Presentation of a novel technology that stabilises Prostate-Specific Antigen (PSA) mRNA directly from urine: proof of concept for multiplexed RNA-based cancer tests in urine.

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INTRODUCTION

PSA protein is produced by cells of the prostate gland. Currently-available PSA tests measure the level of PSA protein in blood and report results as nanograms of PSA per millilitre (ng/mL. PSA levels >4ng/ml prompt further investigation and treatment frequently leading to unnecessary biopsies. Over-expression of PSA coding gene is widely regarded as indicative of tumour development, although current tests are not fit for the clinical setting and require urine samples to be frozen immediately to have any success of measuring mRNA expression.

This work describes a novel technology for processing urine samples that couples homogenisation of the urine specimens with extraction and stabilisation of the genomic contents (RNA and DNA). The proof of concept for this technology shows the viability of identifying the presence of PSA mRNA directly in urine, while providing evidence of stability for all genomic contents through an extended time course. Parallel detection of other genetic markers such as PCA3 and Beta-actin and through qPCR (gDNA) and RTqPCR (mRNA and gDNA) from clinical specimens subjected to this novel technology hints to the next step in prostate cancer diagnosis via the multiplexed detection of synergistic oncogenic marker.

RESULTS

RTqPCR results for extended time courses assayed at 4 °C. and room temperature:

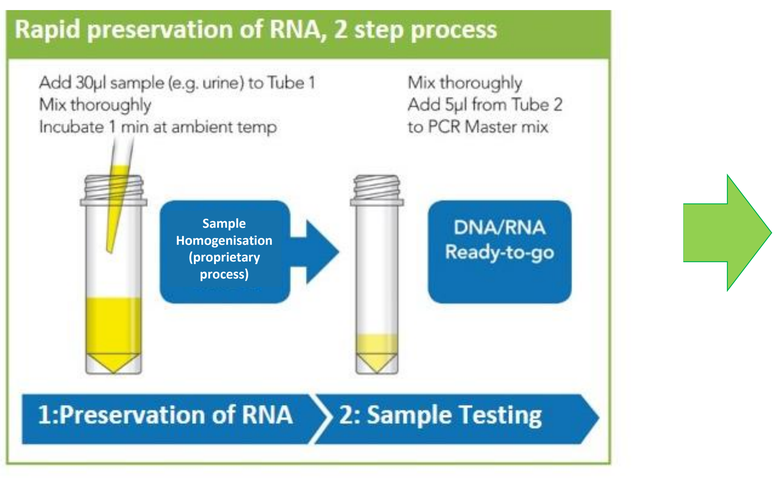

Figure 1: Rapid sample processing. Urine samples were collected in preservation reagent immediately prior to storage for stability assessment

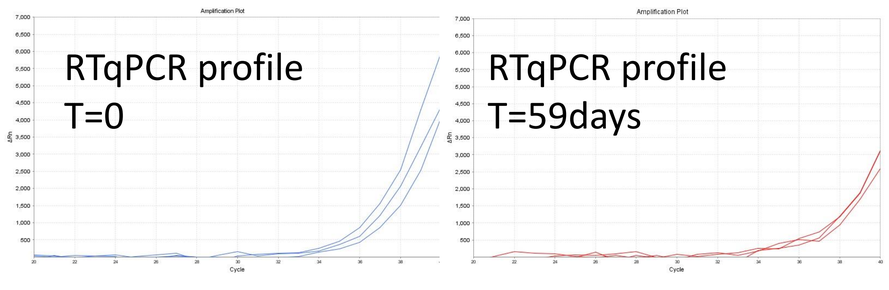

Figure 2: qRT-PCR results
Extracted mRNA kept at room temperature, an illustration of typical results, tested via qRT-PCR (Taqman chemistry)

Table 1: PSA mRNA stability testing Storage at 4°C

Timepoint	qPCR (Ct)	RTqPCR (Ct, avg CT)	
0 hrs.	UND	34.47	35.57(2)
	UND	UND	
	UND	36.67	
1 day	UND	37.19	36.65
	UND	36.14	
	41.4	36.61	
4 days	UND	38.7	36.54
	UND	34.15	
	UND	36.77	
7 days	UND	36.78	37.34
	UND	38.94	
	UND	36.31	
25 days	UND	35.69	35.89
	UND	35.44	
	UND	36.55	

Table 2: PSA mRNA stability testing Storage at ambient temperature (18-22°C)

Stabilisation Buffer	Time Elapsed (days)	Reaction Type	Ct values
Arcis Reagent	0	qPCR	UND
	0	RTqPCR	24.6, 25.76, 25.78, 25.44
Water	0	qPCR	UND
	0	RTqPCR	25.63, 25.9, 25.46, 25.62
Arcis Reagent	6	qPCR	UND
	6	RTqPCR	24.8, 24.57, 25.19, 24.45
Water	6	qPCR	UND
	6	RTqPCR	UND
Arcis Reagent	36	qPCR	UND
	36	RTqPCR	30.6, 31.01, 31.29, 29,78
Water	36	qPCR	UND
	36	RTqPCR	UND

Table 1 and Table 2 show data from Urine samples stored in Arcis preservation reagent. De-protection of the sample for molecular analysis is a single pipette step into Wash Buffer

METHODS

Free mRNA was isolated from fresh urine samples by a variation of the standardised ARCIS 3-minute extraction process, with the inclusion of a proprietary homogenisation step [Arcis Sample Prep Kit Ref UFL002]. Oligonucleotide primers and TaqMan probes for specific RTqPCR reactions were designed for the different gene-markers using proprietary knowledge and ordered from IDT.

All RTqPCR reactions were performed in parallel under the same cycling conditions in the Applied Biosystems STEP ONE Plus system and Ct values were obtained after ROX normalisation with no modification of the automatic thresholds.

All amplicons were subjected to Sanger sequencing, and assessed for specificity via BLAST alignment (BLASTn, NCBI) with 100% homology in human contigs as the default threshold.

CONCLUSIONS

This initial piece of work shows compliance of the ARCIS rapid RNA and DNA extraction process from urine with long term stability of mRNA at 4°C. and room temperature. The sample prep protocol was completed in less than 10 minutes, allowing detection of all genetic markers by standard RTqPCR. Further analysis of PCR products provided both, confirmation of specificity and re-assessment of this approach. Data from this work prompts to envisage a new standard for clinical diagnostic in urine samples.