



Development of Urine-based DNA Methylation Assay for Prostate Cancer Screening

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1. Abstract

Introduction: The best outcome for patients with prostate cancer (PCa) is usually seen for those treated at an early stage of the disease. A digital rectal examination (DRE) and the measurement of serum prostate specific antigen (PSA) levels are the current standards for PCa early detection. However, serum PSA testing lacks both sensitivity and specificity, and histopathological examination of core biopsies frequently fail to identify small foci of PCa. The availability of diagnostic molecular tests that could allow for a more precise detection of malignant prostate cells in asymptomatic men would be of great clinical value to improve early PCa diagnosis.

Study designs: First sample set (for marker identification): 114 men scheduled to undergo a diagnostic prostate biopsy were enrolled in the study. The biopsies were triggered either by a high PSA value (4.0 – 10ng/ml) or by suspicious findings on DRE (PSA range 1.27 - 10ng/ml). Patients with other known or suspected urinary malignancy were excluded from the study. Morning, post-attentive DRE, and post-biopsy urine samples were collected from all individuals. The main goals of the first study were:

a) to determine if attentive DRE can improve the prostate-derived DNA quantity compared to urine collected in the morning or after biopsy, and

b) to evaluate the methylation status of a gene panel (single PCR reactions) in urine samples from subjects with cancer discovered by histopathology versus subjects without cancer (PSA range 1.27 – 10ng/ml)

Second sample set: 255 post-DRE urine samples were collected from 9 clinical sites of which 52 samples were from individuals with PSA levels between 2.5 and 4ng/ml. The main goal of the second study was to evaluate the methylation status of a gene panel (multiplex PCR reactions) in urine samples from subjects with moderately low PSA levels with cancer versus subjects without cancer (PSA range 2.5 – 4ng/ml).

Methods: Gene promoter methylation is associated with prostate cancer and has been successfully used for the molecular detection of adenocarcinoma cells shed into urine. We have developed real-time methylation specific PCR (MSP) assays to define the methylation status of several genes.

Results: First sample set: Evidence of PCa was found in biopsy tissue of 51% of the subjects. Histological diagnosis of the biopsies was compared to DNA methylation results in urine from 102 samples (89% success rate due to low DNA yields for 12 samples). The comparison between different urine sampling techniques showed that attentive DRE provided superior DNA yields and assay results. Best results were obtained in attentive DRE urine samples with a combination of GST-Pi, p14, p16, RARβ2 and RASSF1A resulting in a sensitivity of 74% and a specificity of 75%. Second sample set: PCa was found in 48% of the patients with PSA values ranging from 2.5 – 4ng/ml. Best results were obtained in urine after DRE with a combination of GST-Pi, APC and RARβ2 resulting in a sensitivity of 58% and a specificity of 88%.

2. Materials and methods

Figure 1 shows the workflow used in this study:

The analytes included GST-Pi, RARβ2, APC, p16, p14, RASSF1A methylated promoter sequences.

Sample preparation:

Whole urine samples (50ml) were centrifuged (3000xg, 10min) prior to DNA isolation (Puregene cell and tissue kit from Qiagen, cat#158767).

MSP:

DNA was modified using sodium bisulphite (EZ DNA Methylation™ kit, Zymo Research, cat#D5002). The analyte quantitations were done in real-time PCR assays (single PCR reactions for the first sample set, multiplexed PCR reactions for the second sample set).

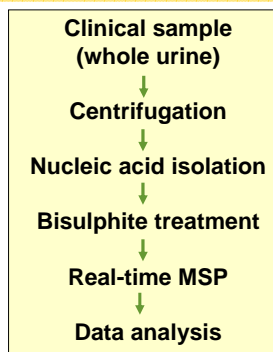


Figure 1: Workflow of the prostate urine methylation assay

3. Results

First sample set (102 individuals, different urine sampling techniques, PSA range 1.27 – 10ng/ml, single PCR reactions):

In Tables 1 and 2 the test results according to Figure 1 are shown. Best results were obtained from urine collected after attentive DRE as compared to morning and post prostate biopsy urine samples (example GST-Pi, Table 1). Combination of GST-Pi, p14, p16, RARβ2 and RASSF1A resulted in a sensitivity of 74% and a specificity of 75% in post-attentive prostate massage urine samples (see Table 2).

GST-Pi (PSA range 1.27 – 10ng/ml)	Sensitivity [%]	Specificity [%]
Post-attentive DRE urine	41	92
Morning urine	18	81
Post biopsy urine	32	90

Table 1: Performance of GST-Pi in post-attentive DRE, morning and post prostate biopsy urine samples from subjects with cancer versus subjects without cancer (PSA range 1.27–10 ng/ml).

Gene panel (PSA range 1.27 – 10ng/ml)	Sensitivity [%]	Specificity [%]
Post-attentive DRE urine	74	75

Table 2: Performance of GST-Pi, p14, p16, RARβ2 and RASSF1A gene panel (single PCR reactions) in post-attentive DRE urine samples from subjects with cancer versus subjects without cancer (PSA range 1.27 – 10ng/ml).

Second sample set (52 individuals, post-DRE urine, PSA range 2.5 – 4ng/ml, Multiplex PCR reactions):

In Table 3 the test results according to Figure 1 are shown. The combination of GST-Pi, RARβ2 and APC using a multiplex PCR reaction resulted in a sensitivity of 58% and a specificity of 88% in post-DRE urine samples (PSA range 2.5 – 4ng/ml).

Gene panel (PSA range 2.5 – 4 ng/ml)	Sensitivity [%]	Specificity [%]
Post-DRE urine	58	88

Table 3: Performance of GST-Pi, RARβ2 and APC gene panel (multiplex PCR reactions) in post-DRE urine samples from subjects with cancer versus subjects without cancer (PSA range 2.5 – 4ng/ml).

4. Conclusions

1. Urine collected after an attentive DRE provides better DNA methylation assay performance compared to post prostate biopsy and morning urine samples
2. A prototype prostate methylation urine assay has been developed using multiplexed real-time MSP
3. In this study the prototype assay demonstrated a sensitivity of 58% and a specificity of 88% in post-DRE urine samples (PSA range 2.5 – 4ng/ml)

Future work: Further refinement and development of the final sample processing and assay procedures followed by large scale prospective clinical validation trials

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