NATIONAL PATHOLOGY ACCREDITATION ADVISORY COUNCIL

# REQUIREMENTS FOR VALIDATION OF SELF-COLLECTED VAGINAL SWABS FOR USE IN THE NATIONAL CERVICAL SCREENING PROGRAM

## (First Edition 2019)

**NPAAC Tier 4 Document**

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# Contents

The National Pathology Accreditation Advisory Council (NPAAC) was established in 1979 to consider and make recommendations to the Australian, state and territory governments on matters related to the accreditation of pathology laboratories and the introduction and maintenance of uniform Standards of practice in pathology laboratories throughout Australia. A function of NPAAC is to formulate Standards and initiate and promote guidelines and education programs about pathology tests.

Publications produced by NPAAC are issued as accreditation material to provide guidance to laboratories and accrediting agencies about minimum standards considered acceptable for good laboratory practice.

Failure to meet these Standards may pose a risk to public health and patient safety.

# Scope

The *Requirements for Validation of Self-Collected Vaginal Swabs for Use in the National Cervical Screening Program (First Edition 2019)* is a Tier 4 NPAAC document and must be read in conjunction with the Tier 2 document *Requirements for Medical Pathology Services*. The latter is the overarching document broadly outlining Standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, laboratory staff and referrers (both for pathology requests and inter-laboratory referrals) are safely and satisfactorily met in a timely manner.

Whilst there must be adherence to all the requirements in the Tier 2 document, reference to specific Standards in the document are provided for assistance under the headings in this document.

The Requirements outline the minimum standards that an Applicant laboratory must meet in order to offer testing of self-collected vaginal swabs (SCVS) for human papillomavirus (HPV) as part of the National Cervical Screening Program (NCSP) as an in-house in vitro diagnostic medical device (IVD). Comparator laboratories must also meet these Requirements, excluding S3.1 in Standard 3 - Validation Performance Requirements.

The actual swab used by a laboratory to enable self-collection of a vaginal sample for HPV testing must be registered on the Australian Register of Therapeutic Goods. However, as yet no manufacturer has validated the use of their HPV assay with this type of specimen and therefore the use of these swabs must be validated as an in-house IVD (i.e., use of this swab type with the HPV assay means the overall test is considered to be an in-house IVD).

Laboratories wishing to offer testing of SCVS can validate use of this specimen type by comparison with health care worker collected swabs from the uterine cervix. Such protocols are not the subject of these Requirements.

# Abbreviations

| Abbreviation | Description |
|---|---|
| ARTG | Australian Register of Therapeutic Goods |
| AS | Australian Standard |
| CLSI | Clinical and Laboratory Standards Institute |
| EQA | External Quality Assurance |
| ISO | International Organization for Standardization |
| HPV | Human Papillomavirus |
| In-house IVD | In-house In Vitro Diagnostic Medical Device |
| LOD | Limit of Detection |
| NAT | Nucleic Acid Test |
| NATA | National Association of Testing Authorities, Australia |
| NCSP | National Cervical Screening Program |
| NPAAC | National Pathology Accreditation Advisory Council |
| QC | Quality Control |
| QS | Quality System |
| RCPA | Royal College of Pathologists of Australasia |
| RMPS | Requirements for Medical Pathology Services |
| SCVS | Self-collected Vaginal Swab |
| TGA | Therapeutic Goods Administration |

# Definitions

| Term | Definition |
|---|---|
| Applicant laboratory | means for the purposes of this document, a laboratory that is seeking to validate the use of SCVS as an in-house IVD short of performing a direct comparison of SCVS against health care worker collected cervical specimens. An applicant laboratory that achieves accreditation to test SCVS using these requirements cannot subsequently function as a Comparator laboratory unless it has performed a direct comparison of SCVS against healthcare worker collected cervical specimens. |
| ARTG registered | means a device included in the Australian Register of Therapeutic Goods by the Therapeutic Goods Administration and approved for supply in Australia. |
| Clinical evidence for an IVD | means all the information that supports the scientific validity and performance for its use as intended by the manufacturer. |
| Clinical utility | means the usefulness of the results obtained from testing with the IVD medical device and the value of the information to the individual being tested and/or the broader population. |
| Clinical performance | means the ability of an IVD medical device to yield results that are correlated with a particular clinical condition/physiological state in accordance with the target population and the intended user. |
| Comparator laboratory | means for the purposes of this document, a laboratory that has validated their assays for SCVS against health care worker collected cervical specimens and been accredited for performing HPV testing on SCVS. |
| Conformity assessment | means a process undertaken by an accreditation body to assess the competence of a laboratory or organisation, based on particular Standard(s) and/or other normative documents, and for a defined scope of accreditation.* |

* Derived from ISO/IEC 17011:2004 – *Conformity assessment - General requirements for accreditation bodies accrediting conformity assessment bodies*

| Term | Definition |
|---|---|
| In vitro diagnostic medical device (IVD) | means the same as the definition in the *Therapeutic Goods (Medical Devices) Regulations 2002* and is a medical device that is:<br>(a) a reagent, calibrator, control material, kit, specimen receptacle, software, instrument, apparatus, equipment or system, whether used alone or in combination with another diagnostic product for in vitro use; and<br>(b) intended by the manufacturer to be used in vitro for the examination of a specimen derived from the human body, solely or principally for:<br>(i) giving information about a physiological or pathological state or a congenital abnormality; or<br>(ii) determining safety and compatibility with a potential recipient; or<br>(iii) monitoring therapeutic measures; and<br>(c) not a product that is:<br>(i) intended for general laboratory use; and<br>(d) not manufactured, sold or presented for use as an IVD medical device. |
| In-house IVD | means the same as the definition in the *Therapeutic Goods (medical Devices) Regulations 2002* and is an IVD medical device that is:<br>(a) within the confines or scope of an Australian laboratory or Australian laboratory network:<br>(i) developed from first principles, or<br>(ii) developed or modified from a published source; or<br>(iii) developed or modified from any other source; or<br>(iv) used for a purpose, other than the intended purpose assigned by the manufacturer; and<br>(b) not supplied for use outside that laboratory or laboratory network. |
| Intended purpose | means the same as the definition in the *Therapeutic Goods (Medical Devices) Regulations 2002* and is the purpose for which the manufacturer of the device intends it to be used, as stated in:<br>(a) the information provided with the device; or<br>(b) the instructions for use of the device; or<br>(c) any advertising material applying to the device; and<br>(d) technical documentation describing the mechanism of action of the device. |

| Term | Definition |
|---|---|
| Medical laboratory network (or Pathology Network) | means, for the purposes of this document, more than one laboratory operating under the same governance structure. |
| Method validation | means the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. |
| Method verification | means procedures to test what extent the performance data obtained by manufacturers during method validation can be reproduced in the environments of end-users. |
| Modified IVD | means any IVD medical device that is:<br>(a) used for a purpose other than that intended by the original manufacturer; or<br>(b) not used in accordance with the manufacturer's instructions for use or the methodology described (i.e, modifications that could affect the performance of the device and would require validation). |
| Requirements for Medical Pathology Services (RMPS) | means the overarching document broadly outlining standards for good medical pathology practice where the primary consideration is patient welfare, and where the needs and expectations of patients, laboratory staff and referrers (both for pathology requests and inter-laboratory referrals) are safely and satisfactorily met in a timely manner. |
| Scientific validity | means the association of an analyte to a clinical condition / physiological state. |
| Validation assessment material | means materials that when tested contribute device performance information useful for the purposes of assessing validation compliance. |

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# Introduction

The *Requirements for Validation of Self-Collected Vaginal Swabs for Use in the National Cervical Screening Program (First Edition 2019)* is an NPAAC Tier 4 document and together with the *Requirements for Medical Pathology Services*, sets out the minimum requirements for best practice for the development and/or use of a self-collected vaginal swab (SCVS) with a commercially supplied HPV assay as an in-house IVD for use in screening as part of the NCSP.

From 1 December 2017, the renewal of the National Cervical Screening Program (NCSP) included a policy for self-collection.[†] Self-collection aims to improve participation in screening by providing an alternative screening process for asymptomatic individuals who are under-screened or never-screened and who have declined conventional screening via invitations and reminders from health care professionals and the National Cancer Screening Register (NCSR), and recognises the vast majority of cervical cancer in Australia occurs among these individuals.

Currently, there are no commercially supplied HPV nucleic acid tests (NATs) that have been approved for primary population screening that have also been validated by the manufacturers for use on SCVS. Therefore, testing of SCVS with these NATs constitutes an in-house IVD. Laboratories must validate use of this specimen type in their HPV assays.

The Requirements distinguish between two types of laboratories – Comparator laboratories and Applicant laboratories. For the purposes of this document, *Comparator laboratory* means a laboratory that has validated their assay/s for SCVS against health care worker collected cervical specimens and been accredited for performing HPV testing on SCVS. An *Applicant laboratory* is a laboratory that is seeking to validate the use of SCVS as an in-house IVD short of performing a direct comparison of SCVS against health care worker collected cervical specimens. An applicant laboratory that achieves accreditation to test SCVS using these requirements cannot subsequently function as a Comparator laboratory unless it has performed a direct comparison of SCVS against healthcare worker collected cervical specimens.

Laboratories wishing to offer testing of SCVS can validate use of this specimen type by comparison with health care worker collected swabs from the uterine cervix. Such protocols are not the subject of these Requirements as it would pose significant operational hurdles including a significant time to the completion of accrual of patient participation. This time delay means that many women will continue not to be screened.

To achieve a balance between the benefits of screening and requirements for validation of the use of SCVS with a HPV assay as an in-house IVD, this document outlines the minimum acceptable standards that need to be met in order to be considered to have validated the use of SCVS with a HPV assay as an in-house IVD short of a direct comparison between health care worker collected cervical swabs and SCVS.

The requirements in this document are specific to the use of SCVS for HPV screening as part of the NCSP and should not be taken to set a precedent for validation of in-house IVDs in other circumstances.

[†] Cancer screening self-collection policy

Applicant Laboratories must obtain approval of their validation protocol as described in ***Appendix B*** from a relevant, appropriately constituted, Human Research Ethics Committee.

The TGA is responsible for ensuring that therapeutic goods available for supply in Australia are safe and fit for their intended purpose. Australian laboratories that develop and use in-house IVDs are considered to be the manufacturer of these devices and as such laboratories are required to meet certain regulatory requirements in order to legally supply[‡] an in-house IVD in Australia. The *Therapeutic Goods (Medical Devices) Regulations 2002* require that a laboratory in which a Class 1-3 in-house IVD is manufactured must be accredited to ISO 15189 for a medical testing laboratory by NATA and meet the NPAAC *Requirements for the Development and Use of In-house IVDs*. Laboratories must also have provided a notification to the TGA of all the Class 1-3 in-house IVDs in use in the laboratory by 1 July 2017. For any new Class 1-3 in house IVDs introduced after this date, laboratories are required to renotify the TGA by 1 July of that financial year.

These Requirements represent a pathway for laboratories to demonstrate compliance with the NPAAC *Requirements for the Development and Use of In House IVDs* and meet the regulatory requirements for in house IVDs.

This Standard is a supplementary Standard to the *Requirements for Laboratories Reporting Tests for the National Cervical Screening Program* and should be read in conjunction with other accreditation requirements.

These Requirements may be subject to change with changes to the Self-Collection policy under the NCSP.

These Requirements have been developed with reference to current Australian legislation and other standards from the International Organization for Standardization (ISO) including:

| | |
|---|---|
| AS ISO 15189 | *Medical laboratories – Requirements for quality and competence* |
| ISO 23640 | *In vitro diagnostic medical devices – Evaluation of stability of in vitro diagnostic reagents* |
| ISO 13485 | *Medical devices – Quality Management Systems – Requirements for Regulatory Purposes* |
| ISO 14971 | *Medical devices – Application of Risk Management to Medical Devices* |

This document should be read within the national pathology accreditation framework including the current versions of the following NPAAC documents:

**All Tier 2 and Tier 3 Documents**

**Tier 4 Document**

- *Requirements for Laboratories Reporting Tests for the National Cervical Screening Program (Second Edition 2019)*

In addition to these Standards, laboratories must also comply with the relevant state and territory legislation (including any reporting requirements).

[‡] Supply in this context means making the test available and reporting patient results of the test.

In each section of this document, points deemed important for practice are identified as either 'Standards' or 'Commentaries.'

- A Standard is the minimum requirement for a procedure, method, staffing resource or facility that is required before a laboratory can attain accreditation — Standards are printed in bold type and prefaced with an 'S' (e.g**. S2.2**). The use of the verb **'must'** in each Standard within this document indicates a mandatory requirement for pathology practice.
- A Commentary is provided to give clarification to the Standards as well as to provide examples and guidance on interpretation. Commentaries are prefaced with a 'C' (e.g. C1.2) and are placed where they add the most value. Commentaries may be either normative or informative, depending on both the content and the context of whether they are associated with a Standard or not. Note that when comments are expanding on a Standard or referring to other legislation, they assume the same status and importance as the Standards to which they are attached. Where a Commentary contains the verb **'must'** then that Commentary is considered to be **normative**.

Please note that any Appendices attached to this document may be either **normative** or **informative** in nature and should be considered to be an integral part of this document. Please note that all NPAAC documents can be accessed at Department of Health.

While this document is for use in the accreditation process, comments from users would be appreciated and can be directed to:


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# 1. Risk Management

**(Refer to Standard 3 in *Requirements for Medical Pathology Services*)**

**S1.1** **The designated person must ensure the laboratory has a documented procedure for the introduction of SCVS for HPV testing.**

**S1.2** **The procedure and results of the validation/verification for SCVS must be approved and authorised as fit for purpose by the designated person.**

**S1.3** **Validation by the Applicant laboratory of the use of SCVS for HPV testing must include statistical correlation against the Comparator laboratory.**

**S1.4** **Laboratories must address the following risk points for testing SCVS:**

- **(a) Specimen integrity: Processes that address common pre-analytical issues and promote safe and timely testing.**
- **(b) Specimen traceability: Processes that enable the traceability of specimens and promote the safety and timeliness of testing.**
- **(c) Specimen analysis: Processes that address failures within the analytical phase of testing.**
- **(d) Quality Control: Processes to monitor assay performance and record corrective actions; and internal audits with a focus on the modified analytical processes (see S6.1)**
- **(e) Quality Assurance: Participation in Quality Assurance Programs, performance reviews and corrective actions where performance is unsatisfactory.**

**S1.5** **Laboratories must have documented procedures for reporting adverse event to the designated person within the laboratory and the TGA.**

# 2. General Requirements

**(Refer to Standard 3 and Standard 4 in *Requirements for Medical Pathology Services*)**

**S2.1 An Applicant laboratory for the purposes of validating the use of SCVS must use the same platform/assay and collection device as the Comparator laboratory.**

C2.1 Specimen stability for SCVS is also required to be validated and should not exceed the transport and handling conditions already validated by the Comparator laboratory.

**S2.2 Once validated to this Requirement, SCVS must only be used in the laboratory that has gained accreditation for this use, or its own Medical Laboratory Network.**

C2.2(i) Individual laboratories within a laboratory network **must** as a minimum verify the analytical performance prior to implementation in the laboratory. Verification **must** be demonstrated through use of the validation assessment material (refer to ***Appendix A)***.

C2.2(ii) The use of SCVS **must** be restricted to use with the particular HPV test platform/assay that the accreditation was assessed against.

**S2.3 Any modifications to the self-collect device must be re-validated according to this Requirement.**

C2.3 For example any variations that a laboratory makes to an in-house IVD, such as a change in swab type or methodology must be re-validated.

# 3. Validation Performance Requirements

### (Refer to Standard 8B in Requirements for Medical Pathology Services)

In accordance with the Standard, *Requirements for the Development and Use of In-House In Vitro Diagnostic Medical Devices (IVDs)*, where a commercially supplied IVD has been modified, validation should focus principally on the effects of that change and demonstrate that the assay continues to perform safely and effectively. For the use of SCVS, laboratories are required to validate that the assay continues to perform effectively at, or near to, the performance characteristics and limit of detection for the HPV assay being used.

**S3.1 The clinical performance of the Applicant laboratory using SCVS for HPV testing must be established by comparison with that of a Comparator laboratory using the Comparator laboratory's collection device and test platform/assay.**

C3.1(i) Applicant laboratories **must** follow the procedure outlined in Appendix B and **must** achieve the level of concordance defined therein.

C3.1(ii) The laboratory **must** produce a method validation report that shows successful completion of the studies.

**S3.2 The accuracy, limit of detection and robustness must be determined by the use of quantified reference materials. Refer to Appendix A.**

C3.2 The applicant laboratory **must** include an assessment of the analytical performance parameters in a method validation report for submission together with clinical performance data derived in **S3.1** of this standard for consideration in accrediting the use of the SCVS with the HPV assay.

**S3.3 There must be a documented investigation to detect possible interference in the performance of the assay from the most likely interfering substance(s), listed in Appendix A.**

C3.3(i) The laboratory **must** be able to show that at least the most likely interfering substances have been reassessed as a source of error when using the SCVS with the HPV assay.

C3.3 (ii) Any increase in interference observed with the use of SCVS as compared to the interference defined in the instructions for use (IFU) provided with the HPV assay **must** be documented.

**S3.4 A successful Applicant Laboratory must not be a Comparator Laboratory for another laboratory to validate their SCVS validation.**

C3.4 An Applicant laboratory who initially validates the use of SCVS by comparison to a Comparator laboratory is unable to subsequently serve as a Comparator laboratory itself unless the use of SCVS is further locally validated by performing a direct comparison of the SCVS against health care worker collected cervical specimens.

# 4. Stability

**(Refer to Standard 8A in Requirements for Medical Pathology Services)**

**S4.1 The stability of the SCVS must be determined under the collection and transport conditions used by the Applicant laboratory.**

C4.1(i) For an Applicant laboratory to extend the conditions for the transport and handling of SCVS beyond that of the Comparator laboratory (refer to **S2.3**), the laboratory **must** demonstrate the stability of the SCVS under known or determined transport conditions in respect to maximum extremes of time, temperature and humidity. This **must** be done using further validation in accordance with ***Appendix C***.

C4.1(ii) A laboratory that has been accredited to perform SCVS HPV testing, may extend the conditions of specimen handling beyond the original accreditation. This would require a separate assessment of stability using quantified reference material to this Requirement. (refer to ***Appendix C***).

**S4.2 The laboratory must document maximum acceptable duration and environmental conditions for specimen handling from the time the specimen is collected until the specimen arrives in the laboratory and is resuspended in the validated media.**

C4.2 The laboratory **must** make this information about these limitations available to the referring practitioner.

# 5. Monitoring

**(Refer to Standard 5 and Standard 8C in *Requirements for Medical Pathology Services*)**

**S5.1 The on-going performance of the assay/instrument combination using the proposed modifications for self-collected specimens must be monitored through the use of relevant quality measures.**

**S5.2 Where available, laboratories must participate in an external quality assurance program for HPV which includes the use of material representative of self-collected specimens[§] and investigate all discordances and document corrective actions.**

[§] *Requirements for Laboratories Reporting Tests for the National Cervical Screening Program (First Edition 2017)*

# 6. Performance Measures

**(Refer to Standard 8B in Requirements for Medical Pathology Services)**

**S6.1 Laboratories must monitor HPV positivity rates and the invalid rates from self-collected specimens.**

# 7. Adverse Event Reporting

**(Refer to Standard and Standard 8C in Requirements for Medical Pathology Services)**

**S7.1 Information relating to an adverse event resulting from the use, or testing, of a self-collected specimen must be reported to the laboratory's designated person and to the Therapeutic Goods Administration.**[**]

[**] Standard 10 of the *Requirements for the Use and Development of In-House In Vitro Diagnostic Medical Devices (Third Edition 2014)*

# Appendix A Use of Validation Assessment Material to Verify Analytical Performance (Normative)

This Appendix describes the minimum testing process that laboratories **must** undertake to evaluate analytical performance with simulated SCVS.

The laboratory procedures described in Parts 1 and 2 **must** be followed and the results **must** be satisfactory in order to be considered to have verified analytical performance using SCVS. Threshold detection can be determined by the repeat analysis of specimens with results near the LOD.

**1. Limit of detection performance assessment**

a. Prepare an inoculum of cells infected by HPV at a concentration which when sampled with the swab and then delivered by the swab device into diluent would represent a final concentration approximating 100x the IVD assay stated LOD in the volume of diluent processed for nucleic acid extraction. Testing of this resuspension **must** produce a HPV Detected result.

b. Dilute the prepared inoculum solution in (a) above tenfold. Sample from this new dilution using the swab and then perform a resuspension into diluent. This resuspension should represent a final concentration approximating 10x the IVD assay stated LOD in the volume of diluent (collection medium) processed for nucleic acid extraction. Testing of this resuspension **must** produce a HPV Detected result in at least 95% of replicates. This testing **must** be performed in replicate.

c. Dilute the prepared inoculum solution in (a) above by 100 fold. Sample from this new dilution using the swab and then perform a resuspension into diluent. This resuspension should represent a final concentration approximating the IVD assay stated LOD in the volume of diluent processed for nucleic acid extraction. Testing of this resuspension may produce a HPV Detected result potentially in >90% of test replicates, however it may produce an invalid result in a percentage of testing events when no HPV is detected due to the absence of internal control materials. This testing **must** be performed in replicate.

**2. Accuracy, specificity, interference and robustness assessment must include the following as a minimum and in replicate if indicated**

a. The swab device should be inoculated with an appropriate amount of the expected internal in-situ derived control material for the assay e.g. human cellular material which could be sourced from commercial cell lines known to not harbour HPV. The swab is then resuspended in the proposed diluent and tested with the assay/platform. Testing should indicate successful detection of the in-situ control and **must** not show any detected result for HPV.

b. Three swab devices are independently inoculated with a different type of non-type 16 or 18 HPV each (i.e. excluding HPV16 or HPV18). The three swabs are then resuspended individually in proposed diluent and each of these three preparations tested with the assay/platform. The testing **must** indicate that these three other HPV types are detectable.

c. To determine the ability for the swab device to carry sufficient HPV in a matrix of other flora and to assess the modified SCVS testing process for elements of interference the following **must** be performed:

i. Prepare a 1mL solution of modified diluent that represents HPV16 (or HPV18) target at a concentration approximately 10000x the stated LOD of the assay in a background of *Neisseria gonorrhoeae* at a concentration of at least $1x10^6$ cells/mL. *N. gonorrhoeae* can be sourced from commercial or locally held bacterial culture collections. A 0.5 McFarland equivalent solution by turbidometry contains a cell density of ~$1.5x10^8$ cells / mL.

ii. Inoculate a swab in this preparation, then resuspend the swab in a new tube of 5mL diluent. The resultant HPV concentration is approximately 40x LOD HPV for swab tips that carry/transfer approximately 20uL by their construction type e.g. a flocked large tip.

iii. Adjust volumes appropriately for a swab device that delivers different volumes from collection into resuspension. Determining the carrying capacity of a swab tip can be achieved by weighing a swab pre and post inoculation with the proposed diluent and determining the differential in weight.

iv. Perform testing of this final resuspension with the assay/platform. The testing **must** produce a HPV Detected result.

d. The following testing **must** be performed to determine the ability of the swab device to carry multiple HPV types from the one collection and to also evaluate the impact of the SCVS modification on the multi-analyte detection of the IVD assay. The testing required is also to assess robustness of the modified processes across operators, reagent lots and where possible multiple instruments. Prepare a solution of 100 µmL of HPV16+HPV18+another HPV type at equal concentrations with all at least 10000x LOD in the proposed diluent. Each of the three HPV types **must** be detected in each of the test events.

e. To assess if the proposed SCVS modification has induced any unexpected changes to the IVD manufacturers' claims for interfering substances, the following testing **must** be performed. Prepare a solution of diluent for testing that represents HPV at 10x the stated LOD of the assay in a matrix containing a known inhibitory substance for the assay, the suggestion is to use whole blood. The amount of inhibitor (e.g. whole blood) in the prepared solution should be adjusted to account for the expected alterations of the modified method. For example, if the proposed SCVS modification resuspends collected material into 5mL, this represents a potential 4 fold increase in the concentration of potential interfering material compared to the normal processing when transferred into a 20mL PreservCyt vial. Examples of known interfering substances are listed in assay manufacturer pack inserts. In this example the prepared diluent would thus need to contain ¼ of the level stated by the IVD package insert to represent a corrected equivalent of the interfering substance. Note that no swab is prepared from this tube and it is to be tested directly in the assay. The HPV **must** remain detectable when this preparation is tested, indicating that the effects of the interfering substance was not exacerbated in any greater amount than the proportional expectations introduced by the in-house assay modification to use the SCVS.

# Appendix B Evaluation of Performance of testing of SCVS by Applicant Laboratories with Clinical Specimens (Normative)

This Appendix describes the process that laboratories **must** undertake to evaluate the performance of the Applicant laboratory against that of the Comparator laboratory when testing actual patient collected samples for HPV.

During the evaluation the Comparator laboratory will be responsible for reporting patient results. Patient results should not be reported by the comparator laboratory until after it has received the result from the applicant laboratory.

1. Clinical specimens for validation of the use of SCVS should be obtained from patients who present for screening, or follow-up investigation, under the NCSP. The swabs used **must** be the same as those for which the Comparator laboratory is accredited.
2. Patients **must** be asked to provide two SCVS specimens using two swabs that is the same as the comparator laboratory. The first specimen will be designated SCVS1 and the second specimen will be designated SCVS2.
3. Sample SCVS1 **must** be forwarded to the Comparator Laboratory for testing. SCVS2 is to be tested by the Applicant laboratory.
4. The maximum extremes of time, temperature and humidity for specimen collection, transport and receipt in the Applicant laboratory for testing **must not** exceed that for which the Comparator laboratory is accredited.
5. Both SCVS1 and SCVS2 **must** be tested using the platform/assay for which the Comparator laboratory is accredited.
6. The Comparator laboratory will provide the SCVS1 testing results to the applicant laboratory. The Applicant laboratory will provide the SCVS2 testing results to the Comparator laboratory.
7. There **must** be at least 30 satisfactory paired samples where the Comparator laboratory has a positive result.

   The National Benchmark HPV Positivity Rate is 8.9%. This rate has been calculated using NCSR data collected from 1 February 2017 to 30 May 2018 using screening specimens only for women aged ≥ 25 years at the time of testing.[††] Based on a HPV positivity rate of 8.9% laboratories may need to test up to 300 specimens in the community setting to achieve at least 30 HPV positive specimens. Higher prevalence settings, such as colposcopy, may not need higher number of samples.
8. Results **must** be tabulated by the Applicant laboratory as follows:

   Data from specimen pairs are to be included only if both SCVS1 and SCVS2 yield satisfactory results (i.e., invalid results should not be included in the table but the total number of samples yielding an invalid result should be separately noted).

[††] NCSR HPV Positivity Rates

**Table 1: Overall HPV detection Rates**

| Comparator | Applicant | | |
|---|---|---|---|
| | HPV negative | HPV positive | Total |
| HPV negative | A | b | a+b |
| HPV positive | C | d (**must** be a minimum of 30) | c+d |
| Total | a+c | b+d | N (=a+b+c+d) |

9. Statistical analysis of the results **must** be performed. Statistical analysis of the results should be limited to the analysis of a binary outcome (i.e. HPV positive/negative) with Cohen's kappa used to determine the degree of agreement. A Kappa value of >0.8 (80% with lower limit of 95% CI) would indicate acceptable agreement provided there is also a negative percent agreement of >90%.

   Discrepant results **must** be reviewed and subject to review outcomes that a maximum of 3 false negatives is acceptable.

   For the purposes of the diagnostic result, the Comparator laboratory's result is that which is reported to the patient.

   The Applicant laboratory **must** review significant discrepant results, where unexpected negative results are obtained or where positive specimens have a lower than expected low Ct value when compared to results from the Comparator laboratory is negative. This does not apply to positive results that have a low Ct value that approaches the positive/negative cut-off of the assay.

10. Laboratories **must** monitor their invalid rate for SCVS specimens.

# Appendix C Evaluation of Stability (Normative)

This Appendix describes the minimum process that laboratories **must** undertake to evaluate specimen stability for SCVS.

There are two distinct considerations around 'Stability testing' as it applies to the processes for validating a SCVS and the chosen swab re-suspension method. Both positive and negative specimens **must** be used in the stability testing evaluation.

The laboratory **must** run a performance validation of SCVS for the known transport conditions from specimen collection prior to resuspension and testing in the laboratory. The assessment **must** include exposure studies to the known (or determined) extremes of transport conditions in respect of time, temperature and humidity. This may require recourse to data loggers or recorded temperatures to define the range required to be validated as per 5.2.2 of ISO23640:2011. The level of validation required will depend on the specimen transport and handling conditions being used and the risks to specimen integrity.

Thresholds for detection can be determined by the repeat analysis of samples with results near the LOD.

The stability evaluation **must** comprise the following:

- Prepare a minimum of ten swabs from a positive suspension of HPV such that the swabs would effectively deliver HPV at a level ten times the claimed LOD into the test volume sampled for nucleic acid extraction. The number of swabs needed will depend on the number of time points being evaluated. There should be replicate samples used to evaluate each time point to obtain a statistically valid result.
- On the day of preparation, resuspend one swab using the laboratory's suspension protocol and perform testing. The Ct values for HPV ($t^1$) and the cellularity control ($t^2$) for this swab will reflect the reference values ($t^1$ and $t^2$ =0).
- Store/incubate the remaining prepared swabs at a temperature above the known highest temperature that the SCVS will be exposed to in the collection and transport processes.
- At time intervals that are relevant to the known transport times to the laboratory (e.g. each day for nine days, every second day for 18 days) remove a number of replicate swabs, resuspend using the laboratory's resuspension protocol and test.
- Prepare a plot with the Ct values for HPV and the cellularity control as Y axis against incubation time in hours/days on the X axis.
- Using an allowable drift limit value on the Y axis that represents the Ct described for the HPV LOD of the test system, determine the point where the upper 95% CI of the plot line intercepts the upper drift limit Ct value. The elapsed time on the X axis at this point describes the stability duration for a sample containing the amount of HPV in the positive suspension for the storage/transport simulation utilised.
- If the plot slope is not nearing the LOD limit to determine an actual stability duration, then the assessment shows that the transport conditions for the SCVS are acceptable and the tolerances of the in-house assay performance exceed any effects of the expected storage/transport conditions.

- If the plot slope does meet the LOD limit prior to the maximum known or determined storage/transport conditions, then this time point (hours/days at X degrees C) is determined to be the maximum acceptable storage/transport conditions for SCVS.

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