January 2023, compiled by:

**Professor Heather Cubie, PhD**  
University of Edinburgh, Scotland  
Senior Advisor, Global Health Academy, Honorary

**MD Marlieke de Fouw, Msc, finalizing PhD**  
Leiden University Medical Center, Netherlands  
Registrar Gynecology and Obstetrics  
Supervisor and technical advisor Female Cancer Foundation  
[info@femalecancerfoundation.org](mailto:info@femalecancerfoundation.org)

### SOP 1  
STANDARD OPERATING PROCEDURE FOR GENEXPERT ANALYSIS

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INTRODUCTION AND SCOPE OF THE SOP

This SOP is to guide the user on how to analyse samples for Human papillomavirus, using the GeneXpert Instrument in ...add country... This SOP is primarily intended for laboratory workers, but it can benefit anyone working in this field.

This practical guide is a part of Prevention and Screening Innovation Project Toward Elimination of Cervical Cancer (PRESHRIP-TEC), which aims to build on and scale up existing screening programs with women-friendly and cost-effective tools, as well as to test whether and why they are effective in increasing cervical cancer screening participation in accessible, affordable, and equitable ways.

This SOP is based on testing with Cepheid Xpert HPV carried out in Nkhoma Hospital Laboratory, Central Malawi between 2013-2017.

While it provides a comprehensive overview of typical practical challenges and solutions linked to GeneXpert analysis, it is not exhaustive.
KEYWORDS

Standard operating procedure, HPV, HPV test, hrHPV test, Screening, sample management, sample collection, sample storage, health care worker, community health worker

ABBREVIATIONS AND ACRONYMS

ASC-US – atypical squamous cells of undetermined significance
DNA – Deoxyribonucleic acid
EMR – Electronic medical record
HIV – Human immunodeficiency virus
HR – High-risk
hrHPV – High-risk human papillomavirus
LEEP – Loop electrosurgical excision procedure
NEG – negative
PCR – Polymerase chain reaction
POS – positive
PRESCRIPT-TEC – Prevention and Screening Innovation Project Toward Elimination of Cervical Cancer
SOP – Standard operating procedure

BACKGROUND

HPV and cervical cancer

Continuing infection with high risk human papillomavirus (hrHPV) is the main cause of cervical cancer and is a precursor to cervical intraepithelial neoplasia (CIN). HPV presence has been implicated in more than 99% of cervical cancers worldwide. HPV is a small, non-enveloped, double-stranded DNA virus, with a genome of approximately 8000 nucleotides. There are more than 118 types of HPV and a subset of approximately 14 of these types is considered high-risk for the development of cervical cancer and its precursor lesions.

PRINCIPLE

Testing platform - the GeneXpert HPV Assay

The GeneXpert HPV Assay is an automated test for qualitative detection and differentiation of HPV DNA. The assay is performed on the Cepheid GeneXpert Instrument System.

The GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for running tests on collected samples and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process.
Because the cartridges are self-contained, cross-contamination between cartridges during the testing process is minimized.

The GenXpert HPV assay includes reagents for the 5' exonuclease real-time PCR detection of HPV. Reagents for the detection of a Sample Adequacy Control (SAC) and a Probe Check Control (PCC) are also included in the cartridge. The SAC reagents detect the presence of a single copy human gene and monitor whether negative samples contains human DNA.

The samples are briefly mixed by inversion 8 to 10 times. Using the supplied transfer pipette, the sample is pipette into the GeneXpert HPV cartridge. The GenXpert cartridge is loaded onto the GenXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time PCR for detection of DNA. Summary of detailed test results are obtained in approximately 60 minutes and are displayed in tabular and graphic formats.

SAMPLE COLLECTION

HPV self-sampling
Samples will be collected by self-sampling using the Floqswab. Collect cervical specimens according to the manufacturer's instructions, see SOP HPV self-sampling.
In the laboratory the sample will be emersed in Mswab Medium tubes of 3 ml, see SOP 3 ‘Receipt of HPV samples and recording test results at the laboratory’.

REAGENTS AND INSTRUMENTS

- Primary samples; dry Floqswabs
- Mswab Medium 3ml tubes
- GeneXpert instrument, computer, barcode reader, and operator manual
- GeneXpert HPV Assay cartridges with integrated reaction tubes
- Disposable gloves and laboratory coat
- Permanent marking pen
- Transfer pipettes for sample processing

PROCEDURE HPV sampling

Before starting
Before starting have the following available:
- freshly made 0.5% chlorine solution to decontaminate and clean spills as they happen (as per decontamination procedure below)
- container for decontamination of used transfer pipettes and other items

Preparing the cartridge
1. Before starting, obtain the following items:
   - GeneXpert HPV Assay cartridge
   - Transfer pipette (provided)
   - Appropriately collected and labeled test sample
2. Inspect the test cartridge for damage. If damaged, do not use.
3. Gently invert the sample vial 8 to 10 times to ensure adequate mixing of the sample.
4. Unwrap the transfer pipette.
5. Open sample container, fill transfer pipette to fill line with sample. (Ensure there are no air bubbles present).
6. Open cartridge lid.
7. Release the pipette's contents gently into the sample chamber of the cartridge, as indicated in the picture below.

8. Close the cartridge lid firmly.

STARTING THE TEST

HPV assay
1. Turn on the GeneXpert instrument. Note: First turn on the GeneXpert instrument, and then turn on the computer. The GeneXpert software will launch automatically.
2. Log on to the GeneXpert System software. Type your user name and password.
3. In the GeneXpert Dx System window, click Create Test.
4. Scan the barcode on the Xpert HPV cartridge. The Create Test window appears. (Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.)
5. In the Patient ID box: Type Patient Name
   In the Sample ID box: Type the Patient Laboratory Number
   (ensuring HPV is always written at the front of number e.g. HPV0002).
6. Click Start Test. Enter your password when requested.
7. Open the instrument module door with the blinking green light and load the cartridge.
   Important: Be sure to load the cartridge into the GeneXpert instrument and start the test within 4hrs of preparing the cartridge.
8. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
9. Complete HPV patient reports (as much as possible)
10. Complete HPV Result Form for lab records (as much as possible)
11. Look at the progress of the test approximately 10 - 20 minutes after starting, to ensure an error or invalid test hasn't occurred.
   - If an invalid test or error has occurred ensure there is enough sample to repeat the test, if so, repeat sample using same Sample ID number as used with initial sample.
   - Document invalid test on HPV Result Form

12. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.

13. Dispose of used cartridges as infectious waste.

Note:
Do not wear gloves while using the computer. This is because of limited supply of gloves which should be worn when handling samples, not when using a computer. In Covid times, this instruction may be to change gloves depending on local policies.

Be sure not to transfer infectious substances from sample to computer, e.g. from gloves or spills.

RESULT INTERPRETATION

The results are interpreted by the GeneXpert Instrument System from measured fluorescence signals and embedded calculation algorithms and will be shown on the Test Results tab of the View Results window.

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>HPV16 POS</td>
<td>HPV16 target DNA sequences are positive.\n  - PCR amplification of the HPV 16 target give Cts within the valid range and fluorescence endpoints above the minimum setting.\n  - SAC: Not applicable. The SAC is ignored because HPV target amplification can compete with this control. PCC: PASS; all probe check results pass</td>
</tr>
<tr>
<td>HPV18_45 POS</td>
<td>HPV18_45 target DNA sequences are positive\n  - PCR amplification of the HPV18_45 target gives Cts within the valid range and fluorescence endpoint above the minimum setting\n  - SAC: Not applicable. The SAC is ignored because Other HR HPV target amplification can compete with this control. PCC: PASS; all probe check results pass</td>
</tr>
<tr>
<td>Other HR HPV POS</td>
<td>Other HR HPV target DNA sequences are positive</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------------</td>
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<tr>
<td></td>
<td>• PCR amplification of the Other HR HPV target gives a Ct within the valid range and a fluorescence endpoint above the minimum setting.</td>
</tr>
<tr>
<td></td>
<td>• SAC: Not applicable. The SAC is ignored because Other HR HPV target amplification can compete with this control.</td>
</tr>
<tr>
<td></td>
<td>• PCC: PASS; all probe check results pass.</td>
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<tr>
<th>HPV NEG</th>
<th>HPV target DNA sequences are negative</th>
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<tr>
<td></td>
<td>• HPV absent or below the assay detection level</td>
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<tr>
<td></td>
<td>• SAC: PASS; PCR amplification of the SAC target gives a Ct within the valid range and a fluorescence endpoint above the minimum setting</td>
</tr>
<tr>
<td></td>
<td>• PCC: PASS; all probe check results pass</td>
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<tr>
<th>ERROR</th>
<th>Presence or absence of HPV target DNA cannot be determined.</th>
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<td></td>
<td>• Retest the procedure SAC: NO RESULT</td>
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<tr>
<td></td>
<td>• PCC: FAIL* all or one of the probe check results fail. The PCC probably failed because the reaction tube was filled improperly or a probe integrity problem was detected.</td>
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<table>
<thead>
<tr>
<th>NO RESULT</th>
<th>Presence or absence of HPV target DNA cannot be determined. Use the instructions in the Retest Procedure section to repeat the test.</th>
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<tr>
<td></td>
<td>Insufficient data was collected to produce a test result (for example the operator stopped a test that was in progress).</td>
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<tr>
<td></td>
<td>SAC: NO RESULT PCC: Not applicable</td>
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<th>INVALID</th>
<th>Presence or absence of HPV target DNA cannot be determined. Repeat the test.</th>
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<tbody>
<tr>
<td></td>
<td>• SAC: FAIL; SAC target result is negative. The SAC Ct is not within valid range and fluorescence endpoint is below the minimum setting</td>
</tr>
<tr>
<td></td>
<td>• PCC: PASS; all probe check results pass</td>
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**Reasons to repeat the test**

Repeat the test using a new cartridge if one of the following test results occurs:

- An **INVALID** result indicates that the SAC failed. The sample was not properly processed, or PCR was inhibited, or sample was inadequate.

  **Important:** An inadequate sample is the most common error and usually reflects a sample with few cells, inadequate mixing before transfer to cartridge or forgetting to add sample to cartridge.

- An **ERROR** result indicates the assay was aborted, possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, pressure limits were exceeded, a probe check failed, or a valve positioning error was detected.
• A NO RESULT indicates that insufficient data was collected. For example, the operator stopped a test that was in progress.

QUALITY ASSURANCE PROCEDURES

Quality control within HPV assay
Each test includes a Sample Adequacy Control (SAC) and a Probe Check (PCC):
  • **Probe Check Control (PCC):** Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.
  • **Sample Adequacy Controls (SAC):** the SAC reagents detect the presence of a single copy human gene and monitor whether the sample contains human DNA.

Positive and negative controls
Positive and negative controls for internal quality assessment are to be run consistently after every 100 patient samples or once per week. These are created from within laboratory resources. For production and storage of control samples, see SOP 4 ’Quality assurance’

SAFETY PRECAUTIONS
  • Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents.
  • Wear protective disposable gloves and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.
  • When processing more than one sample at a time, open only one cartridge, add the Sample Reagent-treated sample (or decontaminated, liquefied sample), and close the cartridge before adding Sample Reagent-treated sample to the next cartridge.

  **Note:** Very important not to have a row of cartridges waiting to be closed all at once.

  • Do not open the Xpert HPV cartridge lid except when adding the treated sample.
  • Do not use a cartridge that has been dropped or shaken after you have added the treated sample.
  • Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.
  • Do not use a cartridge that has a damaged reaction tube.
  • Each Xpert HPV cartridge is used to process one test. Do not reuse processed cartridges.
  • Used cartridges, should be treated as capable of transmitting infectious agents. Dispose used cartridges as hazardous Infectious waste.
  • Do not attempt to clean inside the module chamber. The mechanism is very sensitive. If a module ceases to work, contact Cepheid and follow their instructions.

Decontamination procedure HPV spills
• If sample spills occur decontaminate the spill and immediate area with 0.5% chlorine, leave for 10 minutes.
• Wipe spills after decontamination with an absorbent cloth to remove all chlorine and decontaminated substances.
• Soak (the above used) cloth in 0.5% chlorine to ensure all infectious material has been thoroughly decontaminated.
• With a new absorbent cloth wipe area thoroughly with excess water to get rid of all traces of chlorine. Rinse cloth with water and again wipe the area thoroughly with water.

STORAGE AND HANDLING

• Store the Xpert HPV cartridges and reagents at 2 to 28 °C.
• Do not use reagents or cartridges that have passed the expiration date.
• Do not open a cartridge lid until you are ready to perform testing.
• Process the cartridge within 4 hours after adding the sample to the cartridge.
• The cartridges are stable up to 2 weeks at 2 to 48°C after opening the pouch.

CONCLUSIONS

The standard operating procedure was created as a guide of recommendations for personnel involved in the PRESCRIP-TEC project's cervical cancer screening. The goal of this document is to eliminate possible mistakes and ensure that their tasks are completed without incident. Workers who follow written instructions can easily and confidently perform consistent work with the necessary skills and knowledge.

SOURCES


From the project PRESCRIP-TEC: (2021) Data Management Plan.


## DOCUMENT INFORMATION

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