Guidelines for the use of molecular HPV tests in women and indications for HPV vaccination

Genital infection with human papillomavirus (HPV) is the most common sexually transmitted infection today, and persistent infection with high-risk genotypes is the cause of cervical cancer. Over 40 HPV genotypes infect mucosal surfaces, including the anogenital epithelium (e.g. cervix, vagina, vulva, rectum, urethra, penis and anus). For most of these HPV types, there is sufficient evidence to divide them into “high-risk” (i.e. oncogenic or cancer-associated) types and “low-risk” (i.e. non-oncogenic) types.

<table>
<thead>
<tr>
<th>High-risk (oncogenic or cancer-associated) types</th>
<th>Low-risk (non-oncogenic) types</th>
</tr>
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<tbody>
<tr>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82</td>
<td>6, 11, 40, 42, 43, 44, 54, 61, 72, 73, 81</td>
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HPV 16 is responsible for ~60% of cervical cancer, HPV 18 for ~10%, HPV 45 and HPV 31 for ~4% each, HPV 33, HPV 52, and HPV 58 together account for ~2% of cervical cancer.

These cause benign or low-grade lesions and genital warts.

Natural history of genital HPV infections in women

Most genital HPV infections are transient and asymptomatic. Approximately 70% of women become HPV DNA-negative within one year, and approximately 91% within two years following the initial infection. It is for this reason that HPV DNA assays, while being highly sensitive for detecting HPV infections, are unable to distinguish an infection that will clear spontaneously from one that will become persistent and lead to precancerous lesions and ultimately cervical cancer.

Currently available nucleic acid tests for HPV

In view of the fact that persistent infection with a high-risk HPV is necessary for the development of cervical carcinoma, high-risk HPV testing has become an integral part of cervical cancer screening, triage and the follow-up of treated lesions.

Currently available nucleic acid tests for HPV detect either HPV DNA or RNA. The majority of HPV nucleic acid tests are however DNA tests that are available in various formats, such as a qualitative screening PCR for high-risk HPV DNA without determining the genotype present. The newer generation screening assays not only detect the presence of high-risk HPV DNA, but also provide partial genotyping in that they specifically detect and differentiate between HPV 16 and HPV 18, while they also detect the remaining high-risk HPV genotypes without identifying the specific genotype.

In addition to these screening assays, there are full HPV DNA genotyping assays that are used by laboratories to identify the presence of high, intermediate and low-risk HPV types and to determine the specific genotype present. These full genotyping assays are generally not used as screening tests, but are used largely in epidemiological studies.

RNA-based tests are available that detect messenger RNA (mRNA) from the viral oncogenes E6/E7. The expression of E6/E7 mRNA implies that the virus has integrated into the host’s DNA and disrupted cell cycle control by expressing the oncogenes E6/E7. These have been developed with the hypothesis that they are more predictive of an infection associated with cervical cancer.
The following diagram shows the HPV DNA and E6/E7 mRNA expression in a cervical cancer progression model.

<table>
<thead>
<tr>
<th>Normal cervical epithelium</th>
<th>HPV-infected cervical cells</th>
<th>CIN1 or LSIL</th>
<th>CIN2 or HSIL</th>
<th>CIN3+ or HSIL</th>
<th>Cervical carcinoma</th>
</tr>
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</table>

Increasing E6/E7 mRNA

HPV DNA level high

that will lead to a precancerous lesion or the actual presence of a precancerous lesion, and are thus more specific for cervical disease than HPV DNA tests.

The role of HPV DNA testing in current cervical screening programmes

The three major agreed indications for HPV testing in clinical practice are as follows:

1. Triage of women with equivocal cytology (ASC-US) to determine which patients should be referred for colposcopy. This test can be used to help determine which women (of any age) with ASC-US should be referred for colposcopy (any high-risk HPV DNA-positive) or followed up with cytological screening at 12 months (HPV-negative).

2. Prediction of a therapeutic outcome after treatment of high-grade CIN. HPV DNA testing detects more patients with residual disease with higher sensitivity and similar specificity than follow-up cytology or histology of the resection margins.

3. Primary screening in women 30 years and older to determine the presence of cervical precancer lesions. High-risk HPV DNA testing is significantly more sensitive than cytology in identifying underlying CIN2 or CIN3 lesions. One drawback is the lower specificity, especially in young women where the infection is more likely to be transient. Screening for high-risk HPV without cytology offers similar protection from CIN in comparison to both HPV and cytology (co-testing) and there is now strong evidence to support primary HPV DNA screening. Following a negative high-risk HPV DNA PCR, the rescreening interval should be at least five years. Those who are high-risk HPV DNA-positive require a triage test, such as cytology, to determine which women are more likely to have CIN and would thus need to be referred for colposcopy. Besides cytology, genotyping for HPV 16 or HPV 16 and HPV 18, combinations of HPV 16/18 genotyping and cytology, or HPV retesting to detect persistent infections can be used to triage women who are high-risk HPV DNA-positive.

What about HIV-infected patients?

Due to the transient nature of most HPV infections, the specificity of high-risk HPV DNA PCRs may be less sensitive than follow-up cytology or high-risk HPV DNA testing (co-testing). Theoretically, HPV E6/E7 mRNA assays may have improved specificity over DNA assays. In addition, HPVE6/E7 mRNA assays in the following settings:

1. A quadrivalent HPV 16, 18, 6 and 11 vaccine will protect against precancerous lesions caused by HPV types 16 and 18.

2. A bivalent HPV 16 and HPV 18 vaccine from Merck marketed as GARDASIL®. This vaccine will protect against precancerous lesions caused by HPV types 16 and 18 and genital warts caused by HPV types 6 and 11.

3. A bivalent HPV 16 and HPV 18 vaccine from GSK marketed as CERVARIX®. This vaccine will protect against precancerous lesions caused by HPV types 16 and 18 and cervical dysplasia, cancer of the anogenital tract, as well as genital warts. The following situations:

   • Women older than 21 years with ASC-H, LSIL, CIN1 or LSIL CIN2 or HSIL CIN3+ or HSIL carcinoma, or CIN2 or CIN3 in persons with both ASC-US and LSIL.

   • Adolescents, defined as women who are 20 years and younger (regardless of cytology results).

   • Women with both ASC-US and LSIL.

   • Women with CIN1 or LSIL.

   • Postmenopausal women with LSIL (HPV DNA testing is acceptable in this setting).

   • To check the HPV status of patients with genital warts or another sexually transmitted infection.

   • To check the HPV status of partners of patients with HPV infection.

   • Women with cytological abnormalities. Recent studies have shown that high-risk HPV E6/E7 mRNA testing detects more patients with residual disease with higher sensitivity and similar specificity than follow-up cytology or histology of the resection margins.
The role of HPV mRNA testing in current cervical screening programmes

Due to the transient nature of most HPV infections, the specificity of high-risk HPV DNA testing is low. Several studies have shown that testing for HPV mRNA instead of DNA can be clinically useful as it has a higher specificity for high-grade cervical disease with an equivalent sensitivity of DNA tests. There is sufficient evidence to recommend the use of HPV mRNA assays in the following settings:

1. In conjunction with cytology (co-testing) to guide further management based on the cytology results.
2. As a triage test in patients who have minor cytological abnormalities. Recent studies have shown that high-risk HPV E6/E7 mRNA testing is as sensitive as DNA PCR to detect CIN2 or CIN3 in persons with both ASC-US and LSIL lesions, with significantly better specificity. Thus, the use of mRNA testing as a triage test in both ASC-US and LSIL lesions is recommended over high-risk DNA testing as this will result in fewer unnecessary colposcopy referrals.

While generally as sensitive as high-risk HPV DNA PCRs, there is as yet insufficient evidence to recommend primary screening with HPV E6/E7 mRNA assays. In addition, HPVE6/E7 mRNA assays should not be used to predict therapeutic outcomes after treatment for high-grade CIN as they have been found to be less sensitive than high-risk HPV DNA PCRs in this setting.

What about HIV-infected patients?

HIV-infected persons have a higher rate of incident and prevalent HPV infections and are at higher risk of both precancerous lesions and cervical cancer. HIV-infected persons require more frequent screening with either high-risk HPV DNA tests and/or cytology. Theoretically, HPV E6/E7 mRNA assays may be very useful in HIV-infected women due to their improved specificity over DNA assays. The evidence to support the use of mRNA assays in HIV-infected persons is, however, lacking.

High-risk HPV DNA testing and genotyping is not recommended in the following situations:

- Adolescents, defined as women who are 20 years and younger (regardless of cytology results).
- Women older than 21 years with ASC-H, LSIL or HSIL (HPV DNA testing is acceptable in postmenopausal women with LSIL). HPV E6/E7 mRNA is a better triage test in women with both ASC-US and LSIL.
- Routine screening in women younger than 30 years.
- To check the HPV status of patients with genital warts or another sexually transmitted infection.
- To check the HPV status of partners of patients with genital warts, another sexually transmitted infection, cervical dysplasia or cancer. In particular, the testing of the male partners of women who have tested positive for high-risk HPV is not necessary and results in unnecessary anxiety. Nothing can be inferred or assumed regarding transmission from one partner to the other based on the results of the HPV tests of both sexual partners.

HPV vaccines and indications for vaccination

The prevention of genital HPV infection is important to reduce the prevalence of cervical dysplasia, cancer of the anogenital tract, as well as genital warts. The following two prophylactic vaccines are available:

1. A quadrivalent HPV 16, 18, 6 and 11 vaccine from Merck marketed as GARDASIL®. This vaccine will protect against precancerous lesions caused by HPV types 16 and 18 and genital warts caused by HPV types 6 and 11.
2. A bivalent HPV 16 and HPV 18 vaccine from GSK marketed as CERVARIX®. This vaccine will protect against precancerous lesions caused by HPV types 16 and 18.

Both vaccines are made from non-infectious HPV-like particles known as virus-like particles (VLPs) and do not contain mercury or
thimerosal. They are administered through a series of three intramuscular injections over a six-month period, with CERVARIX® being given at 0, 1 and 6 months, and GARDASIL® at 0, 2 and 6 months.

**Indications for vaccination**

GARDASIL® and CERVARIX® are preventative vaccines and do not treat existing HPV infections, cervical dysplasia, cervical cancer, genital warts or laryngeal papillomatosis.

- **In women:**
  
  HPV vaccination is recommended and licensed in young women aged nine to 26 in certain countries. Ideally, the vaccine should be given prior to the onset of sexual activity so that they are naïve to all vaccine HPV types. Sexually active girls should still be given the vaccine. However, they must be made aware that they will only be protected against those vaccine HPV types to which they have not been exposed. A previous abnormal pap test or positive HPV DNA test does not exclude a woman from receiving the vaccine. It is obligatory that, following vaccination, all women continue to be screened for cervical cancer.

- **In men:**
  
  The quadrivalent vaccine (GARDASIL®) is effective in men to prevent genital warts caused by HPV 6 and HPV 11 and may prevent cancerous lesions caused by HPV 16 and HPV 18, in particular of the anus and oropharynx. In many countries, it is licensed for use in males aged nine to 26, and many would argue for its routine use in both men and women.

**Safety and duration of protection**

Both vaccines are safe and most adverse events are mild pain at the injection site. The quadrivalent vaccine is contraindicated in those with an immediate hypersensitivity reaction to yeast, and the bivalent vaccine contraindicated in those with an anaphylactic latex allergy.

Seroconversion rates are >99% after vaccination. The duration of vaccine protection is unknown, however, current studies, with a five year follow-up, indicate protection for at least five years with no drop in antibody levels.

HIV infection is not a contraindication to vaccination. However, seroconversion rates are likely to be lower than HIV-uninfected persons, with a shorter duration of protection.

**Molecular tests for HPV available at Ampath**

1. **A high-risk HPV DNA PCR.** This is a qualitative PCR for the detection of 14 high-risk HPV genotypes. This assay detects and differentiates between HPV 16, HPV 18 and non-HPV 16/18 genotypes (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). This PCR can be requested on either liquid-based cytology samples or cervical swabs.

2. **A full HPV DNA genotyping assay.** This tests for and determines the genotype of 37 high-risk and low-risk HPVs.

3. **An HPV E6/E7 mRNA assay.** This is a qualitative test for the detection of E6 and E7 mRNA expression from 14 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). This test can only be requested on liquid-based cytology samples.

For further information please contact your local Ampath pathologist.

**References available on request.**

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