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DNA methylation analysis on self-samples for cervical cancer detection

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Registration date 20/04/2026	**Overall study status** Ongoing	
Last Edited 20/04/2026	**Condition category** Cancer	

Plain English summary of protocol
Not provided at time of registration

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Study information

Scientific Title
Validation of DNA methylation analysis on self-collected vaginal specimens collected with Floqswab for cervical cancer detection

Study objectives
The primary objective of the study is to evaluate the clinical performance (i.e., sensitivity and specificity) for CIN3+ of the PreCursor-M Gold assay on residual clinician-taken and self-collected vaginal specimens from women attending an outpatient clinic for routine cervical examination by demonstrating equivalence against a reference method (i.e., PreCursor-M+) tested under the same conditions.

Ethics approval required
Ethics approval required

Ethics approval(s)
Approved 09/02/2026, Ministry of Health of Republic of Uzbekistan Ethics Committee (45 Oybek Street, Tashkent, 100015, Uzbekistan; +998 (0)71 256-37-38; info@ssv.uz), ref: 02-28/3472

Primary study design
Observational

Secondary study design
Observational retrospective multi-center equivalence study

Study type(s)

Health condition(s) or problem(s) studied
Cervical (pre)cancer

Interventions
At least 70 high-risk HPV+ women with CIN3+ and 70 high-risk HPV+ women without evidence of disease (or at maximum CIN1; i.e. ≤CIN1) are required (n = 140 in total). HPV detection is not routinely performed at Tashkent. Therefore the colposcopic impression of women will be used to determine if women are eligible for study inclusion. Eligible women are asked to return for a second visit 2 weeks later to perform the sampling for the study. Eligible are women with a colposcopic impression of CIN3+ and women with a colposcopic impression that is normal or CIN1 (i.e., ≤CIN1). At the second visit of the eligible women both a vaginal self-sample collected with the Floqswab device and a physician-taken cervical smear are collected for high-risk HPV and methylation analysis. After this the women will receive their required diagnostic follow-up according to local guidelines and procedures. At the first visit the eligible women will not undergo biopsy, LEEP or any other interventional procedures as this interferes with the sampling after 2 weeks. Because high-risk HPV and methylation testing will be performed at

Amsterdam University medical center (AUMC, Amsterdam, The Netherlands) the final sample selection for inclusion in the performance study is performed retrospectively based on HPV positivity with the clinically validated QIAscreen HPV PCR Test (Self-screen B.V., The Netherlands). It is estimated that approximately 250 women need to participate to obtain the 140 high-risk HPV-positive women.

First visit:
At first visit colposcopy with acetic acid and Lugol solution is performed (according to routine procedures). When the colposcopic impression of the cervix is CIN3+ or normal or CIN0/1 then women are eligible for study inclusion and are asked to return 2 weeks later for a second visit. These eligible women do not have interventional procedures (i.e., biopsy, LEEP, LLETZ, etc) at the first visit. Eligible women get a unique study ID number that is used for the rest of the study for traceability.

Second visit:
Before colposcopy first the Floqswab self-sample is taken, followed by the clinician-taken cervical smear. The sampling order is as follows:

Self-sampling:
The woman first takes a vaginal self-sample with the Floqswab self-sampling device and the sample is put in the collection tube (without collection media) according to the instructions of the manufacturer. The self-sample is given to the clinician who labels the tube with the study ID. The self-sample is transferred to the lab where it is put in a collection tube holding 5 ml of MSwab medium, the stick of the device is broken at the indicated red mark and the tube is closed. After labelling the tube with the study ID the sample is stored at -20°C until shipment to AUMC.

Clinician-taken cervical smear:
The clinician takes a cervical scrape of the cervix with the routinely used cytobrush. The cytobrush is put into the vial with the Yes Path medium, the stick is removed from the white brush part, releasing the brush into the vial. The vial with the brush is closed and labelled with the study ID and stored at 4°C until shipment to AUMC.

Colposcopy:
The clinician performs colposcopy by routinely used diagnostic procedures (including biopsy /LEEP and use of acetic acid or Lumol when necessary).
These clinician-taken and self-collected samples will be shipped to AUMC for testing for high-risk HPV and methylation within 6 months after collection to assure normal conditions of use of the high-risk HPV and methylation assays. The storage conditions are within the stability claims of the clinician-taken samples and self-samples in MSwab medium. Currently these are 6 months at 2-30°C and 30 months at 2-8°C, being 60 days at -20°C. Prior to the start of the study the storage period for stability can be updated to match that of the study samples.

Study procedures:
The QIAscreen HPV PCR Test and PreCursor-M Gold assay will be performed on the clinician-taken and self-collected sample material according to the instruction for use of the manufacturer (Self-screen B.V.).

Sample:
1. A minimum of 500 µl of the original clinician-collected sample in Yes Path is aliquoted to a new barcode-labelled tube for DNA extraction for high-risk HPV and PreCursor-M Gold analysis.
2. A minimum of 200 µl of the original self-collected sample in MSwab is aliquoted to a new

barcode-labelled tube for DNA extraction for the QIAscreen HPV PCR Test and PreCursor-M Gold analysis.
3. DNA extraction is performed with the QIAamp DNA mini kit according to the IFU and DNA is eluted in 100 µl elution buffer.
4. QIAscreen HPV PCR Test assay will be performed according to the instructions for use. The QIAscreen HPV PCR Test uses 5 µL DNA input in the PCR.
5. The PreCursor-M Gold will be performed according to the instructions for use. 200 ng DNA is first modified using bisulfite conversion with the EZ DNA Methylation Lightning kit (ZYMO Research, USA). The PreCursor-M Gold uses 5 µl input of bisulfite-converted DNA (equivalent to 50 ng inut).
6. Laboratory personnel has been trained on the QIAscreen HPV PCR Test and PreCursor-M Gold. Only laboratory personnel who have been trained can perform the testing.
7. Laboratory testing will be blinded from HPV and clinical information.

The reference method for methylation testing, i.e., PreCursor-M+, will be performed on the same bisulfite-converted DNA sample material as the PreCursor-M Gold has been performed. The PreCursor-M+ will be performed according to the instructions for use of the manufacturer (Self-screen B.V.) using 50 ng bisulfite-converted DNA as input for direct comparison.

Test result interpretation:
Interpretation of QIAscreen HPV PCR test, PreCursor-M+ and PreCursor-M Gold test results will be performed according to their IFU to score a sample positive, negative or invalid for the test. All three assays include an internal sample control with a predefined Ct threshold below which the sample is considered valid for testing. The PreCursor-M Gold result is calculated as a predicted probability value for the ΔΔCt ratios of the targets LHX8 and ASCL1, ranging between 0 and 1. Clinical thresholds for scoring a sample hypermethylation-positive are indicated in the IFU for both cervical scrapes and self-collected samples. The PreCursor-M+ (reference test) result is determined as the ΔΔCt value for both FAM19A4 and miR124-2. The clinical thresholds for each marker are indicated in the IFU for both cervical scrapes and self-collected samples. When one or both markers are below their clinical thresholds a sample is considered hypermethylation-positive. The QIAscreen HPV PCR test result is determined as the combined result for HPV16, HPV18 and other HPV (cumulative signal of 13 other high-risk HPV types). Thresholds for HPV positivity are indicated in the IFU.

Intervention Type
Genetic

Primary outcome(s)
1. Cervical (pre)cancer measured using DNA hypermethylation of promoter regions of ASCL1 and LHX8 measured in self-collected vaginal specimens at study intake

Key secondary outcome(s)

Completion date
01/01/2028

Eligibility

Key inclusion criteria
1. All women aged 25 years or older attending the Waylon women’s health clinic for their routine colposcopic examination of the cervix

2. Women with a colposcopic impression of CIN3+
3. Women with a colposcopic impression that is normal or CIN1
4. Women who consented to participate in the study

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
18 Years

Upper age limit
80 Years

Sex
Female

Total final enrolment
0

Key exclusion criteria
1. Women with insufficient sample volume of clinician-taken and/or self-collected specimen for testing for high-risk HPV and methylation
2. Women who test high-risk HPV negative on their clinician-taken sample
3. Women who objected to the use of their personal data and/or sample material before, during or after the study

Date of first enrolment
01/03/2026

Date of final enrolment
01/03/2027

Locations

Countries of recruitment
Uzbekistan

Sponsor information

Organisation
Self-screen B.V.

Funder(s)

Funder type

Funder Name
Investigator initiated and funded

Results and Publications

Individual participant data (IPD) sharing plan

IPD sharing plan summary
Not expected to be made available