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HEALTH TECHNOLOGY ASSESSMENT REPORT

HPV URINE TEST FOR CERVICAL CANCER SCREENING

KEMENTERIAN KESIHATAN MALAYSIA

MaHTAS

Malaysia Health Technology Assessment Section

MEDICAL DEVELOPMENT DIVISION
MINISTRY OF HEALTH MALAYSIA

DISCLAIMER

This Health Technology Assessment has been developed from analysis, interpretation and synthesis of scientific research and/or technology assessment conducted by other organizations. 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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DISCLOSURE

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EXECUTIVE SUMMARY

Background

According to World Health Organization (WHO) and GLOBOCAN 2012, cervical cancer is the fourth most common form of cancer in women worldwide and the fourth leading cause of cancer-related death globally. While more recent data is yet to be published, according to the National Cancer Registry (NCR) Malaysia 2007, cervical cancer was the third most frequent cancer among women and the fifth most common cancer in the entire general population. Since cervical and female genital infection by specific HPV types is highly associated with cervical cancer, those types of HPV infection have received most attention from scientific studies. The International Agency for Research on Cancer (IARC) has classified **15** HPV types as high risk (**16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82**) and **12** as low risk (**6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108**). In particular, HPV16 and HPV18 are known to cause around 70% of cervical cancer cases worldwide.

For decades, screening using conventional cytological Pap (Papanicolaou) test or Pap smear has been the most widely used strategy for reducing cervical cancer around the world. Subsequently, the molecular methods to detect the HPV present in infected tissues were introduced. The HPV typing is generally done by liquid hybridization (Hybrid Capture 2) and/or conventional and real-time polymerase chain reaction (PCR), using DNA from cervical scrapes/biopsies. Both screening strategies, however, require a pelvic examination, a procedure that is invasive and uncomfortable for the patient, time consuming for healthcare providers and is unlikely to resolve the problem of poor screening uptake. The use of urine, which is straightforward to collect, is claimed to be valuable for this purpose as exfoliated epithelial cells from the cervix and/or vagina is claimed to normally appear in the urine.

In Malaysia, currently all women who are, or who have been sexually active, between the ages of 20 and 65 years, are recommended to undergo Pap smear testing. However, with the significant burden of cervical cancer in Malaysia, and to increase screening uptake as well as the acceptance of the screening procedures, therefore, a Health Technology Assessment (HTA) is required to assess the accuracy, effectiveness, and cost-effectiveness of HPV urine test for cervical cancer screening. This HTA was requested by the Senior Principal Assistant Director of Cancer Unit, Disease Control Division, Ministry of Health Malaysia.

Technical features

Urine would be an appropriate sample for screening large populations as it may increase participation and compliance, since physical scrapes, sometimes unpopular because of the dislike of physical examination or because of religious reasons, are avoided. Efforts have been made to detect the presence of HPV DNA in urine in the most reliable way; using liquid hybridization, and PCR-based methods (using either conventional PCR or real-time PCR).

Policy Question
Should HPV urine test be used as a screening method in the cervical cancer screening programme in Malaysia?

Objective

i. To determine the diagnostic accuracy of HPV urine test for HPV detection
ii. To determine the benefits of cervical cancer screening using HPV urine test compared with conventional cervical cytological specimen, HPV DNA-based using cervical specimen, combination of conventional cytology and HPV DNA-based using cervical specimen, or no screening, with regards to patient outcomes such as detection rate, mortality rate, survival rate, quality of life (QOL), and quality adjusted life years (QALY) gained
iii. To determine the safety of HPV urine test for HPV detection
iv. To determine the economic impacts of HPV urine test for cervical cancer screening
v. To assess the organizational, ethical, and legal aspects related to cervical cancer screening using HPV urine test

Research questions

i. What is the diagnostic accuracy of HPV urine test for HPV detection?
ii. Is cervical cancer screening using urine effective in detecting HPV and reducing mortality?
iii. Is HPV urine test for HPV detection safe?
iv. What is the economic, organizational, ethical, and legal implication/impact related to cervical cancer screening using HPV urine test?

Methods:
Studies were identified by searching electronic databases. The following electronic databases were searched through the Ovid interface: Ovid MEDLINE® In-process and other Non-indexed citations and Ovid MEDLINE® 1946 to present, EBM Reviews - Health Technology Assessment 1st (Quarter 2016), EBM Reviews - Cochrane Database of Systematic Review (2005 to Feb 2016), EBM Reviews - Cochrane Central Register of Controlled Trials (Jan 2016), EBM Reviews - Database of Abstracts of Reviews of Effects (1st Quarter 2016), EBM Reviews - NHS Economic Evaluation Database (1st Quarter 2016). Parallel searches were run in PubMed. Limitations only for female/women were applied to the search. The last search was run on 4th March 2016. Additional articles were identified from reviewing the references of retrieved articles. Studies were selected based on inclusion and exclusion criteria. All full text articles were graded based on guidelines from the U.S./Canadian Preventive Services Task Force or NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4(2nd Edition), March 2001 for test accuracy studies.

Results and conclusion:
A total of 114 titles were identified through Ovid interface, PubMed and references of retrieved articles. A total of 43 abstracts were screened using the inclusion and exclusion criteria. After reading, appraising and applying the inclusion and exclusion criteria to 38 full text articles, 13 full text articles comprising of one systematic review and meta-analysis, nine observational studies (cohort and cross-sectional) and three diagnostic accuracy studies were finally included for this review.

Clinical performance (diagnostic accuracy)
There was limited fair level of retrievable evidence to suggest that:

a. In a combination population of **symptomatic (78%) and asymptomatic (22%)** women, sensitivity and specificity of urine test varies with the types of HPV. Pooled sensitivity and specificity was 87% and 94%, respectively, for urine detection of any HPV. Urine detection of high risk HPV had a pooled sensitivity of 77% and specificity of 88%, while urine detection of HPV 16 and 18 had a pooled sensitivity of 73% and specificity of 98%.
b. In **symptomatic** population, overall sensitivity and specificity has been quite variable, ranging from 44.8% to 90.5% and 34.8% to 85.0%, respectively. Positive predictive value (PPV) ranged from 37.2% to 86.4% whereas NPV ranged from 75.6% to 89.8%.
c. There was no diagnostic study among **asymptomatic** women retrieved

HPV detection and genotyping
There was substantial fair level of retrievable evidence to suggest that:

a. Detection of HPV DNA in urine among screened **asymptomatic** women varies depending on the chosen population. HPV DNA detection ranged from 4.2% to 28.6% in sexually active women, and ranged from 9.2% to 19.2%, particularly in young sexually unexposed girls and healthy tribal girls
b. Detection of HPV DNA in urine was increased among screened **symptomatic** women ranging from 34.5% to 78.1%
c. HPV **type 16** was identified most frequently in both urine and cervical samples

HPV concordance in paired urine and cervical samples
There was substantial fair level of retrievable evidence to suggest that:

a. Overall concordance for HPV positivity and negativity between cervical and urine samples in **symptomatic** women varied from 69.3% to 90.0% (agreement, κ from 0.41 to 0.80)
b. Type specific concordance rates in the paired samples have been very good for invasive cervical cancer (79.0%)
c. There was no study retrieved on concordance between cervical and urine samples among **asymptomatic** women

Safety
There was no retrievable evidence on adverse events or complications associated with HPV urine test used for cervical cancer screening.

Cost / cost-effectiveness / economic evaluation
There was no retrievable evidence on the cost-effectiveness of HPV urine test for cervical cancer screening. However, the average cost per HPV-DNA test for cervical specimen using PCR-based method ranged from RM 91.50 to RM 183.00. Hence, it is assumed that the cost for HPV-DNA detection in urine will most probably the same. The average cost per Pap smear test performed in Malaysia is RM 20.12

Organizational, ethical, and legal considerations
There was evidence to suggest that:

a. The detection of HPV DNA and human DNA (hDNA) in urine sample was significantly improved by a DNA conservation buffer (either in-house or commercial). The difference between the untreated and treated urine was highly significant ($p < 0.001$ for the HPV DNA copies and the hDNA copies).
b. A significantly greater number of HPV DNA and hDNA copies were detected in the first void urine fraction compared with the midstream fraction. The difference was highly significant ($p=0.008$).
c. Urine collection method was highly acceptable and preferred compared to physician-collected cervical samples and brush self-collection among participating women ($p < 0.001$)
d. The barriers for screening may be different in different countries because of the different health-care system structure and cultural acceptance
e. For a mass screening programme to be medically and ethically acceptable, the WHO criteria for mass screening programmes have to be met

Recommendation
Based on the review, there was limited retrievable evidence to support its clinical performance of using urine for HPV DNA detection. Studies that related to diagnostic accuracy were only conducted among symptomatic or in combination of symptomatic and asymptomatic population whereas none in asymptomatic. Similarly, most of the study only tested the concordance rates of HPV DNA in paired urine and cervical samples in symptomatic women but none among asymptomatic. Moreover, there was no evidence retrieved related to the effectiveness or benefits of cervical cancer screening using HPV urine test with regards to patient outcomes such as mortality rate, survival rate, QOL, and QALY gained. The highly acceptance of urine-based programme among participating women, may, however, provide some compensation in term of increased participation and compliance.

HPV urine test may have the potential as one of the screening method to be used in the cervical cancer screening. However, in view of the wide range of sensitivity and specificity in detecting HPV DNA in urine (symptomatic and

combination of symptomatic and asymptomatic population) and no diagnostic accuracy study was retrieved among asymptomatic population, hence, currently HPV urine test is not recommended to be used as one of the screening method in the cervical cancer screening programme in Malaysia until there is more evidence on its diagnostic accuracy.

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Abbreviations

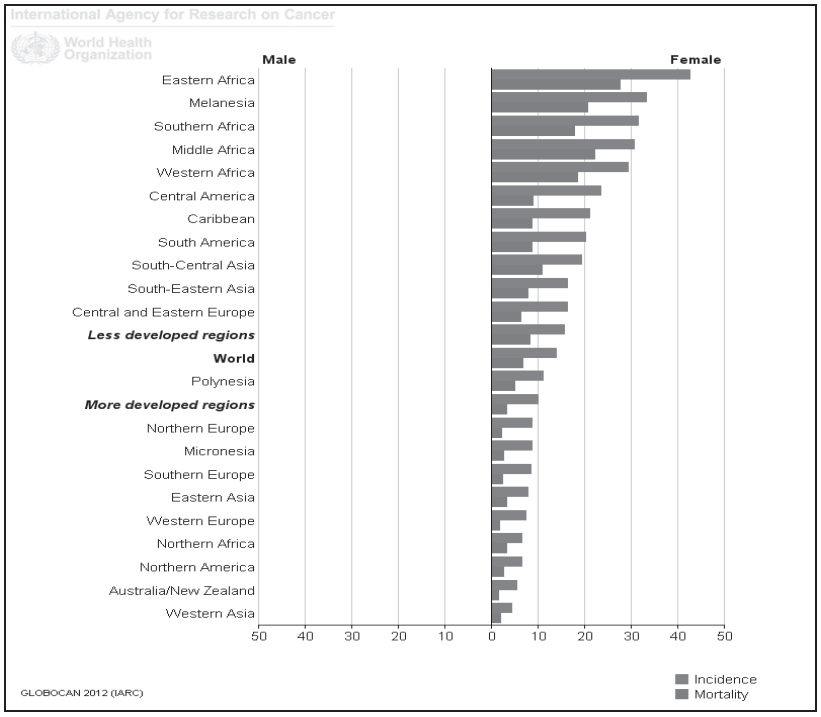
WHO	World Health Organization
NCR	National Cancer Registry
ASR	Age-standardised incidence rate
HPV	Human papilloma virus
DNA	Deoxyribonucleic acid
LSIL	Low-grade squamous intraepithelial lesion
HSIL	High-grade squamous intraepithelial lesion
IARC	International Agency for Research on Cancer
PCR	Polymerase chain reaction
CIN	Cervical intraepithelial neoplastic
STD	Sexually transmitted diseases
HTA	Health Technology Assessment
PPV	Positive predictive value
NPV	Negative predictive value
US FDA	United States Food & Drug Administration
HC	Hybrid Capture™
RNA	Ribonucleic acid
RLU	Relative light units
pg	Pico gram
QOL	Quality of life
QALY	Quality adjusted life years
RCT	Randomised controlled trial
CASP	Critical Appraisal Skills Programme
PRISMA	Preferred reporting items for systematic reviews and meta-analyses
QUADAS	Quality Assessment of Diagnostic Accuracy Studies
CI	Confidence interval
HR	High risk
ASC-US	Atypical squamous cells of unknown significance
ASC-H	Atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion
OR	Odds ratio
SD	Standard deviation
κ	Cohen's kappa
RFLP	Restriction fragment length polymorphism
hDNA	Human DNA
ICC	Invasive cervical cancer

HEALTH TECHNOLOGY ASSESSMENT (HTA)
HPV URINE TEST FOR CERVICAL CANCER SCREENING

1.0 BACKGROUND

According to World Health Organization (WHO) and GLOBOCAN 2012, cervical cancer is the fourth most common form of cancer in women worldwide and the fourth leading cause of cancer-related death globally. The occurrence of cervical cancer varies widely depending on geographic location. The regions of high incidence are Eastern Africa, Melanesia, Southern and Middle Africa, while the incidence is lowest in Australia/New Zealand and Western Asia (**Figure 1**).[1-2] While more recent data is yet to be published, according to the National Cancer Registry (NCR) Malaysia 2007, cervical cancer was the third most frequent cancer among women and the fifth most common cancer in the entire general population. A total of 847 cases were registered with NCR in 2007. The overall age-standardised incidence rate (ASR) of cervical cancer in Malaysia was 7.8 per 100,000 populations. Cervical cancer incidence rate increased with age after 30 years old and peaks at ages 65 to 69 years. Indian women had the highest incidence for cervical cancer followed by Chinese and Malay.[3]

Figure 1: Age-standardised cervical cancer incidence rate by sex and world area. Source: GLOBOCAN 2012



Researchers have clearly identified that infection with specific strains of human papilloma virus (HPV) has been associated with the development of cervical cancer. The HPV is a relatively small, non-

enveloped, double stranded circular deoxyribonucleic acid (DNA) virus, classified in the genus *papillomavirus* of the *Papoviridae* family of viruses.[4] More than 40 HPV types preferentially infect the stratified squamous epithelium of the mucosa of the cervix and vaginal, primarily by sexual intercourse. Up to 80% of sexually active women are infected at some point in their lives and 10% to 20% develop persistent infection.[5]

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 squamous intraepithelial lesion (LSIL). With viral integration, the oncogenic effect of the E6 and E7 proteins is enhanced and cellular changes characteristic of high-grade squamous intraepithelial lesion (HSIL) and ultimately cancer are observed.[6]

Since cervical and female genital infection by specific HPV types is highly associated with cervical cancer, those types of HPV infection have received most attention from scientific studies. More than 120 types of HPV have been identified, and approximately 51 types infect the epithelial membranes of the anogenital tract. The HPV strains are divided into two groups of high risk or low risk based on their oncogenic potential and the 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 **15** HPV types as high risk (**16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82**) and **12** as low risk (**6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108**). In particular, HPV16 and HPV18 are known to cause around 70% of cervical cancer cases worldwide.[7-8]

For decades, screening using conventional cytological Pap (Papanicolaou) test or Pap smear has been the most widely used strategy for reducing cervical cancer around the world. Since the introduction of the Pap test, the incidence and mortality rates from cervical cancer have declined drastically.[9-10] Subsequently the molecular methods to detect the HPV present in infected tissues were introduced. The HPV typing is generally done by liquid hybridization (Hybrid Capture 2, Digene MD, USA) and/or conventional and real-time polymerase chain reaction (PCR), using DNA from cervical scrapes/biopsies.[11] High-risk HPV DNA testing is considered to be added value for an early detection of cervical intraepithelial neoplastic (CIN) lesions in a routine cervical cancer screening programme which facilitates identification of 'high-risk' women for follow-up management. This was based on four randomised controlled trials and pooled analysis of these trials, which showed that HPV detection was more protective against grade 3 CIN and invasive cervical cancer compared with current screening methods.[12-15]

Both screening strategies, however, require a pelvic examination, a procedure that is invasive and uncomfortable for the patient, time consuming for healthcare providers and is unlikely to resolve the problem of poor screening uptake. Therefore, the development of non-invasive self-sample collection methods, which can be incorporated into existing cervical screening programmes, would have the potential of increasing the acceptance of the screening procedures. The use of urine, which is straightforward to collect, is claimed to be valuable for this purpose as exfoliated epithelial cells from the cervix and/or vagina is claimed to normally appear in the urine. Indeed, urine sample collection is use routinely in conjunction with molecular testing approaches in the diagnosis of the most common sexually transmitted diseases (STD) including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Simple and non-invasive, urinary HPV testing may be a pertinent method for providing screening to underprivileged women, to women lacking access to gynaecological specialists, and to women who refuse invasive Pap smears.[16]

In Malaysia, all women who are, or who have been sexually active, between the ages of 20 and 65 years, are recommended to undergo Pap smear testing. If the first two consecutive Pap smear results are negative, screening every three years is recommended.[17]

With the significant burden of cervical cancer in Malaysia, and to increase screening uptake as well as the acceptance of the screening procedures, therefore, a Health Technology Assessment (HTA) is required to assess the accuracy, effectiveness, and cost-effectiveness of HPV urine test for cervical cancer screening. This HTA was requested by the Senior Principal Assistant Director of Cancer Unit Disease Control Division, Ministry of Health Malaysia.

2.0 TECHNICAL FEATURES

Ideally, a screening test should have a high sensitivity to detect disease (low false-negative rate), a high specificity (low false-positive rate), and high positive and negative predictive values (PPV, NPV).[18-19] Efforts have been made to detect the presence of HPV DNA in urine in the most reliable way; using liquid hybridization, and PCR-based methods (using either conventional PCR or real-time PCR).

2.1 Liquid Hybridization: Hybrid Capture™ Assay

Most clinical investigations of HPV testing have used first- or second-generation Hybrid Capture™ (HC), the only HPV test currently approved by the US FDA (**Figure 2**).The HC system is a nucleic acid hybridization assay with signal amplification for the qualitative detection of DNA of high-risk, cancer associated HPV types in urine or cervical specimens. The first HC assay (HC1) is a tube base detection system and probe for only nine of the high-risk HPV types (16, 18, 31, 33, 35, 45, 51, 52 and 56). The second-generation HC system (HC2) has

improved reagents and was based on 96-well microplate format with in-built positive and negative controls. It is an in-solution, hybridization test able to detect 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and five low-risk HPV 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.[20]

Figure 2: Hybrid Capture™ Assay analyzer

To perform the HC2 assay, urine 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 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 results 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.[20-21]

2.2 Polymerase Chain Reaction (PCR)-Based Methods

The PCR is base on the repetitive replication of a target sequence of DNA flanked at each end by a pair of specific oligonucleotide primers, which initiate the polymerase-catalysed chain reaction (**Figure 3**).

Figure 3: Polymerase chain reaction (PCR) analyzer

Because of the exponential increase in the amount of target DNA sequence after a few reaction cycles of denaturation, annealing and extension, PCR has very high levels of molecular sensitivity and permits the detection of less than 10 copies of HPV DNA in a mixture. Therefore, PCR has a lower threshold of molecular detection for HPV DNA than the HC assay. Conventional PCR is based on target amplification with type-specific or consensus or general primers (short DNA fragments) including MY09/11, PGMY09/11, GP5+/6+, and SPF1/2 which are directed to L1 gene, a highly conserved region of the HPV genome. The latter are able to amplify sequences from several different HPV. The amplified DNA products can be revealed by ethidium bromide staining following agarose or acrylamide gel electrophoresis, which permits presumptive verification of the expected molecular weight of the amplified target, thus confirming positivity. Verification can also be done by methods that further probe the post-amplification products for their sequence homology with the target. Dot blot, Southern blot or line strip hybridization are used to this end and generally result in improved molecular sensitivity and specificity as compared with electrophoresis and staining. Finally, the use of restriction enzymes to analyse the fragment length signatures in combination with probe hybridization and direct DNA sequencing, provide the highest possible resolution to distinguish the HPV types present in a biological specimen.[22]

Real-time PCR on the other hand is a technique used to monitor the amplification of a targeted DNA molecule during the PCR in real-time, and not at its end, as in conventional PCR. Two common methods for the detection of PCR products in real-time PCR are *non-specific fluorescent dyes* that intercalate with any double-stranded DNA, and *sequence-specific DNA probes* consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence. In contrast, real-time PCR is less time consuming as it can detect amplifications during the early phases of the reaction, compared with conventional PCR which uses gel electrophoresis to analyse the amplified PCR products. Unlike conventional PCR which is highly sophisticated and labour intensive, automated detection techniques are found in real-time PCR.[23]

2.3 Confirmatory tests / reference / gold standard

Clinical sensitivity and specificity are often used to compare the diagnostic capabilities of a test and they traditionally rely on the performance of a given test, used at a specific test threshold, when compared to a reference or ‘gold standard’ that is supposed to give the ‘true’ diagnosis. It is mandatory that any positive HPV urine test or abnormal Pap smear result be investigated by an appropriate diagnostic procedure. The reference or ‘gold standard’ diagnostic procedure and the procedure of choice for this investigation is colposcopy with cervical biopsy.[24]

3.0 POLICY QUESTION

Should HPV urine test be used as a screening method in the cervical cancer screening programme in Malaysia?

4.0 OBJECTIVE

4.1 To determine the diagnostic accuracy of HPV urine test for HPV detection

4.2 To determine the benefits of cervical cancer screening using HPV urine test compared with conventional cervical cytological specimen, HPV DNA-based using cervical specimen, combination of conventional cytology and HPV DNA-based using cervical specimen, or no screening, with regards to patient outcomes such as detection rate, mortality rate, survival rate, quality of life (QOL), and quality adjusted life years (QALY) gained

4.3 To determine the safety of HPV urine test for HPV detection

4.4 To determine the economic impacts of HPV urine test for cervical cancer screening

4.5 To assess the organizational, ethical, and legal aspects related to cervical cancer screening using HPV urine test

Research questions

i. What is the diagnostic accuracy of HPV urine test for HPV detection?
ii. Is cervical cancer screening using urine effective in detecting HPV and reducing mortality?
iii. Is HPV urine test for HPV detection safe?
iv. What is the economic, organizational, ethical, and legal implication/impact related to cervical cancer screening using HPV urine test?

5.0 METHODS

5.1. Literature search strategy

Literature search was conducted by an *Information Specialist* who searched for published articles pertaining to the use of HPV urine test for cervical cancer screening. The following electronic databases were searched through the Ovid interface: Ovid MEDLINE® In-process and other Non-indexed citations and Ovid MEDLINE® 1946 to present, EBM Reviews - Health Technology Assessment 1st (Quarter 2016), EBM Reviews - Cochrane Database of Systematic Review (2005 to Feb 2016), EBM Reviews - Cochrane Central Register of Controlled Trials (Jan 2016), EBM Reviews - Database of Abstracts of Reviews of Effects (1st Quarter 2016), EBM Reviews - NHS Economic Evaluation Database (1st Quarter 2016). Parallel searches were run in PubMed. **Appendix 4** showed the detailed search strategies. Limitations only for female/women were applied to the search. The last search was run on

4th March 2016. Additional articles were identified from reviewing the references of retrieved articles.

5.2. Study selection

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

5.2.1 Inclusion criteria

a.	**Population**	Female, women
b.	**Intervention**	HPV urine test
c.	**Comparator**	**Test:** HPV DNA-based using cervical specimen **Screening programme:** i. Conventional cytology (Pap smear/liquid-based cytology) ii. HPV DNA-based using cervical specimen iii. Combination of conventional cytology and HPV DNA-based using cervical specimen iv. No screening
d.	**Outcomes**	i. Concordance (test agreement), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HPV urine test ii. Detection rate, mortality rate, survival rate, quality of life (QOL), and quality adjusted life years (QALY) gained iii. Adverse events or complications related to HPV urine test iv. Cost, cost-benefit, cost-effectiveness, and cost utility using HPV urine test in cervical cancer screening v. Organizational, ethical, and legal
e.	**Study design**	HTA reports, systematic review, randomised controlled trial (RCT), diagnostic accuracy, cross-sectional, cohort, case-control, and economic evaluation studies
f.	Full text articles published in English	

5.2.2 Exclusion criteria

a.	**Study design**	Animal study, experimental study, narrative review
b.	Non English full text articles	

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 was absolutely clear from the title and/or abstract that the study was not relevant, it was excluded. Full text article was retrieved for those title and abstract considered as relevant and if it was unclear from the title and/or abstract whether the study was relevant or not. 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 by two reviewers. For systematic review, the criteria assessed include selection of studies, assessment of quality of included studies, heterogeneity of included studies. For cohort study, the criteria assessed were selection of the cohort, accurate measurement of exposure and outcome, confounding factors, follow-up adequacy and length. For diagnostic study, the criteria assess were comparison with appropriate reference standard, all patients get the diagnostic test and reference standard, result of the test influenced by the result of the reference standard, disease status of population clearly described, and methods for performing the test described. All full text articles were graded based on guidelines from the U.S./Canadian Preventive Services Task Force (**Appendix 1**) or NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4(2nd Edition), March 2001 for test accuracy studies (**Appendix 2**).[25-26]

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 6**) and checked by another reviewer. Disagreements were resolved by discussion and the extracted data was presented and discussed with the expert committee. The data extracted was as follows: (1) Details of methods including study design, (2) Study population characteristics including age, trial inclusion and exclusion criteria, (3) Details of intervention and comparator, and (4) Types of outcome measures including diagnostic accuracy (concordance rate, test agreement, sensitivity, specificity, PPV, and NPV), effectiveness of cervical cancer screening (detection rate, mortality rate, survival rate, QOL, and QALY gained), safety (adverse events or complications related to HPV urine test), cost, cost-benefit, cost-effectiveness, cost utility, and economic evaluation of using HPV urine test for HPV detection, and organizational, ethical, and legal issues.

5.5 Methods of data synthesis

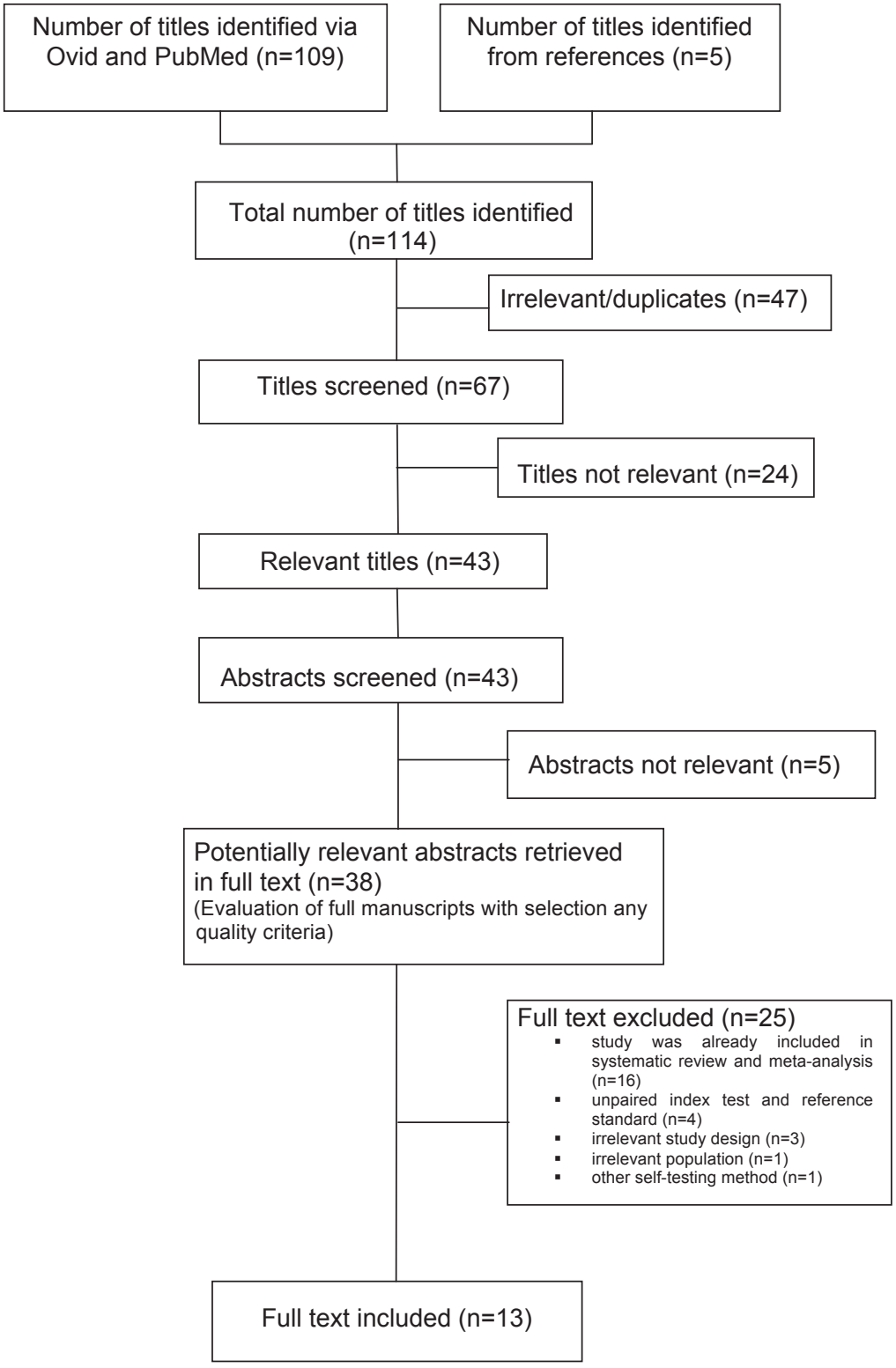
Data on the diagnostic accuracy, effectiveness, safety, cost-effectiveness, organizational, ethical, and legal of HPV urine test for cervical cancer screening were presented in tabulated format with narrative summaries. No meta-analysis was conducted for this review.

6.0 RESULTS

A total of **109** titles were identified through the Ovid interface and PubMed, and **five** were identified from references of retrieved articles. After removal of **47** irrelevant or duplicates, **67** titles were screened. A total of **43** abstracts were found to be potentially relevant and were screened using the inclusion and exclusion criteria. Of these, **38** relevant abstracts were retrieved in full text. After reading, appraising and applying the inclusion and exclusion criteria to the **38** full text articles, **13** full text articles were included and **25** full text articles were excluded. The articles were excluded due to the study was already included in systematic review and meta-analysis (n=16), unpaired index test and reference standard (n=4), irrelevant study design (n=3), irrelevant population (n=1), and other self-testing method (n=1). The excluded articles are listed in **Appendix 7**.

The **13** full text articles finally selected for this review comprised of one systematic review and meta-analysis, nine observational studies (cohort and cross-sectional) and three diagnostic accuracy studies. However, there was no study retrieved on the safety and cost-effectiveness/cost-utility analysis/economic evaluation of using HPV urine test for HPV detection. We also included two articles for ethical and legal consideration (one article by the WHO), and two local studies related to organizational issues.

Flow chart of retrieval of articles used in the results



6.1 DIAGNOSTIC ACCURACY / EFFECTIVENESS OF HPV URINE TEST FOR CERVICAL CANCER SCREENING

6.1.1 Clinical performance: Combination of population (symptomatic and asymptomatic)

Recently, Pathak N et al. 2014 conducted a systematic review and meta-analysis to determine the accuracy of detection of HPV in urine compared with the cervix in sexually active women (general, healthy unmarried college girls, adolescent, undergoing colposcopy, abnormal cytology, CIN2 or worse, low grade dysplasia or worse, and biopsy proved cervical cancer). This review was performed using recommended methods and reported in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement. Several electronic databases were searched from inception to December 2013 - Medline, Embase, the Cochrane Library, Web of Science, BIOSIS, DARE, and SIGLE. Data relating to patient characteristics, study context, risk of bias, and test accuracy were performed. The 2×2 tables were constructed and synthesised by bivariate mixed effects meta-analysis. From the estimates, a summary receiver operating characteristic curve and the following summary accuracy measures with 95% confidence intervals (CI) were derived – sensitivity, specificity, positive and negative likelihood ratio. The reference standard in all studies was a cervical sample taken by a clinician to test for HPV DNA. To visually explore heterogeneity, forest plots for test sensitivity and test specificity with 95% CI for individual studies were generated. To investigate sources of heterogeneity for both sensitivity and specificity, they included in the bivariate mixed effects models whereas sensitivity analyses were conducted to investigate the effect of studies including a narrow patient spectrum. QUADAS-2 tool were applied to all studies and quality assessment involved scrutinising patient selection, conduct of the index test, conduct of the reference standard, and patient flow.[27, level I]

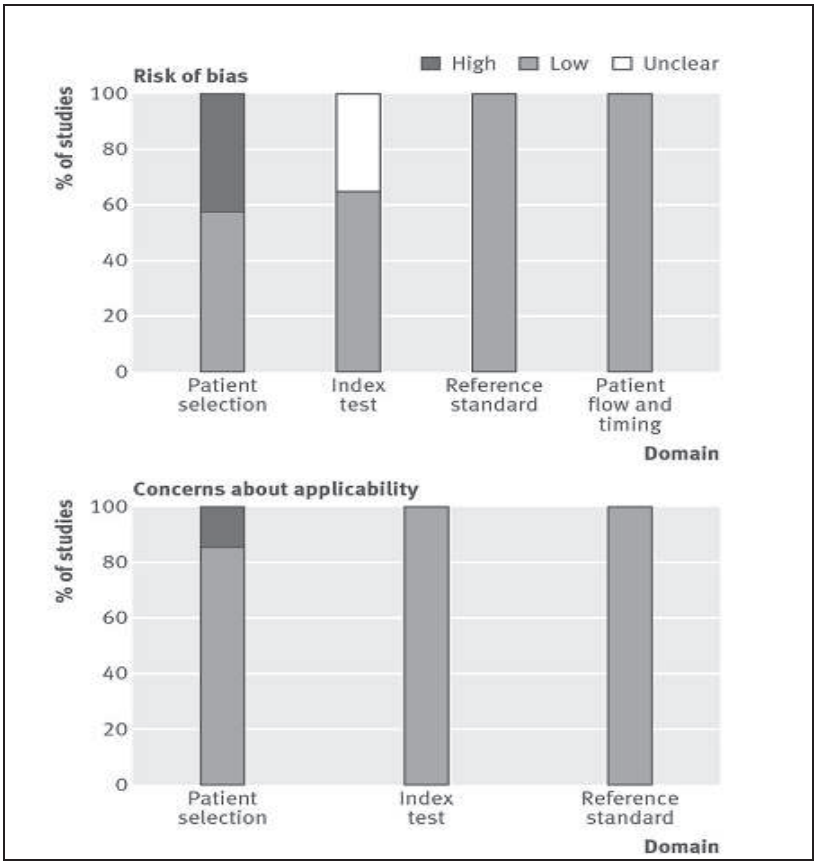
Description of Studies:
A total of 23 articles reporting on 21 studies (2,277 sexually active women) were included in the systematic review. Of these, 16 articles reporting on 14 studies (1,535 women recruited, 1,443 women analysed) were included in the meta-analysis. Twelve out of 21 study populations were recruited from gynaecology or colposcopy outpatient clinics and seven from genitourinary medicine or HIV clinics. For most study populations the purpose of testing was for cervical cancer screening (15/21). The remainder were for HPV surveillance (5/21) or follow-up of CIN (1/21). Four out of the 21 populations were positive for HIV. Of the 11 populations with reported cytology results, 35.9% (304/847) of women had low grade dysplasia or worse. Of the 10 populations with reported biopsy results, 54.1% (385/712) of women had grade 2 or worse CIN and 17.0% (121/712) had biopsy proved cervical cancer.[27, level I]

Most of the studies used conventional PCR (18/21), but testing methods were not uniform. Two of the 21 studies used nested PCR and one of the 21 studies used PCR based DNA microarray. Three studies evaluated quantitative real-time PCR and hybrid capture in addition to conventional PCR. In these cases, only the results for conventional PCR were included in the meta-analysis. The majority of urine sampling was first void (12/21). Other sampling methods included random (2/21), midstream (2/21), morning (1/21), and not specified (4/21). Urine storage temperature ranged from −70°C to 4°C. Sixteen studies used commercial DNA extraction kits and 11 used commercial amplification platforms. The remainder used in-house methods.[27, level I]

Quality of Studies:

Most studies (9/14) used consecutive or random recruitment of participants. All studies had a low risk of bias owing to patient flow and timing. All studies had a low risk of bias for the conduct of the reference standard (**Figure 4**). Five of the 14 studies used in-house methods for the index test and did not specify a threshold. No significant asymmetry in the funnel plot (p=0.62) and hence no evidence of publication bias.[27, level I]

Figure 4: QUADAS-2 quality assessment of 14 studies included in meta-analysis



Sources of Heterogeneity:

Table 1 summarises the results of the bivariate meta-regression based on planned covariates. There was a 22-fold increase in overall accuracy when samples were collected as first void urine compared with random or midstream urine samples (relative diagnostic odds ratio 21.7, 95% CI: 1.3, 376). However, this difference in accuracy is exclusively based on a significant increase in sensitivity of first void urine (relative sensitivity 1.2, 95% CI: 1.06, 1.37, p=0.004). Specificity was not affected by the urine sampling method (p=0.459). Purpose of testing, mean age of participants, HIV status, cytology and biopsy results, detection methods, use of commercial methods, or risk of bias as a result of patient selection did not explain any heterogeneity between indices for study accuracy.[27, level I]

Table 1: Bivariate meta-regression of study characteristics on sensitivity and specificity for detection of HPV in urine compared with cervical detection

Covariate	No of studies	P value*	Categories (sample size)	Sensitivity (95% CI), P value	Specificity (95% CI), P value	Diagnostic odds ratio (95% CI), P value
Urine sampling	12	0.004	First void urine(n=8); random or midstream urine (n=4)	89.0 (75.3 to 95.5); 73.9 (68.3 to 78.8), 0.0042	97.0 (94.1 to 98.5); 81.1 (25.9 to 98.1), 0.4593	264 (76 to 914); 12.2 (0.9, 159), 0.0345
Bias in patient selection	14	0.036	High risk (n=6); low risk (n=8)	91.8 (82.1 to 96.5); 81.3 (68.3 to 89.8), 0.1104	81.5 (52.6 to 94.6); 97.4 (90.0 to 99.4), 0.1696	49 (8 to 311); 162 (29 to 915), 0.3427
Purpose	14	0.708	HPV surveillance (n=5); cervical cancer screening or CIN follow-up (n=9)	89.7 (80.6 to 94.8); 84.2 (71.8 to 91.8)	95.3 (59.4 to 99.6); 93.9 (76.9 to 98.6)	172 (11 to 2569); 82 (15 to 454)
HIV status	14	0.099	Positive (n=3); negative (n=11)	94.3 (71.9 to 99.1); 84.5 (75.3 to 90.7)	71.7 (34.0 to 92.6); 97.0 (86.9 to 99.4)	42 (3 to 571); 175 (31 to 1000)
HPV detection method	14	0.966	Conventional PCR (n=12); real time and nested PCR (n=2)	86.9 (77.3 to 92.8); 85.3 (57.8 to 96.1)	95.2 (82.1 to 98.8); 90.0 (35.1 to 99.3)	131 (26 to 657); 52 (2 to 1259)
DNA extraction method	13	0.996	Non-commercial (n=2); commercial (n=11)	86.0 (47.5 to 97.7); 87.4 (77.3 to 93.4)	96.4 (43.3 to 99.9); 94.7 (79.2 to 98.8)	163 (3 to 8528); 123 (22 to 680)
DNA amplification method	14	0.244	Non-commercial (n=6); commercial (n=8)	86.1 (70.8 to 94.1); 86.9 (75.5 to 93.4)	81.9 (51.3 to 95.1); 97.6 (89.8 to 99.5)	28 (5 to 156); 275 (50 to 1522)

CIN=cervical intraepithelial neoplasia; PCR=polymerase chain reaction.
*Likelihood ratio test.

Sensitivity analysis:

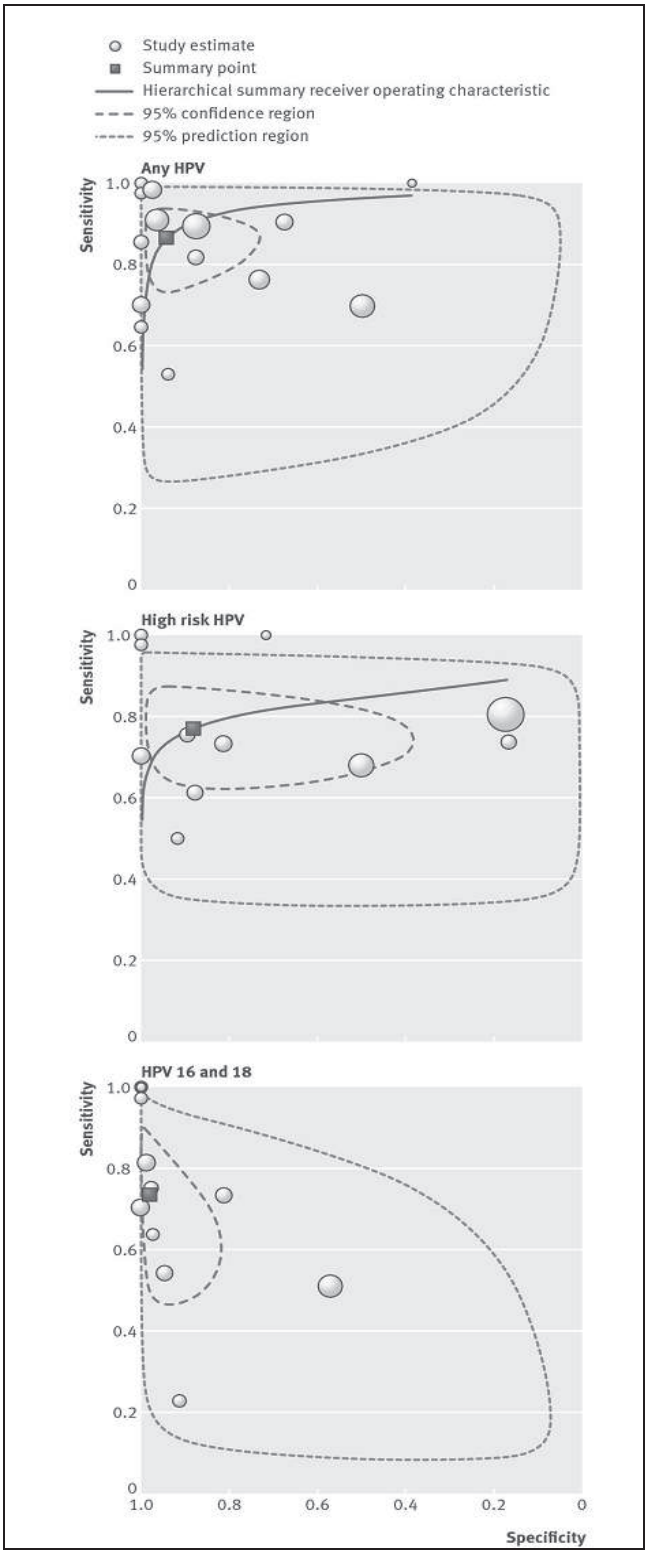
Pooled sensitivity and specificity for detection of any HPV in urine was similar when studies with a narrow spectrum of patients were excluded. Sensitivity was 80% (95% CI: 71%, 88%) and specificity was 98% (95% CI: 89%, 100%).[27, level I]

Meta-analysis:

For urine detection of **any HPV**, individual sensitivities ranged from 53% to 99% and specificities from 38% to 99%. For urine detection of **high risk HPV (HR-HPV)**, individual sensitivities ranged from 50% to 98% and specificities from 17% to 99%. For urine detection of **HPV 16 and 18**, individual sensitivities ranged from 23% to 97% and specificities from 56% to 99%.[27, level I]

Figure 5 summarises the pooled sensitivity and specificities as summary receiver operating curves for the same three groups.[27, level I]

Figure 5: Receiver operating characteristic plots for studies evaluating accuracy of detecting HPV in urine compared with in cervix



Urine detection of **any HPV**:

- Pooled sensitivity of 87% (95% CI: 78%, 92%)
- Pooled specificity of 94% (95% CI: 82%, 98%)
- Positive likelihood ratio was 15.22 (95% CI: 4.56, 50.81)
- Negative likelihood ratio was 0.14 (95% CI: 0.10, 0.20)

Urine detection of **HR-HPV**:

- Pooled sensitivity of 77% (95% CI: 68%, 84%)
- Pooled specificity of 88% (95% CI: 58%, 97%)
- Positive likelihood ratio was 6.33 (95% CI: 1.48, 27.00)
- Negative likelihood ratio was 0.26 (95% CI: 0.16, 0.41)

Urine detection of **HPV16 and HPV18**:

- Pooled sensitivity of 73% (95% CI: 56%, 86%)
- Pooled specificity of 98% (95% CI: 91%, 100%)
- Positive likelihood ratio was 36.97 (95% CI: 6.77, 201.91)
- Negative likelihood ratio was 0.27 (95% CI: 0.15, 0.49)

The authors also mentioned several limitations:
While it is feasible that HPV urine test might be useful for screening, there were many limitations in this meta-analysis. This means its effectiveness as a screening tool is still up for debate and is unproven. A major limitation is the between study variation in pooled sensitivities and specificities. Other issues included:

- The large variation between individual studies for participant characteristics
- The large variation in estimates of test sensitivity and specificity between individual studies
- The lack of standardized methods of urine testing and collection
- The surrogate nature of detecting cervical HPV DNA to predict cervical disease

This ultimately meant a relatively diverse test of screening tests, participants and results were lumped together to give a summary result of test accuracy. This means the pooled result may not actually be a good representation of the underlying studies as they are not a uniform group. This means that all results must be interpreted with caution as they may have been overestimated or underestimated.

If serious consideration is to be given to using urine HPV testing in cervical screening programmes, then further evaluation is essential, including an adequately powered, high-quality prospective study comparing urine testing with vaginal self-sampling and reporting the detection of high grade CIN (pre-cancer) as the primary endpoint.

6.1.2 HPV detection and genotyping: General population (asymptomatic)

Ducancelle et al. 2015 performed the CapU study (the first to evaluate a new strategy involving HPV detection in home-collected urine in complementarity with a cervical cancer screening program) to evaluate the acceptance of a urinary HPV test. Between July 2010 and January 2013, letters proposing a new cervical cancer screening method were sent to 5,000 women (2,000 women in a 55-65 age group; 3,000 in a 40-54 age group) who not had a Pap smear over the past three years. The participating patients had to send their urine samples (first void) to the Angers Hospital Virology Laboratory for analysis using real-time PCR (Abbott Molecular Diagnostics). Cervical cells were obtained using a cervical brush for conventional cytological slides by general practitioners or gynaecologists. Women with cytological abnormalities underwent colposcopy and biopsy. Of the 2,000 letters sent to the 55-65 age group women, 1,940 reached their respective addressees. All 3,000 of the letters sent to women aged 40-54 were successfully delivered. Women in the 55-65 age group and those in the 40-54 age group sent in 259 (12.9%) and 512 (17%) urine samples, respectively. Of the 771 samples received, 687 were suitable for and subjected to analysis, which represented 13.7% of the 5,000 mailings sent. The study indicated that 29 of the 687 analysable samples (4.2%, 95% CI: 2.9%, 6.0%) were HR-HPV positive. Among the 29 HR-HPV positive samples, HR-HPV other than 16 or 18 were the most frequently found types: 22/29 (76%) versus only 2/29 (7%) for HPV 16 single infection, 4/29 (14%) for HPV 16 + HR-HPV coinfection and 1/29 (3%) for HPV 18 + HR-HPV coinfection, (p=0.0001). Human papillomavirus (HPV) 16 or 18 was detected in coinfection with other HR-HPV types in 5/29 (17%) of the patients. Twenty-eight of the 29 HPV-positive women were referred to their physician (follow-up rate of 96.5%) for cytology tests and/or colposcopy. The results showed 19 normal and nine abnormal smears. Among these, there were three atypical squamous cells of unknown significance (ASC-US), one atypical squamous cell-cannot exclude high-grade squamous intraepithelial lesion (ASC-H), two LSIL and three HSIL. The authors concluded that urinary HPV testing may be pertinent to women who do not have cervical Pap smears done and lead to the diagnosis of high-grade cervical lesions.[28, level II-2]

Manhart LE et al. 2006 conducted the first population-level data on HPV detection rate in the United States. A total of 3,262 sexually active women aged 18 to 25 years were included in the WAVE III study (National Longitudinal Study of Adolescent Health). This study was designed to explore health-related behaviours of adolescents and health outcomes in young adulthood, focusing on social contextual influences. Participants completed a computer-assisted survey interview (CASI), collecting extensive data on demographic, social, and behavioural characteristics, and provided a urine specimen (urine sampling method was not mentioned). The HPV positivity was

determined first by PCR amplification and then followed by dot blot hybridization. HPV positive samples were typed using probes to detect types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, 84 using the Roche Diagnostics line blot assay. They found that overall HPV detection was 28.6% (934/3,262). Age-specific incidence was approximately 30% in women aged 18 to 21 years, and declined with age. Race-specific HPV incidence was highest among African and Native Americans and lowest among Asians. A total of 1,985 different combinations of HPV types were identified in the 934 HPV-positive women. HPV 16 was the most commonly identified type (5.8%), followed in frequency by types 84 (3.2%), 51 (3.0%), 62 (3.0%), 54 (2.9%), and 53 (2.8%). Nearly 10% of women with only one lifetime sex partner were infected with a high-risk HPV type. Compared to women without HPV infection, women with infection tended to be younger, single, black, younger at sexual debut, and had more sex partners. There was no difference in HPV positivity by region, educational level, annual personal income, or having signed a virginity pledge.[29, level II-2]

A population based study was developed by Thilagavathi A et al. 2012 to obtain a profile of HPV infection status among young college girls with no sexual exposure. Between August 2009 and July 2010, a total of 238 young sexually unexposed girls, aged from 17 to 25 years with a mean age of 21 (SD = 2.3 years) were randomly selected from a University in Tiruchirappalli district, Tamilnadu, India. The inclusion criteria for the study subjects were no previous history of vaccination, had no treatment for cervical diseases, lack of physical or mental impairments, and no history of previous sexual exposure. First voided mid-stream urine samples were collected and transported to the laboratory on ice and stored at 4°C before initially screened by the L1 consensus primers in a nested PCR format. Positivity for HPV DNA was reported among 9.2% (22/238) of the study subjects. The most frequently detected HPV type was HPV 16 (9.1%; 2/22) followed by HPV 11 (4.5%; 1/22). No co-infection or multiple infections was observed among the study subjects.[30, level II-2]

More recent study by Sharma K et al. 2015 on Indian tribal population examined the status of HPV infection and its genotype distribution in 2,278 healthy tribal girls comprising pre-adolescent (9–12 years), adolescent (13–17 years) and young adult girls (18–25 years) from three different tribal states of India. Random self-collected midstream urine samples along with socio-demographic data were collected by home to home visits and from local schools. Out of these, 2,034 samples which showed adequate DNA and successful amplification of β-globin gene were subjected to HPV detection (PCR using MY09/MY11 and GP5+/GP6+ primers) and genotyping by PCR, PGMY-reverse line blot assay & sequencing. Statistical analysis (univariate and multivariate logistic analysis) was performed to estimate the HPV detection and its association with various risk factors. The detection of HPV in the three sampling states was found to be

almost similar despite distinct geographical locations and ethnicity. Of 2,034 adequate samples, 262 (12.9%) tested positive for one or more HPV genotypes. Out of 262 HPV positive girls 168 (64.1%) were found infected with high risk genotypes, and almost half (132; 50.4%) of them were infected by HPV 16 alone. There was an overall increase in HPV detection with age; thus, young adult girls (18–25 years) were having the highest (19.2%) HPV infection followed by adolescent (11.4%) and pre-adolescent girls (6.6%). Multivariate analysis was applied on four risk factors (menarche, boyfriend, income, and education). There was a strong association between HPV infection and menarche of girls (odds ratio [OR] 3.1, 95% CI: 2.03, 4.73; p<0.001), and who have boyfriend (OR 3.4, 95% CI: 2.11, 4.85; p<0.0001). However, income and education showed weak association with HPV infection.[31, level II-2]

Summary of studies related to HPV detection and genotyping in general population (asymptomatic) is shown in **Table 2**.

Table 2: Studies reporting HPV detection and genotyping in urine samples in general population (asymptomatic)

Study	Patients	Urine sampling method	HPV detection (%)	HPV type
Ducancelle et al. 2015[28]	N=5,000 Aged 40-65 years Had no Pap smear (≤ 3 years)	Type: first void Volume: not mention Storage: 4°C	4.2	Other than 16 or 18
Manhart LE et al. 2006[29]	N=3,262 Aged 18-25 years Sexually active women	Type: not mention Volume: not mention Storage: -70°C	28.6	16, 84, 51, 62, 54, 53
Thilagavathi A et al. 2012[30]	N=238 Aged 17-25 years Young sexually unexposed girls	Type: midstream Volume: 30-40 ml Storage: 4°C	9.2	16, 11
Sharma K et al. 2015[31]	N=2,278 Healthy tribal girls: - pre-adolescent (9–12 years) - adolescent (13–17 years) - young adult girls (18–25 years)	Type: midstream Volume: 20-35 ml Storage: -20°C	 6.6 11.4 19.2	16

6.1.3 Clinical performance, detection, and concordance in paired urine and cervical samples: High risk population (symptomatic)

Ducancelle et al. 2014 conducted a prospective longitudinal multi-centre study (PapU study) involving 245 patients (aged 18 to 55 years) consulting a gynaecologist for cytology in three university hospitals. The main objective was to compare HPV viral loads and genotypes in paired cervical and urine samples, and to assess correlation between virological and cytological results. First-stream urine (5-10 ml) were sampled for each patient and stored at -80°C until analysis. Paired specimens were collected and analysed from 230 of 245 women. The HPV DNA detection and quantification were performed using a real-time PCR method with short fragment PCR primers. Genotyping was carried out using the INNO-LiPA HPV genotyping assay. Cohen's

kappa was used to evaluate agreement for HPV detection between urine and cervical (significant κ value > 0.7). The study showed that for the 230 women with paired samples, cytological examination was normal in 34 patients (15%) and abnormal in 196 (85%). Bethesda system classification was as follows: high-grade squamous intraepithelial lesion (HSIL) in 25 patients, low-grade squamous intraepithelial lesion (LSIL) in 59, and atypical squamous cells of undetermined significance (ASC-US) in 70. Women with cytological abnormalities underwent colposcopy and biopsy. The detection of HPV in the 230 paired urine and cervical smear samples was 42% (98/230) and 49% (113/230), respectively. Overall agreement for HPV positivity and negativity between the paired samples was 90% (κ = 0.80). High HPV viral load in both cervical and urine samples was associated with cytological abnormalities (**Figure 6**). The HPV-positive women were mostly infected with HR-HPV types. The distribution of HPV genotyping was similar across the two sample types (**Figure 7**). The HPV type 16 was identified most frequently in both samples with a detection rate of 38% and 32% in cervical and urine samples, respectively. The agreement between high- and low-risk HPV (LR-HPV) detection in both samples was 97% (κ = 0.95 for HR-HPV and κ = 0.97 for LR-HPV). The authors concluded that urinary HPV DNA testing could be useful for cervical lesion screening based on the high concordance rates for HPV-DNA quantification and genotyping in paired urine and cervical samples.[32, level II-2]

Figure 6: Correlation between HPV viral load in urine and cervical samples and cytology grade

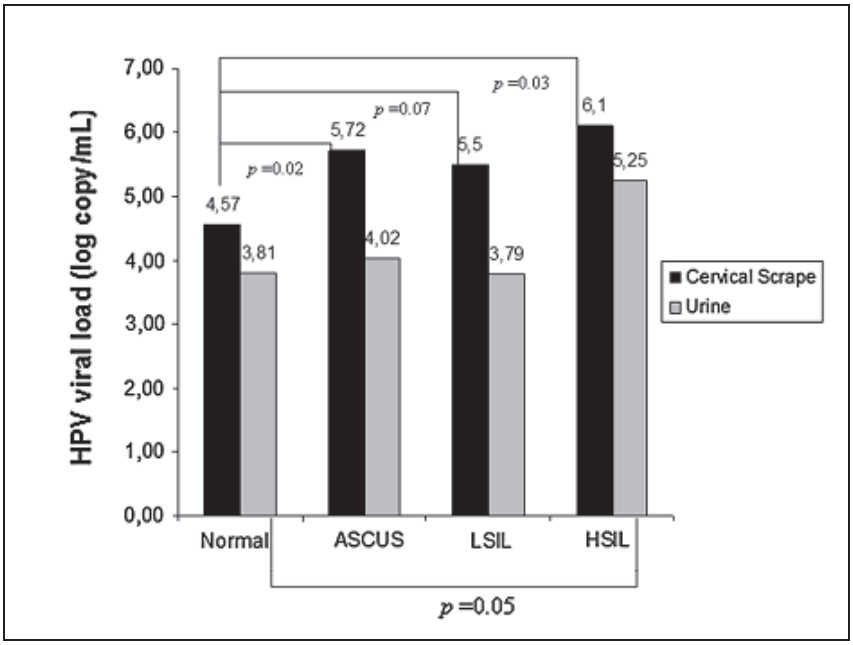
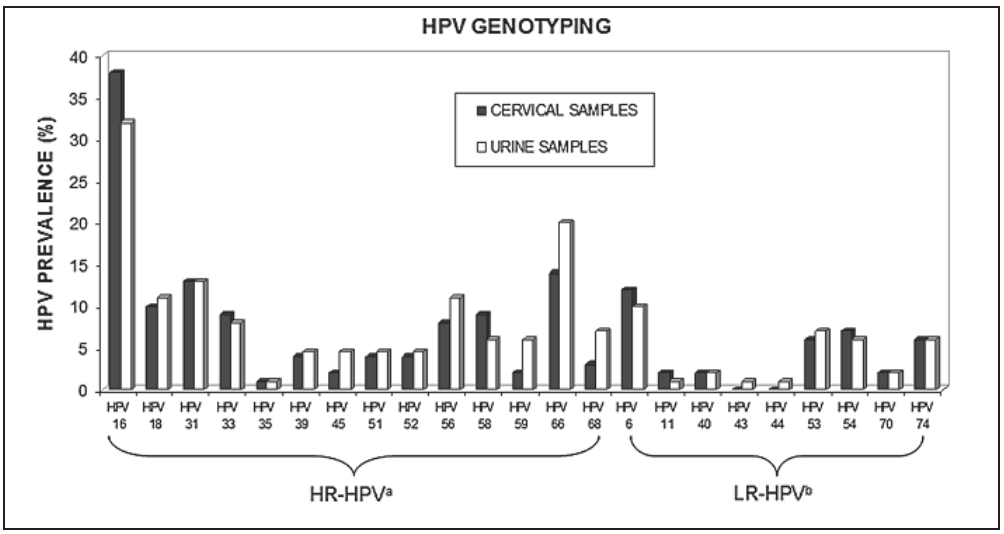


Figure 7: Distribution of HPV genotypes in cervical and urine samples



Bernal S et al. 2014 conducted a diagnostic study to evaluate urine testing for high risk HPV in a high prevalence population. They also evaluated the clinical performance of urine-based sampling compared to cervical sampling and correlated results against histologically confirmed. Paired first voided urine and cervical samples were collected from 125 women (median age 35.5 years) referred to Gynaecology Unit of Valme University Hospital (Seville, Spain) for evaluation of abnormal Pap smear screening results from primary care. For this purpose, Cobas 4800 (Roche Diagnostic, Spain) system features fully automated sample preparation which is an FDA approved real-time PCR assay designed for high risk HPV (HR-HPV) detection and simultaneous HPV-16 and HPV-18 genotyping was used. The sensitivity, specificity, NPV and PPV of HPV detection in urine samples compared to the detection in cervical samples was calculated. Concordance between test was assessed using the Kappa statistic (Cohen's Kappa, κ) and defined as "poor" (κ=0), "slight" ($0.01 < \kappa < 0.20$), "fair" ($0.21 < \kappa < 0.40$), "moderate" ($0.41 < \kappa < 0.60$), "substantial" ($0.61 < \kappa < 0.80$), "almost perfect" ($0.81 < \kappa < 1$) or "perfect" (κ =1). The study demonstrated that 72 out of 125 (57.6%) women tested positive for HPV using at least one of the two samples tested. The detection of HPV carcinogenic types was 52.8% (66/125) in urine samples and 50.4% (63/125) in cervical samples. The overall percent agreement between HPV detection in urine and cervical samples was 88%. A substantial concordance rate of HPV DNA detection in both samples was observed (κ=0.76; 95% CI: 64, 87). In this high prevalence population the sensitivity, specificity, NPV and PPV for detection of HPV DNA from urine versus cervical samples were 90.5% (95% CI: 80, 95%), 85%, (95% CI: 74, 92%), 89.8% (95% CI: 79.5, 95.3) and 86.4% (95% CI: 76.1, 92.7), respectively. Cytological results were available for 122 women, of whom 65 were reported to be

negative for intraepithelial lesions, 21 had ASCUS, 22 had LSIL, and 14 had HSIL. Histological results were available for 80 women, of whom 43 did not have CIN, 17 had CIN1, four had CIN2, and 16 had CIN3. The HPV infection was similar in cervix (62/122) and urine (65/122) regardless of the result of cytology and histology (P>0.05). Compared to histological confirmed CIN2 or 3, the clinical sensitivity and specificity for the detection of high-risk HPV in urine samples were 95% (95% CI: 76, 97%) and 52.4% (95% CI: 40, 64%), respectively. For cervical samples they were 90% (95% CI: 69, 97) and 50.8% (95% CI: 38, 62), respectively.[33, level 2]

From October 1996 to March 1997, a total of 200 women (mean age 31.5 ± 9.4 years) were referred to a colposcopy clinic because of abnormalities upon cervical cytological screening. Sellors JW et al. then determined the sensitivity and specificity of self-collected vaginal, vulvar, and urine (first void) sample compared with physician-collected cervical sample for HPV in detecting HSIL (CIN2 or 3). Presence of HPV was evaluated using the Hybrid Capture 2 assay with a probe cocktail for 13 carcinogenic types. Cervical specimens were also tested for HPV by PCR and hybridization with type-specific probes. Cervical smears for cytological examination were obtained from all women. The sensitivity, specificity, positive and negative predictive values, and positive likelihood ratios of the Hybrid Capture 2 results for the four specimen types were calculated, with the results of colposcopy examination (with directed biopsy as required) as the reference standard. Women with HSIL (CIN 2 or 3) or adenocarcinoma in situ on histological examination were regarded as having a “positive” result. Kappa statistic (Cohen’s Kappa, κ) defined as “poor” (κ=0), “slight” ($0.01 < \kappa < 0.20$), “fair” ($0.21 < \kappa < 0.40$), “moderate” ($0.41 < \kappa < 0.60$), “substantial” ($0.61 < \kappa < 0.80$), “almost perfect” ($0.81 < \kappa < 1$) or “perfect” ($\kappa = 1$). The study revealed that HSIL (CIN grade 2 or 3, and adenocarcinoma) were found in 29.0% (58/200) while the remaining 142 women (71.0%) had normal findings or LSIL (CIN grade 1). The detection of HPV in self-collected urine samples was 34.5% (69/200). The accuracy of the various methods of specimen collection for detecting HSIL (CIN 2 or 3) is shown in **Table 3**. The sensitivity of testing for HSIL (CIN 2 or 3) was progressively lower and the specificity progressively higher with increasing distance from the cervix (vagina, vulva and urine in that order). The sensitivity of cervical specimens was 98.3% (57/58), and for self-collected urine samples it was only 44.8% (26/58). The specificity was 52.1% (74/142) in cervical specimens and 69.7% (99/142) in urine. The likelihood ratios for a positive result with the Hybrid Capture 2 test for the cervical, vaginal, vulvar and urine samples were 2.1, 1.9, 1.7 and 1.5 respectively. Agreement (kappa statistic) between the cervical specimens and the vaginal, vulvar and urine specimens for the presence of HPV was 0.76, 0.55 and 0.41 respectively.[34, level 2]

Table 3: Results of testing for HPV by hybrid capture II assay for various types of specimens, relative to colposcopic results (58 women with biopsy-proven high-grade lesion* and 142 women without a high-grade lesion†)

Specimen	Type of lesion; no. positive for HPV				Test indices; value, % (and 95% confidence interval)‡			
	HSIL	LSIL	Other§	Total	Sensitivity for HSIL	Specificity for HSIL	Positive predictive value	Negative predictive value
Cervical brush sample, collected by physician	57	18	50	125	98.3 (90.8–100.0)	52.1 (43.6–60.6)	45.6 (36.7–54.8)	98.7 (92.8–100.0)
Self-collected vaginal swab	50	19	47	116	86.2 (74.6–93.9)	53.5 (45.0–61.9)	43.1 (33.9–52.6)	90.5 (82.1–95.8)
Self-collected vulvar swab	36	17	36	89	62.1 (48.4–74.5)	62.7 (54.2–70.6)	40.4 (30.2–51.4)	80.2 (71.5–87.1)
Self-collected urine specimen	26	13	30	69	44.8 (31.7–58.5)	69.7 (61.5–77.1)	37.7 (26.3–50.2)	75.6 (67.3–82.7)

*Women in whom high-grade squamous intraepithelial lesion (HSIL; equivalent to cervical intraepithelial neoplasia grade 2 or 3) was diagnosed by cervical biopsy (n = 57) or adenocarcinoma in situ was diagnosed by histologic examination (n = 1).

†Women in whom cervical biopsy did not lead to diagnosis of HSIL and women who did not undergo biopsy.

‡Example calculation of test indices for the physician-collected cervical brush samples: total number positive for HPV = 125; total number negative for HPV = 200 – 125 = 75. *Sensitivity*: Of the 58 women with HSIL, 57 were positive for HPV; sensitivity = 57/58 = 98.3%. *Specificity*: Of the 142 women without HSIL, 68 were positive for HPV (column 5 – column 2) and 74 (142 – 68) were negative for HPV; specificity = 74/142 = 52.1%. *Positive predictive value*: Of the 125 women who were positive for HPV, 57 had HSIL; positive predictive value = 57/125 = 45.6%. *Negative predictive value*: Of the 75 women who were negative for HPV, 74 did not have HSIL = 74/75= 98.7%. Likelihood ratio (positive) is calculated as (sensitivity/(1 – specificity) (98.3/(100 – 52.1) = 2.1

§Women (n = 118) in whom biopsy indicated no squamous intraepithelial lesion and women with a normal cervix on colposcopy.

Stanczuk GA et al. 2003 investigated the presence of HPVs in urine and cervical swab samples collected from 43 indigenous Zimbabwean women's (ages ranged from 24-75 years) with histological confirmed invasive cervical cancer from gynaecological clinic at the university hospital. The second aim of the study was therefore, to determine HPV type-specific concordance between paired cervical and urine samples. Urine samples were obtained before clinical examination while cervical swabs were collected during routine gynaecological examination. The HPV detection was done by means of degenerate primers in a nested PCR. Typing of HPVs was done using restriction fragment length polymorphism (RFLP) analysis. The study demonstrated that HPV DNA was detected in 42 of 43 (98%) cervical sample. The most prevalent type was type 16 (25/42, 59%), followed by type 33 (13/42, 31%). Types 18, 31, and 58 were present in 14% (6/42), 2% (1/42), and 2% (1/42) of samples, respectively. Dual HPV infection was identified in 17% (7/42) of HPV-positive samples (16+33, n=4; 33+18, n=3). On the other hand, HPV DNA was identified in 31 of the 43 (72%) urine samples. Twenty-eight of these samples were typed successfully. HPV 16 was the most prevalent (19/31, 61%), with type 33 present in 16% (5/31) of typed samples. HPV 18 and 31 were present in 13% (4/31) and 3% (1/31) of HPV-positive samples, respectively. Dual infection was present in two (6%) samples (16+18 and 16+33). Type-specific concordance between cervical and urine samples was high (22/28, 79%) and therefore, the HPV types identified in urine samples in most cases represent the same HPV type infecting the cervical epithelium.[35, level II-2]

Song ES et al. 2007 evaluated the clinical efficacy of the urine-based HPV DNA detection using HPV oligonucleotide microarray by comparing the results from matched cervical swab specimens. From January to December 2003, a total of 100 women (mean age 45.2 years) who had adequately analysed cervical swabs for HPV DNA and a biopsy proven histological diagnosis were selected for this study. Twenty three chronic cervicitis patients, 48 patients with CIN, and 29 patients with invasive cervical carcinomas, including three adenocarcinomas, were analysed. The cervical swab samples were collected by scraping the uterine cervical canal with a small cytobrush after a Pap smear. First voided urine samples were collected two weeks after the cervical scraping was performed. Cases showing positive beta-globin bands in both cervical and urine samples after PCR were analysed for the agreement study using kappa index which was defined by a chance corrected proportional agreement rate. It has maximum of 1.00 when agreement is perfect, a value of zero indicates no agreement better than chance, and negative values show worse than chance agreement, which is unlikely. The study indicated that DNA was present in all samples extracted from cervical cytobrush swabs and in 90 of 100 samples (90.0%) extracted from urine. The HPV DNA was detected in 70 of 100 (70.0%) cervical samples; four of 23 (17.4%) chronic cervicitis, 40 of 48 (83.3%) CIN, and 26 of 29 (89.7%) carcinoma samples and were all high risk HPVs. The HPV 16 was the most prevalent type (38 of 70 patients, 54.3%), followed by type 18, 58, 52, 33, 35, 31, and 51. Multiple HPV infection was identified in eight of 70 HPV-positive patients (11.4%). In urine samples, HPV DNA was identified in 47 of 90 (52.2%); three of 23 (13.0%) chronic cervicitis, 27 of 43 (62.8%) CIN, and 17 of 24 (70.8%) carcinoma patients and were all high-risk HPVs. The HPV 16 was the most prevalent type (30 of 45 patients; 63.8%), followed by type 18, 52, 35, 51, 58, 33, and 56. Multiple infection was identified in three of 47 (6.4%) HPV-positive patients. The HPV DNA detection rate of the cervical swab samples increased in accordance with the severity of the cytological and histological diagnosis, and was higher than the rate of urine samples in both CIN and carcinoma patients. The concordance rate for HPV detection between cervical swabs and urine was 69.3%. The type specific agreement of the HPV DNA test between cervical swabs and urine was good ($\kappa > 0.50$) in HPV 16, 18, 52, and 58 and fair ($\kappa < 0.50$) in HPV 33 and 35.[36, level II-2]

Nicolau P et al. 2014 conducted a study to determine the detection rate of HPV in urine samples from women with high-grade cervical lesions. Additionally, they aimed to identify the influence of socio-demographic factors and the different genotypes with urinary HPV positivity. From October 2010 until July 2011, 75 women (mean age 34.8 years) referred to the Cervical Pathology Unit at Obstetrics and Gynaecology Department at Hospital del Mar in Barcelona, with a positive biopsy for CIN2 or CIN3 were included in the study. Socio-demographic characteristics and relevant clinical information were collected from all patients. Cervical intraepithelial neoplasia 2+ (CIN2+)

was defined as lesions of CIN2 and CIN3. Detection and typing of HPV was performed by PCR using the Linear Array HPV Genotyping Test. Cohen's kappa was used to evaluate agreement: "poor" ($0.10 < \kappa < 0.20$), "fair" ($0.21 < \kappa < 0.40$), "moderate" ($0.41 < \kappa < 0.60$), "good" ($0.61 < \kappa < 0.80$). They found that all patients had histological CIN2+, of whom 55% had CIN3. No differences were found among demographic characteristics (age, parity, smoking status or contraceptive method) when comparing patients with positive urine HPV to those with a negative result. The detection of positive urine HPV test was 69.3% in CIN2+ population. For the sub population of CIN3 patients, the detection rose up to 78.1%, but there was no statistically significant difference (p=0.072). Regarding the viral subtype, different 31 genotypes were identified. The most frequent HPV genotype was HPV16. This genotype was positive in 57.7% of urine samples and 53.9% of cervical biopsies with a good level of correlation (kappa coefficient of 0.69).[37, level II-2]

Summary of studies related to clinical performance, detection, and concordance in paired urine and cervical samples in high risk population (symptomatic) is shown in **Table 4**.

Table 4: Studies reporting clinical performance, detection, and concordance in paired urine and cervical samples in high risk population (symptomatic)

Study	Patients	Urine sampling method	Diagnostic accuracy (95% CI)	HPV detection (%)			Concordance (%) (paired cervical + urine)			HPV type
				O	C	U	O	Typ-sp	Agree (k)	
Ducancelle et al. 2014[32]	N=245 Aged 18-55 years Consulting a gynaecologist	Type: NM Volume: NM Storage: -80°C			49.0	42.0	90.0		0.80	16
Bernal S et al. 2014[33]	N=125 Aged 21-65 years Referred to Gynaecology	Type: first void Volume: 30 ml Storage: NM	Sen: 90.5 Spec: 85.0 PPV: 86.4 NPV: 89.8	57.6	50.4	52.8	88.0		0.76	16, 18
Sellors JW et al. 2000[34]	N=200 Mean age 31.5 years Referred to colposcopy	Type: firs void Volume: 30 ml Storage: 4°C	Sen: 44.8 Spec: 69.7 PPV: 37.7 NPV: 75.6		62.5				0.41	16
Stanczuk GA et al. 2003[35]	N=43 Aged 20-70 years Invasive cervical cancer	Type: NM Volume: NM Storage: -20°C			98.0	72.0		79.0		16
Song ES et al. 2007[36]	N=100 Mean age 45.2 years Cervical swab positive Biopsy proven	Type: first void Volume: 30-50 ml Storage: NM			70.0	52.2	69.3		HPV16 (0.64) HPV18 (0.58)	16, 18
Nicolau P et al. 2014[37]	N=75 Aged 24-61 years Biopsy CIN2+ or CIN3+	Type: NM Volume: NM Storage: NM				CIN2+ (69.3) CIN3+ (78.1)			0.69	16

Abbreviation: O, overall; C, cervical; U, urine; Typ-sp, type specific; Agree, agreement; CI. Confidence interval; NM, not mention.

6.2 SAFETY

There was no retrievable evidence from the scientific databases on adverse events or complications associated with HPV urine test used for cervical cancer screening.

6.3 COST / COST-EFFECTIVENESS / ECONOMIC EVALUATION

There was no retrievable evidence on the cost-effectiveness of HPV urine test for cervical cancer screening. However, the average cost per HPV-DNA test for cervical specimen using PCR-based method ranged from RM 91.50 to RM 183.00. Hence, it is assumed that the cost for HPV-DNA detection in urine will most probably the same. The average cost per Pap smear test performed in Malaysia is RM 20.12.[38]

6.4 OTHER CONSIDERATIONS

6.4.1 Factor effecting: urine conservation medium and collection

Detection of HPV DNA in urine, a specimen that can easily be obtained by non-invasive self-sampling and (if necessary) mailed to a laboratory, has been suggested for surveillance and impact studies. Since the lower urinary tract lies in close proximity to the vagina, the vulva and the cervix, it is speculated that like the cervico-vaginal epithelium, the urethral epithelium would also be susceptible to HPV infections. These circumstances provide a rationale for using urine HPV detection as a screening tool for cervical cancer in developing countries. However, the detection of HPV DNA in urine is not as straightforward. A full understanding of how and in what form HPV DNA enters the urine remains lacking.

Vorsters et al. 2014 evaluated the effects of storage, sample preparation, extraction, and sampling on the detection of HPV DNA in the urine of 44 women with a normal cytology but HPV DNA positive cervical sample. For this purpose, they first compared five extraction methods (Qiagen direct, Amicon® + Qiagen, supernatant + Qiagen, Pellet + Qiagen, and EasyMAG) and then analyzed the impact of a urine-conservation medium (UCM1) that was developed in-house based on a phosphate-buffered saline solution with the addition of a chelating agent, a microbicide, a fungicide, and bovine serum albumin. Secondly, they compared the effect of untreated urine stored at room temperature for seven days with the urine mixed with an in-house conservation medium, and urine mixed with a commercially available preservation buffer. They also investigated the impact of testing first-void versus midstream urine on the detection of HPV DNA and human DNA (hDNA). The DNA analysis was performed using real-time PCR to allow quantification of HPV and hDNA. The study demonstrated that:

Effect of a urine-conservation medium (UCM1) and different extraction method

The detection of HPV DNA and hDNA in urine sample was significantly improved by a DNA-conservation medium. Without a preservation buffer, the number of samples that were positive for HPV DNA varied from seven out of 24 (29.2%) for the supernatant to 19 out of 24 (79.2%) for the pellet. Dilution of the urine with 1/3 volume conservation medium increased the number of positive samples. For the supernatant, Qiagen direct, and Amicon® + Qiagen, nine, five, and four additional samples, respectively, were positive in the UCM1 arm. For the detection of hDNA without a conservation buffer, the samples that were positive for hDNA varied from five out of 24 (20.8%) for the supernatant to 23 out of 24 (95.8%) for the pellet. Remarkably, all samples for all extractions, including the supernatant, were positive for hDNA when the urine was mixed with UCM1 (**Table 5**). The difference between the untreated and treated urine was highly significant ($P < 0.001$ for the HPV DNA copies and the hDNA copies) [39, level II-2]

Table 5: Impact of extraction method and urine-conservation medium (UCM0, no medium added; UCM1, in-house urine conservation medium) on the detection of HPV and hDNA in urine, shown as the number of positive/tested samples

Detection	Extraction	UCM0	UCM1
HPV DNA	Qiagen (200 µl)	13/24	18/24
	Amicon®(4 ml) + qiagen	17/24	21/24
	Supernatant (200) µl) + qiagen	7/24	16/24
	Pellet (1 ml)+qiagen	19/24	20/24
	Easy mag (1 ml)	18/24	19/24
hDNA	Qiagen (200 µl)	19/24	24/24
	Amicon®(4 ml)+qiagen	22/24	24/24
	Supernatant (200 µl)+ qiagen	5/24	24/24
	Pellet (1 ml)+ qiagen	23/24	24/24
	Easy mag (1 ml)	21/24	24/24

Comparison of an in-house and a commercial conservation buffer

HPV DNA was detected in the urine samples of 5/13 (38.5%), 12/13 (92.3%), and 11/13 (84.6%) women using the no-treatment, in-house, and commercial-buffer treatments, respectively. A significant difference in detection of HPV DNA between the untreated and treated urine was observed ($p \leq 0.003$, after correcting for volume). In-house buffer and commercial buffer allowed comparable recoveries of HPV DNA. Human DNA was detected in all but one untreated urine sample. The median number of copies of hDNA was 30 in the no-treatment, 3,730 in the in-house-buffer, and 1,200 in the commercial-buffer arm (**Table 6**). Significant differences were observed between the untreated and treated urine ($p \leq 0.001$, after correcting for volume). The in-house and commercial buffer performed similarly regarding the conservation of hDNA. [39, level II-2]

Table 6: HPV and hDNA copies found in urine using no preservative (UCM0), an in-house (UCM1), and a commercial conservation medium (BD Probe Tec™)

Patient number	Copy number HPV 16/18[a]			Copy number hDNA[a]		
	UCM0 no preservative (4 ml)[b]	UCM1 in house (2.66 ml)[c]	BD Probe tec ™ (3.2 ml)[d]	UCM0 no preservative (4 ml)[b]	UCM1 in-house (2.66 ml)[c]	BD Probe tec™ (3.2 ml)[d]
1	0	14	49	0	1,220	9,130
2	0	498	1,090	49	13,700	20,800
3	54,700	46,000	46,100	5	6,130	807
4	0	265	221	102	3,730	1,660
5	46	319	524	6	59	70
6	56	511	747	52	693	1,200
7	0	3	10	75	24,800	22,400
8	0	0	0	2	4,200	440
9	7	409	724	8	2,610	2,410
10	0	2	2	32	8,700	12,400
11	0	6	0	76	230	154
12	0	2	2	4	5,810	160
13	100	245	517	30	195	116
Neg#	8	1	2	1	0	0
Total#	13	13	13	13	13	13

[a] Total DNA eluted in 100 µl; 5 µl used in PCR; copy number expressed as copies per µl DNA extract
[b] 4 ml urine used for ultrafiltration
[c] 4 ml of urine mixed with UCM1 used for Amicon ultrafiltration; the actual volume of urine analyzed is 2.66 ml
[d] 3.2 ml of urine (maximum volume of BD vial) used for ultrafiltration

Impact of the urine fraction on HPV DNA detection

Table 7 shows the detailed results of HPV and hDNA quantification. There were 4.8 to 160 times more HPV DNA copies detected in the first-void fraction compared with the midstream fraction. The difference was highly significant (p=0.008). Human DNA was detected in all samples, but significantly more copies were observed in the first fraction (p=0.007). For all other pairs, between 1.4 and 21.4 times more copies of hDNA were detected in the first part of the urine void compared to the midstream fraction.[39, level II-2]

Table 7: Quantification of HPV DNA and hDNA in the first void (FV) and the midstream urine fraction (MID)

Sample number	HPV copies FV*	HPV copies MID	Ratio HPV copies FV/MID	hDNA copies FV**	hDNA copies MID	Ratio hDNA copies FV/MID	Volume FV ml	Volume MID ml
1	0.6	0	DIV/0	1,880	233	8.1	46	>70
2	1,450	24.4	59.4	1,710	200	8.6	64	64
3	14.4	2.99	4.8	4,110	192	21.4	40	52
4	6,400	626	10.2	552	387	1.4	35	59
5	767	7.6	100	2,830	327	8.7	NR	NR
6	0	0	DIV/0	56.9	142	0.4	24	17
7	2.6	0	DIV/0	204	44.6	4.6	54	89
8	392,000	15,600	25.1	17,300	2,760	6.3	32	37
9	36.4	0.23	160.4	314	64.1	4.9	38	34
10	51	4.18	12.2	5,900	1,500	3.9	60	60

* HPV FV and HPV MID were significantly different (P=0.008); ** hDNA FV and hDNA MID were significantly different (P=0.007)

Between October 2013 and May 2014, Senkomago V et al. conducted a pilot study to examine HR-HPV detection in urine collected at different times (first urination of the day versus initial stream and mid-stream collected later the same day) and in different urine fractions (supernatant, pellet, and unfractionated) using the Trovagene HPV HR test. They also examined the validity of HPV testing in the different urine samples for the detection of histologically-confirmed CIN2+. This study involved 37 non-pregnant women, aged ≥ 30 years, who attended the colposcopy clinic for follow-up of results of abnormal cytology or persistent HPV infection or treatment by loop electrical excision procedure (LEEP). Physician- and self-collected specimens were tested for HR-HPV mRNA using the Aptima HPV assay, which qualitatively detects E6/E7 mRNA of 14 HR-HPV types. Colposcopy was performed and directed biopsies obtained if clinically indicated. Cohen kappa values were calculated to assess agreement between urine samples. Median unbiased estimates and associated mid-P 95% confidence intervals were computed for sensitivity, specificity, PPV, and NPV for CIN2+ detection, stratified by sample type. The study showed that:

<u>High-risk HPV detection: stratified by urine sample type and urine fraction</u>

High-risk HPV detection was similar in unfractionated portions of the three types of urine samples: 64.9% (49.5, 80.2%) in first void, 73.0% (58.7, 87.3%) in initial stream, and 70.3% (55.5, 85.0%) in mid-stream (p-value range for pair wise comparisons: 0.26, 0.80) (**Table 8**). High-risk HPV detection was also similar in all pellet fractions: 67.6% (52.5, 82.7%) in first void, 78.4% (65.1, 91.6%) in initial stream, and 73.0% (58.7, 87.3%) in midstream (p-values range: 0.102, 0.414). In supernatant fractions, HR-HPV detection was similar for first void (73.0% [58.7, 87.3%]) and initial stream (75.7% [61.9, 89.5%]) samples, albeit lower in mid-stream samples (56.8% [40.8, 72.7%]) than in initial stream samples (p-value = 0.035).[40, level 2]

Table 8: High-risk HPV results, stratified by urine collection times and urine fractions tested

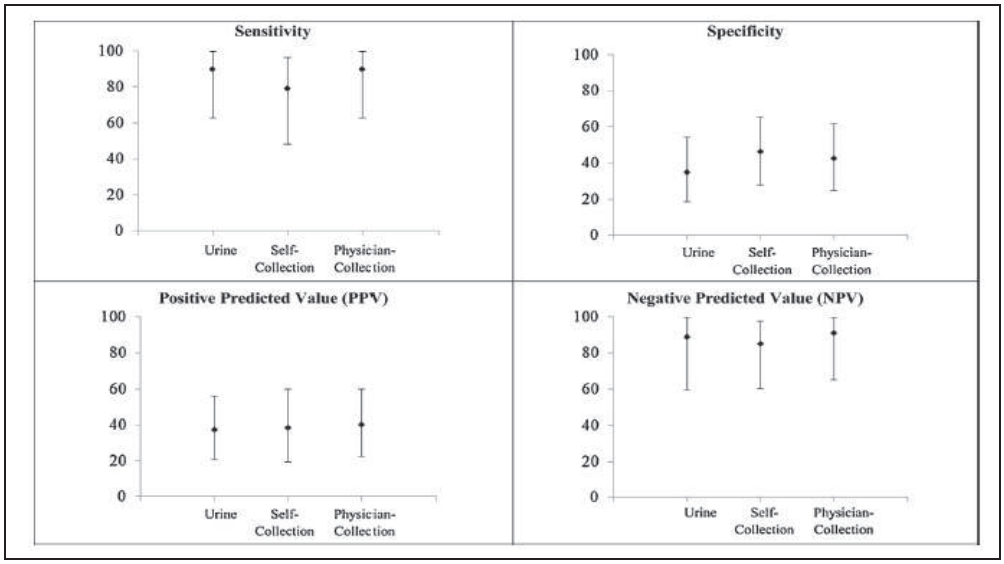
Type of urine sample	HR-HPV positive women, N (%)					
	First morning void at home (*N* = 37)		Initial stream sample at clinic (*N* = 37)		Mid-stream sample at clinic (*N* = 37)	
	No. HR-HPV+	(%) 95% CI	No. HR-HPV+	(%) 95% CI	No. HR-HPV+	(%) 95% CI
Urine fraction						
Unfractionated	24	64. 9 (49.5–80.2)	27	73.0 (58.7–87.3)	26	70.3 (55.5–85.0)
Pellet	25	67.6 (52.5–82.7)	29	78.4 (65.1 91.6)	27	73.0 (58.7–87.3)
Supernatant	27	73.0 (58.7–87.3)	28	75.7 (61.9–89.5)	21	56.8 (40.8–72.7)

[a] A total of nine HR-HPV test results were obtained for each participant: three different fractions (unfractionated (not centrifuged), pellet and supernatant) obtained from each of the three urine sample types (morning void, initial stream and mid-stream) collected per participant.

Detection of high-grade cervical lesions

The validity of HPV testing performance on urine for CIN2+ detection was assessed using the unfractionated initial stream samples, given that HR-HPV detection in unfractionated urine samples was similar to other fractions and detection in initial stream was similar to other sample types. The sensitivity of HR-HPV DNA testing in urine for CIN2+ detection was high (89.9% [95% CI: 62.7, 99.6%]), identical to that of mRNA testing of physician-collected specimens, and comparable to that of self-collected genital specimens (79.1% [48.1, 96.6%]) (**Figure 8**). Specificity of HR-HPV DNA in urine was relatively low (34.8% [18.4, 54.1%]), but comparable to specificity of HR-HPV mRNA testing on physician-collected (42.4% [24.6, 61.6%]) and self-collected genital specimens (46.2% [27.9, 65.2%]). High-risk HPV testing in urine, self-collected, and physician-collected specimens had low, but comparable PPVs of 37.2% (20.6, 56.2%), 38.3% (19.5, 59.8%) and 40.1% (22.4, 59.8%), respectively. Negative predictive value estimates for all tests were high: 88.9% (59.7, 99.5%) for urine, 84.9% (60.3, 97.6%) for self-collected specimens and 90.7% (65.3, 99.6%) for physician-collected specimens.[40, level 2]

Figure 8: Sensitivity, specificity, PPV, and NPV for CIN2+ detection by different tests



6.4.2 Organizational

Cervical cancer screening started with the introduction of the Pap test into clinical practice. In many countries, this occurred as part of family-planning services, so that the target group was younger women. Because such services are frequently not well integrated with secondary levels of care, it was not always possible to ensure adequate diagnosis and treatment of women with a positive test result.

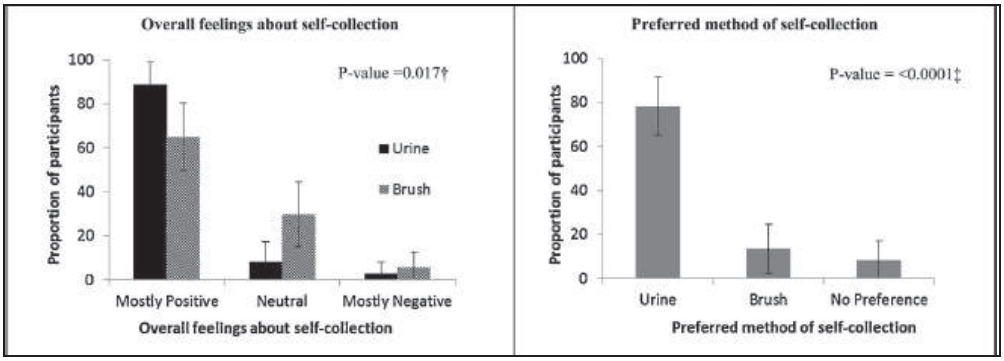
It has now become clear that organized screening programmes have a greater impact than opportunistic screening because they have the potential to achieve greater participation and this can improve equity of access and the likelihood of reaching women at higher risk.[22]

The causal role of high risk types of HPV in cervical carcinogenesis has led to the rigorous evaluation of HPV DNA testing of cervical samples in primary screening. A pooled analysis of four European randomised trials comparing cytology with cytology plus HPV testing over at least two screening rounds, reported that the combination increased protection by 60-70% compared with cytology alone.[12-15] However, most women especially in low socio-economic group consider undergoing cervical cancer screening embarrassing and many consider intravaginal examination with a vaginal speculum painful. These factors can contribute to patients' reluctance to subject themselves to screening programmes contributing to increasing cancer statistics. Therefore, HPV testing is now set to replace cytology in several national screening programmes, and can be performed on self-collected samples, including urine.[41]

Sellors JW et al. evaluated the feasibility of asking women to collect their own samples. The last 128 women who were enrolled were asked to grade the acceptability of the sampling methods on a 5-point *Likert scale*, with "1" indicating that a method was totally acceptable, "3" indicating neutrality and "5" indicating that it was not at all acceptable. Participants were also asked to rank the four methods (vaginal, vulvar, urine and cervical) according to preference (from most preferred to least preferred). The study revealed that the self-sampling methods were generally more acceptable: 98.4% (126/128) found the urine sampling acceptable, 92.9% (118/127) found the vulvar sampling acceptable, and 88.2% (112/127) found the vaginal sampling acceptable whereas only 79.0% (98/124) found cervical sampling by the physician acceptable. The preference rankings indicated that the urine sampling method was the most preferred (ranked first by 105 of 117 [89.7%] women), followed by the vulvar (ranked second by 89 of 116 [76.7%] women), vaginal (ranked third by 89 of 115 [77.4%] women) and cervical (ranked fourth by 88 of 114 [77.2%] women) sampling methods.[34, level 2]

Another study by Senkomago V et al. evaluated the acceptability of urine versus brush self-collection for HR-HPV detection among 37 women attending a colposcopy clinic. They found that a greater proportion of participants reported having mostly positive feelings about urine collection than brush self-collection (89% versus 65%), and more women reported neutral or negative feelings about brush self-collection than urine collection (neutral = 8% versus 30%, and mostly negative = 3% versus 5%, respectively) (p=0.017) (**Figure 9**). Most women (n=29, 78.4%) preferred urine collection compared to brush self-collection (chi-square p value < 0.001) and reported being comfortable with receiving the urine collection kit in the mail (n=32, 86.5%).[40, level 2]

Figure 9: Acceptability of urine versus brush self-collection for HR-HPV detection



Implementation of a national programme requires that there be a national policy that defines the screening age and interval and what method of screening will be used, as well as sufficient political and financial investment. The major issues that have to be considered are:

- The budget to run the programme
- Training of health-care providers in: the logic of the screening policy; carrying out the screening test; patient counselling; and collection and interpretation of monitoring data (participation and follow-up rates)
- Setting up equipment supply systems for the clinic or health centre
- Ensuring that high-quality laboratory services are available
- Establishing a referral pathway for treatment of patients (which may involve training of people at local level and referral for more advanced cases needing specialized treatment)
- Developing the capacity to offer treatment (for in situ disease, definitive treatment and palliative care)
- Setting up national monitoring systems
- Education of the population to ensure participation in the screening programme

Overall, a screening programme should be an integrated system in which, as seamlessly as possible, women are recruited, screened, receive and understand the results, are referred for treatment as required, return for repeat screening as determined by the policy and become advocates for others to participate. This means that all staffs must know, understand and give the same message to patients that services are accessible, equipped and welcoming, and that transport and communications mechanisms with institutions for reading of results and treatment are functional. In other words, a functional health system must operate with sufficient coverage, so that all women in the target group have satisfactory access to services.[22]

In Malaysia, currently all women who are, or who have been sexually active, between the ages of 20 and 65 years, are recommended to undergo Pap smear testing. If the first two consecutive Pap smear results are negative, screening every three years is recommended. However, there were two local studies on the prevalence of HPV using HPV-DNA based testing.

Soon R et al. evaluated the HPV serotype in Malaysian women with invasive cervical cancer (ICC) and CIN. The study showed that HPV DNA was detected in 96.0% of ICC specimens and 95.6% of CIN2 and CIN3 specimens. The HPV-16, -18, and -45 were detected in the majority of Malaysian women with ICC in this study. They concluded that vaccination against oncogenic types including and beyond HPV-16 or -18 is expected to significantly reduce the incidence of cervical cancer in Malaysia.[42]

More recently, Yong CM et al. from Hospital Ampang, Selangor conducted a feasibility study (unpublished) on population based cervical cancer screening in five states of Malaysia (Kelantan, Melaka, Johor, Sabah, and Sarawak). They found that the prevalence of HPV positive among 10,020 healthy women screened was 3.03%.[43]

6.4.3 Ethical and legal consideration

When cancers and tumours have been missed, a negative result will give false reassurance, with the increased possibility that there will be a delay in diagnosis and treatment. The advantages of increased sensitivity have to be weighed against the significant increase in false positive, the consequential number of colposcopy carried out, and the possible reduction in the specificity of the HPV DNA urine test. False positive results expose healthy people to unnecessary intervention and alarm, as well as generating considerable additional costs. The credibility of a screening programme can easily be undermined if the screening tests are considered unreliable. In this review, false positive rates for HPV urine test ranged from 13.6% to 62.8% in high risk population. [33-34, 40, level 2]

Now that studies have clarified the incidence of cervical cancer and strategies that may be effective in detecting and treating it, there is concern about the potential response of insurance companies and employers. Some insurers already require women to state the date and result of their last cervical smear. If a cervical cancer screening programme were introduced, applicants may also be required to supply the date and result of their last HPV urine test or Pap smear or colposcopy, and be charged higher premiums if they have not been screened nor have a positive result. Other issues include whether a person could be refused coverage on the basis of a screening test result or a refusal to have a test, and whether insurance companies would pay the cost of screening tests.

Prorok PC has enunciated the criteria by which any screening/surveillance programme can be judged: [44]

i. The disease must be common and have a substantial mortality and morbidity.
ii. The target population must be easily identifiable, and there must be an expectation that the physicians caring for the population will accept that screening is necessary and that the population will answer the call for screening.
iii. The screening test must have a low morbidity and a high sensitivity and specificity.
iv. There must be standardized recall procedures.
v. The screening test must be acceptable to the target population.
vi. Finally, and most importantly, there must be an acceptable and effective therapy.

In 1968, Wilson and Jungner authored a WHO document entitled "Principles and Practice of Screening for disease (Public Health Papers, No. 34)", which has defined ten criteria to be met by mass screening programmes for it to be medically and ethically acceptable. This criterion has been reviewed in 2003 as in **Appendix 5**. 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.[18]

7.0 DISCUSSION

There was no HTA report related to the accuracy of urine test for detecting HPV required for cervical cancer screening. However, there was recently the first and only systematic review and meta-analysis conducted to determine the accuracy of detection of HPV in urine compared with the cervix in sexually active women. The review showed that detection of HPV DNA in urine has a good accuracy for the presence of cervical HPV. Sensitivity was moderate for detection of any HPV, high-risk HPV, and HPV 16 and 18. The specificity for detection of HPV in urine was especially high for any HPV and the most oncogenic strains, HPV 16 and 18.[27] Compared with existing literatures; three reviews have been published on the detection of HPV DNA in urine. The first concluded that urine HPV detection was worse than cervical HPV detection at predicting CIN.[45] The second focused on surveillance in adolescents rather than in women at an age to be included in cervical cancer screening programmes.[46] The third appraised the potential importance of variations in urine sampling, storage, and testing methods.[47] The latter two reviews concluded that urine HPV detection could be an adequate tool in women, but none of the three reviews included a meta-analysis to support their conclusions.

The HPV detection from asymptomatic population has been strongly associated with age, gradually increasing with sexual activity among adolescents, and generally peaking around 25 years of age.[28-31] Overall, studies conducted among symptomatic patients attending the gynaecology department/cancer clinics or those attending colposcopy clinic have demonstrated a high degree of concordance rates with the same type of HPV in the paired cervical and urine samples.[32-33, 35-37] This fact, combined with a higher viral load in cervical samples (versus urine) suggests the contamination of urine with infected exfoliated cervical cells.[32, 34, 35-36] The chances of such a contamination increase with the higher-grade of lesions/invasive cancers, which carry higher probability of infections with HR-HPV types. Significantly higher viral load has been observed with HR-HPV types, which are more often associated with HSILs and invasive cancers than with the LR-HPV types. [35-37] Therefore, in these settings, urine HPV detection would truly reflect a cervical HPV infection/pathology. In general population screening, however, such high concordance rates and favourable test characteristics may not be obtained.

The HPV DNA positive women were mostly infected with the HR-HPV types which HPV16 was the most frequently identified type in both samples.[28-37] The same results were observed in the majority of Malaysian women with invasive cervical cancer.[42]

The benefits of using urine for the detection of HPV DNA have been evaluated in disease surveillance, epidemiological studies, and screening for cervical cancers in specific subgroups. The need to optimize and standardize sampling, storage, and processing has been reported. Finding showed that the detection of HPV DNA and hDNA in urine sample was significantly improved by a DNA conservation buffer (either in-house or commercial). Additionally, a significantly greater number of HPV DNA and hDNA copies were detected in the first void urine fraction compared with the midstream fraction.[39]

Majority believed that urine testing for HPV in cervical cancer screening needs further evaluation, and that the heterogeneity of urine sampling and testing protocols should be resolved. In particular, attention should be given to the rationale and evidence for using first void urine for HPV testing. During urination, urine is contaminated by impurities, including mucous and the debris of exfoliated cells from the vagina, cervix, and uterus. The initial flow of urine collects most of this debris, which is why in women with cervical HPV infection the first collected part of a urine void contains more HPV DNA than subsequent parts, as concluded by Pathak and colleagues[27] and confirmed by other included studies [28, 33, 34, 36] and recent research.[39] Some studies, however, did not mention which fraction or type of urine was analysed.[29, 32, 35, 37] Midstream urine on the other hand is preferred by some authors, since it is thought to contain less PCR inhibitors.[30-31]

Urine collection method was highly acceptable and preferred compared to physician-collected cervical samples among participating women ($p < 0.001$). A greater proportion of participants was reported having mostly positive feelings about urine collection than brush self-collection (89% versus 65%), and more women reported neutral or negative feelings about brush self-collection than urine collection ($p=0.017$).[34, 40] A better acceptance of urine-based programme by women, may, however, provide some compensation in term of increased participation and compliance, since physical scrapes, sometimes unpopular because of the dislike of physical examination or because of religious reasons, are avoided.

There was no retrievable evidence from the scientific databases on cost-effectiveness and adverse events or complications associated with HPV urine test used for cervical cancer screening. There was also no evidence retrieved related to the effectiveness or benefits of cervical cancer screening using HPV urine test with regards to patient outcomes such as mortality rate, survival rate, QOL, and QALY gained.

Limitations

It should be noted that most of the studies reported in the literature have potential limitations in terms of small sample size and have tested the concordance rates of HPV DNA in paired samples in highly selected subjects (symptomatic or patients referred to colposcopy clinic because of cervical abnormalities). In evaluating the efficacy of screening, it is preferable to have data from randomised screening trials. However, no such data are available with the incidence of clinical invasive cancer of the cervix as the end-point. The data available come from observational studies of screening in different clinic-epidemiological populations which HPV detection rate varies widely across the studies. Moreover, the heterogeneous methods of urine testing and HPV DNA assay systems affected the interpretation of accuracy measures in the individual studies conducted. Studies on the effectiveness of cervical cancer screening using HPV urine test related to mortality rate, survival rate, QOL, and QALY gained was not available, most probably due to unknown pathophysiological or biological behaviour of HPV and its shedding behaviour. Therefore, any new studies on the detection of HPV in urine must assess the feasibility and costs of these pathways. The surrogate nature of detecting cervical HPV DNA to predict cervical disease need further evaluation in prospective data and studies. Although there was no restriction in language during the search, only English full text articles were included in the report.

8.0 CONCLUSION

8.1 Clinical performance (diagnostic accuracy)

There was limited fair level of retrievable evidence to suggest that:

a. In a combination population of symptomatic (78%) and asymptomatic (22%) women, sensitivity and specificity of urine test varies with the types of HPV. Pooled sensitivity and specificity was 87% and 94%, respectively, for urine detection of any HPV. Urine detection of high risk HPV had a pooled sensitivity of 77% and specificity of 88%, while urine detection of HPV 16 and 18 had a pooled sensitivity of 73% and specificity of 98%.

b. In symptomatic population, overall sensitivity and specificity has been quite variable, ranging from 44.8% to 90.5% and 34.8% to 85.0%, respectively. Positive predictive value (PPV) ranged from 37.2% to 86.4% whereas NPV ranged from 75.6% to 89.8%.

c. There was no diagnostic study among asymptomatic women retrieved

8.2 HPV detection and genotyping

There was substantial fair level of retrievable evidence to suggest that:

a. Detection of HPV DNA in urine among screened asymptomatic women varies depending on the chosen population. HPV DNA detection ranged from 4.2% to 28.6% in sexually active women, and ranged from 9.2% to 19.2%, particularly in young sexually unexposed girls and healthy tribal girls.

b. Detection of HPV DNA in urine was increased among screened symptomatic women ranging from 34.5% to 78.1%

c. **HPV type 16** was identified most frequently in both urine and cervical samples

8.3 HPV concordance in paired urine and cervical samples

There was substantial fair level of retrievable evidence to suggest that:

a. Overall concordance for HPV positivity and negativity between cervical and urine samples in symptomatic women varied from 69.3% to 90.0% (agreement, κ from 0.41 to 0.80)

b. Type specific concordance rates in the paired samples have been very good for invasive cervical cancer (79.0%)

c. There was no study retrieved on concordance between cervical and urine samples among asymptomatic women

8.4 Safety

There was no retrievable evidence on adverse events or complications associated with HPV urine test used for cervical cancer screening.

8.5 Cost/cost-effectiveness/economic evaluation

There was no retrievable evidence on the cost-effectiveness of HPV urine test for cervical cancer screening.

8.6 **Organizational, ethical, and legal considerations**

There was evidence to suggest that:

a. The detection of HPV DNA and hDNA in urine sample was significantly improved by a DNA conservation buffer (either in-house or commercial). The difference between the untreated and treated urine was highly significant ($p < 0.001$ for the HPV DNA copies and the hDNA copies).

b. A significantly greater number of HPV DNA and hDNA copies were detected in the first void urine fraction compared with the midstream fraction. The difference was highly significant ($p=0.008$).

c. Urine collection method was highly acceptable and preferred compared to physician-collected cervical samples and brush self-collection among participating women ($p < 0.001$)

d. The barriers for screening may be different in different countries because of the different health-care system structure and cultural acceptance

e. For a mass screening programme to be medically and ethically acceptable, the WHO criteria for mass screening programmes as shown in **Appendix 5** have to be met

9.0 RECOMMENDATION

Based on the review, there was limited retrievable evidence to support its clinical performance of using urine for HPV DNA detection. Studies that related to diagnostic accuracy were only conducted among symptomatic or in combination of symptomatic and asymptomatic population whereas none in asymptomatic. Similarly, most of the study only tested the concordance rates of HPV DNA in paired urine and cervical samples in symptomatic women but none among asymptomatic. Moreover, there was no evidence retrieved related to the effectiveness or benefits of cervical cancer screening using HPV urine test with regards to patient outcomes such as mortality rate, survival rate, QOL, and QALY gained. The highly acceptance of urine-based programme among participating women, may, however, provide some compensation in term of increased participation and compliance.

HPV urine test may have the potential as one of the screening method to be used in the cervical cancer screening. However, in view of the wide range of sensitivity and specificity in detecting HPV DNA in urine (symptomatic and combination of symptomatic and asymptomatic population) and no diagnostic accuracy study was retrieved among asymptomatic population, hence, currently HPV urine test is not recommended to be used as one of the screening method in the cervical cancer screening programme in Malaysia until there is more evidence on its diagnostic accuracy.

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11.0 APPENDICIES

Appendix 1

HIERARCHY 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

HIERARCHY OF EVIDENCE FOR TEST ACCURACY STUDIES

Level	Description	
1.	A blind comparison with reference standard among an appropriate sample of consecutive patients	
2.	Any one of the following	Narrow population spectrum
3.	Any two of the following	Differential use of reference standard
4.	Any three or more of the following	Reference standard not blind Case control study
5.	Expert opinion with no explicit critical appraisal, based on physiology, bench research or first principles.	

SOURCE: NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4 (2nd Edition)

Appendix 3

PTK-FM-02 Pin.1/2016

HEALTH TECHNOLOGY ASSESSMENT (HTA) PROTOCOL
HPV URINE TEST FOR CERVICAL CANCER SCREENING

1.0 BACKGROUND INFORMATION

According to World Health Organization (WHO) and GLOBOCAN 2012, cervical cancer is the fourth most common form of cancer in women worldwide and the fourth leading cause of cancer-related death globally. The occurrence of cervical cancer varies widely depending on geographic location. The regions of high incidence are Eastern Africa, Melanesia, Southern and Middle Africa, while the lowest in Australia/New Zealand and Western Asia. The latest report of the National Cancer Registry (NCR) Malaysia 2007 stated that cervical cancer was the third most frequent among women and fifth most common cancer in the entire general population. A total of 847 cases were registered with NCR in 2007. The overall age-standardised incidence rate (ASR) of cervical cancer in Malaysia was 7.8 per 100,000 populations. Cervical cancer incidence rate increased with age after 30 years old and peaks at ages 65 to 69 years. Compared among the major races, Indian women had the highest incidence for cervical cancer followed by Chinese and Malay.

Researchers have clearly identified that infection with specific strains of human papilloma virus (HPV) has been associated with the development of cervical cancer. HPV is a relatively small, non-enveloped, double stranded circular deoxyribonucleic acid (DNA) virus, classified in the genus *papillomavirus* of the *Papoviridae* family of viruses. More than 40 HPV types preferentially infect the stratified squamous epithelium of the mucosa of the cervix and vaginal, primarily by sexual intercourse. Up to 80% of sexually active women are infected at some point in their lives and 10% to 20% develop persistent infection. 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 squamous intraepithelial lesion (LSIL). With viral integration, the oncogenic effect of the E6 and E7 proteins is enhanced and cellular changes characteristic of high-grade squamous intraepithelial lesion (HSIL) and ultimately cancer are observed.

Since cervical and female genital infection by specific HPV types is highly associated with cervical cancer, those types of HPV infection have received most of the attention from scientific studies. More than 120 types of HPV have been identified, and approximately 51 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 the 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 15 HPV types as high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and 12 as low risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108). In particular, HPV16 and HPV18 are known to cause around 70% of cervical cancer cases, between 41% to 67% of high-grade cervical lesions and 16% to 32% of low-grade cervical lesions worldwide.

For decades, conventional cytological Pap (Papanicolaou) test or Pap smear has been the most widely used strategy for reducing cervical cancer around the world. Since the introduction of the Pap test, the incidence and mortality rates from cervical cancer have declined drastically. Subsequently, the molecular methods to detect the HPV present in infected tissues were introduced. HPV typing is generally done by liquid hybridization (Hybrid

Capture 2, Digene MD, USA) and/or conventional and real-time polymerase chain reaction (PCR), using DNA from cervical scrapes/biopsies. High-risk HPV DNA testing is considered to be added value for an early detection of cervical intraepithelial neoplastic (CIN) lesions in a routine cervical cancer screening programme which facilitates identification of 'high-risk' women for follow-up management. This is based on four randomised controlled trials and pooled analysis of these, which showed that HPV detection is more protective against grade 3 CIN and invasive cervical cancer compared with current screening methods.

Both screening strategies, however, require a pelvic examination, a procedure that is invasive and uncomfortable for the patient, time consuming for healthcare providers and is unlikely to resolve the problem of poor screening uptake. Therefore, the development of non-invasive self-sample collection methods, which can be incorporated into existing cervical screening programs, would have the potential advantage of increasing the acceptance of the screening procedures. The use of urine, which is straightforward to collect, would be valuable for this purpose as exfoliated epithelial cells from the cervix and/or vagina is claimed normally appear in the urine. Indeed, urine sample collection is used routinely in conjunction with molecular testing approaches in the diagnosis of the most common sexually transmitted diseases (STD) including *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Simple and non-invasive, urinary HPV testing may be a pertinent method for providing screening to underprivileged women, to women lacking access to gynaecological specialists, and to women who refuse invasive Pap smears. Urine testing to detect HPV DNA has been previously examined in groups at high-risk infection, such as subjects infected with HIV, adolescents/young women, women with abnormal cervical cytology and cancer, as well as in healthy men and in male patients attending genito-urinary and STD clinics. Studies were included from Canada, Spain, United Kingdom, Greece, Italy, Sweden, India, Netherlands, Colombia, France, Korea, Zimbabwe and USA. However, results of those studies varied significantly and are often inconsistent due to problems associated with the sample collection and processing, the methods used for nucleic acid extraction and HPV DNA detection, and differences between the populations studied.

In Malaysia, all women who are, or who have been sexually active, between the ages of 20 and 65 years, are recommended to undergo Pap smear testing. If the first two consecutive Pap results are negative, screening every three years is recommended.

Technical Description

Ideally, a screening test should have a high sensitivity to detect disease (low false-negative rate), a high specificity (low false-positive rate), and high positive and negative predictive values (PPV, NPV). Alternatively, urine would be an appropriate sample for screening large populations. Urine test may increase participation and compliance, since physical scrapes, sometimes unpopular because of the dislike of physical examination or because of religious reasons, are avoided. Efforts have been made to detect the presence of HPV DNA in urine in the most reliable way; using liquid hybridization, and PCR-based methods (using either conventional PCR or real-time PCR).

Liquid Hybridization: Hybrid Capture™ Assay

Most clinical investigations of HPV testing have used first- or second-generation Hybrid Capture™ (HC), the only HPV test currently approved by the US FDA. The HC system is a nucleic acid hybridization assay with signal amplification for the qualitative detection of DNA of high-risk, cancer associated HPV types in urine or cervical specimens. The first HC assay (HC1) was a tube based detection system and probed for only nine of the high-risk HPV types (16, 18, 31, 33, 35, 45, 51, 52 and 56). The second-generation HC system (HC2) has improved reagents and was based on 96-well microplate format with in-built positive and negative controls. It is an in-solution, hybridization test able to detect 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and five low-risk type (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.

To perform the HC2 assay, urine 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 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 results 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.

Polymerase Chain Reaction (PCR)-Based Methods

The PCR is based on the repetitive replication of a target sequence of DNA flanked at each end by a pair of specific oligonucleotide primers, which initiate the polymerase-catalyzed chain reaction. Because of the exponential increase in the amount of target DNA sequence after a few reaction cycles of denaturation, annealing and extension, PCR has very high levels of molecular sensitivity and permits the detection of less than 10 copies of HPV DNA in a mixture. Therefore, PCR has a lower threshold of molecular detection for HPV DNA than the HC assay.

Conventional PCR is based on target amplification with type-specific or consensus or general primers (short DNA fragments) including MY09/11, PGMY09/11, GP5+/6+, and SPF1/2 which are directed to L1 gene, a highly conserved region of the HPV genome. The latter are able to amplify sequences from several different HPV. The amplified DNA products can be revealed by ethidium bromide staining following agarose or acrylamide gel electrophoresis, which permits presumptive verification of the expected molecular weight of the amplified target, thus confirming positivity. Verification can also be done by methods that further probe the post-amplification products for their sequence homology with the target. Dot blot, Southern blot or line strip hybridization are used to this end and generally result in improved molecular sensitivity and specificity as compared with electrophoresis and staining. Finally, the use of restriction enzymes to analyze the fragment length signatures in combination with probe hybridization and direct DNA sequencing, provide the highest possible resolution to distinguish the HPV types present in a biological specimen.

Real-time PCR on the other hand is a technique used to monitor the amplification of a targeted DNA molecule during the PCR in real-time, and not at its end, as in conventional PCR. Two common methods for the detection of PCR products in real-time PCR are *non-specific fluorescent dyes* that intercalate with any double-stranded DNA, and *sequence-specific DNA probes* consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence. In contrast, real-time PCR is less time consuming as it can detect amplifications during the early phases of the reaction, compared with conventional PCR which uses gel electrophoresis to analyze the amplified PCR products. Unlike conventional PCR which is highly sophisticated and labor intensive, automated detection techniques are found in real-time PCR.

With the significant burden of cervical cancer in Malaysia, and to increase screening uptake as well as the acceptance of the screening procedures, a simple urine test which can detect the HPV could offer women a much less invasive alternative to current cervical cancer screening. Therefore, a Health Technology Assessment (HTA) is required to assess the accuracy, effectiveness, and cost-effectiveness of HPV urine test for cervical cancer screening. This HTA was requested by the Senior Principal Assistant Director of Cancer Unit, Disease Control Division, Ministry of Health Malaysia.

2.0 POLICY QUESTION

Should HPV urine test be used as a screening method in the cervical cancer screening programme in Malaysia?

3.0 OBJECTIVES

3.1 To determine the diagnostic accuracy of HPV urine test for HPV detection

3.2 To determine the benefits of cervical cancer screening using HPV urine test compared with conventional cervical cytological specimen, HPV DNA-based using cervical specimen, combination of conventional cytology and HPV DNA-based using cervical specimen, or no screening, with regards to patient outcomes such as detection rate, mortality rate, survival rate, quality of life, and quality adjusted life years (QALY) gained

3.3 To determine the economic impacts of HPV urine test for cervical cancer screening

3.4 To assess the ethical, legal, and organizational aspects related to cervical cancer screening using HPV urine test

Research Questions

i. What is the diagnostic accuracy of HPV urine test for HPV detection?

ii. Is cervical cancer screening using HPV urine test effective in detecting and reducing mortality?

iii. What is the economic, ethical, legal, and organizational implication/impact related to cervical cancer screening using HPV urine test?

4.0 METHODS

4.1. **Search Strategy**

Electronic database will be searched for published literatures pertaining to HPV urine test for cervical cancer screening

4.1.1 Databases as follows; MEDLINE, EMBASE, PubMed, EBM Reviews-Cochrane Database of Systematic Review, EBM-Reviews-Cochrane Central Register of Controlled Trials, EBM Reviews-Health Technology Assessment, EBM Reviews-Cochrane Methodology Register, EBM Reviews-NHS Economic Evaluation Database, Database of Abstracts of Reviews of Effects (DARE), Horizon Scanning, INAHTA Database, HTA database and FDA database

4.1.2 Additional literatures will be identified from the references of the retrieved articles

4.1.3 General search engine will be used to get additional web-based information if there is no retrievable evidence from the scientific databases

4.1.4 There will be no limitation applied in the search such as year and language

4.1.5 The search strategy will be included in the appendix

4.2 Inclusion and Exclusion Criteria

4.2.1 Inclusion Criteria

a. Population : Female

b. Intervention : HPV urine test

c. Comparators : <u>Test</u>

HPV DNA-based using cervical specimen (Gold standard: cytology/histology)

<u>Screening programme</u>

i. Conventional cytology (Pap smear/liquid-based cytology)

ii. HPV DNA-based using cervical specimen

iii. Combination of conventional cytology and HPV DNA-based using cervical specimen

iv. No screening

d. Outcome :

i. Concordance (test agreement), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HPV urine test

ii. Detection rate, mortality rate, survival rate, quality of life, and quality adjusted life years (QALY) gained

iii. Cost, cost-benefit, cost-effectiveness, and cost utility using HPV urine test in cervical cancer screening

e. Study design : HTA reports, systematic review, randomised controlled trial, diagnostic accuracy studies, cross-sectional, cohort, case-control, and economic evaluation studies

f. English full text articles

4.2.2 Exclusion Criteria

a. Study design : Animal study, experimental study, narrative review

b. Non English full text articles

Based on the above inclusion and exclusion criteria, study selection will be carried out independently by two reviewers. Disagreement will be resolved by discussion.

4.3 Critical Appraisal of Literature

The methodology quality of all retrieved literatures will be assessed using the relevant checklist of Critical Appraisal Skill Programme (CASP)

4.4 Analysis and Synthesis of Evidence

4.4.1 Data extraction strategy

The following data will be extracted:

i. Details of methods and study population characteristics

ii. Detail of intervention and comparators

iii. Details of individual outcomes for accuracy, effectiveness, and cost associated with HPV urine test for cervical cancer screening

Data will be extracted from selected studies by a reviewer using a pre-designed data extraction form and checked by another reviewer. Disagreements will be resolved by discussion.

4.4.2 Methods of data synthesis

Data on the diagnostic accuracy, effectiveness, safety and cost associated with HPV urine test for cervical cancer screening will be presented in tabulated format with narrative summaries. No meta-analysis will be conducted for this Health Technology Assessment.

5.0 Report writing

Appendix 4

Search strategy:

Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>

1. PAPILLOMAVIRIDAE/
2. hpv, human papillomavirus.tw.
3. human papilloma* virus*.tw.
4. human papilloma* virus DNA.tw.
5. papilloma virus*, human.tw.
6. papillomaviridae.tw.
7. virus*, human papilloma*.tw.
8. 1 or 2 or 3 or 4 or 5 or 6 or 7
9. HPV.tw.
10. (HPV adj (urine or genotyping or DNA or DNA test* or DNA urine test or DNA screening)).tw.
11. (human papillomavirus adj (urine test* or DNA test*)).tw.
12. (Urin* adj (HPV or HPV test* or human papillomavirus or human papillomavirus test*)).tw.
13. 9 or 10 or 11 or 12)
14. 8 or 13
15. VAGINAL SMEARS/
16. ((Cervical or vaginal) adj smear*).tw.
17. smear*, cervical.tw.
18. smear*, vaginal.tw.
19. 15 or 16 or 17 or 18
20. PAPANICOLAOU TEST/
21. ((pap or papanicolaou) adj (smear or test)).tw.
22. (cytology adj1 (pap smear or conventional or liquid based)).tw.
23. CPS.tw.
24. LBC.tw.
25. smear, pap.tw.
26. smear, papanicolaou.tw.
27. test, pap.tw.
28. test, papanicolaou.tw.
29. HPV.tw.
30. (HPV adj (genotyping or DNA or DNA test* or DNA screening)).tw.
31. human papillomavirus test*.tw.
32. POLYMERASE CHAIN REACTION/
33. polymerase chain reaction*.tw.
34. reaction*, polymerase chain.tw.
35. ((anchored or inverse or nested) adj (pcr or polymerase chain reaction)).tw.
36. pcr.tw.

37. pcr, anchored.tw.
38. pcr, inverse.tw.
39. pcr, nested.tw.
40. or/20-39
41. 19 and 40
42. cervical cancer screening.tw.
43. 14 and 41 and 42

PubMed

((((cervical cancer screening[MeSH Terms]) OR cervical cancer screening[Title/Abstract])) AND ((PAPILLOMAVIRIDAE[MeSH Terms]) OR ((((((((((((((((((Human Papilloma Virus*[Title/Abstract]) OR Human Papillomavirus Virus*[Title/Abstract]) OR human papilloma* virus DNA[Title/Abstract]) OR Papilloma Virus*, Human[Title/Abstract]) OR papillomaviridae[Title/Abstract]) OR virus*, human papilloma*[Title/Abstract]) OR HPV[Title/Abstract]) OR HPV urine[Title/Abstract]) OR HPV genotyping[Title/Abstract]) OR HPV DNA[Title/Abstract]) OR HPV DNA test[Title/Abstract]) OR HPV DNA urine test[Title/Abstract]) OR HPV DNA Screening[Title/Abstract]) OR human papillomavirus urine test*[Title/Abstract]) OR human papillomavirus DNA test*[Title/Abstract]) OR Urine HPV[Title/Abstract]) OR Urinary HPV test*[Title/Abstract]) OR Urine human papillomavirus[Title/Abstract]) OR Urinary human papillomavirus test*[Title/Abstract]))) AND ((((VAGINAL SMEARS[MeSH Terms]) OR ((((cervical smear*[Title/Abstract]) OR vaginal smear*[Title/Abstract]) OR smear*, cervical[Title/Abstract]) OR smear*, vaginal[Title/Abstract]))) AND ((((((PAPANICOLAOU TEST[[MeSH Terms]) OR (Pap Smear[Title/Abstract] OR Pap Test[Title/Abstract] OR Papanicolaou Smear[Title/Abstract] OR papanicolaou test[Title/Abstract] OR cytology pap smear[Title/Abstract] OR pap smear cytology[Title/Abstract] OR conventional cytology[Title/Abstract] OR liquid based cytology[Title/Abstract] OR CPS[Title/Abstract] OR LBC[Title/Abstract] OR Smear, Pap[Title/Abstract] OR Smear, Papanicolaou[Title/Abstract] OR Test, Pap[Title/Abstract] OR Test, Papanicolaou[Title/Abstract])) OR Human Papillomavirus DNA Tests[MeSH Terms]) OR (hpv[Title/Abstract] OR HPV genotyping[Title/Abstract] OR HPV DNA[Title/Abstract] OR HPV DNA test[Title/Abstract] OR HPV DNA Screening[Title/Abstract] OR human papillomavirus DNA testing[Title/Abstract] OR human papillomavirus test*[Title/Abstract])) OR POLYMERASE CHAIN REACTION[MeSH Terms]) OR (polymerase chain reaction*[Title/Abstract] OR "Reaction*, polymerase chain"[Title/Abstract] OR Anchored PCR[Title/Abstract] OR Anchored Polymerase Chain Reaction[Title/Abstract] OR Inverse PCR[Title/Abstract] OR Inverse Polymerase Chain Reaction[Title/Abstract] OR Nested PCR[Title/Abstract] OR Nested Polymerase Chain Reaction[Title/Abstract] OR PCR[Title/Abstract] OR "PCR, Anchored"[Title/Abstract] OR "PCR, Inverse"[Title/Abstract] OR "PCR, Nested"[Title/Abstract])))

Appendix 5

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.

Appendix 6

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	1. Pathak N, Dodds J, Zamora J et al. Accuracy of human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis. BMJ. 2014; 349:g5264 DOI:10.1136/bmj.g52641
Study Type/Methods	**Systematic review and meta-analysis** **Aim**: To determine the accuracy of detection of HPV in urine compared with the cervix in sexually active women. **Data sources**: Several electronic database from inception to December 2013 - Medline, Embase, the Cochrane Library, Web of Science, BIOSIS, DARE, and SIGLE. **Data extraction and synthesis**: Data relating to patient characteristics, study context, risk of bias, and test accuracy. 2×2 tables were constructed and synthesised by bivariate mixed effects meta-analysis. From the estimates, a summary receiver operating characteristic curve and the following summary accuracy measures with 95% confidence intervals were derived – sensitivity, specificity, positive and negative likelihood ratio. The **reference standard** in all studies was a cervical sample taken by a clinician to test for HPV DNA. To visually explore heterogeneity, forest plots for test sensitivity and test specificity with 95% CI for individual studies we generated. To investigate sources of heterogeneity for both sensitivity and specificity, they included in the bivariate mixed effects models the following planned covariates: **purpose of testing** (HPV surveillance versus cervical cancer screening and follow-up of CIN), **mean age**, **HIV status** (positive versus negative for antibodies to HIV), **prevalence of low grade or worse intraepithelial lesions on cytology**, **prevalence of grade 2 or worse CIN on biopsy**, **urine sampling method** (first void urine versus random and midstream urine), **HPV detection method** (real time polymerase chain reaction (PCR) and nested PCR versus conventional PCR), use of non-commercial versus commercial **DNA extraction methods**, use of non-commercial versus commercial **DNA amplification methods**, and low versus high risk of bias as a result of patient selection. Owing to the restricted number of studies, they entered only one covariate in each analysis. Sensitivity analysis to investigate the effect of studies including a narrow patient spectrum were conducted. **Assessment of study quality**: QUADAS-2 tool to all studies Statistical analyses were performed using STATA (version 13.0) and SAS (version 9.3).
LE	I
Number of Patients & Patient Characteristic	23 articles reporting on 21 studies (2,277 **sexually active women**) were included in the systematic review. Of these, 16 articles reporting on 14 studies (1,535 women recruited, 1,443 women analysed) were included in the meta-analysis. Study population: ▪ General (n=190) ▪ Healthy unmarried college girl (n=100) ▪ Adolescent (80) ▪ Abnormal cytology (n=152) ▪ Undergoing colposcopy (n=336) ▪ Grade 2 or worse CIN (n=385) ▪ Low grade dysplasia or worse (n=304) ▪ Biopsy proved cervical cancer (n=121)
Intervention	Urine sample for HPV-DNA detection
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**Description of Studies:** 12/21 study populations were recruited from gynaecology or colposcopy outpatient clinics and seven from genitourinary medicine or HIV clinics.

Outcome Measures/Effect Size	For most study populations the purpose of testing was for cervical cancer screening (15/21). The remainder were for HPV surveillance (5/21) or follow-up of CIN (1/21). 4/21 populations were positive for HIV. Of the 11 populations with reported cytology results, 35.9% (304/847) of women had low grade dysplasia or worse. Of the 10 populations with reported biopsy results, 54.1% (385/712) of women had grade 2 or worse CIN and 17.0% (121/712) had biopsy proved cervical cancer. Most of the studies used conventional PCR (18/21), but testing methods were not uniform. 2/21 studies used nested PCR and 1/21 used PCR based DNA microarray. Three studies evaluated quantitative real time PCR and hybrid capture in addition to conventional PCR. In these cases, only the results for conventional PCR were included in the meta-analysis. The majority of urine sampling was first void (12/21). Other sampling methods included random (2/21), midstream (2/21), morning (1/21), and not specified (4/21). Urine storage temperature ranged from −70°C to 4°C. Sixteen studies used commercial DNA extraction kits and 11 used commercial amplification platforms. The remainder used in-house methods. **Quality of Studies:** Most studies (9/14) used consecutive or random recruitment of participants. All studies had a low risk of bias owing to patient flow and timing. All studies had a low risk of bias for the conduct of the reference standard. 5/14 studies used in-house methods for the index test and did not specify a threshold. No significant asymmetry in the funnel plot (P=0.62) and hence no evidence of publication bias. **Sources of heterogeneity:** There was a 22-fold increase in overall accuracy when samples were collected as first void urine compared with random or midstream urine samples (relative diagnostic odds ratio 21.7, 95% CI 1.3 to 376). However, this difference in accuracy is exclusively based on a significant increase in sensitivity of first void urine (relative sensitivity 1.2, 95% CI 1.06 to 1.37, P=0.004). Specificity was not affected by the urine sampling method (P=0.46). Purpose of testing, mean age of participants, HIV status, cytology and biopsy results, detection methods, use of commercial methods, or risk of bias as a result of patient selection did not explain any heterogeneity between indices for study accuracy. **Sensitivity analysis:** Pooled sensitivity and specificity for detection of any HPV in urine was similar when studies with a narrow spectrum of patients were excluded. Sensitivity was 80% (95% CI 71% to 88%) and specificity was 98% (95% CI 89% to 100%). **Meta-analysis:** **Individual:** For urine detection of **any HPV**, sensitivities ranged from 53% to 99% and specificities from 38% to 99%. For urine detection of **high risk HPV**, sensitivities ranged from 50% to 98% and specificities from 17% to 99%. For urine detection of **HPV 16 and 18**, sensitivities ranged from 23% to 97% and specificities from 56% to 99%. **Pooled:** Urine detection of **any HPV**: ▪ Pooled sensitivity of 87% (95% confidence interval, CI: 78% to 92%) ▪ Pooled specificity of 94% (95% CI: 82% to 98%) ▪ Positive likelihood ratio was 15.22 (95% CI: 4.56 to 50.81) ▪ Negative likelihood ratio was 0.14 (95% CI: 0.10 to 0.20) Urine detection of **high risk HPV**: ▪ Pooled sensitivity of 77% (95% confidence interval, CI: 68% to 84%) ▪ Pooled specificity of 88% (95% CI: 58% to 97%) ▪ Positive likelihood ratio was 6.33 (95% CI: 1.48 to 27.00) ▪ Negative likelihood ratio was 0.26 (95% CI: 0.16 to 0.41) Urine detection of **HPV 16 and HPV 18**: ▪ Pooled sensitivity of 73% (95% confidence interval, CI: 56% to 86%) ▪ Pooled specificity of 98% (95% CI: 91% to 100%) ▪ Positive likelihood ratio was 36.97 (95% CI: 6.77 to 201.91) ▪ Negative likelihood ratio was 0.27 (95% CI: 0.15 to 0.49)

Outcome Measures/Effect Size	**Limitation:** The use of narrow patient spectrums in six studies including only participants with HIV, adolescents, or participants with high grade cervical intraepithelial neoplasia (CIN) lead towards a high prevalence and could result in biased estimation of test accuracy. Could not perform a multivariable meta-regression analysis owing to the limited number of studies available. A major limitation of this meta-analysis is the between study variation in pooled sensitivities and specificities. This means that all results must be interpreted with caution as they may have been overestimated or underestimated.
General Comments	This review was performed and reported in accordance with the PRISMA

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	2. Manhart LE, Holmes KK, Koutsky LA et al. Human papillomavirus infection among sexually active young women in the United States: Implications for developing a vaccination strategy. Sex Transm. 2006; 33: 502-508
Study Type/Methods	**Cross-sectional** **Objective:** (1) To determine the detection and distribution of HPV types in sexually active females in the United States (2) To identify sociobehavioral correlates of infection in the general population HPV positivity was determined first by PCR amplification and then followed by dot blot hybridization. HPV positive samples were typed using probes to detect types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, 84 using the Roche Diagnostics line blot assay. Post stratification sampling weights generated nationally representative estimates. Univariate and multivariate prevalence ratios (PR) and 95% confidence intervals (CI) were calculated using weighted Poisson regression in Stata version 8.0 for comparisons of categorical characteristics to evaluate factors hypothesized to be associated with HP
LE	II-2
Number of Patients & Patient Characteristic	3,262 **sexually active women**; ages 18 to 25 years National Longitudinal Study of Adolescent Health (Wave III) enrolled subjects from July 2001 to April 2002.
Intervention	Urine specimens were tested and typed for HPV
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HPV Infection:** Overall HPV detection was 28.6% (934/3,262). Age-specific incidence was approximately 30% in women aged 18 to 21 years, and declined with age. Race-specific HPV incidence was highest among African and Native Americans and lowest among Asians. **HPV Types:** A total of 1,985 different combinations of HPV types were identified in the 934 HPV-positive women. HPV 16 was the most commonly identified type (5.8%), followed in frequency by types 84 (3.2%), 51 (3.0%), 62 (3.0%), 54 (2.9%), and 53 (2.8%). Nearly 10% of women with only 1 lifetime vaginal sex partner were infected with a high-risk HPV type. The distribution of HPV types did not differ significantly by geographic region. **Characteristics Associated with HPV Infection:** Compared to women without HPV infection, women with infection tended to be younger, single, black, younger at sexual debut, and had more sex partners. There was no difference in HPV positivity by region, educational level, annual personal income, or having signed a virginity pledge.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	3. Ducancelle A, Legrand M, Pivert A et al. Interest of Human Papillomavirus DNA quantification and genotyping in paired cervical and urine samples to detect cervical lesions. Arch Gynecol Obstet. 2014; 290: 299–308
Study Type/Methods	**Prospective cohort** The PapU study was a prospective longitudinal multi-center study fo: (1) compare HPV viral loads and genotypes in paired cervical and urine samples, and (2) assess correlation between virological and cytological results HPV DNA detection and quantification were performed using a real-time PCR method with short fragment PCR primers. Genotyping was carried out using the INNO-LiPA HPV genotyping assay. Cohen's kappa was used to evaluate agreement (significant κ value > 0.7) for HPV detection between urine and cervical.
LE	II-2
Number of Patients & Patient Characteristic	245 patients (mean age 36 years) **consulting a gynaecologist** for cytology in three university hospitals. Urine and cervical specimens (paired samples) were collected from 230 of these patients. Women with cytological abnormalities underwent colposcopy and biopsy.
Intervention	Urine sample for HPV viral load and genotype
Comparison	Cervical smear sample
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HPV Detection:** For the 230 women with paired samples, cytological examination was normal in 34 patients (15%) and abnormal in 196 (85%). Bethesda system classification was as follows: high-grade squamous intraepithelial lesion (HSIL) in 25 patients, low-grade squamous intraepithelial lesion (LSIL) in 59, atypical squamous cells of undetermined significance (ASCUS) in 70. The detection of HPV in the 230 paired urine and cervical smear samples was 42% (98/230) and 49% (113/230), respectively. **Concordance of HPV DNA Detection in Cervical and Urine Samples:** Overall agreement for HPV positivity and negativity between the paired samples was 90% (κ = 0.80). High HPV viral load in both cervical and urine samples was associated with cytological abnormalities.
Outcome Measures/Effect Size	**HPV Genotyping:** HPV-positive women were mostly infected with HR-HPV types. HPV type 16 was identified most frequently in both samples with a detection rate of 38% and 32% in cervical and urine samples, respectively. The agreement between high- and low-risk HPV (LR-HPV) detection in both samples was 97% (κ = 0.95 for HR-HPV and κ = 0.97 for LR-HPV).
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	4. Sharma K, Kathait A, Jain A et al. Higher prevalence of human papillomavirus infection in adolescent and young adult girls belonging to different Indian tribes with varied socio-sexual lifestyle. PLoS One. 2015; 10(5): e0125693. doi: 10.1371/journal.pone.0125693
Study Type/Methods	**Cross-sectional** **Aim:** To examine the status of HPV infection and its genotype distribution in pre-adolescent, adolescent, and young adult girl from Random self-collected midstream urine samples along with socio-demographic data were collected by home to home visits and from local schools. Out of these, 2,034 samples which showed adequate DNA and successful amplification of β-globin gene were subjected to HPV detection (PCR using MY09/ MY11 and GP5+/GP6+ primers) and genotyping by PCR, PGMY-RLB assay & sequencing. Statistical analysis (univariate and multivariate logistic analysis) was performed to estimate the prevalence of HPV infection and its association with various risk factors.
LE	II-2
Number of Patients & Patient Characteristic	A total of 2,278 **healthy** tribal girls comprising pre-adolescent (9–12 years), adolescent (13–17 years) and young adult girls (18–25 years). These tribes were very poor and are mainly dependent on forest produce and primitive agriculture.
Intervention	Urine sample for HPV detection and genotype
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HPV Infection and Genotype:** The HPV infection in the three sampling states was found to be almost similar despite distinct geographical locations and ethnicity. Of 2,034 adequate samples, 262 (12.9%) tested positive for one or more HPV genotypes. Out of 262 HPV positive girls 168 (64.1%) were found infected with HR genotypes, and almost half (132; 50.4%) of them were infected by HPV 16 alone. **Factors Associated with HPV Infection:** There was an overall increase in HPV detection with age; thus, young adult girls (18–25 years) were having the highest (19.2%) HPV infection followed by adolescent (11.4%) and pre-adolescent girls (6.6%). Multivariate analysis was applied on four risk factors (menarche, boyfriend, income, and education). There was a strong association between HPV infection and menarche of girls (OR 3.1, 95% CI, 2.03–4.73; P<0.001), and who have boyfriend (OR 3.4, 95%CI 2.11–4.85; P<0.0001). However, income and education showed weak association with HPV infection.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	5. Ducancelle A, Reiser J, Pivert A et al. Home-based urinary HPV DNA testing in women who do not attend cervical cancer screening clinics. J Infect. 2015; 71(3): 377-384
Study Type/Methods	**Prospective cohort** **CapU study:** The first to evaluate a new strategy involving HPV detection in home-collected urine in complementarity with a cervical cancer screening program. Between July 2010 and January 2013 **Aim:** To evaluate the acceptance of a urinary HPV test Letters proposing a new cervical cancer screening method using at-home urine self-sampling were sent to 5,000 women aged 40-65 years who had not had a Pap smear over the past three years. The participating patients had to send their urine samples (first void) to the Angers Hospital Virology Laboratory for analysis using real-time PCR (Abbott Molecular Diagnostics).
LE	II-2
Number of Patients & Patient Characteristic	2,000 women in a 55-65 age group and 3,000 in a 40-54 age group **Not had a Pap smear over the past three years**
Intervention	Urine sample for HPV testing and genotype
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**Participation Rate:** Of the 2,000 letters sent to the 55-65 age group women, 1,940 reached their respective addressees. All 3,000 of the letters sent to women aged 40-54 were successfully delivered. Women in the 55-65 age group and those in the 40-54 age group sent in respectively 259 (12.9%) and 512 (17%) urine samples. Of the 771 samples received, 687 were suitable for and subjected to analysis, which represented 13.7% of the 5,000 mailings sent. **HPV Detection and Genotype:** Twenty-nine of the 687 analysable samples (4.2%, 95% CI, 2.9%-6.0%) were HR-HPV positive. Among the 29 HR-HPV positive samples, HR-HPV other than 16/18 were the most frequently found types: 22/29 (76%) versus 2/29 (7%) for HPV 16 single infection, 4/29 (14%) for HPV 16 + HR-HPV coinfection, and 1/29 (3%) for HPV 18 + HR-HPV coinfection, (p=0.0001). HPV 16 or 18 was detected in coinfection with other HR-HPV types in 5/29 (17%) of the patients. **Follow up and Cytological Results:** 28/29 (one refused care) HPV-positive women were referred to their physician (follow-up rate of 96.5%) for cytology tests and/or colposcopy. The results showed **19 normal** and **nine abnormal** smears. Among these latter, there were three ASC-US, one ASC-H, two LSIL and three HSIL.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	6. Sellors JW, Lorincz AT, Mahony JB et al. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. Can Med Assoc J. 2000; 163(5): 513-518
Study Type/Methods	**Cross-sectional (screening/diagnostic type)** **Objective:** To determine the sensitivity and specificity of self-sampling compared with physician-collected cervical samples for HPV in detecting HSIL (CIN 2 or 3), and to evaluate the feasibility of asking women to collect their own samples. From October 1996 to March 1997. First void urine sample. Presence of HPV was evaluated using the hybrid capture II assay with a probe cocktail for 13 carcinogenic types. Cervical specimens were also tested for HPV by PCR and hybridization with type-specific probes. Cervical smears for cytological examination were obtained from all women. The sensitivity, specificity, positive and negative predictive values, and positive likelihood ratios of the hybrid capture II results for the 4 specimen types were calculated, with the results **of colposcopy examination (with directed biopsy as required) as the reference standard**. Women with HSIL (CIN 2 or 3) or adenocarcinoma in situ on histologic examination were regarded as having a “positive” result. Kappa statistic (Cohen’s Kappa, κ) defined as “poor” (κ=0), “slight” (0.01 < κ < 0.20), “fair” (0.21 < κ < 0.40), “moderate” (0.41 < κ < 0.60), “substantial” (0.61 < κ < 0.80), “almost perfect” (0.81 < κ < 1) or “perfect” (κ =1).
LE	2
Number of Patients & Patient Characteristic	200 women referred to a colposcopy clinic because of **abnormalities upon cervical cytological screening**) Mean age 31.5 ± 9.4 years
Intervention	Self-collected vaginal, vulvar, and urine samples for HPV testing to detect HSIL
Comparison	Physician-collected cervical samples
Length of Follow Up (If Applicable)	

Outcome Measures/Effect Size	HSIL (CIN grade 2 or 3, and adenocarcinoma) were found in 58/200 (29.0%). The remaining 142 women (71.0%) had normal findings or LSIL (CIN grade 1). The **sensitivity** of testing for HSIL (CIN 2 or 3) was **progressively lower** and the **specificity progressively higher** with increasing distance from the cervix (vagina, vulva and urine in that order). **Physician-collected cervical samples:** Sensitivity=98.3%; specificity=52.1%; PPV=45.6%; NPV=98.7% **Self-collected vaginal swab:** Sensitivity=86.2%; specificity=53.5%; PPV=43.1%; NPV=90.5% **Self-collected vulvar swab:** Sensitivity=62.1%; specificity=62.7%; PPV=40.4%; NPV=80.2% **Self-collected urine specimen:** Sensitivity=44.8%; specificity=69.7%; PPV=37.7%; NPV=75.6% The likelihood ratios for a positive result with the hybrid capture II test for the cervical, vaginal, vulvar and urine samples were 2.1, 1.9, 1.7 and 1.5 respectively. Agreement (kappa statistic) between the cervical specimens and the vaginal, vulvar and urine specimens for the presence of HPV was 0.76, 0.55 and 0.41 respectively.
Outcome Measures/Effect Size	The self-sampling methods were generally more acceptable: 126/128 (98.4%) found the urine sampling acceptable, 118/127 (92.9%) found the vulvar sampling acceptable, and 112/127 (88.2%) found the vaginal sampling acceptable, whereas only 98/124 (79.0%) found cervical sampling by the physician acceptable.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	7. Stanczuk GA, Kay P, Allan B et al. Detection of human papillomavirus in urine and cervical swabs from patients with invasive cervical cancer. J Med Virol. 2003;71:110-114
Study Type/Methods	**Cross-sectional** **Aim:** (1) To investigate the presence of HPVs in urine and cervical swab samples collected from 43 women who presented with invasive cervical cancer (2) To determine HPV type-specific concordance between paired cervical and urine samples Urine samples were obtained before clinical examination. Cervical swabs were collected during routine gynaecological examination. HPV detection was done by means of degenerate primers in a nested PCR. Typing of HPVs was done using restriction fragment length polymorphism (RFLP) analysis.
LE	11-2
Number of Patients & Patient Characteristic	43 women (indigenous Zimbabweans) with histologically confirmed **invasive cervical cancer** were enrolled from gynaecological clinic at the university hospital. Ages ranged from 24-70 years.
Intervention	Urine sample for HPV detection and type-specific
Comparison	Cervical swab
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HPV Detection and Type-Specific in Cervical sample:** HPV DNA was detected in 42 of 43 (98%). The most prevalent type was type 16 (25/42, 59%), followed by type 33 (13/42, 31%). Types 18, 31, and 58 were present in 6 (14%), 1 (2%), and 1 (2%) of samples, respectively. Dual HPV infection was identified in 7 (17%) of HPV-positive samples (16+33, n=4; 33+18, n=3). **HPV Detection and Type-Specific in Urine sample:** HPV DNA was identified in 31 of the 43 (72%). Twenty-eight of these samples were typed successfully. HPV 16 was the most prevalent (19/31, 61%), with type 33 present in 5 (16%) of typed samples. HPV 18 and 31 were present in 4 (13%) and 1 (3%) of HPV-positive samples, respectively. Dual infection was present in 2 (6%) samples (16+18 and 16+33). **Type-specific concordance** between cervical and urine samples was high (22/28, 79%). Therefore, the HPV types identified in urine samples in most cases represent the same HPV type infecting the cervical epithelium.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	8. Song ES, Lee HJ, Hwang TS. Clinical efficacy of human papillomavirus DNA detection in urine from patients with various cervical lesions. J Korean Med Sci. 2007; 22: 99-104
Study Type/Methods	**Cross-sectional** **Aim:** To evaluate the clinical efficacy of the urine-based HPV DNA detection using HPV oligonucleotide microarray by comparing the results from matched cervical swab specimens. From January to December 2003. First void urine sample. Cases showing positive beta-globin bands in both cervical and urine samples after PCR were analysed for the agreement study. The agreement of the result between HPV detection in cervical swab and urine was evaluated using kappa index which was defined by a chance corrected proportional agreement rate. It has maximum of 1.00 when agreement is perfect, a value of zero indicates no agreement better than chance, and negative values show worse than chance agreement, which is unlikely. “Good” ($\kappa > 0.50$); “Fair” ($\kappa < 0.50$)
LE	11-2
Number of Patients & Patient Characteristic	100 women (mean age 45.2 years) who had adequately analysed cervical swabs for HPV DNA (beta-globin positive) and a **biopsy proven histological diagnosis** were selected for this study. 23 chronic cervicitis patients, 48 patients with CIN, and 29 patients with invasive cervical carcinomas, including three adenocarcinomas, were analysed.
Intervention	Urine sample for HPV detection and type-specific
Comparison	Cervical swab specimens
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HPV Detection and Type-Specific in Cervical swabs:** HPV DNA was detected in 70 (70.0%) of 100 cervical samples; 4 (17.4%) of 23 chronic cervicitis, 40 (83.3%) of 48 CIN, and 26 (89.7%) of 29 carcinoma samples and were all high risk HPVs. HPV 16 was the most prevalent type (38 of 70 patients, 54.3%), followed by type 18, 58, 52, 33, 35, 31, and 51. Multiple HPV infection was identified in 8 (11.4%) of 70 HPV-positive patients. **HPV Detection and Type-Specific in Urine samples:** HPV DNA was identified in 47 (52.2%) of 90 urine samples; 3 (13.0%) of 23 chronic cervicitis, 27 (62.8%) of 43 CIN, and 17 (70.8%) of 24 carcinoma patients and were all high-risk HPVs. HPV 16 was the most prevalent type (30 of 45 patients; 63.8%), followed by type 18, 52, 35, 51, 58, 33, and 56. Multiple infection was identified in 3 (6.4%) of 47 HPV-positive patients. The HPV DNA detection rate of the cervical swab samples increased in accordance with the severity of the cytologic and histologic diagnosis, and was higher than the rate of urine samples in both CIN and carcinoma patients. The **concordance rate** for HPV detection between cervical swabs and urine was 69.3%. The **type specific agreement** of the HPV DNA test between cervical swabs and urine was good in HPV 16, 18, 52, and 58 and fair in HPV 33 and 35.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	9. Thilagavathi A, Shanmughapriya S, Vinodhini K et al. Prevalence of human papillomavirus (HPV) among college going girls using self-collected urine samples from Tiruchirappalli, Tamilnadu. Arch Gynecol Obstet. 2012; 286: 1483-1486
Study Type/Methods	**Cross-sectional** **Aim:** To identify the status of HPV infection among young college girls. Between August 2009 and July 2010. Random midstream urine sample. **Inclusion criteria:** (1) No previous history of vaccination (2) Had no treatment for cervical diseases (3) Lack of physical or mental impairments (4) No history of previous sexual exposure All interviews were conducted by public health nurses. Informed written consent was obtained from all participating subjects prior to the interview. The distribution of HPV genotypes was evaluated by PCR DNA genotyping after self-sampling from study subjects.
LE	II-2
Number of Patients & Patient Characteristic	238 **young sexually unexposed girls**, aged from 17 to 25 years with a mean age of 21 (SD = 2.3 years) were randomly selected from a University in Tiruchirappalli district, Tamilnadu, India.
Intervention	Self-collected urine samples for HPV testing and type-specific
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	Positivity for HPV DNA was reported among 22/238 (9.2%) of the study subjects. The most frequently detected HPV type: ▪ HPV 16 (9.1%; 2/22) ▪ HPV 11 (4.5%; 1/22)
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	10. Bernal S, Palomares J, Artura A et al. Comparison of urine and cervical samples for detecting human papillomavirus (HPV) with the Cobas 4800 HPV test. J Clin Virol. 2014; 61: 548-55
Study Type/Methods	**Cross-sectional (screening/diagnostic type)** **Aim**: Paired first voided urine and cervical samples were collected to evaluate the clinical performance and correlated the results against histologically confirmed cervical disease status. Cobas 4800 HPV test (Roche Diagnostic, Spain), which is an FDA approved real-time PCR assay designed for high risk HPV (HR-HPV) detection and simultaneous HPV-16 and HPV-18 genotyping was used. Results can be obtained approximately 4 hours after receiving the specimen. The sensitivity, specificity, NPV and PPV of HPV detection in urine samples compared to the detection in cervical samples was calculated. Concordance between test was assessed using the Kappa statistic (Cohen's Kappa, κ) and defined as "poor" (κ=0), "slight" (0.01 < κ < 0.20), "fair" (0.21 < κ < 0.40), "moderate" (0.41 < κ < 0.60), "substantial" (0.61 < κ < 0.80), "almost perfect" (0.81 < κ < 1) or "perfect" (κ =1).
LE	2
Number of Patients & Patient Characteristic	125 women (median age 35.5 years) **referred to Gynaecology Unit** of Valme University Hospital (Seville, Spain) for evaluation of **abnormal Pap smear screening results** from primary care
Intervention	Urine sample for HPV detection and type-specific
Comparison	Cervical sample
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	A total of 72/125 (57.6%) women tested positive for HPV using at least one of the two samples tested. The detection of HPV carcinogenic types was 52.8% (66/125) in urine samples and 50.4% (63/125) in cervical samples. The overall percent agreement between HPV detection in urine and cervical samples was 88%. A **substantial** concordance rate of HPV DNA detection in both samples was observed (κ=0.76; 95% CI: 64–87). In this high prevalence population the sensitivity, specificity, NPV and PPV for detection of HPV DNA from urine versus cervical samples were 90.5% (95% CI: 80–95%), 85%, (95% CI: 74–92%), 89.8% (95% CI: 79.5–95.3) and 86.4% (95% CI: 76.1–92.7), respectively. **Clinical performance against cytological and histological endpoint:** Cytological results were available for 122 women, of whom 65 were reported to be negative for intraepithelial lesions, ASCUS (22), LSIL (22), and HSIL (14). Histological results were available for 80 women, of which 43 did not have CIN, CIN1 (17), CIN2 (4) and CIN3 (16). The HPV infection was similar in cervix (62/122) and urine (65/122) regardless of the result of cytology and histology (P>0.05). Compared to histological confirmed CIN 2 or 3, the clinical sensitivity and specificity for the detection of high-risk HPV in urine samples were 95% (95% CI: 76–97%) and 52.4% (95% CI: 40–64%), respectively. For cervical samples they were 90% (95% CI: 69–97) and 50.8% (95% Ci: 38–62), respectively.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	11. Nicolau P, Mancebo G, Agramunt S et al. Urine human papillomavirus prevalence in women with high-grade cervical lesions. Eur J Obstet Gynecol Reprod Biol. 2014: 183: 12-15
Study Type/Methods	**Cross-sectional** **Objective:** (1) To determine the detection of human papillomavirus (HPV) in urine samples from women with high-grade cervical lesions (2) To identify the influence of socio-demographic factors and the different genotypes with urinary HPV positivity From October 2010 until July 2011. Socio-demographic characteristics and relevant clinical information were collected from all patients. CIN2+ was defined as lesions of CIN2 and CIN3. Detection and typing of HPV was performed by PCR using the Linear Array HPV Genotyping Test. All the statistical analysis was performed using STATA SE v10 software (Texas, USA). Cohen's kappa was used to evaluate agreement: "poor" (0.10 < κ < 0.20), "fair" (0.21 < κ < 0.40), "moderate" (0.41 < κ < 0.60), "good" (0.61 < κ < 0.80)
LE	II-2
Number of Patients & Patient Characteristic	75 women (mean age 34.8 years) referred to the Cervical Pathology Unit at Obstetrics and Gynaecology Department at Hospital del Mar in Barcelona, with a **positive biopsy for CIN2 or CIN3**, who were eligible for a conization procedure
Intervention	Urine sample for HPV detection and genotype
Comparison	Cervical sample
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	All patients had histological CIN2+, of whom 55% had CIN3. No differences were found among demographic characteristics (age, parity, smoking status or contraceptive method) when comparing patients with positive urine HPV to those with a negative result. The detection of positive urine HPV test was 69.3% in CIN2+ population. For the sub population of CIN3 patients, the detection rose up to 78.1%, but there was no statistically significant difference although it had a tendency (p =0.072). Regarding the viral subtype, different 31 genotypes were identified. The most frequent HPV genotype was HPV16. This genotype was positive in 57.7% of urine samples and 53.9% of cervical biopsies with a good level of correlation (kappa coefficient of 0.69).
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness **(FACTOR EFFECTING - COLLECTION, STORAGE, EXTRACTION)**

Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	12. Vorsters A, Van den Bergh J, Micalessi I. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect. 2014; 33: 2005–2014
Study Type/Methods	**Cross-sectional** **Aim:** To evaluate the effects of storage, sample preparation, extraction, and sampling on the detection of HPV DNA in the urine of HPV-positive women by: (a) comparing **five extraction methods** (Qiagen direct, Amicon® + Qiagen, supernatant + Qiagen, Pellet + Qiagen, and EasyMAG) and analyzing the impact of a **urine-conservation medium (UCM1)** that was developed in-house based on quantitation of HPV DNA and human DNA (hDNA) (b) comparing the effect of **untreated** urine stored at room temperature for 7 days, urine mixed with an **in-house** conservation medium, and urine mixed with a **commercially** available preservation buffer (c) investigating the impact of testing **first-void** versus **midstream** urine on the detection of HPV DNA and human DNA DNA analysis was performed using real-time PCR to allow quantification of HPV and human DNA.
LE	II-2
Number of Patients & Patient Characteristic	Women (n=44) who were recruited for a phase-one HPV therapeutic vaccine trial were enrolled in the present study. The participants had a cervical sample with **normal cytology that tested positive for HPV 16 or 18 DNA.**
Intervention	Urine of HPV-positive women
Comparison	
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**Effect of a urine-conservation medium (UCM1) and different extraction method:** The use of a DNA-conservation medium had a significant impact for all five extraction methods used. Without a preservation buffer, the number of samples that were positive for HPV DNA varied from 7/24 for the supernatant to 19/24 for the pellet. Dilution of the urine with 1/3 volume conservation medium increased the number of positive samples. For the supernatant, Qiagen direct, and Amicon® + Qiagen, 9, 5, and 4 additional samples, respectively, were positive in the UCM1 arm. For the detection of hDNA without a conservation buffer, the samples that were positive for hDNA varied from 5/24 for the supernatant to 23/24 for the pellet. Remarkably, all samples for all extractions, including the supernatant, were positive for hDNA when the urine was mixed with UCM1. The difference between the untreated and treated urine was highly significant ($P < 0.001$ for the HPV DNA copies and the hDNA copies). **Comparison of an in-house and a commercial conservation buffer:** HPV DNA was detected in the urine samples of 5/13, 12/13, and 11/13 women using the no-treatment, in-house, and commercial-buffer treatments, respectively. A significant difference in detection of HPV DNA between the untreated and treated urine was observed ($p \leq 0.003$, after correcting for volume). In-house buffer and commercial buffer allowed comparable recoveries of HPV DNA.

Outcome Measures/Effect Size	Human DNA was detected in all but one untreated urine sample. The median number of copies of hDNA was 30 in the no-treatment, 3,730 in the in-house-buffer, and 1,200 in the commercial-buffer arm. Significant differences were observed between the untreated and treated urine ($p \leq 0.001$, after correcting for volume). The in-house and commercial buffer performed similarly regarding the conservation of hDNA. **Impact of the urine fraction on HPV DNA detection:** 4.8 to 160 times more HPV DNA copies were detected in the first-void fraction compared with the midstream fraction. The difference was highly significant (P=0.008). Human DNA was detected in all samples, but significantly more copies were observed in the first fraction (P=0.007). For all other pairs, between 1.4 and 21.4 times more copies of hDNA were detected in the first part of the urine void compared to the midstream fraction.
General Comments	

Evidence Table : Diagnostic accuracy and effectiveness **(FACTOR EFFECTING - COLLECTION TIME, TESTING FRACTION)**
Question : What is the diagnostic accuracy of HPV urine test for HPV detection and the effectiveness of cervical cancer screening using HPV urine test?

Bibliographic Citation	13. Senkomago V, Des Marais AC, Rahangdale L et al. Comparison of urine specimen collection times and testing fractions for the detection of high-risk human papillomavirus and high-grade cervical precancer. J Clin Virol. 2016; 74: 26-31
Study Type/Methods	**Cross-sectional (screening/diagnostic type)** Between October 2013 and May 2014, a pilot study to examine HR-HPV detection in urine collected at different times **(first urination of the day versus initial stream and mid-stream collected later the same day)** and in different urine fractions **(supernatant, pellet, and unfractionated)** using the Trovagene HPV HR test. Also examine the validity of HPV testing in the different urine samples for the detection of histologically-confirmed CIN2+. Physician- and self-collected specimens were tested for HR-HPV mRNA using the Aptima HPV assay, which qualitatively detects E6/E7 mRNA of 14 HR-HPV types. Colposcopy was performed and directed biopsies obtained if clinically indicated. Cohen–Kappa values were calculated to assess agreement between urine samples. Median unbiased estimates and associated mid-P 95% CIs were computed for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for CIN2+ detection, stratified by sample type. *GGE: Generalized Estimating Equations
LE	2
Number of Patients & Patient Characteristic	37 non-pregnant women, ≥ 30 years, who attended the colposcopy clinic for follow-up of results of **abnormal cytology** or **persistent HPV infection** or treatment by **loop electrical excision procedure (LEEP).**
Intervention	Urine specimen for the detection of HR-HPV and high-grade cervical precancer
Comparison	Physician- and self-collected a cervico-vaginal sample.
Length of Follow Up (If Applicable)	
Outcome Measures/Effect Size	**HR-HPV Detection - Stratified by Urine Sample Type and by Urine Fraction:** HR-HPV prevalence was similar in **unfractionated** portions of the three types of urine samples: 64.9% (49.5–80.2%) in first void, 73.0% (58.7–87.3%) in initial stream, and 70.3% (55.5–85.0%) in mid-stream (p-value range for pairwise comparisons: 0.26–0.80). HR-HPV detection was similar in all **pellet** fractions: 67.6% (52.5–82.7%) in first void, 78.4% (65.1–91.6%) in initial stream, and 73.0% (58.7–87.3%) in midstream (p-values range: 0.102–0.414). In **supernatant** fractions, HR-HPV prevalence was similar for first void (73.0% [58.7–87.3%]) and initial stream (75.7% [61.9–89.5%]) samples, albeit lower in mid-stream samples (56.8% [40.8–72.7%]) than in initial stream samples (p-value = 0.035). **Detection of High-Grade Cervical Lesions:** The validity of HPV testing performance on urine for CIN2+ detection was assessed using the **unfractionated initial stream** samples, given that HR-HPV prevalence in unfractionated urine samples was similar to other fractions and prevalence in initial stream was similar to other sample types. The sensitivity of HR-HPV DNA testing in urine for CIN2+ detection was high (89.9% [95% CI = 62.7–99.6%]), identical to that of mRNA testing of physician-collected specimens, and comparable to that of self-collected genital specimens (79.1% [48.1–96.6%]). Specificity of HR-HPV DNA in urine was relatively low (34.8% [18.4–54.1%]), but comparable to

Outcome Measures/Effect Size	specificity of HR-HPV mRNA testing on physician-collected (42.4% [24.6–61.6%]) and self-collected genital specimens (46.2% [27.9–65.2%]). HR-HPV testing in urine, self-collected, and physician-collected specimens had low, but comparable PPVs of 37.2% (20.6–56.2%), 38.3% (19.5–59.8%) and 40.1% (22.4–59.8%), respectively. NPV estimates for all tests were high: 88.9% (59.7–99.5%) for urine, 84.9% (60.3–97.6%) for self-collected specimens and 90.7% (65.3–99.6%) for physician-collected specimens. **Acceptability of HR-HPV Testing in Urine:** A greater proportion of participants reported having mostly **positive feelings** about urine collection than brush self-collection (89% versus 65%), and more women reported **neutral or negative feelings** about brush self-collection than urine collection (neutral = 8% versus 30%, and mostly negative = 3% versus 5%, respectively) (GEE p-value = 0.017). Most women (n = 29, 78.4%) preferred urine collection to brush self-collection (chi-square p value < 0.001) and reported being comfortable with receiving the urine collection kit in the mail (n = 32, 86.5%).
General Comments	

Appendix 7

LIST OF EXCLUDED STUDIES

1. Strauss S, Jordens JZ, McBride D et al. Detection and typing of human papillomavirus DNA in paired urine and cervical scrapes. Eur J Epidemiol. 1999; 15: 537-543
2. Jacobson DL, Womack SD, Peralta L et al. Concordance of human papillomavirus in the cervix and urine among inner city adolescents. Pediatr Infect Dis J. 2000; 19: 722-728
3. Prusty BK, Kumar A, Arora R et al. Human papillomavirus (HPV) DNA detection in self-collected urine. Int J Gynecol Obstetr. 2005; 90: 223-227
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10. Bissett SL, Howell-Jones R, Swift C et al. Human papillomavirus genotype detection and viral load in paired genital and urine samples from both females and males. J Med Virol. 2011; 83: 1744-1751
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14. Tanzi E, Bianchi S, Fasolo MM et al. High performance of a new PCR-based urine assay for HPV-DNA detection and genotyping. J Med Virol. 2013; 85: 91-98
15. Mendez K, Romaguera J, Ortiz AP et al. Urine-based human papillomavirus DNA testing as a screening tool for cervical cancer in high-risk women. Int J Gynecol Obstetr. 2014; 124: 151-155
16. Sahasrabuddhe VV, Gravitt PE, Dunn ST et al. Comparison of human papillomavirus detections in urine, vulvar, and cervical samples from women attending a colposcopy clinic. J Clin Microbiol. 2014; 52: 187-192
17. Sehgal A, Gupta S, Parashari A et al. Urine HPV-DNA detection for cervical cancer screening: Prospects and prejudices. J Obstetr Gynaecol. 2009; 29: 583-589
18. D'Hauwers KW, Tjalma WA. Screening for human papillomavirus: Is urine useful? Indian J of Canc. 2009: 46(3): 190-193
19. Brinkman JA, Jones WE, Gaffga AM et al. Detection of human papillomavirus DNA in urine specimens from human immunodeficiency virus-positive women. J Clin Microbiol. 2002; 40: 3155-3161
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21. Vorsters A, Micalessi I, Blicke J et al. Detection of human papillomavirus DNA in urine. A review of the literature. Eur J Clin Microbiol Infect. 2012; 31: 627-640
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23. Burroni E, Bonanni P, Sani C et al. Human papillomavirus prevalence in paired urine and cervical samples in women invited for cervical cancer screening. J. Med. Virol. 2014; 87: 508-515
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HPV URINE TEST FOR CERVICAL CANCER SCREENING

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