Product Profile

# QIAsure Methylation Test

## Identify HPV-positive women at high risk of cervical cancer who need immediate treatment

The QIAsure Methylation Test provides a real-time methylation-specific PCR (qMSP)-based analysis of promoter hypermethylation for the tumor suppressor genes *FAM19A4* and *hsa-mir124-2* in bisulfite-treated DNA of cervical or vaginal specimens. Hypermethylation of these genes, indicated by a positive test result, may correlate with the presence of cancerous cells and advanced transforming cervical intraepithelial neoplasia (CIN), which are lesions with a high short-term progression risk (1–2).

QIAsure Methylation Test can help to detect cervical carcinoma and advanced transforming CIN to objectively discern passive HPV infections from ones that need immediate attention.

- 100% sensitivity in identifying cervical cancer in high-risk HPV-positive samples (1)
- 67% sensitivity for CIN 3* (Table 1a) and 100% sensitivity for advanced transforming CIN 3 (1)

Analysis and interpretation is performed using Rotor-Gene® Q MDx instrument with Rotor-Gene AssayManager® software version 1.0


**Figure 1. QIAsure Methylation Test.** The kit comprises one box containing 2 vials of Master Mix and 2 vials of Calibrator. The master mix is intended for amplification of bisulfite-converted DNA prepared from clinical cervical specimens. The primers in the master mix are specific for the *FAM19A4* and *hsa-mir124-2* promoter regions and include a set of primers for the amplification of β-actin gene (*ACTB*). The latter is the non-methylated reference gene and serves as the internal DNA quality control. The calibrator is a linearized plasmid that contains the sequences of the *FAM19A4*, *hsa-mir124-2*, and *ACTB* amplicons.

## Accurate and robust identification of carcinoma and advanced transforming CIN

### Principle

The presence of high-risk HPV in epithelial cells in the cervix may result in transforming lesions in some women and hence development of cervical cancer (1–9). However, HPV is a common infection and in most cases it does not cause a pre-cancerous or cancerous lesion and is simply cleared by the woman's immune system. Increased expression of the viral oncogenes E6 and E7 in proliferating epithelial cells drives HPV induced carcinogenesis and results in a transforming HPV infection. The associated cervical precursor lesion, also called transforming cervical intraepithelial lesion or transforming CIN, may ultimately progress to cervical cancer (Figure 2). ▷

* Some CIN 3 lesions not identified are likely to regress naturally as they are not advanced transforming CIN.

This process is associated with the accumulation of epigenetic alterations, i.e., increased DNA methylation in specific tumor suppressor genes (Figure 3). By detecting promoter hypermethylation of the tumor suppressor genes *FAM19A4* and/or *hsa-mir124-2*, women with advanced transforming CIN and hence at a high risk of short-term disease progression can be distinguished from women with productive or early transforming CIN who are at low risk of progression to cancer.

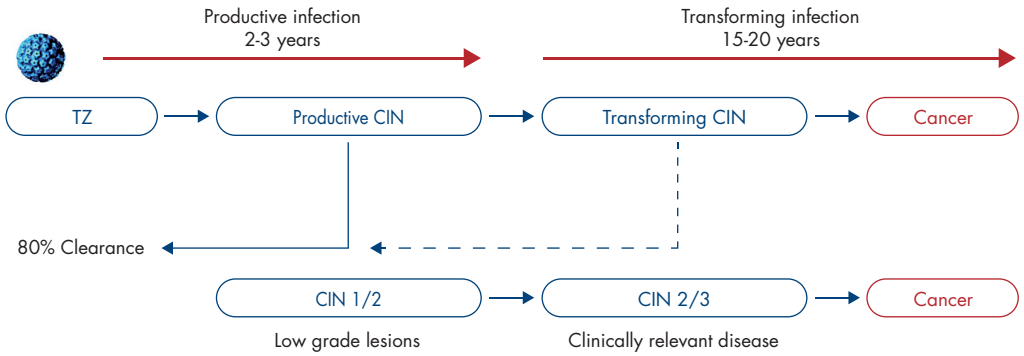


**Figure 2. Natural history of the development of cervical cancer.** Schematic representation of the natural history of cervical cancer and associated at risk populations. **CIN**: cervical intraepithelial neoplasia; **TZ**: transformation zone.

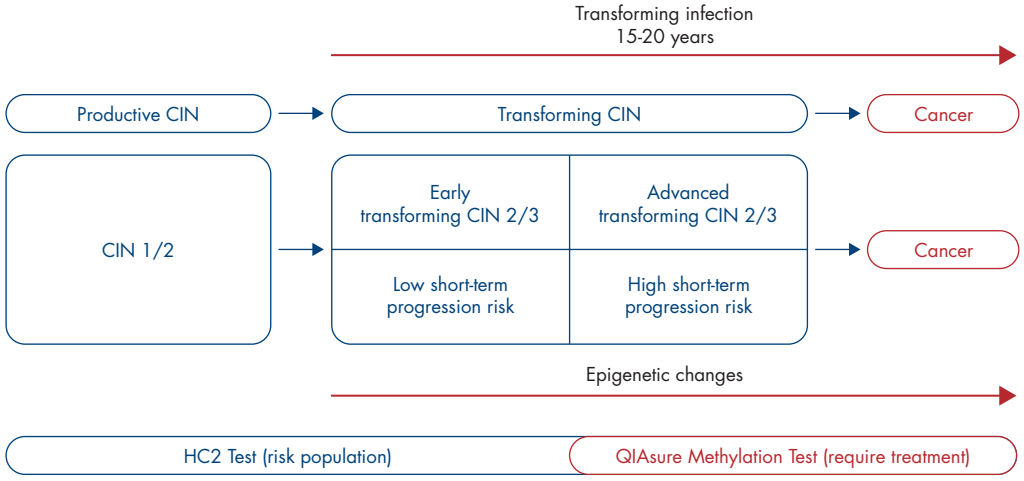


**Figure 3. Heterogeneity seen in CIN and associated risk of disease progression to CIN 3+ and carcinogenic cells.** Schematic representation showing the different lesions seen and associated risk of disease progression and development of cervical cancer. **CIN**: cervical intraepithelial neoplasia.

## Application

The QIAsure Methylation Test can be used to identify hypermethylation of genes *FAM19A4* and *hsa-mir124-2* and is a follow-up test for women with a positive HPV DNA test. It is also indicated for use in women who have a Pap smear showing atypical squamous cells of undetermined

significance (ASC-US). The QIAsure Methylation Test is able to detect CIN 3 at a high risk of short-term progression and cancerous cells with a higher sensitivity compared with cytology or HPV 16/18 genotyping (8). As the QIAsure Methylation Test also has low sensitivity for CIN with low short-term progression risk, it can be used in triage to distinguish women who would benefit from increased surveillance from those who need immediate colposcopy.

## Performance

While cytology detects both early and advanced CIN 2/3 lesions with moderate sensitivity, the QIAsure Methylation Test is a sensitive test for advanced transforming CIN, Tables 1a and b (1, 8). This test uses bisulfite converted DNA from physician collected cervical samples or self-collected vaginal specimens, Figure 4.

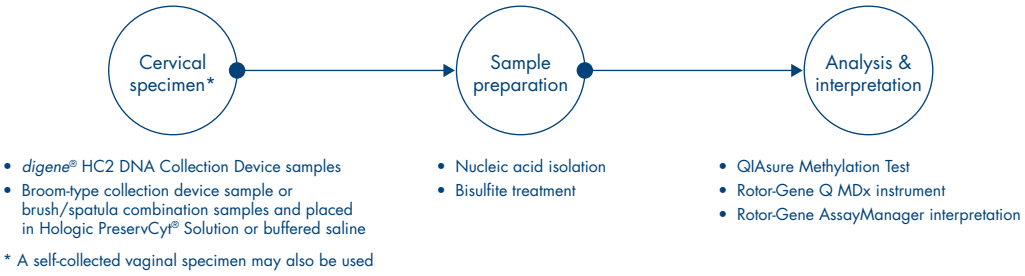


**Figure 4. Workflow from cervical sample to QIAsure methylation result.**

**Table 1a. QIAsure Methylation Test positivity rates for physician collected cervical specimens**

| Clinical endpoint | Fraction | Positivity rate (%) (95% CI) |
|---|---|---|
| ≤CIN 1 | 24/117 | 20.5 (14.1–28.8) |
| CIN 2 | 16/42 | 38.1 (24.8–53.4) |
| CIN 3 | 20/30 | 66.7 (48.4–84.0) |
| Squamous cell carcinoma | 59/59 | 100.0 (94.0–100.0) |
| Adenocarcinoma | 10/10 | 100.0 (69.0–100.0) |
| CIN 3+* | 89/99 | 89.9 (82.2–94.5) |
| All cervical carcinoma*† | 69/69 | 100.0 (94.0–100.0) |

**Table 1b. QIAsure Methylation Test positivity rates in self-collected vaginal brush specimens**

| Clinical endpoint | Fraction | Positivity rate (%) (95% CI) |
|---|---|---|
| ≤CIN 1 | 34/148 | 23.0 (16.9–30.4) |
| CIN 2 | 7/24 | 29.2 (14.6–49.8) |
| CIN 3 | 33/50 | 66.0 (52.0– 77.7) |
| Squamous cell carcinoma | 8/8 | 100.0 (63.1–100.0) |
| Adenocarcinoma | 3/3 | 100.0 (29.2–100.0) |
| CIN 3+* | 44/61 | 72.1 (59.7–81.9) |
| All cervical carcinoma*† | 11/11 | 100.0 (72.0–100.0) |

* Cumulative in this study.

† Squamous cell carcinoma and adenocarcinoma.

**Note**: Hypermethylation of the targets in samples of women harboring advanced CIN lesions and/or cervical cancer might remain undetected due to sampling variability, for example as a result of inadequate sampling.

**References**

1. De Strooper, L.M., et al. (2014) Methylation analysis of the FAM19A4 gene in cervical scrapes is highly efficient in detecting cervical carcinomas and advanced CIN2/3 lesions. Cancer Prev. Res. **7**, 1251–7.
2. Bierkens, M. et al. (2013) CADM1 and MAL promoter methylation levels in hrHPV-positive cervical scrapes increase proportional to degree and duration of underlying cervical disease Int. J. Cancer **133**, 1293–9.
3. Costello, J.F., and Plass, C. (2001) Methylation matters. J. Med. Genet. **38**, 285–303.
4. Wilting, S.M., et al. (2010) Methylation-mediated silencing and tumour suppressive function of has-MiR-124 in cervical cancer. Mol. Cancer **9**, 167.
5. De Strooper, L.M., et al. (2014) CADM1, MAL and miR12-2 methylation analysis in cervical scrapes to detect cervical and endometrial cancer. J. Clin. Pathol. 67, 1067–71.
6. De Strooper, L.M., et al. (2016) Comparing the performance of FAM19A4 methylation analysis, cytology and HPV 16/18 genotyping for the detection of cervical (pre)cancer in high-risk HPV-positive women of a gynecologic outpatient population (COMETH study). Int. J. Cancer **138**, 992–1002.
7. De Strooper, L.M., et al. (2016) Validation of the FAM19A4/mir124-2 DNA methylation test for both lavage- and brush-based self-samples to detect cervical (pre)cancer in HPV-positive women. Gynecol. Oncol. **141**, 341–7.
8. Steenbergen R. D.M., et al (2014). Clinical implications of (epi)genetic changes in HPV-induced cervical precancerous lesions. Nat Rev Cancer **14**, 395–405.
9. Luttmer R. et al. (2016) Management of high-risk HPV-positive women for detection of cervical (pre)cancer. Expert Rev. Mol. Diagn. **16(9)**, 961-74.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAsure Methylation Test Kit | For 72 reactions: 2 vials of Master Mix, 2 vials of Calibrator | 616014 |
| **Related Products** | | |
| Rotor-Gene Q MDx 5plex HRM Platform | Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included | 9002032 |
| Rotor-Gene AssayManager version 1.0 | Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony® RGQ instruments; single license software for installation on one computer | 9022739 |

**Note**: Assay profiles for the QIAsure Methylation Test Kit can be downloaded from **www.qiagen.com**.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

The QIAsure Methylation Test is intended for in vitro diagnostic use.

Self-screen B.V. is the legal manufacturer of the QIAsure Methylation Test.

The QIAsure Methylation Test is manufactured by Self-screen B.V., Biothof 15-1, 1098 RX Amsterdam, the Netherlands and distributed by QIAGEN in Europe.

To find out more, visit **www.qiagen.com/QIAsure1**.