journals.

5.2 Selection Criteria and Method

Based on the policy question the following inclusion and exclusion criteria were used:-

5.2.1 Inclusion criteria:

Studies were selected for inclusion based on certain criteria. The population selected were females. The intervention selected were HPV DNA-based screening test using Hybrid Capture 2 (HC2), Polymerase Chain Reaction (PCR) or type-specific DNA tests. The study design included were systematic review, randomized and non-randomized control trials, cohort and cross sectional

5.2.2 Exclusion criteria:

Cervical cancer screening done with Point-of-Care testing or Test kits. Articles published before year 2000 were excluded.

Based on the above inclusion and exclusion criteria, study selection were carried out independently by two reviewers. The titles and abstracts of all studies were assessed for the above eligibility criteria. If it is absolutely clear from the title and / or the abstract that the study was not relevant, it was excluded. If it was unclear from the abstract and / or the title the full text article was retrieved. Two reviewers assessed the content of the full text articles. Disagreement was resolved by discussion.

5.3. Quality assessment strategy

The methodological quality of all the relevant full text articles retrieved was assessed using the Critical Appraisal Skills Programme (CASP) tool depending on the type of study design.[23] Quality assessment was conducted by three reviewers. Disagreements were resolved by discussion. All full text articles were graded based on guidelines from the U.S./Canadian Preventive Services Task Force (Appendix 1) [19]

5.4. Data extraction strategy

Data were extracted from included studies by a reviewer using a pre-designed data extraction form (evidence table as shown in Appendix 5) and checked by another reviewer. Disagreements were resolved by discussion. The data extracted was as follows:-

- Details of methodology including study design
- study population characteristics including age, trial inclusion and exclusion criteria
- Details of intervention and comparator
- Details of individual outcomes for effectiveness, cost effectiveness mortality rate, detection rate, incidence, quality of life, quality adjusted life years (QALY) gained, adverse events related to screening and treatment, cost, cost-utility and cost-effectiveness of cervical cancer screening using HPV DNA-based screening test, diagnostic accuracy of screening tests used (sensitivity, specificity, PPV, NPV)
- any information on ethical, legal and organizational aspect related to cervical cancer screening using HPV DNA-based screening test

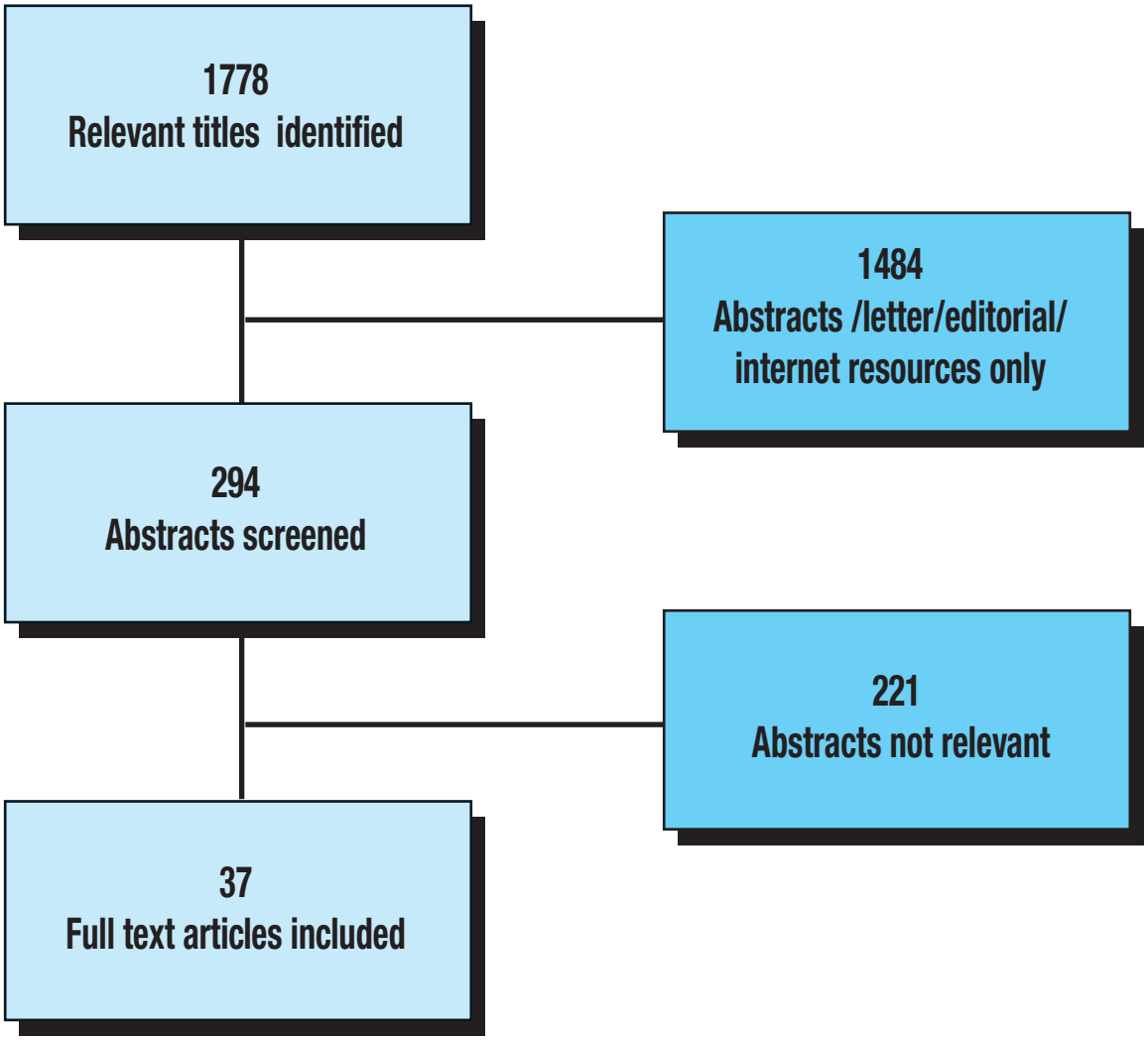
The extracted data was presented and discussed with the expert committee before deciding on the eligibility of articles to be finally included in this report.

6. RESULTS

The search strategy yielded a total of 1778 relevant titles and 294 abstracts were screened using the inclusion and exclusion criteria. After screening, 221 abstracts were found to be irrelevant. In total 37 full text articles which met the inclusion/exclusion criteria and quality of studies were included in this systematic review as shown in Figure 1.

Two systematic reviews, two Meta analysis, six randomised controlled trial, seven cohorts, eleven cross-sectional (screening/diagnostic type), one cross sectional survey on psychological impact of patients undergoing screening for cervical cancer and eight decision analytical modeling (for cost effectiveness) related to the effectiveness of HPV-DNA test for cervical screening were retrieved. However, there was no health technology assessment report retrieved.

Figure 1: Flow chart of retrieval of articles used in the results.



6.1. EFFECTIVENESS OF HPV DNA-BASED SCREENING TEST FOR CERVICAL CANCER

a) Diagnostic Accuracy

In 2007, Mayrand MH et al reported a randomised control trial on a total of 10,154 women ages 30 to 69 years in Montreal and St. John's, Canada to test whether DNA of oncogenic human papillomaviruses (HPV) was superior to the Papanicolaou (Pap) test for cervical-cancer screening. [20 level I] Both tests were performed on all women in a randomly assigned sequence at the same session. The sensitivity of HPV testing for cervical intraepithelial neoplasia of grade 2 or 3 was 94.6% (95% CI, 84.2% to 100%), whereas the sensitivity of Pap testing was 55.4% (95% CI, 33.6% to 77.2%). The specificity was 94.1% (95% CI, 93.4% to 94.8%) for HPV testing and 96.8% (95% CI, 96.3% to 97.3%) for Pap testing. Performance was unaffected by the sequence of the tests. The sensitivity of both tests used together was 100%, and the specificity was 92.5%. Triage procedures for Pap or HPV testing resulted in fewer referrals for colposcopy than did either test alone but were less sensitive. As compared with Pap testing, HPV testing has greater sensitivity for the detection of cervical intraepithelial neoplasia.

Arbyn et al (2006) did a systematic review and meta-analyses on 22,000 patients (from 14 studies) on three possible clinical applications of human papillomavirus (HPV)-DNA testing: triage of women with equivocal or low-grade cytological abnormalities; prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN) lesions, and last not but not least, primary screening for cervical cancer and pre-cancer. [21 level I] Consistent evidence is available indicating that HPV-triage with the Hybrid Capture-2 assay (HC2) is more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex HC2 test does not show a significantly higher sensitivity, but a significantly lower specificity compared to a repeat Pap smear.

- Primary screening with HC2 generally detects 23% (95% confidence interval, CI: 13–23%) more CIN-2, CIN-3, or cancer compared to cytology at cut-off atypical squamous cells of undetermined significance (ASCUS) or LSIL, but is 6% (95% CI: 4–8%) less specific.
- By combined HPV and cytology screening, a further 4% (95% CI: 3–5%) more CIN-3 lesions can be identified but at the expense of a 7% (95% CI: 5–9%) loss in specificity, in comparison with isolated HC2 screening.

Arbyn M et al (2008) evaluated five screening methods, naked eye visual inspection of the cervix uteri after application of diluted acetic acid (VIA), or Lugol's iodine (VILI) or with a magnifying device (VIAM), the Pap smear and human papillomavirus testing with the high-risk probe of the Hybrid Capture- 2 assay (HC2), in 11 studies in India and Africa. [22 level I] More than 58,000 women, aged 25–64 years, were tested with 2–5 screening tests and outcome verification based on colposcopy and histological interpretation of colposcopy-directed biopsies was done on all women independent of the screen test results. The results were as follows:

- VIA showed a sensitivity of 79% (95% CI 73%–85%) for CIN2+ and 83% (95% CI: 77% – 89%) for CIN3+, and a specificity of 85% (95% CI: 81%–89%) for CIN2+ and 84 % (95% CI: 80%–88%) for CIN3+.
- VILI was on average 10% more sensitive and equally specific. The overall pooled sensitivity for VILI (91.2%; CI: 87.8% – 94.6%) was statistically significantly higher (about 10%) than for VIA. On the other hand, the pooled specificity of VILI (84.5%; 95% CI: 81.3% – 87.8%) was not significantly different from that of VIA.
- VIAM showed similar results as VIA. The Pap smear showed lowest sensitivity, even at the lowest cutoff of atypical squamous cells of undetermined significance (57%; 95% CI: 38% – 76%) for CIN2+ but the specificity was rather high (93%; 95% CI: 89%–97%).
- The HC2-assay showed a sensitivity for CIN2+ of 62% (95% CI: 56%–68%) and a specificity of 94% (95% CI: 92%–95%).

The author mentioned that substantial interstudy variation was observed in the accuracy of the visual screening methods. Accuracy of visual methods and cytology increased over time, whereas performance of HC2 was constant. This inconsistency across studies reflects the considerable subjectivity in interpreting visual tests by different providers as a result of different levels of competencies, training methods, monitoring and quality assurance and also reflects the fact that visual inspection methods have low reproducibility.

Lytwyn A, et al (2000) did a community-based randomized trial to compare on 212 women aged 16-50 years with ASCUS or LSIL on cervical cytology screening to test the performance of human papillomavirus (HPV) DNA testing with that of 6-month repeat Papanicolaou (Pap) test in detecting histologically confirmed CIN 2 or 3. [23 level I] A total of 159 women completed the study. The result showed that:

- HPV DNA testing - detected 87.5% (7/8) of the cases of CIN 2 or 3, Repeat Pap smear detected 11.1% (1/9) of cases high-grade intraepithelial neoplasia (HSIL) (p = 0.004), and ASCUS, LSIL or HSIL detected 55.6% (5/9) ASCUS, LSIL or HSIL (p = 0.16).

- Sensitivities:
  - HPV DNA test - 87.5 % (95% CI: 47.4% - 99.7%) (p = 0.004)
  - Repeat Pap smear - 55.6% (95% CI: 21.1% - 86.3%) (p = 0.16)

- Specificities:
  - HPV DNA test - 50.6% (95% CI: 39.1% - 62.1%) (p =0.002)
  - Repeat Pap smear - 55.6% (95% CI: 42.5% - 68.1%) (p = 0.61)

- Loss to follow-up (failed to present for colposcopy) was 17.1% in the HPV test group and 32.7% in the repeat Pap group (p = 0.009)

- In the HPV group 46/87 women (52.9%) were HPV positive. In the repeat Pap test group 29/72 women(40.2%) had ASC US or LSIL and 4/72 (5.6%) had HSIL

- Given the 7 cases of CIN 2 or 3 detected by HPV testing and the 5 cases detected by the repeat Pap smear, the incremental cost of HPV testing was calculated to be $3003 per additional case of CIN identified

The author suggested that the results can be generalizable to a primary care setting. Immediate testing for oncogenic HPV detected significantly more histologically confirmed cases of CIN 2 or 3 than did repeat Pap smear showing HSIL performed at 6 months improved sensitivity of immediate HPV DNA testing compared with repeat Pap. HPV DNA testing was more costly but was associated with significantly less loss to follow-up and may detect more cases of CIN 2 or 3 in women with low-grade cytologic abnormalities.

Gravitt PE et al (2008) compared the performance of Linear Array (LA) to Hybrid Capture 2 (HC 2) for the detection of carcinogenic human papillomavirus (HPV) and cervical precancer. [24 level II-2] LA and HC2 results were compared on baseline specimens collected from women with an atypical squamous cells of undetermined significance (ASCUS) Pap referred into ASCUS and Low-Grade Squamous Intraepithelial Lesion (LGSIL) Triage Study (n = 3,488). HC2 was conducted at the time of the study on liquid cytology specimens. LA was conducted retrospectively on aliquots from a second, stored cervical specimen masked to the HC2 results and clinical data.

- Restricting the analyses to paired results, LA was more likely to test positive for carcinogenic HPV than HC2 (55% versus 53%; P = 0.001), with a percent agreement of 84%, a percent positive agreement of 74%, and a k of 0.68.

- For 2-year cumulative ≥ CIN3, LA and HC2 had similar sensitivities (93.3% versus 92.6%, respectively; P = 1), and LA was marginally less specific than HC2 (48.1% versus 50.6%, respectively; P = 0.05).

- LA and HC2 had similar negative predictive values (98.70% versus 98.64% respectively; P = 0.4), and LA had a slightly lower positive predictive value than HC2 (14.6% versus 15.1%, respectively; P < 0.0001).

The results suggested that LA and HC2 performed similarly in the detection of carcinogenic HPV and identification of CIN3 among women with an ASCUS Pap with CIN 3 lesions.

Lee et al. (2005) evaluated the clinical test performance of HC2 in comparison with HPV DNA Chip (HD-C) in a sample of 400 women referred for follow-up on the basis of abnormal cytology. [25 level II-2] Enrolled women underwent ThinPrep cytology, HPV testing, colposcopy and biopsy. Some HD-C tests were carried out on samples which were frozen after ThinPrep cytology and cytologic diagnoses were made according to the Bethesda system. Histologic diagnoses were made based on the most serious specimen obtained by colposcopy-directed biopsy or loop electrosurgical excision procedure (LEEP).

Clinical test performance for the detection of histologically diagnosed CIN1 or worse was calculated for each of the HPV tests. The sensitivities and specificities of each of the tests was very similar with a high sensitivity (HC2 = 89.9%, HD-C = 86.2%) and low specificity (HC2 = 43.2%, HD-C = 46.2%). Positive predictive values (HC2 = 76.3%, HD-C = 76.5%) and negative predictive values (HC2 = 67.9%, HD-C = 62.2%) were moderate and the two tests performed very similarly. The level of histological diagnosis considered a positive result was CIN1 for this study, whereas most other studies compared the performance of the HPV tests to diagnoses of CIN2 or worse.

Monsonego J et al (2005) assessed the performance of a PCR-based assay in detection of cervical pathology as a part of management for abnormal Pap smear (MAPS) and in women participating in cervical cancer screening. The participants consisted of 270 women referred for colposcopic examination due to an abnormal Pap smear (MAPS Series), and for comparison, another series of 234 women participating in opportunistic cervical cancer screening in Paris, France (Screening Series). [26 level II-2] The mean age of women was 35 years (range 18-75 years). Both series were examined in the same clinic (Institute Alfred Fournier, IAF), during November 2004, by 2 colposcopists.

- The prevalence of HPVMAPS group = (65.9%)
- Screening group (31.2%) (P = 0.0001).
- OR for being HPV positive in a MAPS patient was 4.26 (95%CI 2.936–6.202), as compared with the screening group.
- HPV prevalence was significantly higher among women below 35 years of age (62.8%) as compared with those beyond that age (33.9%) (P = 0.0001) (OR 3.29, 95%CI 2.27–4.75).
- There was a poor concordance between the referral PAP and the current LBC, being only moderate in the screening series, ICC (weighted kappa) = 0.291 (95%CI 0.070–0.459) (P = 0.007), and almost poor in the MAPS Series, with ICC = 0.217 (95%CI 0.04–0.384) (P =0.023).
- AMPLICORR HPV positivity increased linearly with the increasing grade of cervical lesions in detecting high-grade (CIN2–3), whereby AMPLICOR HPV test showed a linear increase of HPV prevalence in parallel with the increasing LBC abnormality, up to 92.6% among the women with HSIL cytology (P = 0.0001 for linear trend).

- Colposcopy was the most sensitive test (96.5%), very similar to AMPLICOR (95.2%) (P = 0.731), while LBC with HSIL cutoff was by far the most specific test (99.5%) and showed the highest PPV (96.1%). NPV of colposcopy (97.2%) and AMPLICOR (96.7%) were similar (P =0.839).

- • The Roche AMPLICOR HPV test had 95.2% (95% CI: 89.9% –100.0%) sensitivity, 42.4% (95% CI: 35.7% – 49.2%) specificity, 33.7% (95% CI: 26.8% – 40.7%) PPV and 96.7% (95% CI: 93.0%– 100.0%) NPV in detecting CIN2–3 lesions among women in the MAPS Series.

Together with abnormal colposcopy and HSIL cytology, the AMPLICOR HPV test was a powerful independent predictor of high-grade CIN2–3, and as such maybe highly suitable as a triage tool used in the management of abnormal PAP test.

Nauclear P et al (2009) did a study using the database from the intervention arm (n = 6257 women) of a population-based randomized trial of double screening with cytology and HPV DNA testing to evaluate the efficacy of 11 possible cervical screening strategies that are based on HPV DNA testing alone, cytology alone, and HPV DNA testing combined with cytology among women aged 32 – 38 years. [27 level II-3] The results were as follows:

- The sensitivity of HPV DNA testing for detecting CIN3+ was 96.0% (95% CI = 86.3% to 99.5%), whereas the sensitivity of cytology was 74.0% (95% CI = 59.7% to 85.4%).

- With CIN3+ as the endpoint, the specificity of cytology was 98.2% (95% CI = 97.9% to 98.5%) and that of HPV testing was 93.6% (95% CI = 93.0% to 94.2%).

- The PPV of cytology for CIN3+ was 25.3% (95% CI = 18.5% to 33.2%), and the PPV of HPV DNA testing for CIN3+ was 11.1 %(95% CI = 8.3% to 14.4%).

- Compared with screening by cytology alone, double testing with cytology and for type-specific HPV persistence resulted in a 35% (95% confidence interval [CI] = 15% to 60%) increase in sensitivity to detect CIN3+, without a statistically significant reduction in the PPV (relative PPV = 0.76, 95% CI = 0.52 to 1.10), but with more than twice as many screening tests needed.

- Several strategies that incorporated screening for high-risk HPV subtypes were explored, but they resulted in reduced PPV compared with cytology.

- Compared with cytology, primary screening with HPV DNA testing followed by cytological triage and repeat HPV DNA testing of HPV DNA – positive women with normal cytology increased the sensitivity for CN3+ detection by 30% (95% CI = 9% to 54%), maintained a high PPV (relative PPV = 0.87, 95% CI = 0.60 to 1.26), and resulted in a mere 12% increase in the number of screening tests (from 6257 to 7019 tests)

Hence, Primary HPV DNA – based screening with cytology triage and repeat HPV DNA testing of cytology-negative women appears to be the most feasible cervical screening strategy in this study.

De Vuyst H et al (2005) assess the test qualities of four screening methods to detect cervical intra-epithelial neoplasia in an urban African setting. [28 level II-2] Six hundred fifty three women, attending a family planning clinic in Nairobi (Kenya), underwent four concurrent screening methods: pap smear, visual inspection with acetic acid (VIA), PCR for high risk human papillomavirus (HR HPV) and cervicography. The presence of cervical intra-epithelial neoplasia (CIN) was verified by colposcopy or biopsy Sensitivity (for CIN2 or higher) and specificity (to exclude any CIN or cancer) was:

- 83.3% (95% CI: 73.6% - 93.0%) and 94.6% (95% CI: 92.6% - 96.5%), respectively, for pap smear;
- 73.3% (95% CI:61.8% - 84.9%) and 80.0% (95% CI: 76.6% - 83.4%) for VIA;
- 94.4% (95% CI: 84.6% - 98.8%) and 73.9% (95% CI: 69.7% - 78.2%) for HR HPV; and
- 72.3% (95% CI: 59.1% - 85.6%) and 93.2% (95% CI: 90.8% - 95.7%) for cervicography.

The Pap smear had the highest specificity (94.6%) while HPV testing had the highest sensitivity (94.4%). The visual methods, VIA and cervicography, were similar and showed an accuracy in between the range of the former two tests.

Gustavson I et al (2009) compare the HC2 with the real-time PCR *hpVIR* assay for detection of HPV in follow-up smears of 398 women diagnosed with atypical squamous cells of unknown significance (ASCUS) or low grade cervical intraepithelial neoplasia (CIN 1) in their initial smear. Total of 391 samples were included in study. [29 level II-2] Thirty-four percent (131) of women were positive with HC2 and 45% (175) with real-time PCR *hpVIR*. HPV 16 was most common single infection. Among those with cytology available 6% (3/52) had a CIN 2. The 3% (13/391) of women positive only with HC2 either contained low-risk HPVs or copy numbers below the cut-off for the real-time PCR *hpVIR* assay.

- The real-time PCR hpVIR assay has a similar sensitivity and specificity as HC2, but real-time PCR hpVIR detect a higher frequency of high-risk HPV infections.
- To detect CIN2 the sensitivity of HC2 was 85% while the sensitivity of real-time PCR hpVIR was 91% and the specificity of HC2 was 73% while the specificity of real-time PCR hpVIR was 60%.
- To detect CIN3 the sensitivity of HC2 and real-time PCR hpVIR was 100% and the specificity of HC2 was 70% while the specificity of real-time PCR *hpVIR* was 57%.

b) Combined test methods

Lorincz AT et al (2003) analyzed 10 large screening studies that used the Hybrid Capture 2 test and 3 studies that used the polymerase chain reaction test in a manner that enabled reliable estimates of accuracy for detecting or predicting highgrade cervical intraepithelial neoplasia (CIN). [30 level I] Most studies allowed comparison of HPV DNA and Papanicolaou testing and estimate of the performance of Papanicolaou and HPV DNA as combined tests. The results were as follows:

- HPV DNA testing by HC2 had a higher sensitivity (in some cases much higher) than cytology.

  - For example, in the study from Reims HC2 HPV DNA testing detected 100% of CIN 2 or 3, as compared to 58% for the conventional Papanicolaou test (Pap test) and 84% for the ThinPrep test. Similar cytology were seen in the studies from Newfoundland, Canada; Seattle, Wash; Morelos, Mexico; and Hannover-Tubingen, Germany
  - (sensitivities for all studies range from 68-100% for HPV DNA test versus 40-86% for pap test)

- The specificity values for HC2 HPV DNA testing were generally lower than the specificity values of the Pap test,
  - (specificities for all studies range from 73-96% for HPV DNA test versus 88-99% for pap test)
- The PPVs of the Pap test were overall a little higher than the PPVs for HPV
- The sensitivity value for CIN 3 or higher using a combination of HPV DNA testing and cytology was greater than 90% in 6 of the 7 studies and was 100% in 3 of the 7 studies.
- The NPVs for the combinations were above 99% for all 7 studies and were 100% in 4 of the 7 studies.

Kitchener HC et al (2009), Women aged 20-64 years who were undergoing routine screening as part of the English National Health Service Cervical Screening Programme in Greater Manchester were randomly assigned (between July, 2001, and September, 2003) in a ratio of 3:1 to either combined liquid-based cytology (LBC) and HPV testing in which the results were revealed and acted on, or to combined LBC and HPV testing where the HPV result was concealed from the patient and investigator. [31 level I] There were 24,510 eligible women at entry (18,386 in the revealed group, 6124 in the concealed group). In the first round of screening 233 women (1.27%) in the revealed group had CIN3+, compared with 80 (1.31%) women in the concealed group (odds ratio [OR] 0.97, 95% CI 0.75-1.25; p>0.2). There was an unexpectedly large drop in the proportion of women with CIN3+ between the first and second rounds of screening in both groups, at 0.25% (29 of 11 676) in the revealed group and 0.47% (18 of 3866 women) in the concealed group (OR 0.53, 95% CI 0.30-0.96; p=0.042). For both rounds combined, the proportion of women with CIN3+ were 1.51% (revealed) and 1.77% (concealed) (OR 0.85, 95% CI 0.67-1.08; p>0.2). LBC combined with HPV testing resulted in a significantly lower detection rate of CIN3+ in the second round of screening compared with LBC screening alone, but the effect was small. Over the two screening rounds combined, co-testing did not detect a higher rate of CIN3+ or CIN2+ than LBC alone. The author mentioned that potential changes in screening methodology should be assessed over at least two screening rounds.

Ekalaksananan T et al (2006) evaluated the value of the combination of p16 and HPV detection in the screening for cervical cancer. 186 patients with previous abnormal cervical lesion were studied. [32 level II-2] After colposcopic examination, two conventional Pap slides were prepared: the first was Papanicolaou-stained and examined by cytologist; the second was immunocytochemically stained for p16. Cervical cells were collected by brush using for HPV detection by Hybrid Capture 2. Biopsy of any colposcopically abnormal lesions was performed. The results were as follows:

- Results of abnormal cervical cell screening by using Pap test, p16 protein and HPV detection
    - p16 was detected in 40 cases. P16 and HPV were found in all high-grade dysplasia and SCC, and in 64% and 27% of low-grade dysplasia, 62% and 0% of ASCUS and 7.4% and 3.4% of normal, respectively.

- Results of p16 protein detection in combination with the detection of HPV.
    - 18 of p16-positive cases (11%) were HPV-negative, 14 of them in the ASCUS and normal group.

- Results when the histological findings are compared with the cytological diagnosis on the Pap smear.
    - All of the 3 CIN 2 or 3 lesions were judged to have HSIL on cytology. 6/8 low grade lesions (squamous metaplasia and CIN I) had normal cytology, 19/27 subjects without dysplastic cells on biopsy.

- Results relation between histological diagnosis and immunocytochemical p16 staining and HPV infection in cervical cells
    - Compared to histological results, all of the p16-positive cases of squamous metaplasia, CIN 2 or 3 and SCC were HR-HPV-positive (5 cases). Therefore, the cases that were positive for both with normal cytology (5 cases) or low-grade dysplasia (3 cases) may comprise a high risk group for neoplastic change.

The author mentioned that the combination of p16 and HPV detection may be useful in cervical cancer screening to identify cervical cells with minor abnormalities and a high risk of progressing to cervical neoplasia and define for those patients requiring an early management or close surveilance.

Howard M et al (2002) did a study to estimate the optimal relative light unit ratio, and correspondingly viral load, of the hybrid capture 2 oncogenic human papillomavirus deoxyribonucleic acid test for detecting cervical intraepithelial neoplasia (CIN). [33] level II-2 Women with abnormal cytology were referred for colposcopy, and a cervical swab or brush specimen was obtained for human papillomavirus testing. Sensitivities, specificities, and likelihood ratios of different relative light unit ratio cutoffs were calculated using a reference standard of colposcopy or biopsy of either CIN 2+ (CIN 2, 3, or carcinoma), or CIN 1+ (CIN 1, CIN 2+). The receiver operating characteristic curve was used to estimate optimal test-positive cutoff points for the hybrid capture 2 test. The analyses were based on the 524 women for whom all relevant data were available, 324 of the 328 from the two randomized trials and all 200 women from the cross-sectional study.

- The presence of any grade of CIN was histologically confirmed in 28.8% (151 of 524), CIN 2 or 3 was present in 18.3% (96 of 524), and squamous cell carcinoma was found in 0.4% (two of 524) of the women
- The area under the ROC curve was 0.82 (95% CI 0.78, 0.87, $P < 0$ .001). The optimal cutoff occurred at a relative light unit ratio (relative light unit is proportional to the amount of DNA in the specimen, and hence is an estimate of viral load) test-positive cutoff of greater than or equal to 15.56.
- CIN 2+ was found in 18.7% (98 of 524) and CIN 1 in 10.5% (55 of 524) of the women. The optimal relative light unit ratio was 15.56, giving a sensitivity and specificity of 82.7% and 73.2% for CIN 2+, and 74.2% and 77.8% for CIN 1+

- In a stratified analysis,
  - a higher relative light unit cutoff (15.19) optimized sensitivity and specificity for CIN 2+ (sensitivity 81.8%, specificity 51.5%) for women with low-grade squamous intraepithelial lesions cytology,
  - whereas the optimal cutoff was 2.36 (sensitivity 100%, specificity 73.0%) for women with atypical squamous cells of undetermined significance, yielding referral rates of 53.3% and 28.7%, respectively.

- For both the 1.0 and 15.56 relative light unit ratio cutoffs, sensitivity is lower and specificity is higher for women older than 30 years compared with women 30 years and younger.

- Likelihood ratios tended to be higher among the older women (age >30).
  - Likelihood ratios were statistically significantly higher for the relative light unit ratio cutoff of 15.56 [4.46, CI (3.27, 6.07)], compared with the cutoff of 1.0 [2.61CI (2.11, 3.22)] in older women for CIN 2+
  - and in all women for CIN 1+ (Likelihood ratios 4.07 for cut off 15.56, and 2.42 with the cutoff of 1.0) $P<0.05$)

Use of a higher cutoff for the relative light unit ratio (higher viral load) of the hybrid capture 2 test may improve the management of women, especially those with low-grade squamous intraepithelial lesions cytology. A test-positive relative light unit ratio cutoff of 15.56 was the optimal cutoff for detecting both the high-grade lesions (CIN 2, 3, or squamous cell carcinoma) and any grade of lesion (CIN 1, 2, 3, or squamous cell carcinoma). Using this higher cutoff (corresponding to a higher viral level), in contrast to the 1.0 cutoff suggested by the manufacturer, specificity for the high-grade outcome increased by 16.4%, with a somewhat smaller reduction in sensitivity of 11.2%. It was also found that likelihood ratios were improved at the 15.56 cutoff compared with the 1.0 cutoff, reflecting the decrease in false-positive results.

c) Age

Tiews S et al (2009) conducted a study on a group of 477 women with a history of known cervical lesions and/or HPV infections (eligibility criterion: HR HPV DNA positive test result with HC2T) and a group of 109 women who were examined as part of their routine cervical cancer screening [34 level II-2]. Baseline HR HPV status was measured at enrollment with the FDA-approved Hybrid Capture 2 HPV DNA Test and the HR HPV 16/18/45 Probe Set Test (HC2T, PST). For risk group, follow-up data was only available for 194 out of 447 women. Data was complete for control group.

At baseline, in the above study, 9 of 109 (8.3%) samples were PST positive in the control group. In the risk group, 292 of 447 (65.3%) samples were PST positive. At follow up, 66.7% of CIN 2 lesion and 88.2% of CIN3 lesions were PST positive. Twenty-five percent of CIN 3 lesions were found in women younger than 30 years. These preliminary results demonstrated that cervical cancer screening at the age of 20 years remains important as

seventeen (25%) of the 68 histologically verified CIN 3 lesions arose in women who were younger than 30 years. The above results also suggested that adding an HR HPV test that detects one or more of the HR HPV types 16, 18 and 45 in conjunction with cytology could help to identify women with an underlying cervical lesion who had an elevated risk of developing severe cervical lesions. This might offer the opportunity of a decrease in incidence and mortality rates that are related with invasive cervical cancer.

Leinonen M et al (2009) compared the age-specific performance of primary HPV DNA screening with that of conventional cytology screening in the setting of an organized population-based cervical cancer screening program in Finland. [35] level I Randomized invitations were sent to women aged 25 – 65 years for routine cervical cancer screening by primary high-risk HPV DNA testing (n = 54 207) with a Hybrid Capture 2 assay followed by cytology triage for women who were HPV DNA positive or by conventional cytology screening (n = 54 218). In both screening arms, cytology results of low-grade squamous intraepithelial lesion or worse triggered a referral for colposcopy. The overall frequency of colposcopy referrals was 1.2% in both screening arms.

- The prevalence of histologically confirmed CIN or cancer was 0.59% in the HPV DNA screening arm versus 0.43% in the conventional screening arm.

- The relative rates of detection for CIN 1,CIN 2, and CIN 3+ for HPV DNA screening with cytology triage versus conventional screening were 1.44 (95%CI = 0.99 to 2.10), 1.39 (95% CI = 1.03 to 1.88), and 1.22 (95% CI = 0.78 to 1.92), respectively.

- The specificity of the HPV DNA test with cytology triage was equal to that of conventional screening for all age groups (99.2% versus 99.1% for CIN 2+).

- Among women aged 35 years or older, the HPV DNA test with cytology triage tended to have higher specificity than conventional screening.
  - the specificity of the HPV DNA testing with cytology triage for CIN 2+ was:
    - 99.0% for 35- to 44-year-olds
    - 99.6% for 45- to 54-year-olds
    - and 99.6% for those aged 55 years or older
  - whereas the specificities of conventional cytology for CIN 2+ in these age groups were
    - 98.9%, for 35- to 44-year-olds
    - 99.3%, for 45- to 54-year-olds
    - and 99.5%, for those aged 55 years or older

- The PPV of the HPV DNA test alone was poor and ranged from 1.6% for CIN 3+ to 8.1% for CIN 1+.

- Women younger than 35 years were referred more often in the HPV DNA screening versus the conventional screening arm (RR = 1.27, 95% CI = 1.01 to 1.60).

- Overall, 7.2% of women in the HPV DNA screening arm versus 6.6% of women in the conventional screening arm were recommended for intensified follow-up, and the percentages were highest among 25- to 29-year-olds (21.9% versus 10.0%, respectively).

Hence, based on the above study, primary HPV DNA screening with cytology triage is more sensitive than conventional screening. Among women aged 35 years or older, primary HPV DNA screening with cytology triage is also more specific than conventional screening and decreases colposcopy referrals and follow-up tests.

d) High Risk HPV Screening

High risk human papillomavirus (HR HPV) have been demonstrated to be the causative agents of cervical cancer [31]. There was an increasing interest in using HR-HPV DNA detection either alone or in addition to the classic cytological examination as a method for primary screening for cervical preneoplastic and neoplastic lesions.

Kotaniemi-Talonen L et al (2005) did a randomised evaluation design to find out whether primary high-risk human papillomavirus (HR HPV) testing implemented into routine screening can bring increase in the programme effectiveness. [37 level I] An equal number of women invited to routine screening was randomly allocated to primary HR HPV screening (n=7060) and to cytological screening (n=7089). In the HR HPV screening arm, after a single positive HR HPV test result, the need of colposcopy referral was determined by a cytological triage test since primary screening with sole HR HPV test would result in a substantial increase in the number of colposcopies (and moreover, increase in total costs and a possible increase in the reported adverse effects). Compared with the conventional arm, more colposcopy referrals were made in the HR HPV screening arm (relative risk 1.51, confidence interval 95% 1.03–2.22). Specificity of the primary screening with sole HR HPV test (91.5–92.1%) was much lower than that with the cytology triage (98.7–99.3%), which was not quite as specific as screening with conventional cytology (99.2–99.6%). Compared with conventional cytology, primary screening with HR HPV test results in increased cross-sectional relative sensitivity at the level of all positive lesions at the cost of substantial loss in specificity. With cytology triage, the specificity improves to the level of conventional cytology. Thus, the author mentioned that they will continue the intake and the followup, and expand the population covered by HR HPV screening, as the results shown in this paper justifed the further evaluation of this specific screening modality.

In 2002 Bory J et al reported a study on 3,091 women with normal smears at the first entry [38 level II-2]. This population was restricted to women who underwent their biennal or triennial routine screening in the Department of Obstetrics and Gynecology of the C.H.U. of Reims. The median follow-up was 12 months (4 to 39 months) for the 659 women with initial HR HPV infection and 27 months (9 to 59 months) for the 2,432 women without any HR HPV detectable at the first smear. Primary endpoint was clinical progression defined as the presence of a high-grade lesion (HGSIL) at the biopsy.

- From the 659 HR-HPV-infected women, 241 (36.6%) had a positive HR HPV test at 2 to 4 examinations with a final histological diagnosis of HGSIL in 51 cases (21.2%) within 4 to 36 months, while women with regressive HPV infection did not develop any lesion during the same period. Hence a recurrent HR HPV infection detected with HC-2 may represent a reliable tool to select populations at risk for the development of HGSIL.

- The incidence of recurrent HR HPV infections was significantly lower ($p<0.02$) for women > 30 years old (32.9%) compared to women < 30 years old (43.3%).

- In the cohort of 2,432 women testing negative for HR HPV infection, only 2 women (0.08%) developed a HGSIL. Both were HR HPV positive 18 and 24 months after the first entry, at the time of diagnosis of disease.

- The RR of incident HGSIL when a HR HPV was detected at enrollment in women with normal smears was 96.7 (CI, 95.8–97.7). The evaluation of the viral load of HR HPV by the HC-2 did not represent a sensitive approach to predict the recurrence of HR HPV infection and/or the apparition of HGSIL.

- However, HPVDNA test cannot be used as a discriminating predictive parameter. At the same time, women with a normal smear and no HR HPV infection have a very low risk for developing a HGSIL. In consequence, in such conditions, the use of HPV testing may allow the screening interval to be safely lengthened to 5 years with a cost-effective benefit.

De Cremoux P et al (2003) conducted a study to evaluate HPV DNA based test by using the Hybrid Capture 2 (HC2 assay) from the residual sample of a liquid-based Pap test (ThinPrep). [39 level II-2] The study population comprised adult women candidates for cervical cancer screening. Two populations were considered: group 1, with 462 consecutive women (25.88%) referred for colposcopy owing to abnormalities detected on previous screening smears, and group 2, with 1,323 consecutive women (74.12%) who were voluntary candidates for the screening of cervical lesions.

- In group 1, 56.9% high risk HPV DNA, 11.9% low risk HPV DNA were detected while in group 2, 16.02 % high risk HPV DNA, 5.97% low risk HPV DNA detected. HPV positivity and viral DNA load increased as a function of histologic grade.

- Combination of smear and HPV DNA had higher sensitivity & specificity compared to HPV DNA test alone:
    - Sensitivity:
        - 85% for combined, 79% HPV alone for group 1,
        - 67% for combined, 64% HPV alone for group 2
    - specificity
        - 82% for combined, 77% HPV alone for group 1,
        - 94% for combined, 86% HPV alone for group 2,
- but not superior to cytologic optimized interpretation of lesions equal or > ASCUS/ AGUS (Both Group 1 & 2)
    - Sensitivity 92% for group 1, 74% for group 2
    - Specificity 80% for group 1, 91% for group 2

Sensitivity of HPV DNA test alone was lower than conventional cytology test. This finding is not in accordance with the results of some studies. According to the author The HC2 assay is a sensitive test to detect HPV DNA sequences in experienced laboratories; however, its use as the only primary test for large-scale screening of cervical neoplasia and, therefore, the management of patients with ASCUS or AGUS, should not be advocated but could be considered a complementary test to the smear, especially for patients with ASCUS or AGUS.

In 2000, Kuhn L et al reported a study that evaluated human papillomavirus (HPV) DNA testing as an alternative screening method. Cervical samples from 2944 previously unscreened South African women aged 35–65 years were tested for high-risk types of HPV with the use of the Hybrid Capture I (HC 1) and Hybrid capture 2 (HC2) assay. [40 level II-2] Women also had a Pap smear, direct visual inspection of the cervix, and Cervicography. Women positive on any screening test were referred for colposcopy.

- The positive Predictive value of detection of HPV DNA testing was 4.6% for low grade SIL or higher and 23.5% for high-grade SIL or higher. In comparison, the PPV of cytology was 58.8% for low grade SIL or higher and 31.9% for high grade SIL or higher.
- With the use of the HC 1 assay, sensitivity of the HC 1 assay for detection of high grade SIL or higher was 73.3% (95% CI=62.6% -82.2%), specificity was 87.8 %(95% CI =86.6%-89.0%)
- The estimated sensitivity of the HC 2 assay for detection of high grade SIL or higher was 88.4% (95% CI =76.9% -92.6%) and the estimated specificity was 81.9% (95% CI=76.5% -86.5%)
- The estimated sensitivity of cytology for detection of high grade SIL or higher was 78.3% (95% CI = 67.9% -86.6%) and the estimated specificity was 96.8% (95% CI =96.1 % -97.4%)
- The area under the ROC curve (higher values indicate better overall performance) was 0.88 for the HC 2 assay and 0.83 for the HCI assay.
- The specificity of cytology was significantly better than either the HCI assay (P<.01) or the HC 2 assay (P<.01) at standard cut-off value.
- HPV DNA testing has a sensitivity equivalent to, or better than that of cytology. Since HPV DNA testing programs may be easier to implement than cytologic screening, HPV testing should be considered for primary cervical cancer screening in low –resource setting.
- HPV DNA testing with the HC 2 assay was more sensitive than cytology for detecting high-grade SIL and invasive cancer.

The author commented that that in many settings, it has proven easier to establish clinical laboratories for large scale HPV DNA than to establish high-quality cytology laboratories. HPV –DNA testing requires less skilled technicians and it was easier to perform than cervical cytology and therefore it may be more feasible to set up HPV DNA testing on site.

e) For Triage

Einstein MH et al (2010) evaluated the clinical performance of the Cervista HPV HR and 16/18 genotyping tests for detection of HPV in cervical cytology specimens. [40 level II-2] DNA was extracted from approximately 4000 residual liquid-based cytology specimens collected during routine liquid-based Papanicolaou tests at standard of care visits and was assessed for the presence of HR HPV and/or HPV types 16 and 18. All women with cytology results of atypical squamous cells of undetermined significance (ASC-US) or greater underwent colposcopic examination and biopsies were collected. Test results were compared with local colposcopy and histology results from a central pathology review panel. There were 1347 subjects with complete data sets of cytology, HR HPV, colposcopy, and histology included in the analysis of the HPV HR test.

- Sensitivity of the HPV HR test for detection of cervical intraepithelial neoplasia (CIN) 2+ among women with ASCUS cytology was 92.8% (95% CI: 84.1% - 96.9%)
- The negative predictive value (NPV) was 99.1% (95% CI: 98.1% - 99.6%).

- Sensitivity for detection of > or =CIN 3 in women with ASC-US was 100% (95% CI:85.1% - 100%)
- The NPV was 100% (95% CI: 99.4% -100%).
- The specificity of the test for detection of > or =CIN 2 and > or =CIN 3 was 44.2% (95% CI: 41.5% -46.9%) and 43% (95% CI: 40.3% - 45.7%), respectively.

The HPV 16/18 genotyping test also performed as expected in women with ASC-US cytology who were positive for HR HPV. The author mentioned that based on the result, the Cervista HPV HR test can be clinically used for detecting HR HPV types in conjunction with cervical cytology for use in triage of women with ASCUS cytology during routine cervical cancer screening.

Castle PE et al (2002) did a study to estimate the risk of developing abnormal cytology during 57 month follow up of subjects with HPV DNA positive but negative cytology on enrolment using HC2. [42 level II-2] A subcohort of 2020 women aged 16 years or older, with a negative Pap test who tested positive at enrollment for oncogenic HPV DNA types using the Hybrid Capture 2 Test were followed for 57 months at Kaiser Permanente (Portland, Oregon). The cumulative incidence for a Pap test interpreted as atypical squamous cells or more severe (≥ ASC) was 16.8% (95% CI = 15.0% – 18.6%), 6.4% (95% CI = 5.2% – 7.6%) for low-grade squamous intraepithelial lesions or more severe, and 2.2% (95% CI= 1.5% – 2.9%) for high-grade squamous intraepithelial lesions or more severe. By comparison, the cumulative incidence of greater than or equal to ASC among HPV-negative women was 4.2% (95% CI = 3.9% – 4.6%). The highest viral load (100 relative light units per the positive control or greater) was associated with a greater risk of an abnormal Pap test (odds ratio= 2.7, 95% CI= 1.7– 4.1) than lower viral loads. The author stated these results suggest that about 15% of women in annual screening programs who concurrently have a negative Pap test and a positive oncogenic HPV test will have a subsequent abnormal Pap test within 5 years.

Pimple S et al compare the utility of cytology and HPV testing in women from Mumbai, India, suspected of having cervical intraepithelial neoplasia (CIN) on visual inspection with acetic acid (VIA), Lugol's iodine (VILI), or both. [43 level II-2] The sensitivity, specificity, and predictive values of these tests for the detection of CIN 2 and/or 3 were evaluated in this cross-sectional study with 756 women suspected of having CIN on visual inspection. There were 25 women with CIN 2, 20 with CIN 3, and 21 with invasive cancer. The accuracy tests results were as follows:

- Sensitivities:
  - Cytology – for CIN 2 or CIN 3 lesions, 64.3% (95% CI, 48.0%–78.4%) for ASCUS, 57.1% (95% CI, 41.0%–72.3%), for LSIL
  - HPV test was 61.0% (95% CI, 44.5%–75.8%).
- The specificity :
  - cytology test using the ASCUS and LSIL thresholds were 95.8% (95% CI, 94.0%–97.2%) and 97.5% (95% CI, 96.0%–98.6%),
  - HPV test was 92.1% (95% CI, 89.6%–94.2%).

- The sensitivity estimates for CIN 3:
  - 85.0% (95% CI, 62.1%–96.8%)cytology at ASCUS
  - 70.0% (95% CI, 45.7%–88.1%)cytology at LSIL
  - 89.5% (95% CI,66.9%–98.7%), for HPV DNA test
- Specificity to detect CIN 3 lesions
  - 94.5% 95% CI, 92.5%–96.1%) cytology at ASCUS
  - 96.1% (95% CI, 94.4%–97.5%) cytology at LSIL
  - 91.1% (95% CI, 88.5%–93.2%) for HPV DNA test

Cytology and HPV testing were both found to be accurate triaging methods for women suspected of having CIN on visual inspection, especially for those with CIN 3 lesions.

Arbyn et al (2006) did a systematic review and meta-analyses on 22,000 patients (from 14 studies) on three possible clinical applications of human papillomavirus (HPV)-DNA testing: triage of women with equivocal or low-grade cytological abnormalities; prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN) lesions, and last not but not least, primary screening for cervical cancer and pre-cancer. [21 level I] Consistent evidence is available indicating that HPV-triage with the Hybrid Capture-2 assay (HC2) is more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex HC2 test does not show a significantly higher sensitivity, but a significantly lower specificity compared to a repeat Pap smear. Results for Triage of minor cytological lesions were as follows:

- On average, in 9.7% (95% CI: 7.7% –11.7%) and 4.3% (95% CI: 2.7% – 5.9%) of cases, underlying CIN-2+ or CIN-3+ was found.
- Overall, HC2 had a sensitivity of 92.5% (95% CI: 90.1% – 94.9%) and 95.6% (95% CI: 92.8%–98.4%) for detecting respectively CIN-2+ or CIN-3+.
- The pooled specificity was 62.5% (95% CI: 57.8% – 67.3%) when the outcome was CIN- 2+ and 59.3% (51.2% – 67.4%) for CIN-3+.
- The sensitivity of HC2 triage of women with an index smear showing LSIL was very high: 97.2% (95% CI: 95.6% – 98.9%), pooled from 10 studies for the outcome of CIN-2+ and 97.0% (95% CI: 93.9% –100%), pooled from five studies for CIN-3+ .
- However its specificity was very low: 28.6% (95% CI: 22.2% – 35.0%) for CIN-2+ and 21.6% (95% CI: 16.6–26.6%) for CIN-3+.
- Histologically confirmed CIN-2+ and CIN-3+ were present in, 18.8% (95% CI: 1.24%–25.2%) and 9.2% (95% CI: 7.0% –11.4%) respectively.

Chen L et al (2008) did a report on a study during a 60-month period from July 2001 to June 2006, whereby all ThinPrep cases with a diagnosis of AGC from the Cleveland Clinic were searched. [44 level II-2] Cases with a cytologic diagnosis of AEC, AGC–favor endocervical origin, or AGC-NOS underwent 'reflex' HPV DNA testing (using either the original liquid based cytology residual specimen or a separate sample co-collected at the initial screening visit for the cytologic diagnosis of AEC). Of a total 332,470 Papanicolaou (Pap) tests performed, 317 cases of AEC had histopathologic follow-up and reflex testing for high-risk HPV infection which may lead to cervical cancer. The results showed that:

- High-risk HPV DNA was detected in 64 of 317 (20.2%) of the patients with AEC lesions. When analyzed by age groups, 21.4% (21 of 98) of the women aged < 30 years and 19.6% (43 of 219) of the women aged ≥30 years tested positive for HPV
- Histopathologic examination of the 64 HPV-positive AEC cases revealed 18 cases (28.1%) of endocervical adenocarcinoma in situ/adenocarcinoma (AIS+) and 22 cases (34.4%) of CIN2+.
- Among 253 of the HPV-DNA negative AEC women, 3 cases (1.2%) had AIS lesion and only 1 case (0.4%) had CIN2+ lesions.
- Cervical AIS+ was found in 28% of the HPV-positive AEC patients and in only 0.9% of the HPV-negative patients (P<0.0001).
- When the significant glandular (AIS1) and squamous (CIN2+) lesions were combined, 62.5% of the lesions were detected in HPV-positive AEC cases compared with 1.6% in the HPV-negative AEC cases (P<0.0001).
- The sensitivity, specificity, positive predictive value, and negative predictive value for high-risk HPV DNA testing to detect clinically significant cervical lesions (CIN2+ and/or AIS+) were 91.0%, 91.2%, 62.5%, and 98.4%, respectively.

Because of a high sensitivity (91.0%) and high specificity (91.2%) in detecting significant cervical lesions, reflex HPV testing for cytologic diagnosis of AEC appears to be a useful ancillary tool in the selection of high-risk patients for colposcopy.

f) Follow-up post treatment

Arbyn et al (2006) did a systematic review and meta-analyses on 22,000 patients (from 14 studies) on three possible clinical applications of human papillomavirus (HPV)-DNA testing: triage of women with equivocal or low-grade cytological abnormalities; prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN) lesions, and last not but not least, primary screening for cervical cancer and pre-cancer. [21 level I] Consistent evidence is available indicating that HPV-triage with the Hybrid Capture-2 assay (HC2) is more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex HC2 test does not show a significantly higher sensitivity, but a significantly lower specificity compared to a repeat Pap smear.

After treatment of cervical lesions, HPV testing easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. Results for Follow up treatment of cervical intraepithelial neoplasia were as follows:

- Sixteen studies were identified that matched inclusion criteria. Studies were heterogeneous with respect to design, timing of visits, choice of HPV testing methods and the assessment of disease status at entry and end of follow-up.
- Treatment failure expressed in terms of residual or recurrent CIN, occurred on average in 10.2% (95% CI: 6.7–13.8) of treated cases.
- The sensitivity of HPV-DNA detection in predicting treatment failure ranged from 67% to 100% and was on average 94.4% (95% CI: 90.9–97.9%).
- The specificity of HPV testing for predicting treatment success was statistically very heterogeneous among studies and varied between 44% and 100%.

Paraskevaidis E et al (2004) did a systematic review whereby eleven studies were ultimately found on evaluating the use of HPV testing after conservative treatment for CIN. Eight studies were prospective, and three studies were retrospective. [45 level I] The total number of women included in these studies were 900, of whom 678 (75.3%) were considered as having a successful treatment, whereas 222 (24.7%) were considered treatment failures. There was a marked heterogeneity in the design, population, intervention and follow-up policy across different studies. The sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in four of them, whereas the specificity of the test differed across the studies, ranging from 44% to 95%. Among the 672 women in whom the treatment was considered to be successful, 566 (84.2%) had a negative postoperative HPV DNA test, whereas 106 (15.8%) had a positive postoperative HPV DNA test. In contrast, among the 204 cases that were considered as treatment failures, only 35 cases (17.2%) had a negative postoperative HPV DNA test, whereas 169 cases (82.8%) were positive for HPV DNA postoperatively. The results of this overview suggest that there might be a role for a HPV DNA test at the follow up period. It seems that a positive HPV test, even in the presence of normal cytology, may pick up early and accurately a treatment failure. Cytology and colposcopy may still be needed in order to rule out false positive and false negative results.

Hernadi Z et al (2005) evaluated the role of human papillomavirus (HPV) testing in post-treatment follow-up of patients after therapeutic excision of the cervix due to positive screening tests. [46 level II-2] A hospital-based retrospective analysis was performed with prospective collection of patient data of women screened for cervical cancer at a Gynecologic Outpatient Clinic. Patients after therapeutic excision due to positive screening results were identified and followed up with HPV testing and serial cytology. The results were:

- A negative HPV test (43 patients) in the post-treatment period excluded not only the recurring CIN but also the development of persisting cytological atypia (negative predictive value (NPV): 100%) during 1201 patient months (median 26 months) and allowed the patient to return back to the population-based screening programs.

- Negative HPV results were detected at median of 6 month (range: 1–24) after therapeutic excision. The median follow up time for negative cytology was 4 months (range: 2–12). It is of note that 10 (23%) of the 43 patients in the HPV negative follow-up group in the post treatment period had equivocal cytology (P3) at single visits during a cumulative 1201 patient months follow-up (median 26 months)

- After 61 treatment for cervicalis intraepithelialis neoplasia (CIN), high-risk HPV infection was detected during the post-treatment follow-up in 18 cases (29.5%), 10 of them had persisting cytological atypia (positive predictive value (PPV): 56% (10/18), 5 developed CIN (PPV: 28% (5/18).

Hence, a negative HPV test eliminates the risk of recurrent disease after treatment for CIN. In a positive HPV test, this may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity. However, as consequence of cases mentioned above, the positive predictive values were relatively low, Sarian LO et al (2004) compared the performance of cervical cytology and HPV DNA test in detection of residual or recurrent disease following the treatment of cervical intraepithelial neoplasia (CIN) 2 or 3 with loop electrosurgical excision procedure (LEEP). 47 level II-2 A series of 107 women subjected to LEEP due to histologically confirmed CIN 2 or 3 between March 2001 and December 2002 were followed-up biannually until January 2004. Follow-up visits consisted of interview and gynecological examination including cervical cytology, hybrid capture 2 (HC2), and colposcopy. Patients presenting with abnormal colposcopy or high-grade squamous intraepithelial lesion (HSIL) smear were subjected to new excision procedure and presence of histologically confirmed CIN 2 or 3 or higher was considered as residual or recurrent disease. Performance indicators were calculated for cytology and HCII assay in detecting residual or recurrent disease. Women age ranged from 20 to 60 years (mean 34 years). The results showed that:

- During the follow-up, eleven (10.2%) women showed residual or recurrent disease during the follow-up. Half of the women with residual or recurrent disease at follow-up presented with complete excision margins of the cone.

- Considering HC2 and Pap smear as standalone tests, both techniques showed similar sensitivity, detecting 100% of CIN 2 or 3 at the first follow-up visit.

- At the second follow-up visit, Pap smear showed better specificity (97% versus 83%) and positive predictive value (PPV- 67% versus 22%) than HC2, and both tests had fairly the same high negative predictive value (NPV- 99% versus 98%) and sensitivity (80% for both).

- The combined positive HC2 and abnormal cytology had the same sensitivity (80%) as each of the tests alone, but specificity (100%) and PPV (100%) were significantly higher than those of single tests.

- When only one of the tests was positive, the sensitivity (100% for 1st follow up and 80%at second follow up) and the NPV (100% at 1st follow-up and 99% at second follow up) of the combination remained the same, but its specificity and PPV were lower than that of the combined two positive tests and that of the individual test, at both follow-up visits

Both tests performed well in detecting residual or recurrent disease after LEEP and combination of the tests did not increase sensitivity of the single tests. HPV testing seems to be a valuable tool in monitoring the therapeutic results conization and to discriminate patients who have a higher risk of disease recurrence HPV testing has a high predictive value in the postconization follow-up, because HPV may act as a marker of undetected residual neoplasia, being a necessary factor for the development of recurrent CIN. Importantly, HPV testing can clarify the referral criteria for colposcopy because HPV detection, even when cervical cytology is normal, might predict an abnormal colposcopy.

g) Mortality

Recently in 2009, the result of a cluster-randomized trial on a total of 131,746 healthy women between the ages of 30 and 59 years had been published and reported by Sankaranarayanan R et al. [48 level I] The study was done in the Osmanabad district in India to measure the effect of a single round of screening by testing for human papillomavirus (HPV) using using the Hybrid Capture 2 test as the index test group, cytologic testing, or visual inspection of the cervix with acetic acid (VIA) as the control group on the incidence of cervical cancer and the associated rates of death. The results were as follows:

- In the HPV-testing group, cervical cancer was diagnosed in 127 subjects (of whom 39 had stage II or higher), as compared with 118 subjects (of whom 82 had advanced disease) in the control group. In the HPV-testing group, the hazard ratio for the detection of advanced cancer was 0.47 (95% CI, 0.32 to 0.69 as compared to the control group)

- There were 34 deaths from cancer in the HPV-testing group, as compared with 64 in the control group. In the HPV-testing group the hazard ratio for death was 0.52 (95% CI, 0.33 to 0.83), as compared with the control group.

- No significant reductions in the numbers of advanced cancers or deaths were observed in the cytologic-testing group or in the VIA group, as compared with the control group.

- PPV for detecting CIN grade 2 or 3 lesions was 11.3% in HPV-testing group, 19.3% in cytologic-testing group, and 7.4% in the VIA group.

- During the 8-year follow-up period, the age standardized incidence of cervical cancer in women with negative HPV test, cytology and VIA was 3.7, 15.5, and 16.0 cases per 100, 000 person-years respectively.

- Hence, the incidence rate of cervical cancer of stage II or higher and death rates from cervical cancer were significantly higher in the cytologic-testing group and the VIA group than in the HPV-testing group.

6.2. SAFETY

a) Psychological effects

McCaffery K et al (2004) did a cross sectional survey to examine the psychosocial impact of testing positive for high risk human papillomavirus (HPV) among women attending primary cervical screening. [49 level II-3] Measures were taken at baseline and one week after the receipt of HPV and cytology screening results. The population consisted of four hundred and twenty-eight women aged 20–64 years. All psychosocial measures were taken prior to colposcopic follow up (which occurred within one month of cervical smear results being given). Anxiety was measured both at baseline and follow up with the widely used short form of Spielberger's State Trait Anxiety Inventory (STAI). The scale consists of six items assessing current levels of anxiety (score range from 6 to 24). A second psychological measure, specific to smear testing, was also used at follow up. The well-validated Cervical Screening Questionnaire (CSQ) assesses psychological distress following cervical screening. The nine-item scale covers perceptions of general and gynaecological health, body image, concerns about fertility, sexual interest, fear of cancer or serious illness and pessimism. Response options were presented on a four-point Likert scale, less than usual, same as usual, rather more than usual, much more than usual, for the first four items, and better than usual, same as usual, worse than usual, much worse than usual, for the remaining five items (score range from 0 to 27). The results were as follows:

- Women with normal cytology who tested positive for HPV (HPV+) were significantly more anxious and distressed than women who were negative (HPV-) using both a state anxiety measure [$F(1,267) = 29$, $P < 0.0001$] and a screening specific measure of psychological distress [$F(1,267) = 69$, $P < 0.0001$].

- Women with an abnormal or unsatisfactory smear result, who tested HPV+, were significantly more distressed than HPV- women with the same smear result [$F(1,267) = 8.8$, $P = 0.002$], but there was no significant difference in state anxiety.

- Irrespective of cytology result, HPV+ women reported feeling significantly worse about their sexual relationships. Approximately one-third of women who tested positive reported feeling worse about past and future sexual relationships compared with less than 2% of HPV- women.

The study suggested that HPV testing may have an adverse psychosocial impact on women who test HPV+ when it is used as a primary screening test alongside conventional cytology. Consideration of the psychosocial consequences of HPV testing is important. Millions of women participate in cervical screening programmes each year and may potentially be affected. The psychosocial impact of HPV testing is currently not well understood and needs further investigation before decisions are made about its introduction into national cervical screening programmes.

6.3. COST/ COST-EFECTIVENESS

Incremental cost-effectiveness ratio (ICERs) from each study was calculated in different ways depending on the assumption, and it was associated with a change from one strategy to a more costly alternative. In this review, included are the studies with alternatives, which involved strategies using HPV DNA testing and these were differentiated by the screening intervals, the starting age for screening and the frequency of visits were compared with the conventional cytology alone as this is the current strategy adopted by the health care system in Malaysia. After considering studies retrieved, eight studies were chosen to be considered for the final review.

By doing the summary as in the table shown, article by Bistolleti *et al* [50 level II-3] and Lwtwyn *et al* [51 level II-3] explained that the strategies using HPV DNA testing were only showing cost saving and not cost saving decision. Bistolleti in his study, in Sweden population based study, found that organized cervical cytology screening between the ages of 32 and 60 years was highly cost-efficient for cervical cancer prevention where if these screening intervals were increased to at least nine years, combined cytology and HPV DNA screening could be a cost saving alternative. Lywtyn *et al* in his surveillance concluded that the combination of repeat Pap testing and Human Pappillomavirus (HPV) testing was more costly, but it may detect more cases of cervical intraepithelial neoplasia (CIN) 2 or 3 than the cytology test alone.

From all the eight studies, four studies were carried out in the developing or low resource regions, and the other four were conducted in developed countries and European Union. From the table, studies carried out in low resource setting countries showed low ICERs regardless the interventions and this was compared to the studies in the developed countries, where the ICERs were extremely high. As this review is done to see whether the interventions associated with HPV testing was cost effective in Malaysia or elsewhere, we then finally excluded the four outliers (with extremely high ICERs and those only showing cost saving and not cost saving). Finally we were left with four alternative strategies which were assembled in the country of South Africa, Thailand and Colombia with the assumptions that they represent almost the same setting as in the middle resource setting like Malaysia with reference to their direct cost, indirect cost, perspective, health system and others. Somehow, the four outlier papers (Bistoletti P *et al* , Kim J J *et al*, [52 level II-3] and Mandelblatt J S *et al*, [53 level II-3] and Lytwyn A et al) summarized that combination of cytology test and HPV testing varies in their strategies in terms of screening intervals, age of screening and others, were found to be the most cost-effective and cost saving alternatives compared to the conventional cytology alone.

From the four studies done in the low resource setting (Vijayaraghavan A *et al* , [54 level II-3] and Goldie S J *et al,* [55 level II-3] and Andres-Gamboa *et a*l , [56 level II-3] and Goldie S J *et al* [57 level II-3] and*)* they were all presented a so much lower ICERs (strategy with lowest ICER means that it is the most cost effective strategy offered), where the lowest ICER found in the study by Colombia (Andreas Gamboa O *et al*) with the intervention being the HPV every five years. This is followed by the single life time screening with HPV DNA testing coupled with immediate cryotherapy once with positive results of HPV (Goldie S J *et al*). Studies in South Africa and Thailand proved that with 1 visit screening with HPV DNA once in a lifetime was the most cost effective strategies (Goldie S J *et al*) while the study assembled in South Africa suggested that conventional cytology followed by HPV triage for equivocal cytology was the most cost effective strategy (Vijayaraghavan A *et al*).

The WHO guideline was applied to elucidate if HPV DNA testing were cost–effective for every country in the world, including Malaysia. The estimation of ICER for HPV DNA testing was based on the four cost-effectiveness studies as discussed above. ICERs from the selected articles were converted into 2007 units to compare with the per capita GDP for Malaysia. Per capita GDP for Malaysia was USD 5151 in 2008 per capita. If the strategy has less than the GDP per capita in Malaysia meant that it was a very cost-effective option that should be adopted in the system. Hence, we took the reading for the Per Capita Gross Domestic Product by countries in 2007 as this will represent the best estimation for the study conducted from 1999 to 2007. Having said that, we concluded that all the four strategies explained in the four included articles were indeed also cost effective options for Malaysia as the ICER was lower than the GDP per capita Malaysia.

Nevertheless, further investigation using the Malaysian own natural history data will be more appropriate to give a more real situation in which between these four strategies could contribute to the less cost and more effective decision. This was due to the transferability factors such as demographics of the population, epidemiology of the disease, clinical practice, experience, education and training of the healthcare professionals; incentives for providers, absolute or relative prices (this was when the relative prices of testing differ between countries than the relative cost-effectiveness will also differ), available resources and services, organization of the delivery system, available treatment options, perspective of the economic evaluation and other study factors. All of these factors contributed to the differences in every setting or countries conducting the studies and thus will influence the results of this cost-effectiveness study.

The average cost per Pap smear test performed in Malaysia was RM 20.12 (USD6.59), the minimum cost was RM 14.16 (USD 4.64) and the maximum cost was RM 34.46 (USD 11.29) (at the rate of USD 1 equivalent to RM3.05).[58] Elsewhere the average cost per HPV DNA test was about RM 91.50- RM183 (USD 30 – USD 62).[54, 55]

Table 1: Summary of the cost-effectiveness analysis of HPV DNA testing for cervical cancer screening

Articles	Vijayaraghavan A et al	Goldie S J et al	Andres-Gamboa O et al	Goldie S J et al	Bistoletti P et al	Kim J J et al	Mandelblatt J S et al	Lytwyn A et al
Main Characteristics Model	A decision analytic Markov model	Computer decision model	Markov model	A state-transition decision model, based on Markov cycles	Markov Model	Computer-based model	A decision analytic model based on a Markov process	Randomized Control Trial
Perspective (from cost data)	Societal perspective Direct costs Clinic visits, diagnostic and screening test, treatment options Indirect costs Patients time costs	Societal perspective Direct cost Direct medical cost (staff, disposable supplies, equipment and specimen transport) Indirect cost Women's time cost, transportation cost, or program related cost	Payers perspective Direct cost Diagnostic, screening test, treatment of cancer an subsequent follow up No indirect cost As this is from the view of payers perspective	Societal perspective Direct cost Screening test ,visits, diagnostic work up, and treatment Indirect cost Time spent on travelling and attending screening and treatment visits	Payers perspective Direct cost Screening test, interventions, treatment and follow up No indirect cost as this is from the view of payers perspective	Societal perspective Direct cost Cost of test, treatment, staff time and office visit Indirect cost Cost of patient time taken for screening and treatment	Societal perspective Direct cost Consumables supplies, personnel, laboratory, procedural cost of the screening test, diagnosis, initial treatment and terminal care Indirect cost Patient time for screening, diagnosis, treatment , travel and waiting	Payers perspective Direct cost Hpv test, repeat pap test, colposcopy visit, endocervical curettage and biopsies, treatments, physician fees No indirect cost was included
benefits/ outcome	QALYs	LYs	LYs	LYs	LYs	LYs	QALYs	Detection Rate
Screening strategies/ Intervention with the most cost effective protocol in the country where study was done.	Conventional cytology followed by HPV triage for equivocal cytology	1 visit HPV DNA once in a lifetime	HPV every 5 years	Single life time screening with HPV DNA testing coupled with immediate cryotherapy once with positive results of HPV	Combination cytology and HPV DNA screening for up to 9 years screening interval	Combination cytology and HPV DNA at 3 years interval	Combination cytology and HPV DNA every 2 years up to the age of 100 years.	Combination of repeat pap test and HPV test
Countries the study was done	South Africa	Five Developing countries: i)South Africa ii)Thailand	Colombia	South Africa	Sweden	4 countries: i)United Kingdom ii)The Netherlands iii)France iv)Italy	United States	Canada
ICER	R2800($398)	i)$467 ii)$170	$44	Less than $50	Cost saving but not cost effective.	i)$75,900 ii)$37,400 iii)$26,300 iv)$25,600	$76,183	Just showing the number of detected high grade CIN with the combination of repeat pap test and HPV and the cost of the interventions. It shows that the combination testing detected more cases of CIN but with more additional cost (not cost saving)
Year based for currency value	2006	2000	2007	1999	2005	2004	2000	2001
Adjusted ICER (to 2007 as 2007 is the latest year where price referred).	$409	i)$467 ii)$170	$44	Less than $62	N/A	i)$83,315 ii)$41,053 iii)$28,869 iv)$28,100	$91,676	N/A

6.4. OTHER COMPETING TECHNOLOGIES

HR-HPV DNA detection has been used in some countries as a triage test for women with equivocal cytology results (ASC-US) [59], as a co-test in addition to cytology and recent data may further support its use for primary screening in women older than 30 years. [60] The accuracy of cervical cancer screening programs may be improved by biomarker assays that specifically highlight transforming HPV infections. These tests may be based on the direct or indirect detection of the viral oncogene E6 and E7 expression in HPV transformed basal keratinocytes. The cellular kinase inhibitor p16INK4a is strongly overexpressed in transforming HR-HPV infections due to the disruption of a pRb controlled negative feedback loop by the viral oncogene E7. P16INK4a-overexpression is thus considered as a surrogate marker for deregulated E7 expression and hence for transforming HPV infections.

Several more recent reports suggested that the detection of p16INK4a-stained cells in cytology samples increased the specificity for diagnosing high grade cervical intraepithelial neoplasia (CIN) compared to HPV DNA testing without losing sensitivity. Alternatively, the detection of HPV E6/E7 mRNA has been proposed as a biomarker for HPV oncogene expression. Reuschenbach. M et al evaluated HPV E6/E7 mRNA detection (APTIMA), p16INK4a-immunocytology (CINtec), and HPV DNA testing (HC2) to identify women with high grade cervical neoplasia in a cohort. 61 Liquid based cytology specimens were collected from 275 patients. All assays were performed from these vials. Detection rates of each test were evaluated against conventional H&E based histopathology alone and stratified by p16INK4a-immunohistochemistry (IHC).

- All assays yielded a high sensitivity for the detection of CIN3+
  - 96.4% (95% CI: 90.4% – 98.8%) for HC2,
  - 95.5% (95% CI: 89.2% – 98.3%) for APTIMA and CINtec)
- and CIN2+
  - 91.5% (95% CI: 85.8% – 95.1%) for HC2,
  - 88.4% (95% CI: 82.3% – 92.7%) for APTIMA, 86.6% (95% CI: 80.2% – 91.2%) for CINtec).
- The specificity to detect high grade dysplasia was highest for CINtec p16INK4a-cytology
  - 60.6% (95% CI: 52.7% – 68.0%) in CIN3+ and 74.8% (95% CI: 65.5% – 82.3%) in CIN2+),
  - followed by APTIMA 56.4% (95% CI: 48.4% – 64.0%) in CIN3+ and 71.2% (95% CI: 61.7% – 79.2%) in CIN2+
  - and HC2 49.1% (95% CI: 41.3% – 56.9%) in CIN3+ and 63.4% (95% CI: 53.7% –72.1%) in CIN2+.
  - All tests had higher sensitivity using p16INK4a-IHC-positive CIN2+ lesions as endpoint.

Biomarkers that detect HPV induced dysplastic changes in the transforming stage are promising tools to overcome the current limitations of cervical cancer screening.
Dockter J et al evaluated APTIMA HPV Assay performance for detection of high-risk HPV and high-grade cervical intraepithelial neoplasia (CIN) compared to Hybrid Capture 2 HPV DNA (HC2) test. Liquid Pap specimens were collected from 800 women referred for colposcopy were tested with the APTIMA HPV Assay and the HC2 test. [62] Complete results were available for 753 subjects. A subset of samples (n = 393) were typed using Roche's Linear Array HPV Genotyping Test.

- Sensitivity and specificity for detection of high-risk HPV were >92% and 99% for the APTIMA HPV Assay and 93% and 82% for the HC2 test.
- Clinical sensitivity and specificity were 91% and >55% for detection of CIN 2+, and 98% and 53% for detection of CIN 3+ for the APTIMA HPV Assay;
- Values for the HC2 test were 95% and 47% for CIN 2+, and 99% and 44% for CIN 3+.

The APTIMA HPV Assay is sensitive and very specific for detection of high-risk HPV. The APTIMA HPV Assay had similar clinical sensitivity for disease detection but higher clinical specificity than the HC2 test, which may improve patient management and reduce the cost of care.

6.5 OTHER CONSIDERATIONS

a) Organizational

In Malaysia, Pap Smear Screening Programme has started since 1969. Initially it was introduced to all family planning acceptors. Later in 1995, during the Healthy Lifestyle Campaign on cancer, the services were expanded to all eligible women between 20 to 65 years old, once every 3 years.

There were two major restrictions noted that may impede the use of current HPV testing technologies in screening programs: (1) the methods and instrumentation required to process cervical specimens, and (2) the technical equipment requirements for interpreting test results. All cervical cancer screening approaches faced common challenges to successful implementation. Cytology, visual inspection with acetic acid (VIA), HPV DNA-Based testing, and other screening approaches faced barriers such as logistic and infrastructure inadequacies, cost concerns, poor follow-up, and sociocultural constraints. Health care planners who are considering implementing any type of cervical cancer screening must develop clinical protocols that are responsive to the natural history of cervical disease, the diagnostic characteristics of the screening technology, disease prevalence in the target population, and women's as well as providers' needs and concerns.

b) Ethical and legal consideration

In 1968, Wilson and Jungner authored a WHO document entitled "Principles and Practice of Screening for disease (Public Health Papers, No. 34)" has defined ten criteria to be met by mass screening programmes for it to medically and ethically acceptable. This criterion has been reviewed in 2003 as in Appendix 4.

Ethical analysis in this context weighs the probable or expected value of mass screening in the population concerned against the assumed or probable risks of adverse physical or psychological effects for those affected if mass screening is or is not done.

7 LIMITATIONS

Our study has several limitations. Although we only included RCTs for effectiveness, we also included cohort and cross sectional studies for adverse events and accuracy of tests. Although there was no restriction in language during the search but only English full text articles were included in the report.

8 DISCUSSION AND CONCLUSION

An article (American Journal for Clinical Pathology *highlights* HPV Test Utilization Policies June 11, 2009) stated that Excessive testing for the human papillomavirus (HPV) may result in over-management and harmful treatment for benign conditions while minimally reducing cancer incidence, according to a recent article in the June 2009 issue of *American Journal of Clinical Pathology* (*AJCP*). [63] Overtesting and testing women who are at virtually no risk of cancer adds cost without benefit.

Developed by the Cytopathology Education and Technology Consortium, and endorsed by American Society for Clinical Pathology (ASCP), the American Cancer Society, and several other professional medical societies, the Statement on HPV DNA Test Utilization delineates the appropriate and inappropriate uses of HPV: [64]

- *When is HPV testing useful and appropriate?*
  HPV testing should be used only for high-risk HPV types: The test should be FDA-approved or clinically validated with the supporting data subject to peer review. Testing for low-risk HPV serves no clinical purpose and cannot be justified. HPV testing is clinically indicated for the triage of patients with equivocal cytology results (atypical squamous cells). In addition, co-testing with the Pap test in women over 30 years of age provides predictive safety for at least three years in women who are negative on both tests. Therefore, women who choose this form of combined testing should not be screened more frequently.

- *When is HPV testing inappropriate?*
  HPV testing should not be done in women younger than 30 in routine cervical cancer screening. It also should not be done more frequently than every three years as a screening test in women over 30. Also, it should not be the automatic follow-up test or "reflex" to abnormal test results in adolescents (women younger than 20). If HPV testing is inadvertently administered to adolescents, the results should not be used to influence patient management.

The AJCP article stated that HPV is common, but cervical cancer is not. Some types of HPV are associated with precancer and cancer which are considered high risk, whereas others are considered low risk. According to the U.S. Centers for Disease Control and Prevention, at least half of sexually active people will have HPV in their lives, but few will get cancer.

Beyond the cost issues that arise out of unnecessary testing, another point to consider is the physical discomfort and anxiety that a woman suffers in anticipation of an often unnecessary, invasive procedure.

Although differences in a woman's lifetime cancer risk associated with alternative screening approaches are small, the difference in colposcopy referrals is 3-fold. Combined screening with 2 tests, cytologic testing and HPV DNA testing, leads to the highest number of false-positive results and excessive referrals across all screening frequencies, even when restricted to women older than 30 years. Although the sensitivity of combined cytologic and HPV testing is highest, expected CIN 2 or 3 diagnoses are similar for all 4 strategies. Combined cytologic and HPV testing also resulted in high numbers of CIN 1 diagnoses. For younger women, nearly half of all colposcopies resulted in a CIN 1 diagnosis regardless of strategy. Due to the fact that most CIN 1 is likely to regress, this potential overdiagnosis may also be of particular concern, especially if conservative management guidelines are not followed and overtreatment occurs and/or if a woman's quality of life is compromised by the need for repeated visits and more frequent follow-up screening. [65]

There was good level of evidence to suggest that HPV DNA-Based Screening Test for Cervical Cancer has moderate accuracy if used alone but much higher sensitivity if used in combination with Pap smear. The sensitivity of HPV testing for cervical intraepithelial neoplasia of grade 2 or 3 was 94.6% (95% CI: 84.2% to 100%), compared to Pap testing which had a sensitivity of 55.4% (95% CI: 33.6% to 77.2%). The sensitivity of both tests used together was 100%, and the specificity was 92.5%. Compared to the other tests, The Hybrid Capture 2 assay showed a sensitivity for CIN2+ of 62% (95% CI: 56% – 68%) and a specificity of 94% (95% CI: 92% –95%) whereas VIA and VIAM had a sensitivity of 79%, specificity of 85%, while VILI had a sensitivity of about 89%, specificity 85%. Visual inspection is an alternative low-technology screening tests usually done in low resource settings with potential difficulties in implementing cervical cytology-based screening. However a clear understanding of the anatomy, physiology and pathology of the cervix is absolutely essential to understand the basis and to interpret the outcome of screening using VIA, VILI and VIAM.

There was good level of evidence to show that HPV DNA-Based testing may be able to decrease the incidence and mortality rates related to invasive cervical cancer. Ams mentioned by Sankaranarayanan R et al. [48] in the HPV-testing group, the hazard ratio for the detection of advanced cancer was 0.47 (95% CI, 0.32 to 0.69 as compared to the control group). There were 34 deaths from cancer in the HPV-testing group, as compared with 64 in the control group. In the HPV-testing group the hazard ratio for death was 0.52 (95% CI, 0.33 to 0.83), as compared with the control group. Hence, the incidence rate of cervical cancer of stage II or higher and death rates from cervical canc er were significantly higher in the cytologic-testing group and the VIA group than in the HPV-testing group.

There was moderate to good level of evidence to show that HPV-triage using the Hybrid Capture 2 assay was more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. Among women aged 35 or older, the HPV DNA test with cytology triage tended to have higher specificity than conventional screening and decreases colposcopy referrals and follow-up test.[35] False negatives would be reduced, double negative patients could be safely screened at longer intervals (reducing costs) and patients as being at high risk but not having identifiable cervical cancer could be monitored closely. The specificity of the HPV DNA testing with cytology triage for CIN 2+ was 99.0% for 35- to 44-year-olds, 99.6% for 45- to 54-year-olds, and 99.6% for those aged 55 years or older. Compared with cytology, primary screening with HPV DNA-Based test followed by cytological triage and repeat HPV DNA-Based test of HPV DNA– Based positive women with normal cytology increased the sensitivity for CN3+ detection by 30% (95% CI = 9% to 54%), and resulted in a mere 12% increase in the number of screening tests.

Castle et al [45] mentioned in their study that about 15% of women in annual screening programs who concurrently have a negative Pap test and a positive oncogenic HPV DNA-Based test may have a subsequent abnormal Pap test within 5 years, hence indicating triaging with HPV DNA-Based test may be important within five years intervals.[45]

There was good to fair level of evidence to suggest that after treatment of cervical lesions, HPV DNA-Based test easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. Treatment failure expressed in terms of residual or recurrent CIN, occurred on average in 10.2% (95% CI: 6.7% –13.8%) of treated cases. The sensitivity of HPV DNA-Based test detection in predicting treatment failure ranged from 67% to 100% and was on average 94.4% (95% CI: 90.9% – 97.9%). There was also good to fair level of evidence on the role of HPV DNA-Based test in post-treatment follow-up of patients after therapeutic excision of the cervix due to positive screening tests. A negative HPV DNA-Based test in the post-treatment period excluded not only the recurring CIN but also the development of persisting cytological atypia (negative predictive value (NPV): 100%) a negative HPV DNA-Based test eliminates the risk of recurrent disease after treatment for CIN. In a positive HPV DNA-Based test, this may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity.

There was fair level of evidence with the assumption that the countries (South Africa, Thailand and Peru) in the studies mentioned represent almost the same resource setting as Malaysia suggesting cost effective options in Malaysia. The studies showed that the ICER was lower than the GDP per capita in Malaysia which was noted to be about USD 5151 in 2008 (According to the International Commission on Macroeconomics and Health guidelines, interventions with an ICER between one and three times gross domestic product (GDP) per capita are considered cost effective). The four strategies derived from four different studies were:

- Using HPV DNA testing every five years as a screening strategy in Colombia (Gamboa A *et al*). The ICER was USD$44 in Colombia.

- A single life time screening with HPV DNA testing coupled with immediate cryotherapy once with positive results of HPV (Goldie S J *et al*). The ICER was less than $62 in South Africa.

- With at least 1 visit screening with HPV DNA once in a lifetime was the most cost effective strategies (Goldie S J *et al*). The ICER was USD$467 for South Africa, USD $170 for Thailand and USD $152 for Peru.

- Conventional cytology followed by HPV triage for equivocal cytology was the most cost effective strategy (Vijayaraghavan A *et al*). The ICER was USD$409 for South Africa

Beyond the cost issues that arise out of unnecessary testing, another point to consider is the physical discomfort and anxiety that a woman suffers in anticipation of an often unnecessary, invasive procedure. HPV testing may have an adverse psychosocial impact on women who test HPV positive when it is used as a primary screening test alongside conventional cytology. Consideration of the psychosocial consequences of HPV testing is important.

Health care planners who are considering implementing any type of cervical cancer screening must develop clinical protocols that are responsive to the natural history of cervical disease, the diagnostic characteristics of the screening technology, disease prevalence in the target population, and the Malaysian needs and concerns.

As recommended by the Cytopathology Education and Technology Consortium, and endorsed by American Society for Clinical Pathology (ASCP), the American Cancer Society, and several other professional medical societies, HPV DNA-based testing should be used only for high-risk HPV types and co-testing with the Pap test in women over 30 years of age provides predictive safety for at least three years in women who are negative on both tests

9 RECOMMENDATION

Based on the above review, HPV DNA-based testing may be incorporated in the cervical screening program. HPV DNA-based testing may be done every five years as a primary screening strategy or combined with Pap test in women over 30 years of age for an interval / frequency of at least three to five years in women who are negative on both tests in the annual screening. Although HPV DNA-based test is expensive (about RM 91.50- RM183 while Pap smear costs about RM 14.16 per test), it has higher sensitivity than Pap smear. For the primary screening strategy, it is suggested that HPV DNA-based testing may be done every five years since the test is expensive for the moment.

Alternatively a single life time screening using HPV DNA-based test was one of the most cost effective strategies carried out in South Africa, Thailand and Peru which Malaysia may emulate. However, local economic evaluation and research should be conducted with due consideration for our Malaysian healthcare systems as well as local costing that will further provide more evidence to support the above strategies.

HPV DNA-based test can be used to triage patients for atypical squamous cells of undetermined significance (ASCUS) in women aged 35 or older, whereby these women will undergo HPV DNA-based testing after conventional cytology. This strategy is recommended since it has been shown that this strategy is less expensive and more effective with higher specificity than screening using repeated cytology alone.

HPV DNA-based testing may be recommended as a follow up screening for post treatment cases since HPV DNA-based test easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. A negative HPV DNA-based test in the post-treatment period eliminates the risk of recurrent disease after treatment for CIN while a positive HPV DNA-based test, may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity.

A standard guideline needs to be developed for cervical cancer screening and management of abnormal findings if HPV DNA-based testing is adopted as a screening test for cervical cancer screening in Malaysia. Organisational issues such as training, manpower, good referral system, and funding need to be addressed at all levels.

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Appendix 1

HIERACHY OF EVIDENCE FOR EFFECTIVENESS STUDIES DESIGNATION OF LEVELS OF EVIDENCE

I Evidence obtained from at least one properly designed randomized controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

Appendix 2

HEALTH TECHNOLOGY ASSESSMENT (HTA) PROTOCOL
HPV DNA-BASED SCREENING TEST FOR CERVICAL CANCER

1. BACKGROUND INFORMATION

Cancer of the uterine cervix is a leading cause of mortality and morbidity among women worldwide. In developing countries it is the most common gynaecological cancer and one of the leading causes of cancer deaths amongst women. In 2003, cervical cancer was reported to be the second most common cancer among Malaysian women. The overall age-standardized incident rate (ASR) of cervical cancer in Malaysia was 19.7% per 100,000 populations.

Research worldwide has clearly shown that virtually all cervical cancer is caused by human papilloma virus (HPV) infection. HPV is a sexually transmitted infection (STI) that is very common among young men and women. Women persistently infected with certain carcinogenic types are at increased risk of developing severe dysplasia and cervical cancer.

HPV is a double-stranded DNA virus. The virus is transmitted to the cervix and vaginal tissues primarily by sexual intercourse. HPV can infect and persist in vulvar, vaginal, and cervical tissue throughout a lifetime. This family of viruses includes those responsible for genital condylomata or warts, squamous cell carcinomas of the genital tract including vaginal and vulvar cancers, and cervical cancer. There are over 50 viral types of HPV that infect the genital tract but only a small portion appears to cause most cervical neoplasias and cancers. Of the 15 to 20 types associated with cervical cancer, a worldwide study determined that four types; 16, 18, 31, and 45 accounted for 80 percent of cervical cancers. Other types identified as high-risk are 33, 35, 39, 51, 52, 56, 58, 59, and 68.

To promote cervical cancer abnormalities, the virus must become integrated into the host genomic DNA. This event, which is essential for cancer progression, appears to be rare. In the absence of viral integration, the normal viral lifecycle produces morphologic changes in the cervical epithelium characteristic of low-grade dysplasia (LSIL). With viral integration, the oncogenic effect of the E6 and E7 proteins is enhanced and cellular changes characteristic of high-grade dysplasia and ultimately cancer are observed. Inter-related host factors such as age, nutritional status, immune function, smoking, and possibly silent genetic polymorphisms modulate incorporation of viral DNA.

HPV infection is most common in younger women. Although prevalence varies among regions, it reaches a peak of at least 20 percent among women between the ages of 20 and 24 years of age, with a subsequent decline to approximately three percent among women over 30 years of age. Despite a decline in HPV prevalence among women over the age of 25 years, the risk for cervical cancer increases until women reach their fifties, probably due to risks associated with persistent HPV infection. Women over 30 years of age who are infected with high-risk HPV may be up to 116 times more likely to develop severe dysplasia than similar, uninfected women.

Screening programs for cervical cancer have been instituted in developed countries for decades and over a period of time have been shown to be effective in reducing the overall mortality from this disease. Such programs however can only be made to work provided the necessary infrastructure and funds are available.

1.1 Technology description

Generally, the cervical cancer screening programs have relied on cytological testing using the Papanicolaou (Pap) smear test which has well recognized limitations. A variety of screening tests have therefore been developed in an attempt to overcome the innate limitations of conventional cytology. HPV cannot be cultured reliably in a laboratory setting; therefore, HPV diagnostics rely on molecular technologies that detect HPV DNA in cervical/vaginal samples. There are various techniques available for HPV-DNA testing of which the Hybrid capture II assay and Polymerase chain reaction are the most common techniques.

Study involves literature review on the effectiveness, safety and cost effectiveness of HPV DNA-based screening test currently used in medical practice.

2. POLICY QUESTION

a) Is HPV DNA-based test suitable as a primary screening test for cervical cancer?
b) What is the role of HPV DNA based test in clinical management

3. OBJECTIVE

To undertake a systematic review on the safety, effectiveness/efficacy (diagnostic accuracy), cost effectiveness, social, organizational and legal implications of using HPV DNA-based screening test as a primary screening test for cervical cancer and as a tool for clinical management for cervical cancer.

4. METHODOLOGY

4.1 Search Strategy

Electronic database will be searched for published literatures pertaining to HPV DNA based screening test for cervical cancer. The following sources will be searched:

i. Databases as follows: MEDLINE, Pubmed, EBM Reviews – Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, HTA Database EBM Reviews – NHS Economic Evaluation Database, EBM Full Text- Cochrane DSR, ACP journal Club and DARE.

ii. Google will be used to search as additional web-based information

iii. Additional articles will be identified from reviewing the bibliographies of retrieved articles.

4.2 Inclusion and exclusion criteria

Inclusion criteria

i. Study design: experimental studies, cross sectional, cohort, case control, randomized control trials and systematic reviews

ii. Population: female with sexual exposure.

iii. Setting: all healthcare setting

iv. Intervention: HPV DNA-based screening test using either Hybrid Capture 2 (HC2) or Polymerase Chain Reaction (PCR), type specific DNA test,

v. Comparators: cytology test (conventional smears, liquid base) , Visual inspection with acetic acid (VIA), visual inspection with Lugol's iodine (VILI)

vi. Outcomes:

- Primary outcome: sensitivity, specificity, positive and negative predictive value (diagnostic accuracy)
- CIN2+(surrogate marker), mortality reduction, incidence, quality of life
- Adverse events, safety

vii. Articles from year

Exclusion criteria

Based on these inclusion criteria, study selection will be carried out independently by two reviewers. Disagreements will be resolved by discussion. A third person, whose decision is final, will be consulted when disagreements persists after discussion.

4.3 Data extraction strategy

The following data will be extracted:

- Details of methods and study population characteristics
- Details of intervention and comparator
- Details of individual outcomes for effectiveness, safety, cost effectiveness
- Details of social and legal implications related to use of HPV DNA-based screening

Data will be extracted from included studies by a reviewer using a pre-defined data extraction form and checked by another reviewer. Disagreements will be resolved by discussion. A third person, whose decision is final, will be consulted when disagreements persists after discussion.

4.4 Quality assessment strategy

The methodological quality of all relevant articles will be assessed by using Critical Appraisal Skills Programme (CASP) depending on the type of study design. Quality assessment will be conducted by a reviewer and checked by a second reviewer.

4.5 Methods of analysis / synthesis

Data on clinical effectiveness, safety, and cost effectiveness will be presented in tabulated format with narrative summaries. A decision on whether to pool efficacy, safety and accuracy outcomes will be taken following the updated search and based on clinical and statistical heterogeneity and the range of outcome measures reported. Data will be pooled using fixed model unless statistical heterogeneity between studies is found, in which case random effect model will be used.

5. Report writing

Appendix 3

SEARCH STRATEGY

SEARCH TERMS

papillomavirus infections, papilloma virus, human papillomavirus type 11-13, human papillomavirus type 16, human papillomavirus type 18, human papillomavirus type 31, 45, hpv, papilloma$, ASCUS, ASC-US, ASC-H, LSIL, HSIL, papillomavirus human, , invader HPV, p16, cervical intraepithelial neoplasia, squamous intraepithelial, squamous intra-epithelial, cin-1, cin-2, cin-3, cin1, cin2, cin3, dyskaryosis, low grade, high grade, cervical cancer, cervix neoplasma, cervix uteri, cervix dysplasia, atypical squamous cells, squamous intraepithelial or squamous intra-epithelia, atypical squamous cells, dyskaryotic, dyskaryosis, uterine cervical neoplasms

HPV DNA based testing, polymerase chain reaction, amplicor, roche, hybrid capture, real time assay, screening assay, surepath, thinprep, GenID, Hybrid Capture™ assays , Hybrid Capture-II (HC2)cytodiagnosis, vaginal smear, papsmear (conventional / liquid base cytological smear); VIA (visual inspection acetic acid); visual inspection lugol's Iodine, Papanicolaou (Pap) test, co-testing

sensitivity, specificity, predictive value of tests, false negative reactions, false positive reactions, likelihood functions, roc curve, reference standards, diagnosis, positive result, predictive validity, senstiv$, specific$, comparative, comparison accuracy, ppv, npv, positive predictive value, negative predictive value, likelihood ratio$, accuracy, diagnostic error; false negative reactions; or false positive reactions, reference standard, CIN 2+, mortality, incidence rate, quality of life, adverse event , safety, Mass screening, cervical screening, primary screening test, triage test, follow-up test, Cost, cost effective, cost benefit, "Client-Participation", acceptability or acceptance, "Stress, Psychological" psychological aspect, social, legal, organizational

1. MEDLINE (OVID) 1950 to August Week 2 2010

01 - ("papillomavirus infaction" or "papilloma virus" or "human papillomavirus type 11" or "human papillomavirus type 12" or "human papillomavirus type 13" or "human papillomavirus type 16" or "human papillomavirus type 18" or "human papillomavirus type 31" or "human papillomavirus type 45" or hpv or papillomavirus$ or ASCUS or ASC-US or ASC-H or LSIL or HSIL)

02 - (hpv or papillomavirus$ or ASCUS or ASC-US or ASC-H or LSIL or HSIL).

03 - ("papillomavirus human" or "invander HPV" or p16 or "cervical interaepithelial" or neoplasia or "squamaus intrapithelial" or "squamous intra-epithelial").

04 - (cin-1 or cin-2 or cin-3 or dyskaryosis or "low grade" or "high grade" or "cervical cancer" or "cervix neoplasma" or "cervix uteri" or "cervix dysplasia").

05 - ("atypical squamous cells" or "squamous intraepithelial" or "squamous intra-epithelia" or "uterine cervical neoplasms").

06 - ("HPV DNA base testing" or "polymerase chain reaction" or amplicor or cyctcy or roche or "imaging system").

07 - ("hybrid capture" or biotools or "realtime assay" or surepath or thiprepn or ginID or "hybrid capture assays" or hybrid caputre-II)

08 - (cytodiagnosis or "vaginal smear" or "papsmear conventional" or "papsmear liquid base cytological" or VIA or "visual inspection lugol's" or Iodine or "papanicolaou test" or co-testing)

09 - (sensitivity or specificity or "predictive value of test" or "false negative reactions" or "false positive reactions" or "likelihood functions" or "roc curve" or "reference standards" or diagnosis)

10 - ("positive result" or "predictive validity" or senstiv$ or specifics$ or comparative or "comparison accurancy" or ppv or npv or "positive predictive value" or "negetive predictive value" or "likelihood ratio$").

11 - (accuracy or "diagnosis error" or "cin 2+" or mortality or "incidence rate" or "quality of life" or "adverse event" or safety or "mass screening" or "cervical screening" or "primary screening test" or "triage test" or "follop-up test" or cost or "cost effective" or "cost benefit").

2. PubMed – August 2010

01 - ("papillomavirus infection" OR "papilloma virus" OR "human papillomavirus type 11-13" OR "human papillomavirus type 16" OR "human papillomavirus type 18" OR "human papillomavirus type 31" OR "human papillomavirus type 45")

02 - (hpv OR papillomavirus$ OR ASCUS OR ASC-US OR ASC-H OR LSIL OR HSIL)

03- ("papillomavirus human" OR "invader HPV" OR p16 OR "cervical interaepithelial neoplasia" OR "squamous intrapithelial" OR "squamous intra-epithelial")

06 - (cin-1 OR cin-2 OR cin-3 OR cin-3 OR dyskaryosis OR "low Grade" OR "high grade" OR "cervical cancer" OR "cervix neoplasma" OR "cervix uteri" OR "cervix dysplasia")

07 - ("atypical squamous cells" OR "squamous intraepithelial" OR "Squamous intra-epithelia" OR dyskaryosis OR "uterine cervical neoplasms")

08 - #1 OR #2 OR #3 OR #6 OR #7

09 - ("HPV DNA base testing" OR "polymerase chain reaction" OR amplicor OR cyctcy OR roche OR "imaging system")

10 - ("hybrid capture" OR biotools OR "real time assay" OR surepath OR thinprep OR GenID OR "hybrid capture assays" OR "hybrid capture-II")

11 - #9 OR #10

12 - (cytodiagnosis OR "vaginal smear" OR "Papsmaer conventional" OR "papsmear liquid base cytological smear" OR VIA OR "visual insoectionn lugol,s Iodine" OR "papanicolaou test OR co-testing)

13 - sensitivity OR specificity OR "predictive value of tests" OR "false negative reactions")

14 - (sensitivity OR specificity OR "predictive value of test" OR "false negative reactions" OR "false positive reactions" OR "likelihood fuctions" OR "roc curve" OR reference standards OR diagnosis)

15 - ("positive result" OR "predictive validity" OR comparative OR "comparison accurancy" OR ppv OR npv OR "positive predictive value" OR "negative predictive value")

16 - ("likelihood ratio$" OR accuracy OR "diagnosis error" OR "CIN 2+" OR mortality)

19 - ("incidance rate" OR "Quality of life" OR "adverse events" safety OR "Mass screening" OR "cervical screening")

20 - ("primary screening test" OR "triage test" OR " follow-up test OR cost OR "cost effective" OR "cost benefit")

21 - #13 OR #14 OR #15 OR #16 OR #19 OR # 20

22 - #8 AND #11 AND #12 AND #21

23 - #8 AND #11 AND #12 AND #21 Limits: Humans, Female

3. EBM Reviews – Cochrane Database of Systematic Review (OVID)

01 – Hpv DNA test for screening
02 – Hpv DNA based screening
03 – Hpv DNA testing
04 – Human papillomavirus

4. EBM Reviews – Cochrane Central Registry of Controlled Trials

01 – Hpv DNA test for screening
02 – Hpv DNA based screening
03 – Hpv DNA testing
04 – Human papillomavirus

5. EBM Reviews – DARE

01 – Hpv DNA test for screening
02 – Hpv DNA test
03 – Hpv DNA screening
04 – Hpv DNA test screening and testing

6. EBM Reviews – NHS

01 – Hpv DNA test for screening
02 – Hpv DNA test
03 – Hpv DNA screening
04 – Hpv DNA test screening and testing

7. HTA

01 – Hpv DNA test for screening
02 – Hpv DNA test
03 – Hpv DNA screening
04 – Hpv DNA test screening and testing

Appendix 4

Screening criteria
The Wilson-Jungner criteria for appraising the validity of a screening programme

1. The condition being screened for should be an important health problem
2. The natural history of the condition should be well understood
3. There should be a detectable early stage
4. Treatment at an early stage should be of more benefit than at a later stage
5. A suitable test should be devised for the early stage
6. The test should be acceptable
7. Intervals for repeating the test should be determined
8. Adequate health service provision should be made for the extra clinical workload resulting from screening
9. The risks, both physical and psychological, should be less than the benefits
10. The costs should be balanced against the benefits

World Health Organisation 1968

Criteria for appraising the viability, effectiveness and appropriateness of a screening programme 2003

The condition

1. The condition should be an important health problem.
2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.
3. All the cost-effective primary prevention interventions should have been implemented as far as practicable.
4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

The test

5. There should be a simple, safe, precise and validated screening test.
6. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed.
7. The test should be acceptable to the population.
8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.
9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested for, should be clearly set out.

The treatment

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.
11. There should be agreed evidence-based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.
12. Clinical management of the condition and patient outcomes should be optimised in all healthcare providers prior to participation in a screening programme.

The screening programme

13. There should be evidence from high-quality randomised controlled trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an 'informed choice' (for example, Down's syndrome and cystic fibrosis carrier screening), there must be evidence from high-quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.
14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/intervention) is clinically, socially. and ethically acceptable to health professionals and the public.
15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).
16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (ie value for money).
17. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.
18. Adequate staffing and facilities for testing, diagnosis, treatment, and programme management should be available prior to the commencement of the screening programme.
19. All other options for managing the condition should have been considered (for example, improving treatment and providing other services), to ensure that no more cost-effective intervention could be introduced or current interventions increased within the resources available.
20. Evidence-based information, explaining the consequences of testing, investigation, and treatment, should be made available to potential participants to assist them in making an informed choice.
21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.
22. If screening is for a mutation, the programme should be acceptable to people identified as carriers and to other family members.

http://www.gp-training.net/training/tutorials/management/audit/screen.htm.

Appendix 5

Evidence Table : HPV DNA screening for Cervical cancer

Question : Is HPV DNA - based test effective?

Bibliographic citation	1) Mayrand MH, Franco ED, Rodrigues I, et al. Human papillomavirus DNA versus Papanicolaou screening tests for cervical cancer. NEJM. 2007; 357(16): 1579-1588
Study type	Randomized control trial Compare HPV DNA testing with conventional Pap testing as a screening method to identify high-grade cervical intraepithelial neoplasia
LE	I
Number of patients & Patient characteristics	10 154 women aged 30 to 69 years randomly assigned to a)'focus on Pap' – index test is pap smear b) 'focus on HPV' – index test is HC2
Intervention	Hybrid capture 2, conventional pap smear
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	Conservative definition: Sensitivity: 55.4% (Pap), 94.6% (HPV) ; P=0.01 Specificity : 96.8% (Pap), 94.1% (HPV); P<0.001 Liberal Definition: Sensitivity: 43.4% (Pap), 45.9% (HPV) ; P=0.01 Specificity : 96.9% (Pap), 94.2% (HPV); P<0.001 The sensitivity of both tests used together was 100%, and the specificity was 92.5%.
General comments	

Bibliographic citation	2)Arbyn M, Sasieni P, Meijer CJLM, et al. Chapter 9: Clinical applications of HPV testing: A summary of Meta –Analyses. *Vaccine*. 2006; 24S3: 78-79
Study type	Meta Analyses
LE	I
Number of patients & Patient characteristics	Systematic review and meta-analysis Only studies relating to unselected populations and using either biopsy or surgery as the reference standard were included. 22,000 patients (from 14 studies) MEDLINE search from 1983 to 1995 Methodological aspects of all the studies were assessed using a list of criteria proposed by the Cochrane Methods Working group on meta-analysis of diagnostic and screening tests.
Intervention	
Comparison	
Length of follow up	

Outcome measures/ Effect size	To see on the performance of HPV DNA testing on three possible clinical application: i)triage of women with equivocal or low-grade cytological abnormalities; ii)prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (CIN) lesions iii)primary screening for cervical cancer and pre cancer **Result for Triage of minor cytological lesions** **Result of low grade** On average, in 9.7% (95% CI: 7.7–11.7%) and 4.3% (95% CI: 2.7–5.9%) of cases, underlying CIN-2+ or CIN-3+ was found. • Overall, HC2 had a sensitivity of 92.5% (95% CI: 90.1–94.9%) and 95.6% (95% CI: 92.8–98.4%) for detecting respectively CIN-2+ or CIN-3+. • The pooled specificity was 62.5% (95% CI: 57.8–67.3%) when the outcome was CIN-2+ and 59.3% (51.2–67.4%) for CIN-3+. • The sensitivity of HC2 triage of women with an index smear showing LSIL was very high: 97.2% (95% CI: 95.6–98.9%), pooled from 10 studies for the outcome of CIN-2+ and 97.0% (95% CI: 93.9–100%), pooled from five studies for CIN-3+ . • However its specificity was very low: 28.6% (95% CI: 22.2–35.0%) for CIN-2+ and 21.6% (95% CI: 16.6–26.6%) for CIN-3+. • Histologically confirmed CIN-2+ and CIN-3+ were present in, 18.8% (95% CI: 1.24–25.2) and 9.2% (95% CI: 7.0–11.4) respectively. **Results for Follow up treatment of cervical intraepithelial neoplasia** • Sixteen studies were identified that matched inclusion criteria. Studies were heterogeneous with respect to design, timing of visits, choice of HPV testing methods and the assessment of disease status at entry and end of follow-up. • Treatment failure expressed in terms of residual or recurrent CIN, occurred on average in 10.2% (95% CI: 6.7–13.8) of treated cases. • The sensitivity of HPV-DNA detection in predicting treatment failure ranged from 67% to 100% and was on average 94.4% (95% CI: 90.9–97.9%). • The specificity of HPV testing for predicting treatment success was statistically very heterogeneous among studies and varied between 44% and 100%. **Results of Primary screening** • Overall, the sensitivity of HC2 was 23% (95% CI: 13–23%) higher. The pooled specificity of HC2 was overall 6% lower than cytology • The combination of cytology with HC2 was respectively 45% (95% CI: 31–60%) and 39% (95% CI: 11–73%) higher for the detection of respectively CIN-2+ or CIN-3+ than cytology alone (at cut-off ASCUS+), whereas the specificity was 7% lower (95% CI: 6–8%). • Adding a Pap smear to the HC2 test and considering ASCUS or worse as a positive cytological result increasedthe sensitivity of HC2 for CIN-2+ or CIN-3+ with 7% and 4%, respectively, but resulted in a loss in specificity of 5% (95% CI: 4–6%) and 7% (95% CI: 5–9%). Conclusion: • Consistent evidence is available indicating that HPV-triage with the Hybrid Capture-2 assay (HC2) is more accurate (significantly higher sensitivity, similar specificity) than repeat cytology to triage women with equivocal Pap smear results. • When triaging women with low-grade squamous intraepithelial lesions (LSIL), a reflex HC2 test does not show a significantly higher sensitivity, but a significantly lower specificity compared to a repeat Pap smear. • After treatment of cervical lesions, HPV testing easily detects (with higher sensitivity and not lower specificity) residual or recurrent CIN than follow-up cytology. • The evidences showed that HPV testing in triage of women with atypical cytology and in surveillance after treatment of CIN lesions may be recommended.
General comments	

Bibliographic citation	3) Arbyn M, Sankaranarayanan R, Muwonge R, et al. Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India. *Int J. Cancer*. 2008; 123: 153-160
Study type	Meta analyses
LE	I
Number of patients & Patient characteristics	58,679 24510 eligible women (18386 in revealed group and 6124 assigned the the concealed group) Characteristic of the patient: Women aged 25 to 64, from 11 urban settings in India and Africa
Intervention	HPV (HC2), VIA,VILI,VIAM, pap smear
Comparison	Colposcopy
Length of follow up	N/A
Outcome measures/ Effect size	**Accuracy test of VIA:** • Pooled sensitivity was 79.2%, Pooled specificity was 84.7% • Showed a sensitivity of 79% (95% CI 73–85%) and 83% (95% CI 77–89%), and a specificity of 85% (95% CI 81–89%) and 84% (95% CI 80–88%) for the outcomes CIN2+ or CIN3+, respectively. **Accuracy test of VILI:** • The overall pooled sensitivity for VILI (91.2%; CI 87.8–94.6%) was statistically significantly higher (about 10%) than for VIA. On the other hand, the pooled specificity of VILI (84.5%; CI 81.3–87.8%) was not significantly different from that of VIA. VIAM showed similar results as VIA. The Pap smear showed lowest sensitivity, even at the lowest cutoff of atypical squamous cells of undetermined significance (57%; 95% CI 38–76%) for CIN2+ but the specificity was rather high (93%; 95% CI 89–97%). The HC2- assay showed a sensitivity for CIN2+ of 62% (95% CI 56–68%) and a specificity of 94% (95% CI 92–95%). Substantial interstudy variation was observed in the accuracy of the visual screening methods
General comments	Limitation: - Inclusion and exclusion criteria not mentioned. Heterogeneity test not available

Bibliographic citation	4) Lytwyn A, Sellors JW, Mahony JB et al. Comparison of human papillomavirus DNA testing and repeat Papanicolaou test in women with low-grade cervical cytologic abnormalities: a randomized trial. *CMAI* 2000;163(6):701-707
Study type	Randomised control trial.
LE	I
Number of patients & Patient characteristics	212 women aged 16-50 years with ASCUS or LSIL on cervical cytology screening It is a pragmatic management trial, with women recruited and managed in, primary care practices.
Intervention	Immediate HPV DNA testing using hybrid Capture II assay or a repeat Pap test in 6 months.
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	1. HPV DNA testing - detected 87.5% (7/8) of the cases of CIN 2 or 3, Repeat Pap smear detected 11.1% (1/9) of cases high-grade intraepithelial neoplasia (HSIL) (p = 0.004), and ASCUS, LSIL or HSIL detected 55.6% (5/9) ASCUS, LSIL or HSIL (p = 0.16). 2. Sensitivities: HPV DNA test - 87.5 % (47.4-99.7) (p = 0.004) Repeat Pap smear - 55.6(21.1-86.3) (p = 0.16). 3. Specificities: HPV DNA test - 50.6% (39.1-62.1) (p =0.002) Repeat Pap smear - 55.6% (42.5-68.1) (p = 0.61). 4. Loss to follow-up (failed to present for colposcopy) was 17.1% in the HPV test group and 32.7% in the repeat Pap group (p = 0.009). 5. In the HPV group 46/87 women (52.9%) were HPV positive. In the repeat Pap test group 29/72 women(40.2%) had ASC US or LSIL and 4/72 (5.6%) had HSIL 6. Given the 7 cases of CIN 2 or 3 detected by HPV testing and the 5 cases detected by the repeat Pap smear, the incremental cost of HPV testing was calculated to be $3003 per additional case of CIN identified. Interpretation: 1. the results can be generalizable to a primary care setting 2. immediate testing for oncogenic HPV detected significantly more histologically confirmed cases of CIN 2 or 3 than did repeat Pap smear showing HSIL performed at 6 months. 3. improved sensitivity of immediate HPV DNA testing compared with repeat Pap HPV testing was more costly than delayed Pap test.but was associated with significantly less loss to follow-up. It may detect more cases of CIN 2 or 3 in women with low-grade cytologic abnormalities.
General comments	

Bibliographic citation	5) Gravitt PE, Schiffman M, Solomon D et al. Comparison of Linear Array and Hybrid Capture 2 for Detection of Carcinogenic Human Papillomavirus and Cervical Precancer in ASCUS-LSIL Triage Study. Cancer Epidemiol Biomarkers Prev. 2008; 17(5): 1248–1254
Study type	Cross sectional (screening/ diagnostic type of study)
LE	II-2
Number of patients & Patient characteristics	Linear Array (LA) and HC2 results were compared on baseline specimens collected from women with an atypical squamous cells of undetermined significance (ASCUS) Pap referred into ASCUS and Low-Grade Squamous Intraepithelial Lesion Triage Study (n = 3,488). HC2 was conducted at the time of the study on liquid cytology specimens. LA was conducted retrospectively on aliquots from a second, stored cervical specimen masked to the hc2 results and clinical data. Paired LA and HC2 results (n = 3,289; 94%), were compared for the detection of carcinogenic HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 2-year cumulative cervical intraepithelial neoplasia (CIN) grade ≥3 as diagnosed by the quality-control pathology review.
Intervention	HPV DNA testing using the hybrid capture 2 (HC2) assay
Comparison	cytology testing, VIA, and VILI
Length of follow up	
Outcome measures/ Effect size	1. Restricting the analyses to paired results, LA was more likely to test positive for carcinogenic HPV than HC2 (55% versus 53%; P = 0.001), with a percent agreement of 84%, a percent positive agreement of 74%, and a k of 0.68. 2. For 2-year cumulative ≥ CIN3, LA and HC2 had similar sensitivities (93.3% versus 92.6%, respectively; P = 1), and LA was marginally less specific than HC2 (48.1% versus 50.6%, respectively; P = 0.05). 3. LA and HC2 had similar negative predictive values (98.70% versus 98.64% respectively; P = 0.4), and LA had a slightly lower positive predictive value than HC2 (14.6% versus 15.1%, respectively; P < 0.0001). Conclusion: LA and HC2 performed similarly in the detection of carcinogenic HPV and identification of CIN3 among women with an ASCUS Pap with CIN 3 lesions.
General comments	

Bibliographic citation	6) Lee GY, Kim SM, Rim SY, et al. Human papillomavirus (HPV) genotyping by HPV DNA chip in cervical cancer and precancerous lesions. *Int J Gynecol Cancer*. 2005; 15: 81-87
Study type	Cross sectional (screening/ diagnostic type study) Study done on women referred to O&G dept due to abnormal cervical cytology or cervicogram. First clinical test performed was cytology, followed by HPV test, colposcopy and biopsy. The results of each test were compared with results of histological diagnosis (Bethesda System).
LE	II-2
Number of patients & Patient characteristics	400 patients
Intervention	Hybrid Capture 2, HPV DNA chip(HD-C), Cytology, ThinPrep cytology
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	Rates of HPV positivity (positive predictive value) in the HC-II test & HD-C test according to the histological diagnosis were: - 56.8 and 53.8% in cervicitis; - 91.5 and 91.5% for CIN I; - 88.1 and 81.0% for CIN II; - 88.6 and 84.2% for CIN III; - 92.5 and 88.7% for cancer. Most prevalent types of HPV according to the HPV tests were types 16, 58, 18, 52. Type 16 was detected in the more advanced lesions. *Sensitivity:* - 88.4% for ThinPrep cytology, - 89.9% for HC-II for the high-risk group, - 86.2% for HD-C test. *Specificity:* - 54.5% for ThinPrep cytology, - 43.2% for HC-II for the high-risk group, - 46.2% for t HD-C test.
General comments	

Bibliographic citation	7) Monsonego J, Bohbot JM, Pollini G et al. Performance of the Roche AMPLICOR Human papillomavirus (HPV) test in prediction of cervical intraepithelial neoplasia (CIN) in women with abnormal PAP smear. *Gynecol Oncol*. 2005; 99:160–168
Study type	Cross sectional (screening/ diagnostic type of study)
LE	II-2
Number of patients & Patient characteristics	270 women referred for colposcopic examination due to an abnormal PAP smear (MAPS Series), and for comparison, another series of 234 women participating in opportunistic cervical cancer screening in Paris, France (Screening Series). Both series were examined in the same clinic (Institute Alfred Fournier, IAF), during November 2004, by 2 colposcopists (JM, GP). The mean age of women was 35 years (range 18-75 years)
Intervention	Roche AMPLICOR Human papillomavirus (HPV) test – a type of PCR test
Comparison	Colposcopic biopsy and/or LEEP cone biopsy was used as the gold standard in the triage group, while liquidbased Cytology (LBC) was the reference test in the screening group.
Length of follow up	
Outcome measures/ Effect size	1. The prevalence of HPVMAPS group = (65.9%) Screening group (31.2%) (P = 0.0001). OR for being HPV positive in a MAPS patient was 4.26 (95%CI 2.936–6.202), as compared with the screening group. 2. HPV prevalence was significantly higher among women below 35 years of age (62.8%) as compared with those beyond that age (33.9%) (P = 0.0001) (OR 3.29, 95%CI 2.27–4.75). 3. There was a poor concordance between the referral PAP and the current LBC, being only moderate in the screening series, ICC (weighted kappa) = 0.291 (95%CI 0.070–0.459) (P = 0.007), and almost poor in the MAPS Series, with ICC = 0.217 (95%CI 0.04–0.384) (P =0.023). 4. AMPLICORR HPV positivity increased linearly with the increasing grade of cervical lesions in detecting high-grade (CIN2–3), whereby AMPLICOR HPV test showed a linear increase of HPV prevalence in parallel with the increasing LBC abnormality, up to 92.6% among the women with HSIL cytology (P = 0.0001 for linear trend). 5. Colposcopy was the most sensitive test (96.5%), very similar to AMPLICOR (95.2%) (P = 0.731), while LBC with HSIL cutoff was by far the most specific test (99.5%) and showed the highest PPV (96.1%). NPVof colposcopy (97.2%) and AMPLICOR (96.7%) were similar (P =0.839). 6. The Roche AMPLICOR HPV test had 95.2% (89.9–100.0) sensitivity, 42.4% (35.7–49.2) specificity, 33.7% (26.8–40.7) PPV, and 96.7% (93.0– 100.0) NPV in detecting CIN2–3 lesions among women in the MAPS Series. Interpretation: Together with abnormal colposcopy and HSIL cytology, the AMPLICOR HPV test is a powerful independent predictor of high-grade CIN2–3, and as such highly suitable as a triage tool used in the management of abnormal PAP test. However, more data are clearly needed on the performance of AMPLICOR test in studies where it is directly compared with HC2 as a triage tool, and particularly as a screening tool, based on biopsy confirmed cervical pathology as the gold standard.
General comments	

Bibliographic citation	8) Lorincz AT and Richart RM. Human Papillomavirus DNA Testing as an Adjunct to Cytology in Cervical Screening Programs. *Arch Pathol Lab Med*. 2003; 127:959–968.
Study type	Systematic review: respect to HPV DNA testing, 10 of the 12 studies used HC210,21,22,24–28,30,31 and 3 used PCR,10,20, 29 of which the Seattle study provided both HC2 and PCR data.
LE	I
Number of patients & Patient characteristics	Large screening studies of 1000 women or more that employed HC2 or PCR in a manner that allowed reliable estimates of accuracy for detecting High-grade CIN or cancer. The studies spanned a broad range of geographic, ethnic, and socioeconomic groupings, representing many of the major populations worldwide. The studies varied widely in population size, from 1365 women in Cape Town, South Africa, to 20, 810 women in Portland. Overall, the studies included more than 77, 000 women and more than 1000 cases of CIN 2 or 3, spanning 4 continents and 11 countries. The disease reference standard was CIN 2 or 3, because it allowed assessment of test performance on the basis of the ability to detect all reasonable suspicion of potentially malignant disease.
Intervention	Either the Hybrid Capture 2 (HC2) test or a polymerase chain reaction (PCR) test performed in an expert laboratory
Comparison	Cytology
Length of follow up	
Outcome measures/ Effect size	1. HPV DNA testing by HC2 had a higher sensitivity (in some cases much higher) than cytology. a. For example, in the study from Reims HC2 HPV DNA testing detected 100% of CIN 2/3, as compared to 58% for the conventional Papanicolaou test (Pap test) and 84% for the ThinPrep test. Similar cytology were seen in the studies from Newfoundland, Canada; Seattle, Wash; Morelos, Mexico; and Hannover-Tubingen, Germany b. (sensitivities for all studies range from 68-100% for HPV DNA test versus 40-86% for pap test) 2. The specificity values for HC2 HPV DNA testing were generally lower than the specificity values of the Pap test, a. (specificities for all studies range from 73-96% for HPV DNA test versus 88-99%for pap test) 3. The PPVs of the Pap test were overall a little higher than the PPVs for HPV 4. the sensitivity value for CIN 3 or higher using a combination of HPV DNA testing and cytology was greater than 90% in 6 of the 7 studies and was 100% in 3 of the 7 studies. 5. The NPVs for the combinations were above 99% for all 7 studies and were 100% in 4 of the 7 studies. Interpretation: 1. From the present review HPV DNA testing using HC2 or PCR can identify almost all patients with CIN 3 or higher. Adding a fluid-based cytology test to the HPV DNA test increases sensitivity by approximately 5%. 2. More importantly, the NPVs for the combinations were above 99% for all 7 studies and were 100% in 4 of the 7 studies. If a patient is negative for HPV DNA and has a negative Pap test, the clinician can state with reasonable certainty that “negative means negative.” 3. It seems likely, if combined HPV DNA and Papanicolaou testing is widely adopted, that the results would be salutary. False negatives would be expected to be dramatically reduced, double-negative patients could safely be screened at longer intervals (offsetting increased testing costs), and patients identified as being high risk but not having identifiable disease could be monitored closely. These outcomes would benefit patients, doctors, and the health care system. Consistent with these ideas we may want to incorporate adjunctive HPV DNA testing in women older than age 30 years at 3 yearly or longer intervals
General comments	Does not mention clearly the databases used to retrieve the published articles. For some studies the raw data were furnished by the principal investigators themselves.

Bibliographic citation	9) Kitchener HC, Almonte M, Thomson. C et al. HPV testing in combination with liquid –based cytology in primary cervical screening (ARTISTIC): a Randomised Controlled Trial. *Lancet Oncology.* 2009;10:672-682
Study type	Randomised Controlled Trial Aim of the study: To determine whether combined testing would result in a reduced incidence of high-grade disease in the second screening round compared with LBC alone
LE	I
Number of patients & Patient characteristics	25078 Characteristic of the patient: Women aged 20-64 years were recruited in GP and family –planning clinics All women had both cytology and HPV testing, were randomly assigned at a ratio of 3:1 (between July 2001 and September 2003)
Intervention	HPV testing and LBC
Comparison	LBC alone
Length of follow up	3 years (2 screening rounds 2001-03 and 2004-07)
Outcome measures/ Effect size	Results: 24510 eligible women at entry (18 386 in the revealed group and 6124 in the concealed group). Overall CIN3+ rates in round 1 were 1.27% (233 of 18386) in the revealed group, including ten CIN3+ cases in cytologically normal women, and 1.31% (80 of 6124) in the concealed group (OR 0.97, 95% CI 0.75-1.27; p>0.2). The proportion of women with CIN3+ in round 2 was 0.25% (29 of 11676) in the revealed group and 0.47% (18 of 3866) in the concealed group (OR 0.53, 95% CI 0.30 -0.96; p=0.042) But when round 1 and 2 were combined, the overall detection rates in the 2 groups of the trial were similar for both CIN3+ (1.51% revealed, 1.77% concealed (OR 0.85,95% CI 0.67 -1.08; p>0.2) Interpretation: LBC combined with HPV testing resulted in a significantly lower detection rate of CIN3+ in the second round of screening compared with LBC alone, but the effect was small. Over the 2 screening rounds combined, co testing did not detect a higher rate of CIN3+/ CIN2+ compared to LBC alone.
General comments	

Bibliographic citation	10) Gustavson I, Juko-Pecirep I, Backlund I, et al. Comparison between the Hybrid Capture 2 and the hpVIR real-time PCR for detection of human papillomavirus in women with ASCUS or low grade dysplasia. *J Clin Virol.* 2009; 45: 85-89.
Study type	Cohort Cervical smears from women diagnosed with ASCUS or CIN 1 in regular screening program were analysed using HC2 and real time PCR (after 3 months of initial screening).
LE	II-2
Number of patients & Patient characteristics	398 women in Uppsala, Sweden.
Intervention	Hybrid capture 2 & real time PCR
Comparison	
Length of follow up	3 months
Outcome measures/ Effect size	Total of 391 samples were included in study. 34% (131) of women were positive with HC2 and 45% (175) with hpVIR. HPV 16 was most common single infection. Among those with cytology available 6% (3/52) had a CIN 2. The 3% (13/391) of women positive only with *HC2* either contained low-risk HPVs or copy numbers below the cut-off for the *hpVIR* assay. *Conclusion:* The *hpVIR* assay has a similar sensitivity and specificity as *HC2*, but *hpVIR* detect a higher frequency of high-risk HPV infections Sensitivity and specificity of both assays are similar for detection of CIN 2 or more severe lesion. hpVIR higher detection rate of high-risk HPV. Specificity of HC2 is higher than hpVIR.: - **CytologyCIN 2+** : Sensitivity 85% HC2, 91% hpVIR Specificity 73% HC2, 60% hpVIR - **Cytology CIN 3** : Sensitivity 100% for both Specificity 70% HC2, 57% hpVIR
General comments	

Bibliographic citation	11) Ekalaksananan T, Pientong C, Chamsai P, et al. Usefullness of combining testing for p16 protein and human papillomavirus (HPV) in cervical carcinoma screening. Gynaecol Oncol. 2006;103: 62-66
Study type	A Prospective cohort study Objective: To investigate the utility of the simultaneous combination of testing for p16 protein and HPV DNA in screening for cervical cancer
LE	II-2
Number of patients & Patient characteristics	The study subjects of 186 women (aged 35-64) with mean of 50 years, who had an abnormal Pap smear. Most of them had cervical cell diagnosed as ASCUS and Low grade lesion who had not received definite treatment for cervical cancer.
Intervention	HPV DNA (Hybrid Capture 2)
Comparison	Combination of P16 and HPV DNA
Length of follow up	2-5 years
Outcome measures/ Effect size	Results of abnormal cervical cell screening by using Pap test, p16 protein and HPV detection • p16 was detected in 40 cases. P16 and HPV were found in all high-grade dysplasia and SCC, and in 64% and 27% of low-grade dysplasia, 62% and 0% of ASCUS and 7.4% and 3.4% of normal, respectively. Results of p16 protein detection in combination with the detection of HPV. • 18 of p16-positive cases (11%) were HPV-negative, 14 of them in the ASCUS and normal group. Results when the histological findings are compared with the cytological diagnosis on the Pap smear. • All of the 3 CIN 2 or 3 lesions were judged to have HSIL on cytology. 6/8 low grade lesions (squamous metaplasia and CIN I) had normal cytology, 19/27 subjects without dysplastic cells on biopsy. Results relation between histological diagnosis and immunocytochemical p16 staining and HPV infection in cervical cells • Compared to histological results, all of the p16-positive cases of squamous metaplasia, CIN 2 or 3 lesions and SCC were HR-HPV-positive (5 cases). Therefore, the cases that were positive for both with normal cytology (5 cases) or low-grade dysplasia (3 cases) may comprise a high-risk group for neoplastic change. conclusion: The combination of p16 and HPV detection may be useful in cervical cancer screening to identify cervical cells with minor abnormalities and a high risk of progressing to cervical neoplasia and define for those patients requiring an early management or close surveilance.
General comments	

Bibliographic citation	12) Howard M, Sellors J and Kaczorowsk J et al. Optimizing the Hybrid Capture 2 Human Papillomavirus Test to Detect Cervical Intraepithelial Neoplasia. *Obstet Gynecol.* 2002; 100: 972– 980.
Study type	Cross sectional study (screening/ diagnostic type of study)
LE	II-2
Number of patients & Patient characteristics	Women with abnormal cytology were referred for colposcopy, and a cervical swab or brush specimen was obtained for human papillomavirus testing. Sensitivities, specificities, and likelihood ratios of different relative light unit ratio cutoffs were calculated using a reference standard of colposcopy or biopsy of either CIN 2+ (CIN 2, 3, or carcinoma), or CIN I + (CIN I, CIN 2 +). The receiver operating characteristic curve was used to estimate optimal test-positive cutoff points for the hybrid capture 2 test. Women aged 16-50 years participated. Median age was 30 years.
Intervention	HPV DNA testing using the hybrid capture 2 (HC2) assay
Comparison	colposcopy or biopsy of either CIN II+ (CIN II, III, or carcinoma), or CIN I + (CIN I, CIN II +)
Length of follow up	
Outcome measures/ Effect size	The analyses were based on the 524 women for whom all relevant data were available, 324 of the 328 from the two randomized trials and all 200 women from the cross-sectional study. 1. The presence of any grade of CIN was histologically confirmed in 28.8% (151 of 524), CIN 2 or 3 was present in 18.3% (96 of 524), and squamous cell carcinoma was found in 0.4% (two of 524) of the women. 2. The area under the ROC curve was 0.82 (95% CI: 0.78 to 0.87, P <0 .001). The optimal cutoff occurred at a relative light unit ratio (relative light unit is proportional to the amount of DNA in the specimen, and hence is an estimate of viral load) test-positive cutoff of greater than or equal to 15.56. 3. CIN 2+ was found in 18.7% (98 of 524) and CIN I in 10.5% (55 of 524) of the women. The optimal relative light unit ratio was 15.56, giving a sensitivity and specificity of 82.7% and 73.2% for CIN 2+, and 74.2% and 77.8% for CIN I+ 4. In a stratified analysis, - a higher relative light unit cutoff (15.19) optimized sensitivity and specificity for CIN 2+ (sensitivity 81.8%, specificity 51.5%) for women with low-grade squamous intraepithelial lesions cytology, - whereas the optimal cutoff was 2.36 (sensitivity 100%, specificity 73.0%) for women with atypical squamous cells of undetermined significance, yielding referral rates of 53.3% and 28.7%, respectively. 5. For both the 1.0 and 15.56 relative light unit ratio cutoffs, sensitivity is lower and specificity is higher for women older than 30 years compared with women 30 years and younger. 6. Likelihood ratios tended to be higher among the older women (age >30). - Likelihood ratios were statistically significantly higher for the relative light unit ratio cutoff of 15.56 [4.46, CI (3.27, 6.07)], compared with the cutoff of 1.0 [2.61CI (2.11, 3.22)] in older women for CIN 2+ - and in all women for CIN I+ (Likelihood ratios 4.07 for cut off 15.56, and 2.42 with the cutoff of 1.0) P<0.05) Conclusion: 1. Use of a higher cutoff for the relative light unit ratio (higher viral load) of the hybrid capture II test may improve the management of women, especially those with low-grade squamous intraepithelial lesions cytology 2. A test-positive relative light unit ratio cutoff of 15.56 was the optimal cutoff for detecting both the high-grade lesions (CIN II, III, or squamous cell carcinoma) and any grade of lesion (CIN I, II, III, or squamous cell carcinoma). 3. Using this higher cutoff (corresponding to a higher viral level), in contrast to the 1.0 cutoff suggested by the manufacturer, specificity for the high-grade outcome increased by 16.4%, with a somewhat smaller reduction in sensitivity of 11.2%. It was also found that likelihood ratios were improved at the 15.56 cutoff compared with the 1.0 cutoff, reflecting the decrease in false-positive results.
General comments	

Bibliographic citation	13), Tiews S, Steinberg W, Schneider W, et al. Determination of the diagnostic accuracy of testing for high risk (HR) human papillomavirus types 16, 18 and 45 in precancerous lesions: preliminary data. J Clin Virol. 2009; 46(S3): S11-S15.
Study type	Cohort Evaluate association between HR-HPV test results and risk of concurrent CIN 2-3 lesions. HR-HPV DNA test done on all specimens at enrolment. Specimens positive with HC2 were retested with HPV 16/18/45 Probe Set Test (PST). Surrogate endpoint- diagnosis of CIN 2-3
LE	II-2
Number of patients & Patient characteristics	586 women (109 control group, 477 risk group). Mean age: 32.8 years Age range from 18 – 60 years **Risk gp**: women who demonstrated cervical lesions/ or had history of HPV infection **Ctrl gp:** women attending routine cervical cancer screening
Intervention	Hybrid capture 2
Comparison	1.5 years
Length of follow up	
Outcome measures/ Effect size	For risk gp, follow-up data was only available for 194 out of 447 women. Data was complete for control gp. At baseline: **Ctrl** - 9 of 109 (8.3%) samples were PST positive. **Risk** – 292 of 447 (65.3%) samples were PST positive At follow-up: 66.7% of CIN 2 lesion and 88.2% of CIN3 lesions were PST positive. 25% of CIN 3 lesions found in women younger than 30 years. Viral load has no influence on severity of cervical lesion
General comments	

Bibliographic citation	14) Kotaniemi-Talonen L, Nieminen P, Anttila A, et al. Routine cervical screening with primary HPV testing and cytology triage protocol in a randomised setting. Br J Cancer. 2005; 93: 862-867.
Study type	RCT
LE	1
Number of patients & Patient characteristics	Women invited to routine screening were randomly allocated to 2 groups: a) Screening arm: hrHPV test with cytology triage (7060 women) Mean age: 45.8 years b) Conventional cytology test (7089 women) Mean age: 45.9 years Age range from 30 to 60 years
Intervention	hrHPV DNA test (Hybrid Capture 2), cytology
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	- In the hrHPV arm, specificity for the sole primary screening test was 92.1% for any lesion (CIN1+), 91.7% for moderate to severe lesions (CIN2+) and 91.5% for severe lesions (CIN3+). - For the hrHPV screening with cytology triage, specificity estimates were 99.3, 98.9 and 98.7%, - For the conventional arm 99.6, 99.3 and 99.2%, respectively Primary screening with hrHPV test had better sensitivity but lower specificity than cytology. Specificity improved in hrHPV screening with cytology triage.
General comments	

Bibliographic citation	15) Bory J , Cucherousset J , Lorenzato M et al. Recurrent human papillomavirus infection detected with the Hybrid capture ii assay selects women with normal cervical Smears at risk for developing high grade cervical lesions: A longitudinal study of 3,091 women. Int. J. Cancer: 102, 519–525 (2002)
Study type	Cohort
LE	II-2
Number of patients & Patient characteristics	3,091 women with normal smears at the first entry. This population was restricted to omen who underwent their biennal or triennial routine screening in the Department of Obstetrics and Gynecology of the C.H.U. of Reims. All of these women had a cervical smear within normal limits at baseline.
Intervention	Hybrid Capture 2 (HC 2), cytology
Comparison	Diagnosis Confirmed by ciolposcopy
Length of follow up	The median follow-up was 12 months (4 to 39 months) for the 659 women with initial HR-HPV infection and 27 months (9 to 59 months) for the 2,432 women without any HR-HPV detectable at the first smear.
Outcome measures/ Effect size	Primary endpoint was clinical progression defined as the presence of a high-grade lesion (HGSIL) at the biopsy. 1. From the 659 HR-HPV-infected women, 241 (36.6%) had a positive HR-HPV test at 2 to 4 examinations with a final histological diagnosis of HGSIL in 51 cases (21.2%) within 4 to 36 months, while women with regressive HPV infection did not develop any lesion during the same period. 2. The incidence of recurrent HR-HPV infections was significantly lower ($p<0.02$) for women > 30 years old (32.9%) compared to women < 30 years old (43.3%). 3. In the cohort of 2,432 women testing negative for HR-HPV infection, only 2 women (0.08%) developed a HGSIL. Both were HR-HPV positive 18 and 24 months after the first entry, at the time of diagnosis of disease. 4. The RR of incident HGSIL when a HR-HPV was detected at enrollment in women with normal smears was 96.7 (CI, 95.8–97.7). The evaluation of the viral load of HR-HPV by the HC-II did not represent a sensitive approach to predict the recurrence of HR-HPV infection and/or the apparition of HGSIL. Interpretation: 1. A recurrent HR-HPV infection detected with HC-II represents a reliable tool to select populations at risk for the development of HGSIL. 2. At the present time, however, HPVDNA test cannot be used as a discriminating predictive parameter. 3. It has been well established that the mean infection duration for HR-HPV infection is of 13.5 months (12 months in our experience). Consequently, women with normal smears and HR-HPV infection may be controlled every 6 months with cytological examination and HPV testing until HPV infection regresses. At the same time, women with a normal smear and no HR-HPV infection have a very low risk for developing a HGSIL. In consequence, in such conditions, the use of HPV testing may allow the screening interval to be safely lengthened to 5 years with a cost-effective benefit.
General comments	

Bibliographic citation	16) Leinonen M, Nieminen P, Kotaniemi-Talonen L, et al. Age specific evaluation of primary human papillomavirus screening versus conventional cytology in a randomized setting. J Natl Cancer Inst. 2009; 101: 1612-1623.
Study type	RCT A randomized study comparing the age-specific performance of primary HPV DNA screening with that of conventional cytological screening that was incorporated into the routine screening practice of Finland.
LE	1
Number of patients & Patient characteristics	Randomized invitations were sent to women aged 25 – 65 years for routine cervical cancer screening by primary high-risk HPV DNA testing (n = 54 207) with a Hybrid Capture 2 assay followed by cytology triage for women who were HPV DNA positive or by conventional cytology screening (n = 54 218)
Intervention	HPV DNA testing (HC2), cytology
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	The overall frequency of colposcopy referrals was 1.2% in both screening arms. • Women younger than 35 years were referred more often in the HPV DNA screening vs the conventional screening arm (RR = 1.27, 95% CI = 1.01 to 1.60). • The prevalence of histologically confirmed CIN or cancer was 0.59% in the HPV DNA screening arm vs 0.43% in the conventional screening arm. • The relative rates of detection for CIN 1,CIN 2, and CIN 3+ for HPV DNA screening with cytology triage vs conventional screening were 1.44 (95%CI = 0.99 to 2.10), 1.39 (95% CI = 1.03 to 1.88), and 1.22 (95% CI = 0.78 to 1.92), respectively. • The specificity of the HPV DNA test with cytology triage was equal to that of conventional screening for all age groups (99.2% vs 99.1% for CIN 2+, $P = .13$). • Among women aged 35 years or older, the HPV DNA test with cytology triage tended to have higher specificity than conventional screening. • The PPVs for HPV DNA screening with cytology triage were consistently higher than those for conventional screening. • In both screening arms, the test specificities increased with increasing age of the women being screening, whereas the highest PPVs were observed among the youngest women being screened. • Overall, 7.2% of women in the HPV DNA screening arm vs 6.6% of women in the conventional screening arm were recommended for intensified follow-up, and the percentages were highest among 25- to 29-year-olds (21.9% vs 10.0%, respectively). Conclusions Primary HPV DNA screening with cytology triage is more sensitive than conventional screening. Among women aged 35 years or older, primary HPV DNA screening with cytology triage is also more specific than conventional screening and decreases colposcopy referrals and follow-up tests.
General comments	

Bibliographic citation	17) Nauclear P, Ryd W, Tornberg S, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst. 2009; 101: 88-89.
Study type	Cross sectional to evaluate the efficacy of 11 possible cervical Screening strategies that are based on HPV DNA testing alone, cytology alone, and HPV DNA testing combined with cytology among women aged 32 – 38 years.
LE	II-3
Number of patients & Patient characteristics	6257 women aged 32-38 years.
Intervention	HPV DNA test (PCR), cytology
Comparison	
Length of follow up	
Outcome measures/ Effect size	7.1% women were HPV DNA positive. Prevalence of HPV DNA increased with increasing cytological severity. HPV DNA & cytology – detecting CIN3+ Sensitivity : 96% (HPV), 74% (cyto) Specificity: 93.6% (HPV), 98.2% (cyto) Results of comparison of different HPV DNA based screening strategies for detecting CIN2+ and CIN3+ with that of screening by cytology only: Primary screening with HPV DNA test followed by cytological triage and screening for persistent HPV type-specific had higher sensitivity (34% and 30% increase in sensitivity to detect CIN2+ and CIN3+) without decreasing PPV. This strategy also reduced number of screening tests required per detected case of CIN2+ or CIN3+.
General comments	

Bibliographic citation	18). De Cremoux, Coste J, Sastre-Garau X, *et al.* Efficiency of the Hybrid Capture 2 HPV DNA Test in cervical cancer screening. A study by the French Society of Clinical Cytology. *Am J Clin Pathol*. 2003; 120: 492-499
Study type	Cross sectional screening /diagnostic comparative type of study Conventional pap smear performed first followed by monolayer technique. Residual sample used for HPV DNA sequencing. All women then underwent colposcopic examination. All cytologic & pathologic interpretation done in blinded condition
LE	II-2
Number of patients & Patient characteristics	1785 women with mean age 34.5 years **Gp 1** : 462 women referred for colposcopy due to abnormalities in previous screening **Gp 2**: 1323 voluntary candidates for screening
Intervention	Hybrid Capture 2 Pap smear & monolayer technique
Comparison	Confirmed by colposcopy
Length of follow up	
Outcome measures/ Effect size	Gp 1: 56.9% high risk HPV DNA, 11.9% low risk HPV DNA detected Gp 2: 16.02 % high risk HPV DNA, 5.97% low risk HPV DNA detected HPV positivity and viral DNA load increased as a function of histologic grade. Combination monolayer smear- HPV DNA had higher sensitivity & specificity compared to HPV DNA test alone Sensitivity: - 85% for combined, 79% HPV alone for gp 1 - 67% for combined, 64% HPV alone for gp 2 specificity - 82% for combined, 77% HPV alone for gp 1 - 94% for combined, 86% HPV alone for gp 2 but not superior to cytologic optimized interpretation of lesions equal or > ASCUS/AGUS (Both Gp 1 & 2) - Sensitivity 92% for gp 1, 74% for gp 2 - Specificity 80% for gp 1, 91% for gp 2 Conclusion: Sensitivity of HPV DNA test alone was lower than conventional cytology test.
General comments	

Bibliographic citation	19) .Kuhn .L, Denny.L,Pollack.A, et al Human Papillomavirus DNA testing for cervical cancer screening in Low –Resource Setting Journal of the National Cancer Institute.2000;92(10):818-825
Study type	Cross Sectional Study, (screening /diagnostic comparative type) Objective: To evaluate HPV DNA testing as an alternative screening method.
LE	II-2
Number of patients & Patient characteristics	2944 patient Aged 35-36, previously not having Paps, enrolled at a primary care clinical site in Khayelitsha, all women were volunteers and were informed well of the studies. Written consent was obtained and the women enrolled were then administered a questionnaire
Intervention	HPV (Hybrid Capture I (HCI and HC 2) assay), Pap smear, Direct visual Inspection (DVI) and Cervicography
Comparison	Confirmed by colposcopy
Length of follow up	N/A
Outcome measures/ Effect size	**HPV DNA prevalence** • High risk HPV DNA was detectable with the use of the HCI assay in 16.2% (95% CI) and high levels (>10x the positive control) were measured in 6.1% (95% CI) of 2943 women screened with the use of HCI assay. • Prevalence of HPV DNA positivity with the use of HCI assay was lowest in women aged 40-49 years. **Positive Predictive Value of HPV DNA** The positive Predictive value of detection of HPV DNA testing was 4.6% for low grade SIL or higher and 23.5% for high-grade SIL or higher. In comparison, the PPV of cytology was 58.8% for low grade SIL or higher and 31.9% for high grade SIL or higher. **Estimated sensitivity and specificity,** With the use of the HCI assay, **sensitivity of the HCI assay** for detection of high grade SIL or higher was 73.3% (95% CI=62.6% -82.2%), specificity was 87.8%(95% CI =86.6%-89.0%) **The estimated sensitivity of the HC2 assay** for detection of high grade SIL or higher was 88.4% (95% CI =76.9% -92.6%) and the estimated specificity was 81.9% (95% CI=76.5% -86.5%) **The estimated sensitivity of cytology** for detection of high grade SIL or higher was 78.3% (95% CI = 67.9% -86.6%) and the estimated specificity was 96.8% (95% CI =96.1 -97.4%) ROC Curves • The area under the ROC curve (higher values indicate better overall performance) was 0.88 for the HC2 assay and 0.83 for the HCI assay. • At an estimated specificity of 95% (i.e., when the HPV DNA prevalence was 5% in women at low risk for having cervical disease), either test could achieve estimated sensitivities of 57% to identify women with high-grade SIL or cancer. conclusion: • The specificity of cytology was significantly better than either the HCI assay (P<.01) or the HC2 assay (P<.01) at standard cut-off value. • HPV DNA testing has a sensitivity equivalent to, or better than that of cytology. Since HPV DNA testing programs may be easier to implement than cytologic screening, HPV testing should be considered for primary cervical cancer screening in low –resource setting. • HPV DNA testing with the HC2 assay was more sensitive than cytology for detecting high-grade SIL and invasive cancer
General comments	Limitations of the particular study : i)Criterion standard for detection of cervical disease i.e colposcopy followed by pathology diagnosis was not applied to all study participants ii)Colposcopy was not performed in women with four negative screening tests (verification bias) Authors from his study, commented that in many settings, it has proven easier to establish clinical laboratories for large scale HPV DNA than to establish high-quality cytology laboratories. HPV –DNA testing requires less skilled technicians and it is easier to perform than cervical cytology and therefore it may be more feasible to set up HPV DNA testing on site. Furthermore, he suggested that in low resource setting, HPV DNA testing identifies not only women who currently have high grade cervical disease but for women who are at greatest risk of developing the disease in the future.

Bibliographic citation	20) De Vuyst H, Claeys P, Njiru S, et al. Comparison of pap smear, visual inspection with acetic acid, human papillomavirus DNA-PCR testing and cervicography. *Int J Gynecol Obstet.* 2005; 89:120 -126
Study type	Cross –Sectional Studies (screening/ diagnostic type of study) Objectives: This study was designed to compare the test qualities of alternative screening methods for the detection of CIN and invasive cervical cancer which could be used in poor resource regions.
LE	II-2
Number of patients & Patient characteristics	Between January 1998 and July 2000, non pregnant women aged 25-55 attending a family planning clinic in Nairobi, Kenya. Of the 816 women presenting at Visit 1, 653 attended the clinic for visit 2 and were included in the study. The first eight consecutive clients presenting at the family planning clinic were invited to participate in the study. The study nurse was blinded to the clinical background (referred patients or women attending for family planning). All examiners interpreting pap smears, HPV DNA PCR, or cervicographies were blinded to the clinical background and to other screening test results
Intervention	HPV DNA test
Comparison	Pap smear, visual inspection with VIA and cervicography,
Length of follow up	N/A
Outcome measures/ Effect size	For cases of CIN2 or worse: • Pap smear showed a sensitivity of 83.3% and a specificity of 90.3% • VIA showed a sensitivity of 73.3% and a specificity of 77.6% • HPV(any type) showed a sensitivity of 96.3% (the highest sensitivity occurred) but with the lowest specificity of 55.8% (with the rate of false positives was considerably higher) • But HPV HR with sensitivity of 94.4% (not much different from the HPV but it did increase the specificity of 69.3% • Cervicography with a sensitivity of 72.3% and specificity of 90.5% • Sensitivity (for CIN2 or higher) and specificity (to exclude any CIN or cancer) were • 83.3% (95% CI: 73.6% - 93.0%) and 94.6% (95% CI: 92.6% - 96.5%), respectively, for pap smear; • 73.3% (95% CI: 61.8% - 84.9%) and 80.0% (95% CI: 76.6% - 83.4%) for VIA; • 94.4% (95% CI: 84.6% to 98.8%) and 73.9% (95% CI: 69.7% - 78.2%) for HR HPV; • And 72.3% (95% CI: 59.1% - 85.6%) and 93.2% (95% CI: 90.8% - 95.7%) for cervicography. Conclusion: The pap smear had the highest specificity (94.6%) and HPV testing with the highest sensitivity (94.4%). The visual methods, VIA and cervicography, were similar and showed an accuracy in between the 2 tests. But however, from the perspective of the need for a simple and widely applicable screening test for cervical (pre) cancer in poor resource countries, this study showed the evidence of the effectiveness of VIA as a primary screening tool.
General comments	i)The test of pap smear was performed at a referral and training centre, which cautions that these results cannot be generalized for other cytology laboratories in the region

Bibliographic citation	21) Chen L. and Yang B. Assessment of reflex human Papillomavirus DNA testing in patients with atypical endocervical cells on cervical cytology. Cancer (Cancer Cytopathol). 2008; 114: 236–241.
Study type	Cohort
LE	II-2
Number of patients & Patient characteristics	During a 60-month period from July 2001 to June 2006, all ThinPrep cases with a diagnosis of AGC from the Cleveland Clinic were searched. Cases with a cytologic diagnosis of AEC, AGC–favor endocervical origin, or AGC-NOS underwent 'reflex' HPV DNA testing (using either the original liquid based cytology residual specimen or a separate sample co-collected at the initial screening visit for the cytologic diagnosis of AEC).
Intervention	HPV DNA with the hybrid capture II
Comparison	Cytology/ Pap smear
Length of follow up	45 months with a range of 15 to 74 months.
Outcome measures/ Effect size	Of a total 332,470 Papanicolaou (Pap) tests performed, 317 cases of AEC had histopathologic follow-up and reflex testing for high-risk HPV. 1. High-risk HPV DNA was detected in 64 of 317 (20.2%) of the patients with AEC lesions. When analyzed by age groups, 21.4% (21 of 98) of the women aged < 30 years and 19.6% (43 of 219) of the women aged ≥30 years tested positive for HPV 2. Histopathologic examination of the 64 HPV-positive AEC cases revealed 18 cases (28.1%) of endocervical adenocarcinoma in situ/adenocarcinoma (AIS1) and 22 cases (34.4%) of CIN2+. 3. Among 253 of the HPV-DNA negative AEC women, 3 cases (1.2%) had AIS lesion and only 1 case (0.4%) had CIN21 lesions. 4. Cervical AIS1 was found in 28% of the HPV-positive AEC patients and in only 0.9% of the HPV-negative patients (P<0.0001). 5. When the significant glandular (AIS1) and squamous (CIN21) lesions were combined, 62.5% of the lesions were detected in HPV-positive AEC cases compared with 1.6% in the HPV-negative AEC cases (P\.0001). 6. The sensitivity, specificity, positive predictive value, and negative predictive value for high-risk HPV DNA testing to detect clinically significant cervical lesions (CIN21 and/or AIS1) were 91.0%, 91.2%, 62.5%, and 98.4%, respectively. Conclusion: 1. Because of a high sensitivity (91.0%) and high specificity (91.2%) in detecting significant cervical lesions, reflex HPV testing for cytologic diagnosis of AEC appears to be a useful ancillary tool in the selection of high-risk patients for colposcopy.
General comments	

Bibliographic citation	22) Einstein MH, Martens MG, Garcia FAR, et al. Clinical validation of the Cervista® HPV HR and 16/18 genotyping tests for use in women with ASCUS cytology. *Gynecol Oncol*. 2010; 118: 116-122.
Study type	Cohort, multicenter Evaluation of clinical performance of Cervista HPV HR and Cervista 16/18 test for detection of CIN 2 and CIN 3 in women with ASCUS.
LE	II-2
Number of patients & Patient characteristics	1514 women with ASCUS cytology results. Age 18 or older.
Intervention	Cervista HPV HR and Cervista 16/18 test
Comparison	
Length of follow up	
Outcome measures/ Effect size	<u>Detection of CIN</u> HPV HR Sensitivity: 92.8% (≥CIN 2) , 100% (≥CIN 3) Specificity: 44.2% (≥CIN 2) , 43% (≥CIN 3) 16/18 genotyping Sensitivity: 68.8% (≥CIN 2) , 77.3% (≥CIN 3) Specificity: 69.3% (≥CIN 2) , 67.3% (≥CIN 3) For both test, sensitivity decreased with increasing age
General comments	

Bibliographic citation	23) Castle PE, Wacholder S, Sherman ME, et al. Absolute risk of a subsequent abnormal pap among oncogenic human papillomavirus DNA positive, cytologically negative women. *Cancer*. 2002; 95(10): 2145-2151
Study type	Cohort Study to estimate absolute risk of developing abnormal cytology during 57 month follow up of subjects with HPV DNA positive but negative cytology on enrolment using HC2
LE	II-2
Number of patients & Patient characteristics	2020 women with negative cytology and positive f---or HPV DNA on enrolment. Age 16 years or older.
Intervention	Hybrid capture 2
Comparison	57 month
Length of follow up	
Outcome measures/ Effect size	Cumulative incidence of ≥ASC was 16.8%, ≥LSIL was 6.4%, ≥HSIL was 2.2%. In HPV negative, cumulative incidence of ≥ASC was 4.2%. Risk of abnormal Pap test increased with increasing level of viral load. The highest viral load (100 relative light units per the positive control or greater) was associated with a greater risk of an abnormal Pap test (odds ratio 2.7, 95% CI 1.7– 4.1) than lower viral loads. These results suggest that about 15% of women in annual screening programs who concurrently have a negative Pap test and a positive oncogenic HPV test will have a subsequent abnormal Pap test within 5 years. This risk estimate will be useful to the many clinicians and patients likely to be diagnosed with an HPV infection and negative cytology if HPV DNA is added to general screening.
General comments	

Bibliographic citation	24) Pimple S, Muwonge R, Amin G et al. Cytology versus HPV testing for the detection of high-grade cervical lesions in women found positive on visual inspection in Mumbai, India. *Int J Gynecol Obstet.* 2010; 108: 236–239
Study type	Cross sectional (screening/ diagnostic type of study) It is part of a larger multicenter International Agency for Research on Cancer (IARC) study involving the concurrent evaluation of cytology testing, HPV testing, VIA, and VILI as screening tools for the early detection of cervical cancer precursors in India and Africa.
LE	II-3
Number of patients & Patient characteristics	A total of 4039 women were screened by VIA and/or VILI. Of these women, 756 (18.7%) were found to be positive having CIN on VIA and/or VILI and had simultaneously undergone cytology and/or HPV testing. All the analyses that follow are based on findings concerning these 756 women. They were aged between 30 and 60 years, had an intact uterus, and no known history of CIN. The final diagnosis was the histopathology result unless it was inconclusive. In this case, it was the colposcopic finding.
Intervention	HPV DNA testing using the hybrid capture 2 (HC2) assay, cytology testing, VIA, and VILI
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	1. Sensitivities: Cytology – for CIN 2 or CIN 3 lesions, 64.3% (95% CI, 48.0%–78.4%) for ASCUS, 57.1% (95% CI, 41.0%–72.3%), for LSIL HPV test was 61.0% (95% CI, 44.5%–75.8%). 2. The specificity : cytology test using the ASCUS and LSIL thresholds were 95.8% (95% CI, 94.0%–97.2%) and 97.5% (95% CI, 96.0%–98.6%), HPV test was 92.1% (95% CI, 89.6%–94.2%). 3. The sensitivity estimates for CIN 3: 85.0% (95% CI, 62.1%–96.8%)cytology at ASCUS 70.0% (95% CI, 45.7%–88.1%)cytology at LSIL 89.5% (95% CI,66.9%–98.7%), for HPV DNA test 4. specificity to detect CIN 3 lesions 94.5% 95% CI, 92.5%–96.1%) cytology at ASCUS 96.1% (95% CI, 94.4%–97.5%) cytology at LSIL 91.1% (95% CI, 88.5%–93.2%) for HPV DNA test Interpretation: 1. The HC2 assay has a higher sensitivity to detect CIN 3 lesions than cytology testing at the 2 thresholds used in this study, but the specificity of HPV testing was significantly lower. Cytology and HPV testing were both found to be accurate triaging methods for women suspected of having CIN on visual inspection, especially for those with CIN 3 lesions.
General comments	

Bibliographic citation	25) Paraskevaidis E, Arbyn M, Sotiriadis A. et al. The role of HPV DNA testing in the follow-up period after treatment for CIN: a systematic review of the literature. *Cancer Treatment Reviews*. 2004; 30: 205–211.
Study type	Systematic Review
LE	I
Number of patients & Patient characteristics	Eleven studies were ultimately found on evaluating the use of HPV testing after conservative treatment for CIN. Eight studies were prospective, and three studies were retrospective. The total number of women included in these studies were 900, of whom 678 (75.3%) were considered as having a successful treatment, whereas 222 (24.7%) were considered treatment failures.
Intervention	HPV DNA testing, cytology,
Comparison	colposcopy and/or biopsy
Length of follow up	
Outcome measures/ Effect size	1. There was a marked heterogeneity in the design, population, intervention and follow-up policy across different studies. 2. The sensitivity of HPV DNA testing in detecting treatment failures was quite good in most studies, reaching 100% in four of them, whereas the specificity of the test differed across the studies, ranging from 44% to 95%. 3. Among the 672 women in whom the treatment was considered to be successful, 566 (84.2%) had a negative postoperative HPV DNA test, whereas 106 (15.8%) had a positive postoperative HPV DNA test. In contrast, among the 204 cases that were considered as treatment failures, only 35 cases (17.2%) had a negative postoperative HPV DNA test, whereas 169 cases (82.8%) were positive for HPV DNA postoperatively. Interpretation: 1. The results of this overview suggest that there might be a role for a HPV DNA test at the follow up period. It seems that a positive HPV test, even in the presence of normal cytology, may pick up early and accurately a treatment failure. Cytology and colposcopy may still be needed in order to rule out false positive and false negative results.
General comments	Most of the studies are relatively small. There was different initial evaluation of HPV DNA status, different CIN grades are included and different treatment modalities for CIN were used With regards to follow-up, there was marked heterogeneity in starting point, duration and methods. Colposcopy, which was considered to be the gold standard, was not blindly and consistently employed for all patients. So, elective colposcopic evaluation for patients with positive cytology could lead to a strong verification bias. For all these reasons, a formal meta-analysis was not feasible at the present point as the trials' designs were heterogeneous. Nonetheless, all studies showed a similar direction of effect, demonstrating a relationship between a positive postoperative HPV DNA test and treatment failure.

Bibliographic citation	26) Hernadi Z, Szoke K, Tama´s Sapy T, et al. Role of human Papillomavirus (HPV) testing in the follow-up of patients after treatment for cervical precancerous lesions. *Eur Jour Obstet Gynecol Reproductive Biol*. 2005; 118: 229–234
Study type	Cross sectional (screening/ diagnostic type study) A hospital-based retrospective analysis was performed with prospective collection of patient data of women screened for cervical cancer at a Gynecologic Outpatient Clinic.
LE	II-2
Number of patients & Patient characteristics	Patients after therapeutic excision due to positive screening results were identified and followed up with HPV testing and serial cytology. Out of the 76 patients treated by surgical excision due to positive cytology, 61 patients had a CIN histology. The distribution of patients according to the grade of CIN was as follows: CIN1: 12, CIN2: 18, CIN3: 31 patients, respectively. As the main focus in this study was on the recurrence of CIN after surgical excision of the lesion, these patients were followed-up both with HPV testing and serial cytology (median interval: 5 months, range: 1.5–12 months). Women were aged between 20-53 years (median age of 33 years)
Intervention	HPV DNA with the hybrid capture, cytology
Comparison	Colposcopy
Length of follow up	median interval: 5 months, range: 1.5–12 months).
Outcome measures/ Effect size	1. A negative HPV test (43 patients) in the post-treatment period excluded not only the recurring CIN but also the development of persisting cytological atypia (negative predictive value (NPV): 100%) during 1201 patient months (median 26 months) and allowed the patient to return back to the population-based screening programs. 2. Negative HPV results were detected at median of 6 month (range: 1–24) after therapeutic excision. The median follow up time for negative cytology was 4 months (range: 2–12). It is of note that 10 (23%) of the 43 patients in the HPV negative follow-up group in the post treatment period had equivocal cytology (P3) at single visits during a cumulative 1201 patient months follow-up (median 26 months) 3. After 61 treatment for cervicalis intraepithelialis neoplasia (CIN), high-risk HPV infection was detected during the post-treatment follow-up at 18 cases (29.5%), 10 of them had persisting cytological atypia (positive predictive value (PPV): 56% (10/18), 5 developed CIN (PPV: 28% (5/18). Interpretation: 1. A negative HPV test eliminates the risk of recurrent disease after treatment for CIN. 2. In a positive HPV test this may indicate a significant risk for the recurrence of persistent cytological atypia and CIN with high sensitivity. However, as consequence of cases mentioned above the positive predictive values are relatively low
General comments	

Bibliographic citation	27) Sarian LO, Sophie FM, Derchain SFM, et al. HPV DNA test and Pap smear in detection of residual and recurrent disease following loop electrosurgical excision procedure of high-grade cervical intraepithelial neoplasia. *Gynecol Oncol.* 2004; 94: 181–186
Study type	Cross sectional (screening/ diagnostic type study)
LE	II-2
Number of patients & Patient characteristics	A series of 107 women subjected to LEEP due to histologically confirmed CIN 2 or 3 between March 2001 and December 2002 were followed-up biannually until January 2004. Follow-up visits consisted of interview and gynecological examination including cervical cytology, hybrid capture 2 (HC 2), and colposcopy. Patients presenting with abnormal colposcopy or high-grade squamous intraepithelial lesion (HSIL) smear were subjected to new excision procedure and presence of histologically confirmed CIN 2 or 3 or higher was considered as residual or recurrent disease. Performance indicators were calculated for cytology and HC 2 assay in detecting residual or recurrent disease. Women age ranged from 20 to 60 years (mean 34 years)
Intervention	HPV DNA with the hybrid capture 2, Cytology/ Pap smear
Comparison	Colposcopy
Length of follow up	
Outcome measures/ Effect size	1. During the follow-up, eleven (10.2%) women showed residual or recurrent disease during the follow-up. Half of the women with residual or recurrent disease at follow-up presented with complete excision margins of the cone. 2. Considering HC 2 and Pap smear as stand alone tests, both techniques showed similar sensitivity, detecting 100% of CIN 2 or 3 at the first follow-up visit. 3. At the second follow-up visit, Pap smear showed better specificity (97% versus 83%) and positive predictive value (PPV- 67% versus 22%) than HC 2, and both tests had fairly the same high negative predictive value (NPV- 99% versus 98%) and sensitivity (80% for both). 4. The combined positive HC 2 and abnormal cytology had the same sensitivity (80%) as each of the tests alone, but specificity (100%) and PPV (100%) were significantly higher than those of single tests. 5. When only one of the tests was positive, the sensitivity (100% for 1st follow up and 80%at second follow up) and the NPV (100% at 1st follow-up and 99% at second follow up) of the combination remained the same, but its specificity and PPV were lower than that of the combined two positive tests and that of the individual test, at both follow-up visits Interpretation: 1. HPV testing seems to be a valuable tool in monitoring the therapeutic results of conization and to discriminate patients who have a higher risk of disease recurrence 2. HPV testing has a high predictive value in the postconization follow-up, because HPV may act as a marker of undetected residual neoplasia, being a necessary factor for the development of recurrent CIN. Importantly, HPV testing can clarify the referral criteria for colposcopy because HPV detection, even when cervical cytology is normal, might predict an abnormal colposcopy
General comments	

Bibliographic citation	28) Sankaranarayanan R, Nene BM, Shastri SS, et al. HPV screening for cervical cancer in rural india. NEJM. 2009; 360(14): 1385-1393.
Study type	Cluster-randomized control trial To evaluate effectiveness of single round of HPV testing, cytologic testing or VIA in reducing incidence of cervical cancer as compared to control group that received usual care
LE	I
Number of patients & Patient characteristics	131 746 healthy women between ages 30 and 59 years from 52 clusters of villages. Subjects were randomly assigned to four groups of 13 clusters each. The groups were randomly assigned to undergo screening by HPV testing (34,126 women), cytologic testing (32,058), or VIA (34,074) or to receive standard care (31,488, control group). Women who had positive results on screening underwent colposcopy and directed biopsies, and those with cervical precancerous lesions or cancer received appropriate treatment
Intervention	HPV DNA test (Hybrid capture 2), cytology, VIA
Comparison	Control group (standard care)
Length of follow up	8 years
Outcome measures/ Effect size	PPV for detecting CIN grade 2 or 3 lesions was 11.3% in HPV-testing group, 19.3% in cytologic-testing group, and 7.4% in the VIA group. Incidence rate of cervical cancer of stage II or higher and death rates from cervical cancer were significantly higher in the cytologic-testing group and the VIA group than in the HPV-testing group. In the HPV-testing group, the hazard ratio for the detection of advanced cancer was 0.47 (95% CI, 0.32 to 0.69) and the hazard ratio for death was 0.52 (95% CI, 0.33 to 0.83), as compared with the control group. During the 8-year follow-up period, the age standardized incidence of cervical cancer in women with negative HPV test, cytology and VIA was 3.7, 15.5, and 16.0 cases per 100 000 person-years respectively.
General comments	

Bibliographic citation	1) Vijayaraghavan A, Efrusy M, Lindeque G *et al.*Cost Effectiveness of high-risk HPV DNA testing for cervical cancer screening in South Africa. Gynecologic Oncology.2009;112:377-383
Study type	A decision analytic Markov model was used to model the costs, survival and quality of life associated with screening and treating cervical cancer. Objective: To determine the cost effectiveness of several cervical cancer strategies using human papillomavirus (HPV) testing in South Africa Type of study: Cost Utility analysis and from the view of societal perspective. Setting: Primary care in South Africa Perspective: Societal Perspective
LE	II-3
Number of patients & Patient characteristics	The model had a hypothetical cohort of 100,000 South African women from age 13 years
Intervention	HPV DNA with strategies as follows: a) no screening b) conventional cytology b) conventional cytology followed by HPV testing for triage of equivocal cytology results c) HPV testing followed by colposcopy for HPV-positive women; d) HPV testing followed by cytology for triage of HPV-positive women e) combined screening with cytology and HPV testing
Comparison	Compared between the strategies in intervention
Length of follow up	-
Outcome measures/ Effect size	a) Quality adjusted life years saved (QALYs); b) Incremental Cost-Effectiveness ratios(ICER); c) Lifetime risk of cervical cancer When the ICER of below strategies compared to the current clinical practice of using conventional cytology alone: i) conventional cytology followed by HPV triage for equivocal cytology results:R2800 ii) HPV DNA testing followed by cytology for HPV-positive women :R8286 iii) HPV DNA testing followed by colposcopy for all HPV positive women:R6534 iv) Simultaneous HPV DNA testing and conventional cytology co screening:R8040 Overall Conclusion: i) Conventional cytology with use of HPV testing for triage of abnormal cytology is less expensive and more effective than screening using cytology alone and is thus a dominant strategy(with the least ICER) ii) For South African women, use of HPV testing to triage ASCUS pap smears was less expensive and more effective than cytology testing alone
General comments	Limitations a) data were combined from multiple sources with varied study designs b) HPV found cost effective in SA, but not applicable in other countries in the world because of the infrastructure which is not sufficient (the result is not generalize) c) The long screening interval in SA, the author chose to model only strategies that improved upon the sensitivity of conventional cytology d) The results may not be generalizable to countries other than South Africa since the model used are relied on country-specific data, assumptions regarding epidemiology, infrastructure and costs

Bibliographic citation	2) Goldie S J, Gaffikin L, Goldhaber-Fiebert J D *et al*. Cost Effectiveness of cervical cancer screening in five developing countries. The New England Journal of Medicine.2005;353:20
Study type	A computer based, state transition, decision model was constructed to simulate the natural history of cervical cancer and the clinical and economic impact of the alternative screening strategies in hypothetical cohorts of women in the five countries Objective: To assess the cost effectiveness of the alternative screening strategies for cervical cancer, compared with no screening in the five developing countries(India, Kenya, Peru, South Africa and Thailand) Type of study: Cost Effectiveness Analysis Setting: Primary care setting Perspective: Societal perspective
LE	II-3
Number of patients & Patient characteristics	The study population comprised women eligible for cervical cancer screening.
Intervention	The 3 testing strategies were: i) VIA ii) Paps smear iii) HPV DNA differentiated as below: i) 1 visit VIA ii) 2 visit VIA iii) 1 visit HPV iv) 2 visit HPV v) 3 visit HPV vi) 2 visit Pap vii) 3 visit Pap viii) 2 visit HPV and VIA With targeted ages: i) no screening at all ii) once per life time(at age 35) iii) twice per life time(at age 35 and 40) iv) three times per life time(at age 35, 40 and 45)
Comparison	
Length of follow up	
Outcome measures/ Effect size	The incremental cost effectiveness ratios (ICER) were calculated to combine the costs and benefits of the alternative screening strategies. Each strategy was compared with the next less expensive option. The results were captured as below: i) In SA, incremental cost per LY gained was $467 with 1 visit HPV DNA once , $1093 with 1 visit HPV DNA twice and $2458 with 1 visit HPV DNA three times compared with the next less expensive options. ii) In Thailand, the incremental cost per LY gained was $170 with 1 visit HPV DNA once,$277 with 1 visit VIA twice,$310 with 1 visit HPV DNA twice and $658 with 1 visit HPV DNA three times iii) In India, the incremental cost per LY gained was $10 with 1 visit VIA once, $91 with 1 visit VIA twice, $268 with 1 visit VIA three times and $591 with 1 visit HPV DNA three times iv) In Kenya, the incremental cost per LY gained was $134 with 1 visit VIA once, $91 with 1 visit VIA twice,$268 with 1 visit VIA three times and $591 with 1 visit HPV DNA three times. v) In Peru, the incremental cost per LY gained was $124 with 1 visit VIA once,$152 with 1 visit HPV DNA once, $453 with 1 visit HPV DNA twice and $1,145 with 1 visit HPV DNA three times Thus, this study concludes that the most cost effective strategies were those that required the fewest visits, resulting in improved follow up testing and treatment. Overall conclusions: Screening strategies for cervical cancer that incorporate VIA (India, Kenya and Peru) or HPV DNA testing (SA and Thailand) in 1 or 2 clinical visits were cost effective alternative as compared to the conventional 3 visits cytology-based screenings.
General comments	

Bibliographic citation	3)Andres-Gamboa O, Chicaiza L, Garcia-Molina M *et al* Cost Effectiveness of conventional cytology and HPV DNA testing for cervical cancer screening in Colombia. Salud Publica de Mex.2008;50:276-285
Study type	Markov model to simulate the natural history of cervical neoplasia over a life time. Objective: To examine the cost-effectiveness of conventional cytology and human papillomavirus(HPV) deoxyribonucleic acid (DNA) for cervical cancer screening. Type of Study: Cost Effectiveness Analysis Perspective : Payer perspective. Setting: Primary care in Colombia
LE	II-3
Number of patients & Patient characteristics	
Intervention	The strategies compared were: i) Annual conventional cytology until 2 consecutive negative results and every 3 years afterwards. Women with atypical squamous cells of uncertain significance (ASCUS) received HPV testing. Those with a low-grade intraepithelial lesion or more and those with a positive HPV test received colposcopy. ii) Annual cytology until three consecutive negative results and every 3 years afterwards. Follow up was as for annual cytology until 2 negative results. iii) HPV testing every 3 years, with cytology for positive results , and women with ASCUS or more received colposcopy iv) HPV testing every 5 years, with cytology for positive results and women with ASCUS or more received colposcopy. Notes: i) All these strategies were compared with no screening ii) Cytology screening was performed between the ages of 21 and 69 years iii) HPV DNA testing was performed from age 30 to 69 years.
Comparison	ICER compared between the strategies in the intervention.
Length of follow up	
Outcome measures/ Effect size	Results: The mean discounted lifetime costs: i) $130.90 with no screening ii) $293.90 with HPV every 5 years iii) $338.60 with cervical cytology annually until 2 negative results iv) $361.80 with cervical cytology annually until 3 negative results v) $367.60 with HPV every 3 years The Life Years: i) 28.4 with no screening ii) 32.10 with HPV every 5 years iii) 28.5 with cervical cytology (2 negatives result) iv) 28.6 with cervical cytology (3 negatives) v) 32.11 with HPV every three years ICER: All strategies were cost effective in comparison with no screening. i) Both cervical cytology strategies were dominated by, which means they were more expensive and less effective than HPV every five years. ii) HPV every five years (which had an incremental cost per LY gained of $44 over no screening) is the most cost effective strategy available, as compared with the Colombia GDP. iii) HPV every 3 years was more costly, with ICER of $7,370 (The threshold for the ICER was the Colombian yearly per capita gross domestic product of $3,200, so if the ICER is less lower than the GDP, it is considered cost effective) Overall conclusion : HPV DNA testing every five years with an ICER $44 was a cost effective alternative compared with other conventional cytology strategies for screening in Colombia especially at high coverage and high rates of follow up.
General comments	Limitation : a) Data were combined from various sources, different designs (cohort, clinical trials, population cancer registries) and also different eligibility criteria for participating women. b) The study adapted assessed result in short periods of time projected to long periods in the model c) Lack of country-specific information

Bibliographic citation	4)Goldie S J, Kuhn L, Denny L *et al* Policy analysis of cervical cancer screening strategies in low-resource settings: Clinical Benefits and Cost-effectiveness JAMA.2001;285(24):3107-3115
Study type	A state-transition decision model, based on Markov cycles, was constructed to simulate he natural history of human papillomavirus (HPV) infection-induced cervical neoplasia and cervical cancer screening, diagnosis, and treatment. The objective: Was to assess the cost effectiveness of the screening strategies for cervical cancer (South Africa is a low resource setting and this may represent of many developing countries) Type of study : Cost Effectiveness analysis Perspective: Societal perspective. Setting: community in developing countries.
LE	II-3
Number of patients & Patient characteristics	Hypothetical cohort of 30 year old black South African women.
Intervention	Screening strategies examined were: i) DVI (direct visual inspection) and HPV, 1 visit ii) self collected HPV iii) DVI followed by HPV iv) clinician collected HPV v) cervical cytology, 2 visits were performed vi) cervical cytology , 3 visits Where : 3 visits includes an initial screening examination, biopsy for positive result , and the last visit for treatment 2 visits includes a first visit on screening and 2nd for treatment (for women without colposcopy/ biopsy) 1 visit with screening and immediate treatment in all screening positive women (on the same day)
Comparison	ICER compared between the strategies in the intervention.
Length of follow up	Incremental cost results: An incremental cost –effectiveness analysis was conducted to combine costs and benefits of the screening strategies. Here costs and LYS. When assessing the relative efficiency of the different screening strategies, 5 strategies dominated the remaining options these being: i) One –visit DVI lifetime (cost saving) ii) 2 visit DVI lifetime ($70 per LYs) iii) every 5 year DVI ($140 per LYs) iv) every 3 year DVI ($460 per LYS);and v) every 3 year HPV ($11500 per LYs) When screening strategies were performed once in a life time, comparing each strategy with the no screening option: i) 1 visit DVI was cost saving, ii)cost effectiveness ratio was $14 with one visit HPV testing iii) $26 with two visit self collected HPV iv) $44 with 2 visits DVI followed by HPV v) $39 with 2 visits clinician collected HPV vi) $81 with 2 visits cervical cytology vii) $147 with 3 visits cervical cytology Overall conclusion: By looking at the ICER In the population of SA women, a single life time screening with DVI / HPV DNA testing coupled with immediate cryotherapy cost less than $50 per woman and were generally more cost effective than other screening strategies in conventional cytology.
Outcome measures/ Effect size	Limitations a) data were combined from multiple sources that varied in study design and entry criteria b) short term clinical studies were used to extrapolate long –term consequences c) The upfront costs of initiating new screening programs or of providing ongoing training and supervision of clinicians practicing DVI were not included d) The effectiveness of interval screening using DVI or HPV testing has not been fully evaluated(but it was minimized by focus only on 1 visit screening strategies) e) Result may not be generalizabled to countries other than South Africa(assumptions will need to be incorporated into independent analyses) f) All screening tests may not be equally available in low-resource settings, and certain screening tests may be selected for programmatic reasons(political matters, ethics and cultural issue) g) CE strategy is always differ according to the CE threshold of given setting (different setting/ countries)
General comments	

Bibliographic citation	5)Bistoletti P, Sennfalt K,Dillner.J, Cost – effectiveness of Primary Cytology and HPV DNA cervical screening Int.J.Cancer.2008;122;372-376
Study type	A markov model was used to simulate the progress of cervical cancer and pre-cancerous lesions in women aged 32 to 60 years. Objective: to estimate the life expectancy and per woman costs of four screening strategies for HPV. Type of Study: Cost Effectiveness analysis Perspective: National Health Services, Sweden. Setting : Primary care
LE	II-3
Number of patients & Patient characteristics	4 strategies were compared : i) cervical cytology screening once every 3 years for women aged 32,35,38,41,44,47,50,55 to 60 years ii) strategy 1 + HPV-deoxyribonucleic acid (DNA) testing at age 32 years iii) Combined HPV DNA testing and cervical cytology screening 3 times during life time only at ages 32,41 and 50 years. iv) No screening
Intervention	
Comparison	Comparison done between strategies
Length of follow up	
Outcome measures/ Effect size	The health outcomes and costs were presented for three discount rates. At a discount rate of 3%, the life expectancy (Lys) and costs were as below: i) strategy 1:129.67 years, cost:$245 ii) strategy 2: 29.67 years, cost:$284 iii) strategy 3:29.69 years, cost:$210 iv) strategy 4:29.29 years,cost:$523 (No ICER result was presented, the result were revealed in term of cost and benefits only) At all discount rates, strategy iii) dominated (combined HPV DNA testing and cervical cytology screening at ages 32,41 and 50 years) dominated the other 3 strategies (more effective and less expensive). Overall conclusion : i) For population based, organised cervical cytology screening between the ages 32 and less than 60 years was highly cost-efficient for cervical cancer prevention. ii) If screening intervals were increased to at least nine years, combined cytology and HPV DNA screening appeared to be more effective and less costly
General comments	Limitations of the study: a) The study did not include hysterectomy rates in the model so they have opted to use actual population-based transition probabilities as input data, as this reflects the impact of the screening program as a whole b) Only the provider/ health service perspective was chosen, however no significant cost shifting can be expected between health care providers c) The uncertainty regarding the societal perspectives of cervical cancer screening make economic analyses attempting to capture all the effects and consequences of screening unreliable. d) Cost estimates are on average slightly lower than cost estimates from the US, the UK and the Netherlands (this is because these activities are in Sweden were mainly performed by midwives), furthermore higher prevalence of positive cervical cytology screening test can be observed in UK and USA than in Sweden, then this contributed to a better cost-effectiveness showed in our model compared to studies from the UK and the USA, the costs for treatment of early and curable invasive cancer as well as for continuing care and terminal care e) The model starts at age 32 and therefore the costs and effects of screening before that age were not analyzed.

Bibliographic citation	6) Kim.J J, Wright T C, Goldie S J, Cost Effectiveness of Human Papillomavirus DNA testing in the United Kingdom, The Netherlands, France, and Italy Journal of the National Cancer Institute.2005;97(12):888-895
Study type	Created computer-based model of the natural history of cervical carcinogenesis for each using country specific data on cervical cancer risk Aim: The study sought to compare the CE of current cytology screening strategies in the UK, Netherlands, France and Italy with other 2 new strategies (HPV as triage and combination testing) Type of study:Cost Effectiveness analysis Perspective: Societal perspective Setting: hypothetical community setting in one of the countries studied (the UK, Netherlands, France and Italy)
LE	II-3
Number of patients & Patient characteristics	The hypothetical populations comprised women currently eligible for screening in the UK, Netherlands, France and Italy
Intervention	The study examined the use of HPV DNA testing in screening programmes for cervical cancer. i) Cytology throughout a woman's lifetime and using HPV testing as a triage strategy for equivocal cytology results ii) Cytology until age 30 years and followed by HPV testing in combination with cytology thereafter.
Comparison	Current cytology screening strategies in the UK, Netherlands, France and Italy
Length of follow up	
Outcome measures/ Effect size	Outcome measured: i) Benefits:total discounted life years ii) total average lifetime costs Synthesis of costs and benefits: The costs and benefits were combined to calculate the cost per life –year saved. Cost-effectiveness ratios were calculated and strategies were eliminated according to the conventional rules of dominance and extended dominance. The remaining strategies were considered cost effective if the incremental cost-effectiveness ratio was less than three times the country-specific gross domestic product per capita. The preferred strategy in the every country was estimated to be combination testing at 3 year intervals. The country specific ICER for this strategy were: i) $75,900 per life year saved in the UK, ii) $37,400 in the Netherland iii)$26,300 in France iv)$25,600 in Italy. Overall conclusion : HPV DNA testing has the potential to improve health benefits at a reasonable cost compared with current screening policies (solely conventional cytology) in the four European countries.therefore the HPV testing may be more cost effective than conventional cytology screening for cervical cancer.
General comments	Limitations of the study: i) Country specific data were not available for all of the input parameters ii) No empiric country specific data suitable for inclusion in this model on the cost and quality of life decrements iii) Long term outcomes associated with different strategies for managing HPV DNA –positive woman are not known

Bibliographic citation	7)Mandelblatt J S; Lawrence W F; Womack S M *et al*. Benefits and Costs of using HPV testing to Screen for Cervical Cancer JAMA.2002;287(18):2372-2381
Study type	A decision analytic model based on a Markov process was constructed in order to simulate the natural history of a cervical cancer. Objective: To compare the cost-utility of the eighteen screening strategies for cervical cancer in the average Type of study: Cost Utility Analysis Perspective: Societal perspective Setting: Community
LE	II-3
Number of patients & Patient characteristics	The study population comprised the average US population of women, aged 20 years and over. Women with human HIV infection were excluded from the analysis.
Intervention	3 screening strategies for cervical cancer were examined. The strategies were Paps test, HPV and their combinations with different screening intervals. The details of the strategies were compared as below: i)pap every 3 years with screening stopped at 65,75 and 100 years ii)HPV every 3 years with screening stopped at 65, 75 and 100 years iii)Pap every 2 years with screening stopped at 65,75 and 100 years iv)HPV every 2 years with screening stopped at 65,75 and 100 years v)Pap plus HPV every 3 years with screening stopped at 65, 75 and 100 years vi)Pap plus HPV every 2 years with screening stopped at 65, 75 and 100 years vii)no screening (but this is not standard of care)
Comparison	Compared between the screening strategies listed in the intervention
Length of follow up	
Outcome measures/ Effect size	Synthesis of costs and benefits An incremental cost –utility analysis was carried out to combine the costs and QALYs. Each alternative was compared with the next most effective non-dominated option. The ranking was as follows: 1) Pap every 3 years and screening stopped at 65 years, not cost effective 2) Pap every 3 years and screening stopped at 75 years, $11,830 per QALY 3) Pap every 3 years and screening stopped at 100 years, not cost effective 4) HPV every 3 years and screening stopped at 65 years, dominated 5) HPV every 3 years and screening stopped at 75 years, not cost effective 6) HPV every 3 years and screening stopped at 100 years, not cost effective 7) Pap every 2 years and screening stopped at 65 years, not cost effective 8) Pap every 2 years and screening stopped at 75 years,$29,871 per QALY 9) Pap every 2 years and screening stopped at 100 years,$56, 440 per QALY 10) Pap plus HPV every 3 years and screening stopped at 65 years, dominated 11) HPV every 2 years and screening stopped at 65 years, dominated 12) Pap plus HPV every 3 years and screening stopped at 75 years, dominated 13) pap plus HPV every 3 years and screening stopped at 100 years, dominated 14) HPV every 2 years and screening stopped at 75 years, dominated 15) HPV every 2 years and screening stopped at 100 years, not cost effective 16) Pap plus HPV every 2 years and screening stopped at 65 years, not cost effective 17) Pap plus HPV every 2 years and screening stopped at 75 years,$70,347 per QALY 18) Pap plus HPV every 2 years and screening stopped at 100 years, $76,183 per QALY In this analysis, the benefits of the screening can be achieved by screening up until ages 65 to 75 years old. Beyond this(like in this study 100 years), the benefits are very small and need to be weighed against the harms. Conclusion : i) Screening with HPV+ Pap tests every 2 years up to 100 years appears to save additional years of life at an incremental cost of $76,183 per QALY compared with Pap testing alone every 2 years (maximum savings). ii) Applying age limits to screening is a viable option to mantain benefits while reducing costs. In this study proved that screening more than age 75, is not cost effective and the screening should stopped at age 75 years the most. iii) The optimum screening ages starts at 20 years .
General comments	Limitations of the study: i) Infrastructure issues, model assumptions, choice of technologies, short term disutility, use of modelling and generalizability ii) Model used assumes that screening occurs in an existing system (does not include infrastructure development cost) iii) Model combines HPV infection and LSIL into one state, it biases the results to make HPV screening appear less favourable relative to Pap Screening(due to higher rates of workup of transient HPV infection)

Bibliographic citation	8)Lytwyn A, Sellors J W, Mahony J B *et a.* Adjunctive Human Papillomavirus Testing in the 2-Year Follow up of Women With Low-Grade Cervical Cytologic Abnormalities :A Randomized Trial and Economic Evaluation Archive Pathol Lab Med.2003;127:1169 -1175
Study type	Modelling from Randomized Controlled Trial Aim of the study: To evaluate the effectiveness and costs of the repeated Pap test and oncogenic HPV test for the detection of histologically confirmed CIN 2 or 3. Type of study: Cost Effectiveness analysis Perspective: Ministry of Health, Ontario(health service) Setting: Primary care
LE	II-3
Number of patients & Patient characteristics	The study population comprised 257 women aged between 16 years and 50 years who were members of community-based family practices and who had ASCUS or LSIL on screening for cervical cytology.
Intervention	Repeated HPV and Pap test (combination)
Comparison	Repeated pap test alone (current practice)
Length of follow up	Every 6 months for 2 years
Outcome measures/ Effect size	Estimated benefits used in the economic analysis: Over the 2 year period, the combined pap test and HPV test detected 11 of 11 cases (100%), while the pap test alone detected 7 of 11 caes (63%). Therefore the difference between the 2 groups was not statistically significant. Costs results: For the 2 year period, the total cost of combined repeat pap test and HPV test to detect 11 cases of high grade CIN is Can$57,916. The total cost of repeat pap test to detect 7 cases is Can$40,094. Synthesis of costs and benefits: The cost effectiveness ratio of combined testing compared with Pap test alone was Can $4,456 per additional case of high grade CIN. (it is not actually presented in ICER) Overall conclusion: The combination of repeat Paps testing and HPV was more costly, but it may detect more cases of CIN 2 or 3 than the pap test alone. It also stated in the study that poor adherence limits the usefulness of a strategy that requires repeated follow up.
General comments	i. The issue of generalisability to other settings was not addressed ii. The authors did not present their results selectively and their conclusions reflected the scope of the analysis

Question : Is HPV DNA - based test safe?

Bibliographic citation	1) McCaffery K,Waller J, Forrest S.Testing positive for human papillomavirus in routine cervical Screening: examination of psychosocial impact. an International Journal of Obstetrics and Gynaecology December 2004, Vol. 111, pp. 1437–1443
Study type	Cross sectional survey To examine the psychosocial impact of testing positive for high risk human papillomavirus (HPV) among women attending primary cervical screening.
LE	I
Number of patients & Patient characteristics	Four hundred and twenty-eight women aged 20–64 years.
Intervention	Postal questionnaire survey.
Comparison	
Length of follow up	
Outcome measures/ Effect size	• Women with normal cytology who tested positive for HPV (HPV+) were significantly more anxious and distressed than women who were negative (HPV-) using both a state anxiety measure [F(1,267) = 29, P < 0.0001] and a screening specific measure of psychological distress [F (1,267) = 69, P < 0.0001]. • Women with an abnormal or unsatisfactory smear result, who tested HPV+, were significantly more distressed than HPV- women with the same smear result [F(1,267) = 8.8, P= 0.002], but there was no significant difference in state anxiety. • Irrespective of cytology result, HPV+ women reported feeling significantly worse about their sexual relationships. Approximately one-third of women who tested positive reported feeling worse about past and future sexual relationships compared with less than 2% of HPV- women. The study suggests that HPV testing may have an adverse psychosocial impact on women who test HPV+ when it is used as a primary screening test alongside conventional cytology.
General comments	

Appendix 6

LIST OF EXCLUDED STUDIES

1. Stephenson M, & Doughty C. Performance of Commercially Available HPV Tests NZHTA TECHNICAL BRIEF June 2007 Volume 6 Number 7.

2. Dixon EP, Lenz KL, Doobay H, et al. Recovery of DNA from BD SurePath cytology specimens and compatibility with the Roche AMPLICOR Human Papillomavirus (HPV) Test. Journal of Clinical Virology. 2010; 48: 31–35.

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