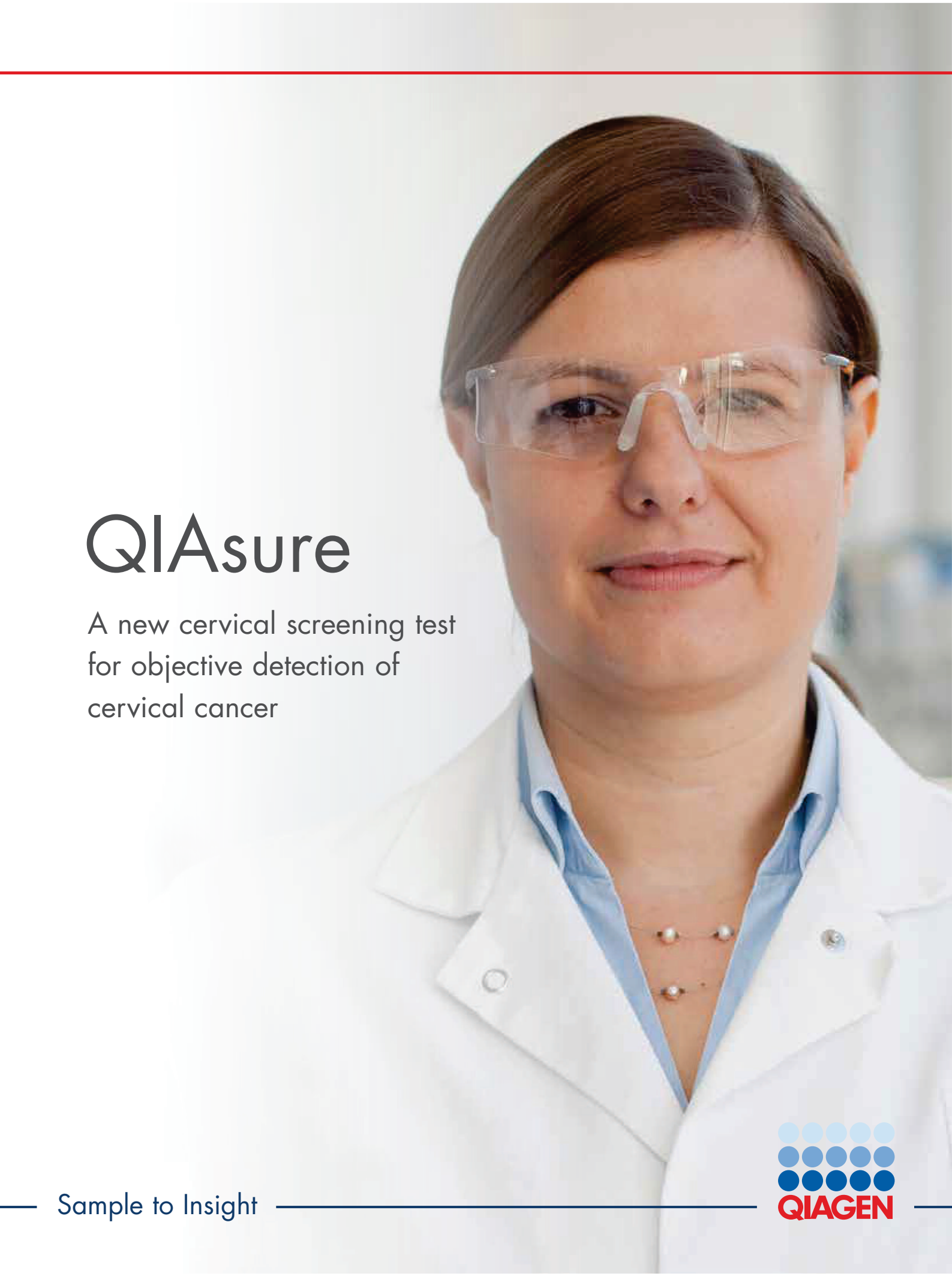


QIAsure

A new cervical screening test for objective detection of cervical cancer

Sample to Insight

QIAGEN

QIAsure: A breakthrough solution in cervical cancer screening

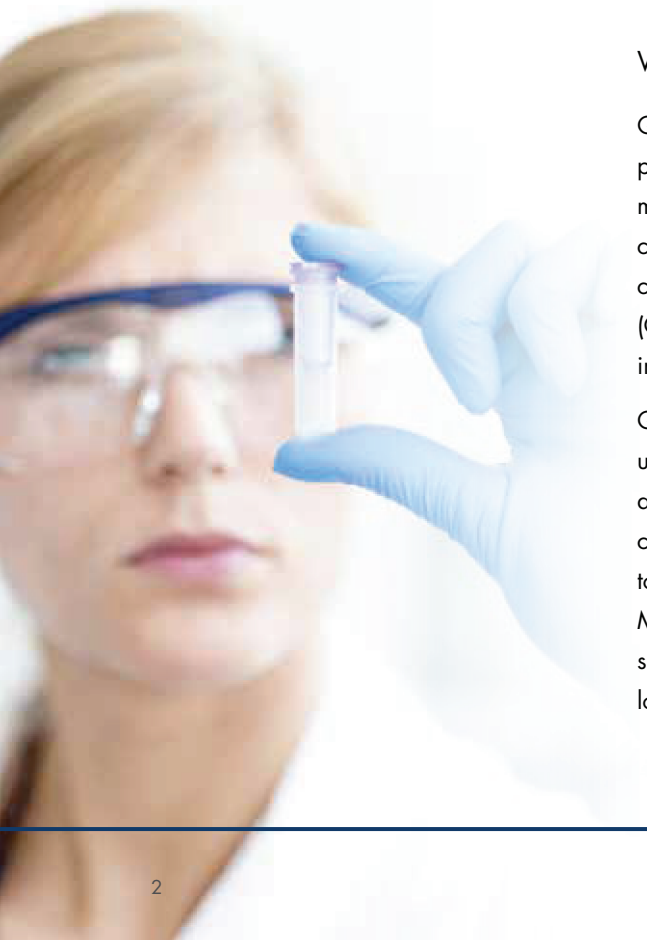
We are at a turning point in the battle against cervical cancer. The global human papillomavirus (HPV) epidemic has led to a rise in cervical cancer incidence worldwide. It is now the second most common cancer among women aged 15 to 44, affecting more than 500,000 women a year (1). Fortunately, cervical cancer is nearly 100% preventable through early detection of precancer before the disease becomes a danger to a patient's life or reproductive health.

QIAGEN, the developer of the gold-standard HPV screening test *digene*® HC2 High-Risk HPV DNA Test®, brings to market the QIAsure Methylation Test—a new cervical cancer screening test that gives you molecular insight into whether an HPV infection is actively transforming cervical cells into cancer.

Why QIAsure?

Today, women have an 80% chance in their lifetimes of contracting high-risk HPV (hrHPV), the causal agent of cervical cancer. Advances in technology have created effective methods for detection of hrHPV. However, only 10% of women with hrHPV will develop long-lasting HPV infections that put them at risk for development of cervical cancer (2). The current clinical focus on hrHPV testing and genotyping lets clinicians know whether a patient *could* develop cervical cancer; however hrHPV infection is a risk factor, not an indicator of cervical cancer. Cytology is a valuable standard practice of care to look for abnormal cells in cervical specimens, but due to its moderate sensitivity and subjective results, cytology can produce false-positive or false-negative results and sometimes miss advanced transforming cervical precancer and cancer.

QIAsure provides an effective triage test in cervical screening to fulfill the need for more actionable molecular insight into whether a patient has cervical precancer or cancer.



What is QIAsure?

QIAsure is a quantitative methylation specific PCR (qMSP) test that lets lab professionals see what's happening at a molecular level so they can determine whether a hrHPV patient is at short-term risk of developing cervical cancer. QIAsure detects the presence of biomarkers associated with cervical carcinoma and advanced transforming cervical intraepithelial neoplasia (CIN), to objectively discern passive HPV infections from ones that warrant immediate attention.

QIAsure can be used to triage a positive hrHPV test on the same specimen used for an HPV test or liquid-based cytology (LBC). It can also be used as a confirmatory test after an atypical squamous cells of undetermined significance (ASC-US) cytology result. QIAsure looks for methylation of the promotor regions of host cell genes *FAM19A4* and *miR124-2* in the cervical cells. Methylation of these genes indicates carcinogenic cell transformation and high short-term risk of developing cervical cancer; absence of methylation indicates low short-term risk of developing cervical cancer.

DNA methylation for objective detection of CIN and carcinoma

DNA methylation is a biochemical process that is important for normal development in higher organisms (3). It involves the addition of a methyl group to the 5′ position of the cytosine pyrimidine. Abnormal patterns of DNA methylation have been implicated in various cancers, including cervical cancer where promoter hypermethylation of the tumor suppressor genes *FAM19A4* and/or *miR124-2* has been reported (4–10).

Independent research from leading cervical cancer scientists confirms the effective use of DNA methylation analysis for detection of cervical carcinomas and advanced CIN 2/3 lesions (4–10). Researchers have distinguished *FAM19A4* as an effective triage marker for hrHPV-positive women, with a high sensitivity in the detection of cervical carcinoma and advanced CIN 2/3 lesions (4).

Transforming CIN 2/3 can be divided into early and advanced transforming CIN based on the level of genetic and epigenetic alterations, which QIAsure measures through analysis of *FAM19A4* and *miR124-2* hypermethylation. QIAsure detects 100% of carcinomas (squamous cell carcinoma and adenocarcinoma), with variation in detection of other grades of CIN, from 88.9% in CIN 3+ to 52.4% of CIN 2 and 28.2% of <CIN 1 (see Table 1 on page 4). In a subgroup of women over 30 years (n=287), the CIN 3+ sensitivity and specificity for *FAM19A4* methylation (88.3% and 62.1%) was greater than that of cytology (85.0% and 47.6%) and HPV 16/18 genotyping (70% and 57.7%) (9).

Lab Workflow

QIAsure was developed in partnership with renowned pathologists Prof. Dr. Chris Meijer and Prof. Dr. Peter Snijders at VU University Medical Center in Amsterdam, Netherlands. QIAsure uses a multiplex real-time methylation-specific PCR assay for the detection of promoter hypermethylation of *FAM19A4* and *miR124-2*. QIAsure measures the methylation status of these genes in bisulfite-converted DNA isolated from specimens collected in the following ways:

- Cervical specimens collected with the *digene* HC2 DNA Collection Device (physician collected)
- Cervical specimens collected using a brush/broom-type collection device and placed in PreservCyt® Solution (physician collected)
- Vaginal specimens collected with *digene* HC2 DNA Collection Device (self-collected)

QIAsure can be performed in the PCR lab through industry-standard methods of sample prep/extraction for bisulfite-converted DNA, and assay automation platforms for analysis (clinically validated for Rotor-Gene® Q MDx instrument).

QIAsure positive result: Hypermethylation of *FAM19A4* and/or *miR124-2* genes indicates presence of cervical carcinoma and/or advanced transforming CIN and high short-term risk of cervical cancer progression.

QIAsure negative result: Absence of hypermethylation of *FAM19A4* and/or *miR124-2* genes may indicate a lack of carcinogenic transformation of cervical cells and low short-term risk of cervical cancer progression.

With the results, lab professionals can provide clinicians with vital information that can protect a patient's reproductive health and inform clinical decisions on follow-up surveillance or treatment.

Clinical trials

QIAsure transforms information into insight through DNA methylation analysis that objectively determines the presence of biomarkers associated with carcinogenic cervical cells and advanced transforming CIN lesions. QIAsure has been clinically proven to detect the transformation of cervical cells and advanced CIN 2/3 lesions, even in patients with normal cytology.

In clinical trials, QIAsure testing was performed on physician-collected cervical specimens from 258 hrHPV-positive women including 117 without evidence of CIN 2 or worse after 18 months follow-up (CIN≤1), 42 with CIN 2, 30 with CIN 3, 50 with squamous cell carcinoma, and 10 with adenocarcinoma. QIAsure detected 100% of carcinomas (squamous cell carcinoma and adenocarcinoma) in these samples, but varied in detection of other grades of CIN, from 88.9% in CIN 3+ to lower sensitivity for CIN 1/2. *FAM19A4* and *miR124-2* methylation analysis specifically detects advanced transforming CIN lesions. CIN 2/3 with low levels of *FAM19A4* and *miR124-2* methylation have low short-term progression risk for cancer. These patients can be managed by close surveillance rather than treated.

Table 1. QIAsure assay positivity rates

Clinical endpoint	Fraction	Positivity rate (95% CI)
All carcinomas	69/69	100.0% (94.0–100.0)
Adenocarcinoma	10/10	100.0% (69.0–100.0)
Squamous cell carcinoma	59/59	100.0% (94.0–100.0)
CIN 3+	88/99	88.9% (81.0–93.7)
CIN 3	19/30	63.3% (45.1–78.4)
CIN 2	22/42	52.4% (37.5–66.8)
≤CIN 1	33/117	28.2% (20.8–37.0)

QIAsure detects 100% of cervical carcinomas*

*QIAsure has 100% accuracy in detecting biomarkers associated with cervical carcinoma in patients.

Table 2. *FAM19A4* sensitivity rates in hrHPV positive women >30 years (n=287) (9)

Detection Method	CIN 3 + sensitivity (%)	Positivity rate (95% CI)
FAM19A4 methylation	88.3	55.8-68.4% (CI)
Cytology	85.0	41.1-54.1% (CI)
HPV16/18 genotyping	70.0	51.3-64.1% (CI)

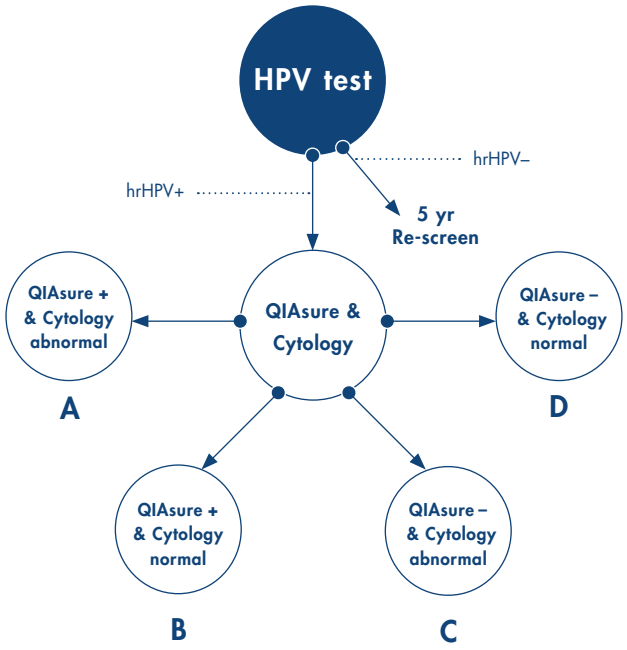
Improved cervical screening

QIAsure can be performed in the PCR lab with the same automation and the same sample used for a primary screening HPV test. It can also be performed on the same sample collected for liquid-based cytology. This makes the lab's workflow simpler and easier, reduces hands on time, and improves time to result.

QIAsure has been developed as a triage test to fit into current cervical cancer screening algorithms. The flowchart diagram on the top right illustrates the test sequence following a HPV primary screening test. The flowchart diagram on the bottom right illustrates the test sequence following cytology primary screening test.

This innovative qMSP triage test can be integrated into standard screening algorithms to add objective clinical assurance, so that patients are effectively screened for dangerous cervical precancer and cancer. It can separate out patients with hrHPV infections that are transforming into cancer from patients who have low short-term risk of developing cancer. By including this test as part of the clinical assessment, QIAsure can help guide treatment decisions and prevent women with non-transforming infections from receiving unnecessary treatments.

HPV Primary Screening



Cytology Primary Screening

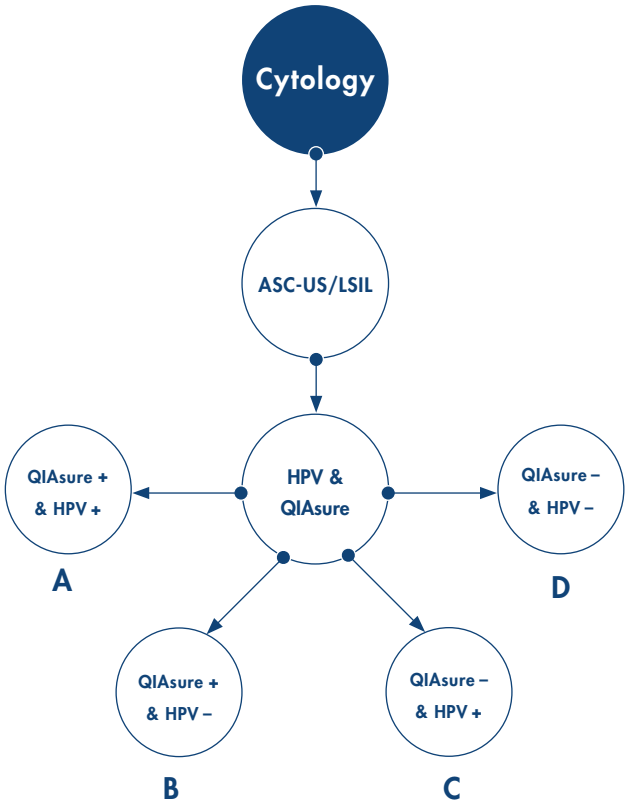


Figure 1. Screening flowcharts.
Proposed guidelines for primary screening scenarios

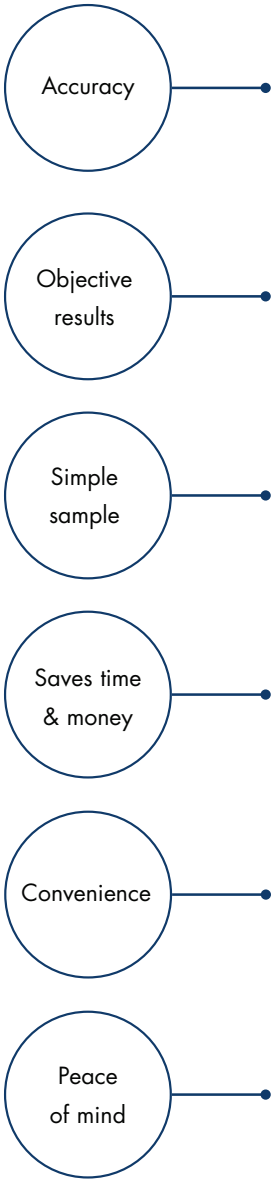
A. High risk of advanced transforming CIN, refer to colposcopy.

B. High risk of advanced transforming CIN, refer to colposcopy.

C. Low risk of advanced transforming CIN, refer to colposcopy—be conservative in treatment vs. surveillance decision.

D. Low risk of advanced transforming CIN, recommend increased surveillance.

Key benefits:



Clinical implications

QIAsure delivers objective assurance that women are safe from cervical cancer following a positive hrHPV result or an abnormal cytology result. The high sensitivity and specificity of QIAsure offers reassurance for absence of cervical cancer in test negative women; a positive test identifies women who need immediate treatment. Unlike cytology, *FAM19A4/miR124-2* methylation analysis on hrHPV-positive cervical specimens detects 100% of biomarkers associated with cervical cancer, and identifies advanced transforming CIN2/3.

QIAsure is not intended to replace cytology. It can however be used in combination with or instead of cytology after an HPV primary screening test or as an additional test post cytology to gain a higher certainty that advanced lesions and cervical cancer are not present.

QIAsure bridges a gap in cervical screening as an accurate and objective triage test that can be performed on the same sample used for hrHPV testing or LBC. It is a unique and effective method to see beyond HPV and gain molecular insights into whether a hrHPV infection is actively transforming into cervical cancer. It provides clinicians with a new way to detect the presence of cervical precancer and make informed decisions about follow-up or surveillance.

Moreover, QIAsure provides peace of mind for patients so they can live their lives safe, confident, and sure of their reproductive health.

QIAGEN and cervical cancer prevention

QIAGEN is a worldwide leader in innovative Sample to Insight® solutions for molecular diagnostics, applied testing, advanced genomics, and clinical and academic research. QIAGEN is committed to the highest standards of performance and service excellence to create real-world value for our customers and real-world human impact for millions of patients across the world.

Since the launch of the *digene* HPV Test in 1999, QIAGEN has been at the forefront of advanced HPV screening and cervical cancer prevention. Considered the gold standard for HPV testing, the *digene* HPV Test uses advanced molecular technology to provide a highly accurate means of identifying women at risk for cervical cancer. For almost 20 years, clinicians around the world have used the *digene* HPV Test to routinely screen more than 100 million women for the presence of HPV. QIAGEN is deeply committed to helping women in developing nations around the world gain access to essential cervical cancer screening through its QIAGEN*cares* and *care*HPV® initiatives.

QIAGEN is excited to bring its newest Women's Health solution to market—QIAsure, a qMSP triage test for objective detection of cervical precancer and cancer.

Ordering Information

Product	Content	Cat no.
QIAsure Methylation Test	For 72 reactions: 2 Master Mixes, 2 Calibrators.	616014
Related Products		
Rotor-Gene® Q MDx 5plex HRM System	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	9002033
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®	Software for routine testing in combination with the Rotor-Gene Q and QIAsymphony® RGQ instruments; single license software for installation on one computer	9022739

For more information about QIAsure, visit **www.qiagen.com/qiasure**.

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