



# Human papillomavirus testing as part of the renewed National Cervical Screening Program



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## Background

On 1 December 2017, Australia moved to a new National Cervical Screening Program (NCSP), which uses primary human papillomavirus (HPV) nucleic acid testing (NAT) followed by reflex liquid-based cytology for women aged between 25 and 74 years.

## Objectives

The aim of this article is to provide an overview of the different HPV NAT assays that satisfy the requirements for use in the renewed NCSP.

## Discussion

Australia has adopted innovative, evidence-based criteria for the inclusion of HPV NAT assays in the renewed NCSP. These include the requirements for detection of all 12 designated oncogenic HPV types, including separate detection and reporting of HPV 16 and 18; validation against reference assays showing sufficient sensitivity and specificity for the detection of underlying high-grade cervical disease; reproducibility; and the presence of cellularity and inhibition controls. Practitioners can feel assured that HPV NAT undertaken as part of the renewed NCSP will produce high-quality results irrespective of location or pathology provider.

**THE RENEWED** Australian National Cervical Screening Program (NCSP) began on 1 December 2017. Recommendations made in 2014 by the Medical Services Advisory Committee (MSAC), which examined new evidence including technologies, form the basis of the renewed NCSP. While the Pap smear at two-yearly intervals has been the basis of the Australian NCSP for over 25 years, the key recommendation of the MSAC was that the Pap smear be replaced by a cervical screening test (CST) that would use a human papillomavirus (HPV) test as the primary screening test followed by reflex cytology of HPV-positive specimens.<sup>1</sup> The renewed NCSP also includes an increase in the screening entry age from 18 to 25 years and screens women until the age of 69 years, with an exit test between 70 and 74 years. The renewed NCSP has also introduced the option for women who are under-screened or who have never participated in cervical screening to access HPV self-collection in a clinical setting.

## HPV NAT

There is a wealth of evidence showing the increased sensitivity of HPV-based screening, compared with cytology-based screening.<sup>2-4</sup> A population-based HPV primary cervical screening program began in the Netherlands earlier in 2017. In the Netherlands there was a tender process to determine a single HPV nucleic acid testing (NAT) assay that would be used throughout the country. Australia has taken a different approach by using a quality-based requirements framework

for testing, which allows different pathology providers to select the assay that best fits their needs as long as it meets the performance standards and characteristics required by the program.

## Requirements for the use of human papillomavirus nucleic acid HPV NAT assays in the National Cervical Screening Program

In Australia, the type of HPV NAT that can be undertaken in the NCSP is governed by the National Pathology Accreditation Advisory Council (NPAAC) requirements, specifically those for laboratories reporting tests for the NCSP.<sup>5</sup> These requirements have been designed to reduce the risk of false results occurring, either positive or negative.

## Oncogenic HPV

There are 12 HPV types classified as definitely oncogenic (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) according to the World Health Organization.<sup>6</sup> NPAAC requires that all assays used in the NCSP are commercially supplied assays that can detect all designated 12 oncogenic HPV types. HPV assays must also be able to separately identify HPV 16 and HPV 18 in order to identify women who are at higher risk of developing cervical cancer on the basis solely of the HPV result. Some assays give a combined result for HPV 18 and HPV 45 (18/45), and these assays are regarded as satisfying the requirements, with women who test positive for HPV 18/45 undertaking the same clinical pathway as women who test positive for HPV 18.

## Clinical sensitivity, specificity and reproducibility

HPV NAT assays have a clinical cut-off point for positivity, which has been set specifically to facilitate their use as a risk-stratification test for primary screening. HPV NAT assays are not a diagnostic test for cervical disease, nor are they a diagnostic test for the presence of any HPV at all; that is, the cut-off point requires a threshold amount of HPV to be present. This threshold amount is associated with the presence of a high-grade squamous intraepithelial lesion (HSIL). Many HPV infections either resolve without treatment or may persist but never lead to cervical abnormalities. Additionally, there is currently no treatment for HPV infections per se, other than the removal of HPV-positive lesions. The clinical sensitivity, specificity and reproducibility of HPV NAT assays are assessed using a protocol known as the Meijer criteria.<sup>7</sup> Briefly, an HPV NAT assay must show clinical sensitivity and specificity for detecting the underlying presence of a cervical HSIL of not less than 90% and 98% respectively of a validated reference assay. To satisfy the Meijer criteria, an HPV NAT assay must also show intra-laboratory and inter-laboratory agreement with a lower confidence bound of 87%.

## HPV NAT assay controls

In addition to showing clinical sensitivity, specificity and reproducibility, the NPAAC requirements state that an HPV NAT assay must contain a control to monitor for inhibition and/or assay failure and a control for cellularity to detect inadequate or empty cervical samples. The control for inhibition/assay failure is critically important, as contaminants of cervical samples, such as blood or lubricant, can inhibit the ability of an assay to detect HPV. The cellularity control is also important, as an assay lacking a cellularity control would report an empty liquid-based cytology vial with insufficient or absent cellular material as negative, and a recommendation to rescreen in five years may then be incorrectly assigned to a woman. Unsatisfactory samples – those

with low cellularity (or inhibition) – occur at a rate of 0.1–0.2% in clinician-collected samples, but this rate has been observed to be as high as 10% in some self-collection studies from overseas. Within the Australian environment, a recent pilot study of the renewed NCSP guidelines for self-collection in Victoria found the rate of unsatisfactory self-collected samples, resulting from low cellularity, was 2.5%.<sup>8</sup> A recent examination of unsatisfactory results in cytology-based CSTs in Victoria found a rate of 2.7%.<sup>9</sup>

## HPV NAT for self-collected samples

As part of the renewed NCSP, an alternative screening pathway will be offered to eligible under-screened or never-screened women attending a healthcare setting to overcome the barriers some women experience with having a clinician-collected CST. Eligible women must:

- be over 30 years of age and at least two or more years overdue for a CST
- have declined a speculum examination
- be under the supervision of a health professional that routinely offers cervical screening.

There is a wealth of data showing that a self-collected sample tested for HPV NAT<sup>10</sup> is of similar sensitivity for detecting HSIL as a clinician-collected sample. However, it has also been noted that PCR-based tests have stronger evidence of equivalence to clinician-collected samples and, as such, the NPAAC requirements clearly state that any self-collected specimens must be tested on a PCR-based HPV test. Currently, only one pathology laboratory in Australia is validated for testing of self-collected samples for HPV NAT.<sup>11</sup>

## Other quality measures

In addition to the requirements that an HPV NAT assay must meet for use within the NCSP described above, there are a number of additional quality control measures in place. Each laboratory has to undertake a minimum number of tests in order to accurately monitor the variation in HPV-positive results being produced at

other laboratories and ensure sufficient consistency with national rates. It is hoped that this will ensure that any potential quality issues that could adversely affect assay performance – for example, variation in liquid-based cytology media or storage/transport conditions – will be detected prior to disposal of patient specimens.

The HPV NAT assays are also subject to a range of quality-control processes. For example, the NPAAC requires that controls not supplied by the manufacturer are run on every day that HPV is being tested for. Additionally, laboratories performing HPV NAT assays must participate in an external quality assurance program (eg the Royal Australasian College of Pathologists Quality Assurance Program), and any discrepancies must be investigated.

## HPV NAT assays used in the renewed National Cervical Screening Program

At the time of writing, there were six HPV NAT assays that had met the requirements for use in the renewed NCSP (Table 1). The Roche cobas 4800 and Roche cobas 6800 HPV NAT assays are the most widely used throughout Australia. All six are in use in Australian laboratories in some form. The Australian requirements are based on an assay satisfying certain quality measures and, as such, a number of other assays are likely to be available for use in the near future, including assays from Hologic, AusDiagnostics and Euroimmun. This flexibility will allow pathology laboratories to choose HPV NAT assays that best meet the needs of their referring practitioners and workflows.

## Conclusion

Australia has adopted innovative, evidence-based criteria for the inclusion of HPV NAT assays in the renewed NCSP. In addition, the quality of HPV testing in the program is further supported by a comprehensive quality program, which includes monitoring of HPV positivity rates in CSTs and the daily testing of quality control samples. Practitioners

**Table 1. NPAAC requirements for HPV assays for use in the renewed National Cervical Screening Program**

HPV assay	NPAAC requirements				
	Meijer criteria	Screening	Controls		Self-collection
			Cellularity	Inhibition	PCR-based
Roche cobas 4800	✓	✓	✓	✓	✓
Abbott RealTime High-Risk HPV	✓	✓	✓	✓	✓
Becton Dickinson Onclarity	✓	✓	✓	✓	✓
Seegene Anyplex II	✓	✓	✓	✓	✓
Cepheid GeneXpert	✓	✓	✓	✓	✓
Roche cobas 6800	✓	✓	✓	✓	✓

HPV, human papillomavirus, NPAAC, National Pathology Accreditation Advisory Council

can feel assured that HPV NAT undertaken as part of the renewed NCSP will produce high-quality results irrespective of location or pathology provider.

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