Development of a Bisulfite-free DNA methylation marker-based diagnostic test for cervical cancer diagnostics

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Objectives

Currently, cervical cancer screening is done by HPV-testing and cytology-based diagnostic measures (Pap test). Clarification of abnormal findings is important to limit invasive diagnostics, overtreatment and watchful waiting periods. ScreenYu Gyn® is a reliable CE-IVD test based on the detection of DNA methylation in one single marker (ZNF671). In a new development, we aim to avoid the tedious DNA bisulfite treatment, by using methylation-sensitive restriction enzymes (MSREs) to discriminate methylated from non-methylated DNA. Based on this technique, an MSRE qPCR assay was developed for the analysis of DNA methylation in the marker region ZNF671 and a control marker region, ACTB.

Methods

For this study, cervical smears from 204 women with the cytology findings NILM (n=100), CIN I (n=17), CIN II (n=17), CIN III (n=66) and CxCa (n=4) were selected and tested with the developed MSRE qPCR assay (workflow is shown in Figure 1.). For comparison, ScreenYu Gyn® test results were available for all samples.

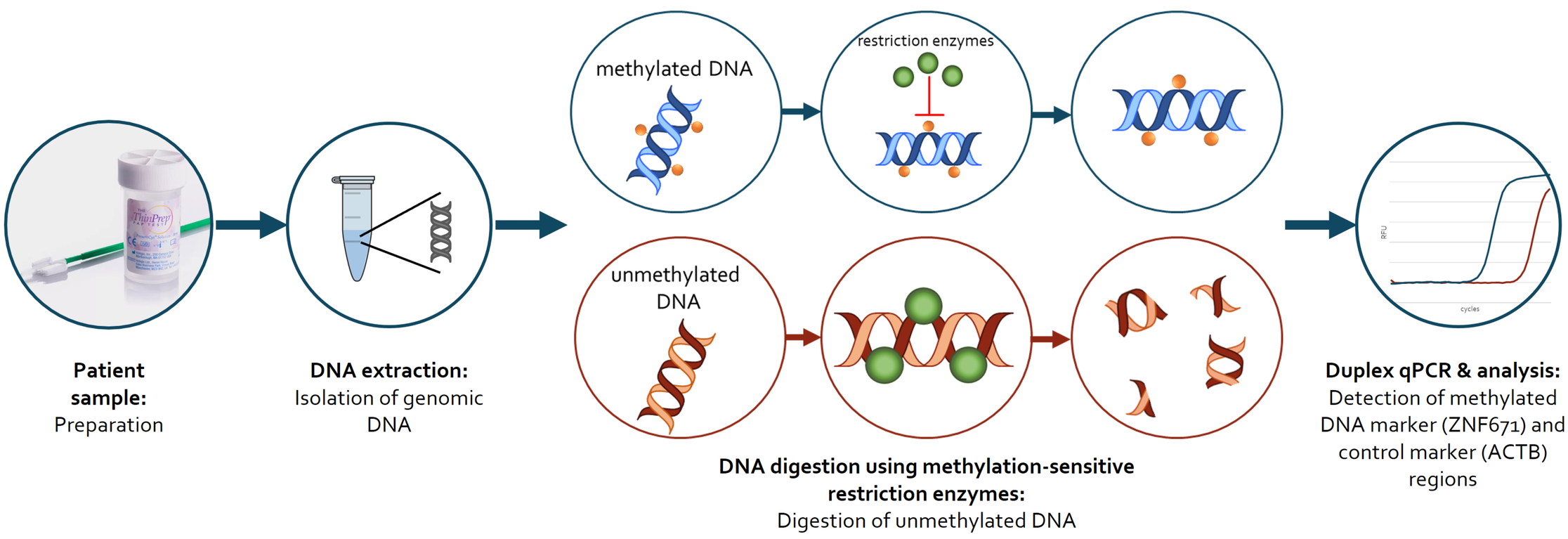

Figure 1: Workflow of the MSRE qPCR-based assay.

Results

All 204 cervical smears were tested in the ScreenYu Gyn® test and the developed MSRE qPCR-based assay. The sensitivity of both assays for CIN3+ were comparable (63% and 64%), whereas the specificity was higher for the MSRE qPCR assay (88%) compared to the ScreenYu Gyn® test (81%). The detection rate on NILM samples was lower (4%) in the MSRE assay than in the ScreenYu Gyn test (10%). On the other hand, the ScreenYu Gyn test showed higher validity for the tested samples (99% compared to 95% in the MSRE qPCR-based assay).

Table 1: Performance indicators including sensitivity, specificity, false positive rates and validity, based on the 204 cervical smear samples and shown for the MSRE qPCR-based assay and ScreenYu Gyn® test.

	Sensitivity (CIN III+)	Specificity (CIN II-)	False positive rate (NILM)	Validity
MSRE	64%	88%	4%	95%
ScreenYu Gyn®	63%	81%	10%	99%

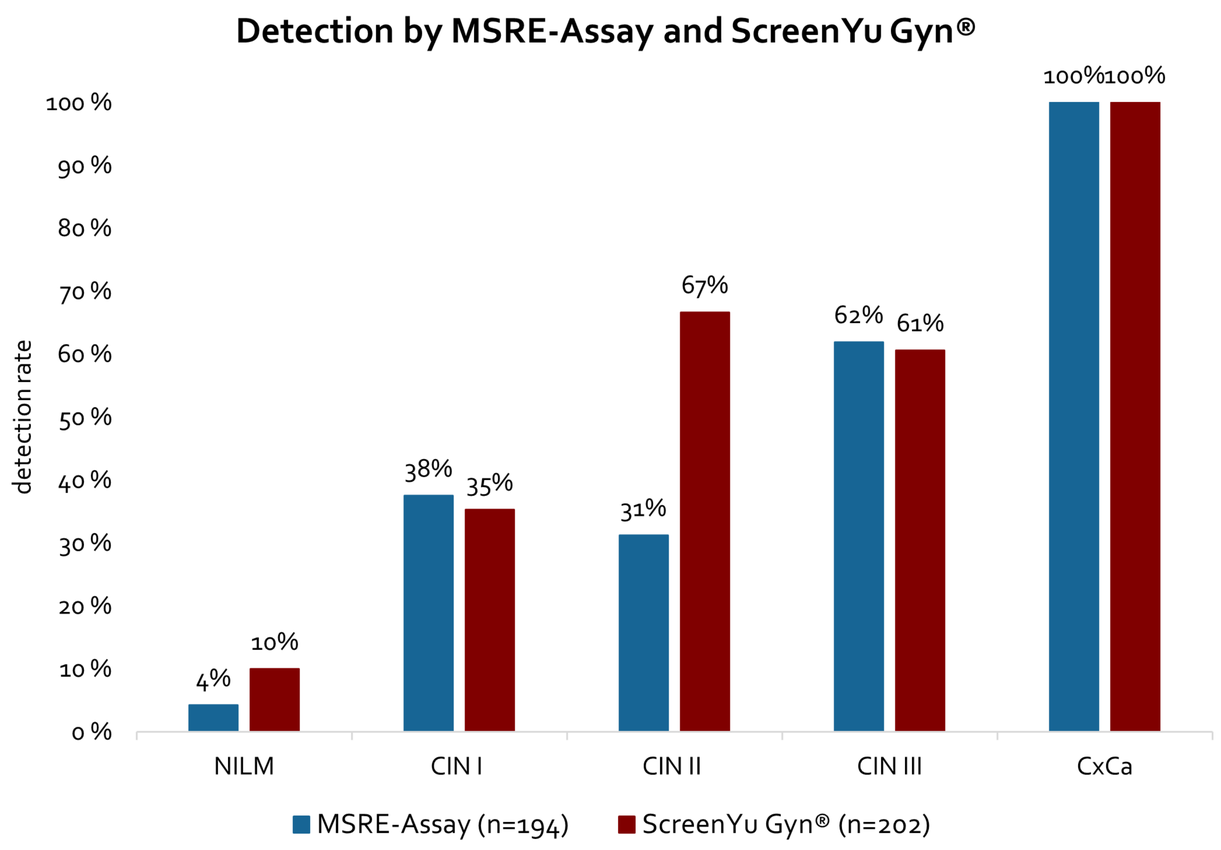

Figure 2: Detection rates [%] of the 204 cervical smears with different cytological findings. Illustrated for the MSRE qPCR-based assay and ScreenYu Gyn® test.

Conclusion

First tests using the developed MSRE qPCR-based assay showed promising results, emphasizing its potential as an alternative method for the detection of DNA methylation. In contrast to established assays based on DNA bisulfite conversion, MSRE-based methylation assays provide the opportunity for further developments such as fully automated tests for high throughput in diagnostic laboratories or Point of Care tests.

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