QIAsure® Methylation Test

The following summaries represent peer-reviewed studies published from 2019–2022, evaluating the use of *FAM19A4* and *miR124-2* methylation analysis as a triage strategy in different patient populations and screening scenarios following an hrHPV positive result.

Author	Article	Journal	Year
Kremer, W.W., et al.	The use of molecular markers for cervical screening of women living with HIV in South Africa	AIDS	2019
Dick, S., et al.	Long-term CIN3+ risk of HPV positive women after triage with FAM19A4/miR124-2 methylation analysis	Gynecol Oncol	2019
Vink, F.J., et al.	FAM19A4/miR124-2 methylation in invasive cervical cancer: A retrospective cross-sectional worldwide study	Int J Cancer	2019
Bonde, J., et al.	Methylation markers FAM19A4 and miR124-2 as triage strategy for primary papillomavirus screen positive women: A large European multicenter study	Int J Cancer	2020
Kremer, W.W., et al.	The use of host cell DNA methylation analysis in the detection and management of women with advanced cervical intraepithelial neoplasia: a review	BJOG	2020
Dick, S., et al.	Risk-stratification of HPV-positive women with low grade cytology by FAM19A4/miR124-2 methylation and HPV genotyping	Br J Cancer	2021
Albulescu, A., et al.	Epigenetic approaches for cervical neoplasia screening (Review)	Exp Ther Med	2021
Vink, F.J., et al.	Classification of high-grade cervical intraepithelial neoplasia by p16ink4a, Ki-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management	Int J Cancer	2021
Kremer, W.W., et al.	Clinical regression of high-grade cervical intraepithelial neoplasia is associated with absence of FAM19A4/miR124-2 DNA methylation (CONCERV Study)	J Clin Oncol	2022
Hampl, M., et al.	Evaluation of FAM19A4/miR124-2 methylation performance in the management of CIN3 diagnosed pregnant women	Int J Cancer	2022
Vink, F.J., et al.	FAM19A4/miR124-2 methylation testing and HPV16/18 genotyping in HPV-positive women under the age of 30 years	Clin Infect Dis	2022
Kaliff, M., et al.	Full genotyping and FAM19A4/miR124-2 methylation analysis in high-risk human papillomavirus-positive samples from women over 30 years participating in cervical cancer screening in Orebo, Sweden	PLoS One	2022
Verhoef, L., et al.	Direct bisulphite conversion of cervical samples for DNA methylation analysis	Epigenetics	2022

The use of molecular markers for cervical screening of women living with HIV in South Africa

(Kremer, W.W., et al., AIDS 2019)

Background: For women with HIV who are at an increased risk for cervical cancer, it is important to have effective cervical screenings to lower morbidity and mortality rates that may result. The objective of this study is to determine the performance of molecular screening strategies for detection of cervical intraepithelial neoplasia (CIN) grade 3 or worse (CIN3+) in comparison with cytology screening in women living with HIV.

Methods: Cytology and human papillomavirus (HPV)-based strategies were evaluated, including single test and QIAsure Methylation Test triage strategies. The participants were put through cytology screening and a colposcopy-directed biopsy. Valid results on cytology, HPV status, 16/18 genotyping and histology were available for 318 women. Detection of HPV and QIAsure hypermethylation was performed on DNA obtained from cervical scrapes. Histological diagnosis of CIN3+ was used as outcome.

Results: Cytology had the highest specificity (91.6%) while having the lowest sensitivity (59.3%). A single HPV test provided highest sensitivity (83.1%), but lowest specificity (66.4%). Combining cytology and methylation did not improve the performance compared with cytology alone: a small increase in sensitivity was seen at the cost of a decrease in specificity. Triage of high-risk HPV positive women with methylation increased specificity (76.1%) compared with a single HPV or cytology test, while maintaining acceptable sensitivity (72.9%). A similar level of performance was observed for HPV16/18 with methylation triage (sensitivity 79.7%, specificity 74.8%).

Conclusion: Molecular screening strategies using HPV, with or without HPV16/18 genotyping, and the QIAsure Methylation Test showed a higher sensitivity with an acceptable loss in specificity compared with current cytology screening and are efficient for the detection of CIN3+ in South African women with HIV.

Long-term CIN3+ risk of HPV positive women after triage with FAM19A4/miR124-2 methylation analysis

(Dick, S., et al., Gynecol Oncol 2019)

Background: Since HPV testing provides better protection against cervical cancer compared with cytology, new cervical cancer screening guidelines recommend high-risk HPV testing as a primary screening tool. This study evaluates the long-term risk for CIN3+ among HPV positive women triaged with *FAM19A4/miR124-2* methylation analysis.

Methods: In a post hoc analysis, data on QIAsure Methylation Test, cytology, and HPV16/18 genotyping of HPV positive women (n = 1025) from a large population-based screening cohort with 14-year follow-up were evaluated. Cumulative CIN3+ incidences over three screening rounds (5-year intervals) of four triage strategies were compared: QIAsure Methylation Test analysis, cytology, HPV16/18 genotyping with QIAsure Methylation Test, and HPV16/18 genotyping with cytology.

Results: Kaplan-Meier estimates of 14-year cumulative CIN3+ incidence of HPV positive women with a negative methylation and a negative cytology triage test were comparable (16.3% and 15.6%, respectively). The cumulative CIN3+ incidence of methylation positive and cytology positive women were 39.8% and 46.5%, respectively. HPV16/18 genotyping with methylation and HPV16/18 genotyping with cytology resulted in the lowest 14-year cumulative CIN3+ incidence among triage negative women (10.7% and 10.0%, respectively), but cumulative CIN3+ incidence among triage positive women was lower (33.4% and 35.7%, respectively) compared with triage by methylation alone and cytology alone.

Conclusion: Among HPV-positive women ages 30 and older, a negative QIAsure Methylation Test triage result provides a similar long-term CIN3+ risk compared with a negative cytology triage test. Due to their high CIN3+ risk, women with a positive methylation triage test could be referred for colposcopy. Therefore, QIAsure Methylation Test analysis is a promising alternative to cytology for triage of HPV positive women.

FAM19A4/miR124-2 methylation in invasive cervical cancer: A retrospective cross-sectional worldwide study

(Vink, F.J., et al., Int J Cancer 2019)

Background: Widespread adoption of primary HPV-based screening has encouraged the search for a triage test which retains high sensitivity for the detection of cervical cancer and precancer, but increases specificity to avoid overtreatment. This study evaluates Methylation analysis of *FAM19A4* and *miR124-2* genes for the triage of high-risk (hr) HPV-positive women.

Methods: This study assessed the consistency of QIAsure Methylation Test (a quantitative methylation-specific PCR (qMSP)-based assay) analysis in the detection of cervical cancer in a series of 519 invasive cervical carcinomas (n = 314 cervical scrapes, n = 205 tissue specimens) from over 25 countries. Positivity rates stratified per histotype, FIGO stage, hrHPV status, hrHPV genotype, sample type and geographical region were calculated.

Results: In total, 510 of the 519 cervical carcinomas (98.3%; 95% CI: 96.7–99.2) tested methylation-positive. Test positivity was consistent across the different subgroups based on cervical cancer histotype, FIGO stage, hrHPV status, hrHPV genotype, sample type and geographical region.

Conclusion: Analysis of the QIAsure Methylation Test detects nearly all cervical carcinomas, including rare histotypes and hrHPV-negative carcinomas. These results indicate that a negative QIAsure Methylation Test result is likely to rule out the presence of cervical cancer.

Methylation markers FAM19A4 and miR124-2 as triage strategy for primary papillomavirus screen positive women: A large European multicenter study

(Bonde, J., et al., Int J Cancer 2020)

Background: With its superior sensitivity for ≥CIN2 detection and an improved protection against cervical cancer, hrHPV-based cervical screening has replaced or is scheduled to replace cytology as primary screening method in several countries with several other expected to follow shortly. Recently, the QIAsure Methylation Test has been introduced as a commercial CE-IVD triage test for screening and diagnostic purposes. VALID-SCREEN is an EU-multicenter, retrospective study conducted to evaluate the clinical performance of the *FAM19A4/miR124-2* methylation-based molecular triage test (QIAsure) as a substitute or addition to cytology as reflex testing of HPV-screen-positive women.

Methods: The QIAsure Methylation Test was evaluated in 2384 HPV-positive cervical screening samples, from women 29-76 years old, derived from four EU countries. Specimens were collected in ThinPrep® or SurePath® media, HPV-status, concurrent cytology, and histology diagnosis were provided by the parent institutes. The control population consisted of women with no evidence of disease within two years of follow-up.

Results: A total of 899 histologies were retrieved; 527 showed no disease, 124 CIN2 (5.2%), 228 CIN3 (9.6%) and 20 cervical cancers (0.8%); 19 of 20 screen-detected cervical cancers were found methylation-positive (sensitivity 95%). Overall specificity of the QIAsure Methylation Test was 78.3% (n = 2013; 95%CI: 76-80). The negative predictive value of hrHPV positive, methylation-negative outcomes were 99.9% for cervical cancer (N = 1694; 95%CI: 99.6-99.99), 96.9% for ≥CIN3 (95%CI: 96-98), and 93.0% for ≥CIN2 (95%CI: 92-94). Overall sensitivity for CIN3 using the QIAsure Methylation Test was 77% (n = 228; 95%CI: 71-82). CIN3 sensitivity was uniform between centers independent of sample collection medias, DNA extraction methods and HPV screening tests.

Conclusion: In perspective, these conclusions support practical pilot implementation of the QIAsure Methylation Test into cervical screening programs to provide further data and experiences to inform on how a fully molecular cervical screening program can be designed to the benefit of women and health care services both.

The use of host cell DNA methylation analysis in the detection and management of women with advanced cervical intraepithelial neoplasia: a review

(Kremer, W.W., et al., BJOG 2020)

Background:

This paper briefly reviews the role of hypermethylation of host cell genes in cervical carcinogenesis and discusses potential clinical applications of methylation analysis in the management of hrHPV-positive women.

Summary: The review highlights the role of DNA methylation in cervical carcinogenesis and discusses the potential clinical applications of DNA methylation analysis in the detection and management of women with cervical neoplastic lesions. The review focused on methylation markers that have been clinically validated for the detection of cervical cancer (CIN3 and CIN2) in cervical scrapes and self-collected cervico-vaginal swabs, and that are currently available (either commercial or research assays). The four clinical applications of methylation analysis for hrHPV positive women are 1) it can be used for primary triage of hrHPV-positive women to detect cervical cancer and advanced cervical intraepithelial neoplasia, 2) as a secondary triage for women with minor cytological abnormalities to identify those with the highest risk of CIN3, 3) as an exit test for women leaving the screening program to identify cervical cancer and advanced CIN, and 4) to support the management of CIN.

Risk-stratification of HPV-positive women with low grade cytology by FAM19A4/miR124-2 methylation and HPV genotyping

(Dick, S., et al., Br J Cancer 2021)

Background: The introduction of primary HPV screening has doubled the number of colposcopy referrals because of the direct referral of HPV-positive women with a borderline or mild dyskaryosis (BMD) cytology (ASC-US/LSIL) triage test. Further risk stratification is warranted to improve the efficiency of HPV-based screening.

Methods: This study evaluated the discriminative power of the QIAsure Methylation Test, HPV16/18 genotyping and HPV16/ 18/31/33/45 genotyping in HPV-positive women with BMD (n = 294) in two Dutch screening trials. Absolute CIN3+ risks and colposcopy referrals within one screening round were calculated.

Results: Methylation analysis discriminated well, yielding a CIN3+ risk of 33.1% after a positive result and a CIN3+ risk of 9.8% after a negative result. HPV16/18 and HPV16/18/31/33/45 genotyping resulted in a 27.6% and 24.6% CIN3+ risk after a positive result, and a 13.2% and 9.1% CIN3+ risk after a negative result. Colposcopy referral percentages were 41.2%, 43.2%, and 66.3% for the QIAsure Methylation Test, HPV16/18 and HPV16/18/31/33/45 genotyping, respectively. The CIN3+ risk after a negative result could be lowered to 2.8% by combining methylation and extended genotyping, at the cost of a higher referral percentage of 75.5%.

Conclusion: The use of the QIAsure Methylation Test and/or HPV genotyping in HPV-positive women with BMD has the potential to lead to a reduction in the number of direct colposcopy referrals.

Epigenetic approaches for cervical neoplasia screening (Review)

(Albulescu, A., et al., Exp Ther Med 2021)

Background: HPV infection is the leading cause of cervical cancer. The Papanicolaou cytology test is the usually employed type of screening for this infection; however, its sensitivity is limited. Only a small percentage of women infected with high risk HPV develop cervical cancer with an array of genetic and epigenetic modifications. Thus, it is necessary to develop rapid, reproducible, and minimally invasive technologies for screening. DNA methylation has gained attention as an alternative method for molecular diagnosis and prognosis in HPV infections.

Methods: Review of an article representing the potential of DNA methylation in cervical neoplasia screening for clinical purposes.

Results: It was observed that the methylation human and viral genes was correlated with high grade lesions and cancer. Methylation biomarkers have shown a good capacity to discriminate between high grade lesions with a transformative potential and cervical cancer, being able to detect these modifications at an early stage.

Conclusion: With further research, the epigenetic profiles and subtypes of the tumors could be elaborated, which would aid in therapy selection by opening avenues in personalized precision medicine. Response to therapy could also be evaluated through such methods and the accessibility of liquid biopsies would allow a constant monitoring of the patient's status without invasive sampling techniques.

Classification of high-grade cervical intraepithelial neoplasia by p16ink4a, Ki-67, HPV E4 and FAM19A4/miR124-2 methylation status demonstrates considerable heterogeneity with potential consequences for management

(Vink, F.J., et al., Int J Cancer 2021)

Background: High-grade cervical intraepithelial neoplasia (CIN2 and CIN3) represents a heterogeneous disease with varying cancer progression risks. Biomarkers indicative for a HPV infection (HPV E4) and a transforming HPV infection (p16ink4a, Ki-67 and host-cell DNA methylation) could provide guidance for clinical management in women with high-grade CIN.

Methods: This study evaluates the cumulative score of immunohistochemical expression of p16ink4a (Scores 0-3) and Ki-67 (Scores 0-3), referred to as the "immunoscore" (IS), in 262 CIN2 and 235 CIN3 lesions derived from five European cohorts in relation to immunohistochemical HPV E4 expression and the QIAsure Methylation Test in the corresponding cervical scrape.

Results: The immunoscore classification resulted in 30 lesions within IS group 0-2 (6.0%), 151 lesions within IS group 3-4 (30.4%) and 316 lesions within IS group 5-6 (63.6%). E4 expression decreased significantly from CIN2 to CIN3 ($P < .001$) and with increasing immunoscore group ($P_{trend} < .001$). Methylation positivity increased significantly from CIN2 to CIN3 ($P < .001$) and with increasing immunoscore group ($P_{trend} < .001$). E4 expression was present in 9.8% of CIN3 (23/235) and in 12.0% of IS group 5-6 (38/316). In a minority (43/497, 8.7%) of high-grade lesions, characteristics of both transforming HPV infection (DNA hypermethylation) and productive HPV infection (E4 expression) were found simultaneously. Next, we stratified all high-grade CIN lesions, based on the presumed cancer progression risk of the biomarkers used, into biomarker profiles.

Conclusion: In conclusion, a considerable amount of heterogeneity in biomarker expression in a large series of high-grade CIN lesions evaluated with p16ink4a, Ki-67 and E4 immunohistochemical staining and the QIAsure Methylation Test was found. Biomarker profiles were identified that may help clinicians in a more personally tailored management of women with high-grade CIN and thereby preventing overtreatment, and particularly in younger women.

Clinical regression of high-grade cervical intraepithelial neoplasia is associated with absence of FAM19A4/miR124-2 DNA methylation (CONCERV Study)

(Kremer, W.W., et al., J Clin Oncol 2022)

Background: Cervical screening can prevent cancer by detection and treatment of CIN2/3. However screening also results in considerable overtreatment because many CIN2/3 lesions show spontaneous regression when left untreated. In this multicenter longitudinal cohort study of women with untreated CIN2/3, the prognostic value of the QIAsure Methylation Test was evaluated for clinical regression.

Methods: Women with CIN2/3 were prospectively followed for 24 months. Surgical excision was replaced by a wait-and-see policy. The QIAsure Methylation Test was evaluated on all clinician-collected samples and self-collected samples collected at baseline. Every six months, HPV testing and cytology were conducted on a clinician-collected sample, and a colposcopic examination was performed by a gynecologist to exclude progression. At the final study visit, two biopsies were taken. Clinical regression was defined as histologically confirmed absence of CIN2/3 or an HPV-negative clinician-collected sample with normal cytology. Regression incidences were estimated using the Kaplan-Meier method.

Results: 114 women (median age, 30 years; range, 20-53 years) were included, 80 of whom were diagnosed with CIN2 and 34 with CIN3. During the study, 65.8% of women (75/114) did not receive surgical treatment. Women with a negative QIAsure Methylation Test result on the baseline clinician-collected sample showed more clinical regression (74.7%) than women with a positive methylation result (51.4%, P = .013). Regression in women with a negative QIAsure Methylation Test result was highest when cytology was atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion (88.4%) or HPV16 was negative (85.1%).

Conclusion: Most of the women with untreated CIN2/3 and a negative baseline QIAsure Methylation Test result showed clinical regression. In conclusion, methylation, in combination with cytology or HPV genotyping, can ultimately be used to support a "wait-and-see" policy in women with CIN2/3.

Evaluation of FAM19A4/miR124-2 methylation performance in the management of CIN3 diagnosed pregnant women

(Hampl, M., et al., Int J Cancer 2022)

Background: Pregnant women diagnosed with CIN3 have high regression rates after delivery. Biomarkers are needed to only identify pregnant women with progressive CIN requiring treatment to reduce over referral and overtreatment. *FAM19A4* and *hsa-miR124-2*, commercially available as QIAsure Methylation Test, has been identified as a promising biomarker for management of women with high-grade CIN and has the advantage of a quantitative and objective test result. Application of the *FAM19A4/miR124-2* methylation test could therefore help the clinician in the management of pregnant women with CIN2/3. The aims of this German multicenter study were to retrospectively evaluate the performance of the *FAM19A4/miR124-2* methylation test as a molecular triage test.

Methods: In this German multicenter retrospective study, biopsy material was collected from pregnant women diagnosed with cervical cancer (n = 16), with CIN3 that progressed to cancer during pregnancy (n = 7), with CIN3 that regressed to CIN1 or less within

six months after delivery (n = 41), without CIN (n = 16), CIN3 covering 3-4 quadrants (n = 14) and randomly selected CIN3 (n = 41). *FAM19A4/miR124-2* methylation analysis was performed blinded on first diagnosis.

Results: All pregnant women with cervical cancer and with CIN3 progressing to cancer tested positive for *FAM19A4/miR124-2* methylation (100%, 22/22). In the regressing CIN3 group 47.5% and in the group without CIN 21.6% tested methylation positive. High-volume CIN3 and random selected CIN3 were methylation-positive in 91.7% and 82.1%, respectively. Methylation levels were significantly higher in progressive CIN3 and cancer compared to the controls (P < .0005). The likelihood ratio of a negative methylation test (LR) for progressive CIN3+ was 0 (95% CI: 0-0.208).

Conclusion: A negative QIAsure Methylation Test result can rule out progressive CIN disease in pregnant women diagnosed with CIN3. This can help the clinician by managing these pregnant women with conservative follow up until after delivery.

FAM19A4/miR124-2 methylation testing and HPV16/18 genotyping in HPV-positive women under the age of 30 years

(Vink, F.J., et al., Clinical Infect Dis 2022)

Background: High-grade squamous intraepithelial lesions (HSIL) or CIN grade 2/3 lesions in HPV–positive women under 30 years of age have high spontaneous regression rates. To reduce overtreatment, biomarkers are needed to delineate advanced CIN lesions that require treatment. We analyzed the *FAM19A4/ miR124-2* methylation test and HPV16/18 genotyping in HPV-positive women aged less than 30 years, aiming to identify CIN2/3 lesions in need of treatment.

Methods: A European multicenter retrospective study was designed evaluating the *FAM19A4/miR124-2* methylation test, also referred to as QIAsure Methylation Test, and HPV16/18 genotyping in cervical scrapes of 1,061 HPV-positive women aged 15–29 years. A subset of 62 CIN2 and 103 CIN3 were immunohistochemically characterized by HPV E4 expression, a marker for a productive HPV infection, and p16ink4a and Ki-67, markers indicative for a transforming infection. CIN2/3 lesions with low HPV E4 expression and high p16ink4a/Ki-67 expression were considered as nonproductive, transforming CIN, compatible with advanced CIN2/3 lesions in need of treatment.

Results: FAM19A4/miR124-2 methylation positivity increased significantly with CIN grade and age groups (<25, 25–29, and ≥30 years), while HPV16/18 positivity was comparable across age groups. FAM19A4/miR124-2 methylation positivity was HPV type independent. Methylation-positive CIN2/3 lesions had higher p16ink4a/Ki-67-immunoscores (P = .003) and expressed less HPV E4 (P = .033) compared with methylation-negative CIN2/3 lesions. These differences in HPV E4 and p16ink4a/Ki-67 expression were not found between HPV16/18–positive and non-16/18 HPV–positive lesions.

Conclusion: Compared with HPV16/18 genotyping, the QIAsure Methylation Test detects nonproductive, transforming CIN2/3 lesions with high specificity in women aged <30 years, providing clinicians supportive information about the need for treatment of CIN2/3 in young HPV-positive women.

Full genotyping and *FAM19A4/miR124-2* methylation analysis in high-risk human papillomavirus-positive samples from women over 30 years participating in cervical cancer screening in Orebo, Sweden

(Kaliff, M., et al., PLoS One 2022)

Background: HPV as a primary screening test has favorably higher sensitivity compared to cytology, which allows for longer screening intervals than cytology. However, since the specificity is low, a triage method is needed. However, cytology is a resource-intensive and subjective method of analysis, not optimal for a screening using self-sampling, which has been introduced on a larger scale. Other triage methods have been discussed, and DNA hypermethylation analysis in certain host cell genes has been suggested as a promising molecular triage method comparable to cytology. The aim of this project was to evaluate what HPV genotypes were present together with the *FAM19A4/miR124-2* methylation status in the 500 first hrHPV-positive samples from women over 30 years participating in cervical cancer screening after conversion to primary HPV testing in Örebro, Sweden.

Methods: Between October 2016 and April 2017, 7673 women over age 30 years (30–58 years) participated in cervical cancer screening in Örebro, Sweden. HPV mRNA-positive screening samples (n = 529) were included and subjected to genotyping targeting a broad range of both low-risk and high-risk genotypes in addition to hypermethylation analysis of the two human genes *FAM19A4/miR124-2*. QIAsure (QIAGEN), a multiplex-methylation–specific rtPCR method, was used to evaluate the hypermethylation status of two human host cell genes (FAM19A4 and *miR-124-2*), including one internal control gene (ACTB). Cycle threshold values of the two targets in each sample were reported in relation to the internal control and a low copy number plasmid control, the calibrator. The assay was run on the Rotor-Gene Q MDx 5plex HRM (QIAGEN) system and results were automatically analyzed with the Rotor-Gene Assay Manager software. Detected hypermethylation in any of the two targets resulted in a positive test result.

Results: LBC samples were available for hypermethylation analysis in 504 cases, and data could be obtained in 487 samples. Analytical results were positive for hypermethylation in one or both targets in 32% (158/487) and negative in 68% (329/487). Among hypermethylation-positive cases, a substantial number of positive cases were also found in the group of women with no evidence of disease (115/156, 74%). The proportion of methylation positivity increased by severity of screening outcome and probability of a positive methylation result increased with increasing age of the woman. There was a statistically significant difference in hypermethylation-positive proportion between the three age groups ($p < 0.001$).

Conclusion: HPV genotyping in this study shows evidence that a relatively large proportion of histological ≥HSILs will remain, even after age cohorts vaccinated with the quadrivalent, as well as the nonavalent, vaccine enter screening. Except for age, no HPV-related independently predictive factors for hypermethylation were found. Accordingly, age needs to be considered in development of future screening algorithms, if including triage with hypermethylation and HPV genotyping.

Direct bisulphite conversion of cervical samples for DNA methylation analysis

(Verhoef, L.,et al., Epigenetics 2022)

Background: In recent years, HPV-based screening has been adopted in several countries given a better protection against cervical cancer and precancer than cytology. With the implementation of HPV-based screening, triage testing has become important to increase specificity and positive predictive value, while retaining accurate identification of women with high-grade CIN, who require follow-up management. Epigenetic biomarkers have a strong potential to be implemented as molecular triage tool [for women that test hrHPV-positive]. Studies have reported a gradual increase in DNA methylation of specific host–cell genes with higher grade of CIN, reaching highest levels in cervical cancer. A well-studied methylation marker panel with host–cell genes *FAM19A4* (currently known as *TAFA-4*) and *miR124-2* showed good triage performance in HPV-positive women. In this current study, they compared results of ACTB control gene and methylation of *FAM19A4* and *miR124-2* genes on bisulphite-treated DNA derived from direct cell conversion to those from a protocol involving prior DNA isolation and normalization (reference protocol). Direct cell conversion protocols could further improve efficiency and considerably enhance the practicability and operations of methylation analysis in diagnostic and screening settings.

Methods: Clinician-collected cervical samples were obtained from the Scottish HPV Archive of the University of Edinburgh, Scotland. Clinician-collected cervical samples (n = 120) were subjected to a direct conversion protocol, or genomic DNA was isolated with a fixed amount used for subsequent bisulphite conversion. Converted samples were compared for ACTB control gene and methylation of *FAM19A4* and *miR124-2* genes using quantitative methylation-specific *PCRFAM19A4/miR124-2*. Methylation analysis was performed using the QIAsure Methylation Test. The housekeeping gene ACTB was used to verify DNA quality and successful bisulphite conversion. A sample was considered to have a valid test result when Cq value of ACTB was below 26.4. ΔCq values were calculated for each target separately (i.e., *FAM19A4* and *miR124-2*) as the difference between the Cq value of the target and the Cq value of the reference (ACTB). For normalization, the ΔCq value of a calibrator sample that is included in the QIAsure Methylation Test was subtracted from the ΔCq of the target resulting in a ΔΔCq value. A lower ΔΔCq value corresponds to a higher methylation level of the respective target.

Results: All 120 samples had a valid test result, with ACTB Cq values ranging from 22.53–26.11. ΔΔCq values of *FAM19A4* and *miR124-2* were significantly correlated between both protocols, with particularly strong correlation in CIN2+. Overall, there was a good agreement between ΔΔCq values obtained using the direct cell conversion protocol and the reference protocol for both *FAM19A4* and *miR124-2*. When we exploratively applied the assay's cut-off to score a sample hypermethylation-positive or -negative, the direct cell conversion protocol also showed a high agreement (108/119) with the reference protocol. The percent agreement and kappa value are well in line with an earlier study reporting on the intra- and inter-laboratory agreement of the *FAM19A4/miR124-2* methylation test.

Conclusion: We showed that a direct cell conversion protocol demonstrates a high success rate and good analytical performance on cervical samples as compared with a protocol using normalized genomic DNA as input. Direct cell conversion provides a practical workflow, and the results shown here may form the basis for effective high-throughput DNA methylation analysis to support a fully molecular solution to cervical cancer screening.

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