Human Papilloma Virus (HPV) Sequencing Using a Cytobrush (code 41416)

Notice of Assessment

June 2013
1 GENERAL INFORMATION

1.1 Requestor: CHU Sainte-Justine.

1.2 Application Submitted: August 3, 2012.

1.3 Notice Issued: April 12, 2013.

Note:
This notice is based on the scientific and commercial information (submitted by the requestor[s]) and on a complementary review of the literature) according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCE(S)

2.1 Name of the Technology: HPV sequencing using a cytobrush.

2.2 Brief Description of the Technology
An in-house PCR amplification technique targeting a specific HPV region as well as a direct sequencing (PCR-DS) method, developed at CHU Ste-Justine. Direct sequencing uses two primer pairs specific to HPV (L1 gene): MY09/MY11 and GP5/GP6 as well as $^{33}$P-labelled dideoxynucleotide. The MY09/MY11 pair (second tier) is used in the event of negative results with GP5/GP6 (first tier). If results are positive, reamplification of the GP5/GP6 pair is conducted. Subsequently, direct sequencing for HPV typing is performed on positive PCRs. Reading a 34-nucleotide sequence from the L1 gene region is sufficient for typing a new or known sequence. Sequencing is performed with a Thermo Sequenase kit from Amersham Canada, Baie d’Urfé, Quebec (Feoli-Fonseca et al., 1998a; 1998b).

2.3 Company or Developer
Testing procedure developed in-house, as detailed in an article published by Feoli-Fonseca et al. in 1998.

2.4 Licence(s): Not applicable.

2.5 Patent, If Applicable: Not applicable.

2.6 Approval Status (Health Canada, FDA)
In-house procedure; no Health Canada license.

2.7 Weighted Value: 47.0 (however, the requestor has indicated that an updated estimate has been provided to the MSSS; the updated weighted value is reported to be 25.0).

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patient Group
According to the requestor, the test targets women with cervical lesions detected by colposcopy, as well as women older than 30 who are part of the screening process.
3.2 Targeted Disease(s)

The human papilloma virus (HPV) infection is one of the most common sexually transmitted infections. The majority of people infected are asymptomatic, and 90% will no longer be infected after one or two years. As for the remaining 10%, the infection will become persistent and the risk of developing cancer will increase (Zandberg et al., 2013). Cervical cancer is the 13th most common form of cancer in Canadian women (1,350 new cases in Canada and 280 in Quebec in 2012) (SCC, 2012). While there are approximately 40 HPV genotypes that can infect the human genital mucosa, only 15 of them are associated with a high risk of cervical dysplasia or cancer in women. Any persistent high-risk HPV infection increases the risk of cancer, although it can take up to several decades before the infection progresses into cancer (INSPQ, 2011). During such time, screening can detect cell abnormalities for close monitoring or early treatment. HPV type 16 is reportedly responsible for 50% of cases of cervical cancer, HPV 18 for 10% to 15%, HPV 45 for 7%, and HPV 31 for approximately 3% (Khan et al., 2005). Most HPV genital infections self-heal; the risk of cancer is associated solely with persistent infections. Moreover, HPV 16 is reportedly linked to certain forms of oropharynx cancer (squamous cell cancer of the head and neck) (Psyrri et al., 2009).

3.3 Number of Patients Targeted

A total of 400 tests per year (information provided by the requestor).

3.4 Medical Specialties Involved (and Other Professionals, If Any)

Family medicine, gynecology and oncohematology.

3.5 Testing Procedure

Conventional Pap smear using plastic brushes (cytobrush). Swabs or small sticks may also be used. Additionally, the analysis may be conducted on formalin-fixed and paraffin-embedded (FFPE) biopsy specimens.

4 TECHNOLOGY BACKGROUND

4.1 Nature of the Diagnostic Technology: Complementary.

4.2 Brief Description of the Current Technological Context

Three types of analyses are available for HPV diagnostic testing: 1) detection of oncogenic groups; 2) targeted genotyping; and 3) extensive genotyping or sequencing.

Four codes are found in the Index that correspond to HPV diagnostic testing:

- **41407**: Human Papilloma Virus (HPV) (Detection of oncogenic groups using commercial kits) (WA: 34.0)
- **41408**: Human Papilloma Virus (HPV) (Sequencing for typing) (WA: 61.0)
- **41416**: Human Papilloma Virus (HPV) (Sequencing using a cytobrush) (WA: 47.0), (WA: 25.0 according to the requestor)
- **41395**: Human Papilloma Virus (HPV) (Detection) (In situ hybridization on paraffin-embedded blocks) (WA: 60.0)
While the analysis technique is similar for codes 41408 and 41416, the sample is different. Sequencing for typing (41408) requires a paraffin-embedded sample, which involves more handling and increases the weighted average. For test code 41416, the sample may be collected using a cytobrush, swabs or small sticks. However, it is not formalin-fixed and paraffin-embedded, nor is it a liquid sample.

Code 41407 could be used for the proposed test; it is less specific, as the proposed testing allows for detecting oncogenic and non-oncogenic groups.

4.3 **Brief Description of the Advantages Cited for the New Technology**

The proposed test displays a level of sensitivity and specificity clearly superior to hybrid capture testing (not available in Quebec). Methodological changes when performing the technique have allowed costs to be cut by half (information retrieved from the form filled out by the requestor). Additional changes have also allowed for the significant reduction of the time for implementation (simpler DNA template preparation, PCR amplification followed by direct sequencing, etc.) (Feoli-Fonseca et al., 1998b).

As for line blot assays, theoretically, PCR-DS can detect all HPV types, thereby eliminating the risk of cross-hybridization. It can also detect new virus types (Feoli-Fonseca et al., 2001).

4.4 **Cost of Technology and Options**: Not analyzed.

5 **EVIDENCE**

5.1 **Clinical Relevance**

5.1.1 **Other Tests Replaced**

This test is complementary to other HPV diagnostic and screening procedures.

5.1.2 **Diagnostic or Prognostic Value**

No information was found on the relationship between knowledge of the virus type and mortality or survival. The link between genotyping on the one hand and morbidity and quality of life on the other has not been studied.

Changes to the treatment based on test results: No information available.
5.1.3 Therapeutic Value

Confirmation of the presence of any high-risk HPV type allows for early treatment or close monitoring. However, evidence on the usefulness of type-specific genotyping is not available for the indications specified in the application; i.e., cervical lesions detected through colposcopy and screening in women over 30.

5.2 Clinical Validity

Feoli-Fonseca et al. (2001) have tested their procedure on 691 biopsy samples collected from patients following a clinical or histological assessment that suggested a possible HPV infection. Samples were collected from 11 tissues and organs, including four from the anogenital region. All samples were examined by anatomical pathologists and classified in five categories (CIN I to III, CIS and invasive cancer). From the 691 specimens, a total of 519 (75%) were classified under CIN I, II, or III (no case of invasive carcinoma), PCR-DS results were HPV positive in 70% of cases (484/691); more than one HPV type was found in a few cases (531 isolates). The detection rate varied based on the biopsy site and the type of pathology (from 6% in cases of cervical inflammation to 100% in cervical CIS cases). Furthermore, the detection rate was 73% (406/554) in all cases of cervical lesions taken together and 64% (88/137) in all samples collected from tissues other than the cervix. The authors also noted a tendency for PCR-based techniques to underestimate co-infections. In the presence of two or more HPV types within one sample, PCR-based techniques may amplify one sequence while masking the others.

The false-negative issue associated with PCR due to infections with multiple viral types of low copy number has also been mentioned in the report (Abreu et al., 2012).

5.2.1 Clinical Utility

In a literature review, Chan et al. (2012) report four main clinical applications of HPV detection (without necessarily stating the technique involved):

1. primary screening (more sensitive than cytology, more objective interpretation, high NPV, enables long-term risk stratification, less variation from one centre to another), but specificity and PPV are low;
2. co-testing with cytology for primary screening (improves sensitivity for CIN II+ lesions, double negative results bring down the risk of CIN II+ significantly, possibility of extending intervals between screening rounds);
3. screening in cases of abnormal cytology (in case of ASC-US cytology, which is the most common application);
4. post-treatment follow-up (more sensitive than cytology, better NPV for recurrent cases and residual lesions). To conclude their report, the authors have highlighted the importance of these tests in the future, given that although the incidence of HPV and cervical cancer will decrease with vaccination, so will the efficiency of Pap smear cytology.

5.3 Analytical (or Technical) Validity

There are no data on technical validity specific to the technique used for the requested test.

5.4 Recommendations for Listing in Other Jurisdictions

For cervical cancer screening in women aged 30 and above who have had a negative cytology and for whom HPV DNA results are positive (for any of the 13 or 14 high-risk HPV types), targeted genotyping for the detection of HPV type 16 and 18 is recommended. If positive, these women
should be sent for colposcopy, and if negative, they should be followed up with cytology and HPV testing after 12 months (Saslow et al., 2012; ASCCP, 2009). There is no mention of a specific genotyping test.

In Quebec, cervical cancer screening is opportunistic. In its 2011 report, the INSPQ recommended screening using cytology (on slides or liquid-based) for any woman who is sexually active or who has been in the past. Among other things, the report recommends that in the case of women aged 30 and above who have had equivocal screening test results (ASC-US), an HPV screening test and a colposcopy should be suggested if results are positive, or another cytology should be scheduled after 12 months if HPV test results are negative. The report states that the tests administered must be approved by Health Canada (INSPQ, 2011).

The Canadian recommendations for screening of cervical cancer published in January 2013 did not address the issue of HPV screening tests due to a lack of evidence on the effects of these tests on mortality and the incidence of invasive cancer. These recommendations will be updated when sufficient data are available (Pollock et al., 2013).

6 ANTICIPATED OUTCOMES FOLLOWING THE INTRODUCTION OF THE ANALYSIS

6.1 Impact on Material and Human Resources: Not assessed.

6.2 Economic Consequences of Introducing Test Into Quebec’s Health Care and Social Services System: Not assessed.

6.3 Main Organizational, Ethical, or Other (Social, Legal, Political) Issues: Not assessed.
7 INESSS NOTICE IN BRIEF

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<th>Status of the Diagnostic Technology:</th>
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<tr>
<td>☐ Established</td>
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<td>☒ Innovative</td>
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<tr>
<td>☐ Experimental (for research purposes only)</td>
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<tr>
<td>☐ Replacement for technology: __________________, which becomes obsolete</td>
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<th>INESSS Recommendation:</th>
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<tr>
<td>☐ Keep test in the Index</td>
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<td>☒ Remove test from the Index</td>
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<td>☐ Reassess test</td>
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<th>Additional Recommendation:</th>
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<tr>
<td>☐ Draw connection with listing of drugs, if companion test</td>
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<td>☐ Production of an optimal use manual</td>
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<td>☐ Identify indicators, when monitoring is required</td>
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Note
The analysis featured previously was developed locally and enables extensive genotyping of specific HPV types. According to the Screening Guidelines for the Detection of Cervical Cancer in Québec (Lignes directrices sur le dépistage du cancer du col utérin au Québec), published by the INSPQ in June 2011, any generic test must be approved by Health Canada. However, this test could prove useful from a public health perspective (monitoring in a context of universal vaccination) or in research projects.
REFERENCES


