DNA isolation from Whatman FTA® Mini Cards as a high-throughput method for genotyping of Glutathione S - transferase P1 polymorphisms in workers with asbestosis

Franko A[1], Toplak N[2], Dodić-Fikfak M[1], Dolžan V[3]

[1]Clinical Institute of Occupational Medicine, University Medical Centre, Ljubljana, Slovenia
[2]Omega d.o.o., Dolinškova 8, Ljubljana, Slovenia
[3]Institute of Biochemistry, Faculty of Medicine, Ljubljana, Slovenia

INTRODUCTION AND THE AIM OF THE RESARCH

Molecular-epidemiological studies in which a large number of samples needs to be analysed require a rapid and high-throughput method for DNA isolation from biological samples. Different approaches are used for collection of samples for molecular-epidemiological studies such as buccal smears, mouth washes or filter cards. In our study we used Whatman FTA® Card (Whatman Bioscience) technology, which allows room temperature collection, shipment, archiving and purification of nucleic acid from biological samples for PCR analysis. We tested different protocols for DNA extraction from the Whatman FTA® cards using BloodPrep chemistry on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems) for amplification efficiency of extracted DNA and for compatibility with a downstream high sample throughput SNP genotyping with real-time PCR analysis.

Our aim was to develop a rapid and high-throughput method for DNA isolation from Whatman FTA® Card (Whatman Bioscience) suitable for genotyping of Glutathione S-transferase P1 (GSTP1) polymorphisms in workers with asbestosis. Asbestos related diseases are among the most extensively studied occupational diseases and although the causal relationship between asbestos exposure and pulmonary diseases was well demonstrated, relatively little is known about the genetic factors involved in the susceptibility of the exposed individuals to development of these conditions.

DISCUSSION

As Whatman FTA® Cards are very convenient for collecting and archiving blood samples our aim was to develop a rapid and high-throughput method for DNA isolation from Whatman FTA® Card suitable for genotyping of Glutathione S-transferase P1 (GSTP1) polymorphisms in workers with asbestosis. We developed and validated a method for DNA extraction from the Whatman FTA® Cards using BloodPrep chemistry on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems) that allowed for rapid DNA isolation in a 96-well plate format compatible with a high- throughput SNP analysis.
Other SNPs that may modify the risk for asbestosis can be analyzed by conventional or real-time PCR from the eluted DNA stored at 20°C without the need for the repeated DNA isolation.

MATERIAL AND METHODS

Blood samples
For isolation of DNA and subsequent genotyping capillary blood samples from finger tips have been collected on Whatman FTA® Card from 700 study subjects employed in the asbestos cement manufacturing plant of Salonit Anhovo, Slovenia. Among them 350 subjects were diagnosed with asbestosis while the control group of 350 sex and age matched exposed subjects did not develop asbestosis.

Extraction of DNA from Whatman FTA® Cards
For each subject 15 excision circles were punctured from the corresponding Whatman FTA® Card. We tested six different protocols for DNA extraction from the Whatman FTA® Cards and two different protocols for negative control. All were using BloodPrep chemistry on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems). The protocols are outlined in Table 1. In two protocols (3 and 5) the excision circles were included during the extraction with the Genomic DNA Purification Tray 2. In other protocols only nucleic acids eluted from the excision circles during the Pre-Step procedure were applied on Genomic DNA Purification Tray 2. Our approach allowed for rapid DNA isolation in a 96-well plate format.

Protocol No.	Method BloodPrep	1	2	3	4	5	6	7	8
Pre-step	15 cut of circles from Whatman FTA® Cards in 1.5ml microcentrifuge tube	+	+	+	+	+	+	-	-
	Add Proteinaze K solution (20mg/ml) and PK Digestion Buffer	+	+	+	+	+	+	-	-
	Incubation 10min -58şC	+	-	-	+	+	-	-	-
	Add BloodPrepDNA Purification Solution	+	+	+	+	+	+	-	-
ABIPRISM™ 6100 Nucleic Acid PrepStation	Genomic DNA Purification Tray 2	-	+	+	+	+	-	-	+
	Original excision circles from Whatman FTA® Cards added to tray	-	-	+	-	+	-	-	-
	Add BloodPrep DNA Purification Solution	+	+	+	+	+	+	-	-
	3 X Add BloodPrep DNA Wash Solution	+	+	+	+	+	+	-	-
	Elution Solution 1	+	+	+	+	+	+	+	+
	Elution Solution 2	+	+	+	+	+	+	+	+
	The final volume of isolated DNA was 100µl.								

Tabela 1: The protocol of DNA isolation from Whatman FTA® Cards using BloodPrep chemistry on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation.
Legend:
+ : used solutions, step of extraction or instrument
- : non-used solutions, step of extraction or instrument

The efficiency of different isolation protocols was analyzed by real-time PCR using Applied Biosytems ABI PRISM Pre-Developed TaqMan® Assay Reagent - Ribosomal RNA Control, using a reaction volume of 10µl with ABI PRISM 7900HT instrument (Applied Biosystems).

Discrimination Assays Work
GSTP1 genotyping for Ile105Val and Ala114Val polymorphisms was carried out using Custom TaqMan® SNP Genotyping Assays according to the manufacturer's instructions using a reaction volume of 5µl in 384-well plate on ABI PRISM 7900HT instrument .

RESULTS

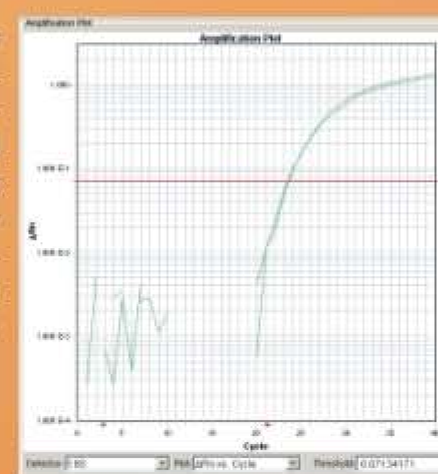
The efficiency of DNA isolation using the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation

The results of the amplification of Ribosomal RNA control from DNA samples isolated with different extraction protocols using BloodPrep chemistry on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation are shown in Table 2. Four protocols yielded amplifiable DNA. Among them protocol No 4 was chosen as the most suitable for a rapid isolation in a 96-well plate format as this protocol used only the eluate from the Pre-Step Procedure and did not include the transfer of excision circles from Whatman FTA® Cards to the Genomic DNA Purification Tray 2 AB tray. The amplification plots of a representative sample extracted by protocols 4 and 8 (negative control) are shown in Figures 1a and 1b, respectively. The succesful amplification of the Ribosomal RNA control proved that the developed extraction protocol allows a simple removal of potential PCR inhibitors and elution of DNA from Whatman FTA® Card for downstream real-time PCR analysis.

Protocol	1	2	3	4	5	6	7	8
Amplification of Ribosomal RNA Control	-	+	++	++	++	-	-	-

Table 2. The results of amplification of Ribosomal RNA Control from DNA extracted with BloodPrep Chemistry from Whatman FTA® Cards using different protocols.
Legend: The numbers of the protocols corrspond to the protocols outlined in Table 1.
+ : positive amplification
- : negative amplification

Figure 1a) Positive result of real-time PCR using PDAR - Ribosomal RNA Control with isolated DNA using BloodPrep chemistry (No. 4) on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation.



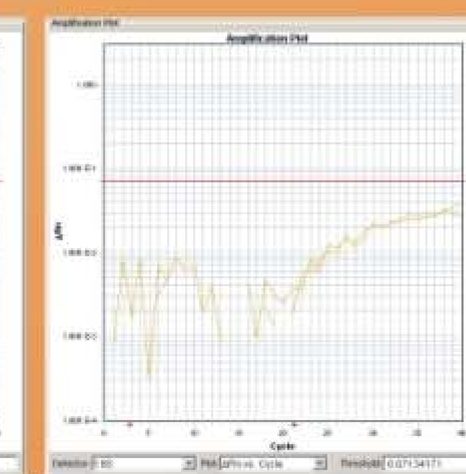


Figure 1b) Negative result of real-time PCR using PDAR - Ribosomal RNA Control with isolated DNA using BloodPrep chemistry (No. 8) on the semi-automated ABI PRISM™ 6100 Nucleic Acid PrepStation.

Genotyping of Glutathione S-transferase P1 polymorphisms

So far 540 asbestos exposed workers were genotyped for Ile105Val (GSTP1) and Ala114Val (GSTP3) polymorphisms. The genotype distribution for both polymorphisms is shown in Table 3. No amplification product was obtained in 7 (1.3%) samples, while GSTP1 alone did not amplify in 25 (4.6%) and GSTP3 alone did not amplify in 33 (6.1%) samples.

GSTP1	*N*	*%*	GSTP3	*N*	*%*
Ile/Ile	242	47,0	Ala/Ala	407	80,3
Ile/Val	225	43,7	Ala/Val	92	18,1
Val/Val	48	9,3	Val/Val	8	1,6
Total	515		*Total*	507	

Table 3: GSTP1 genotypes of asbestos exposed workers

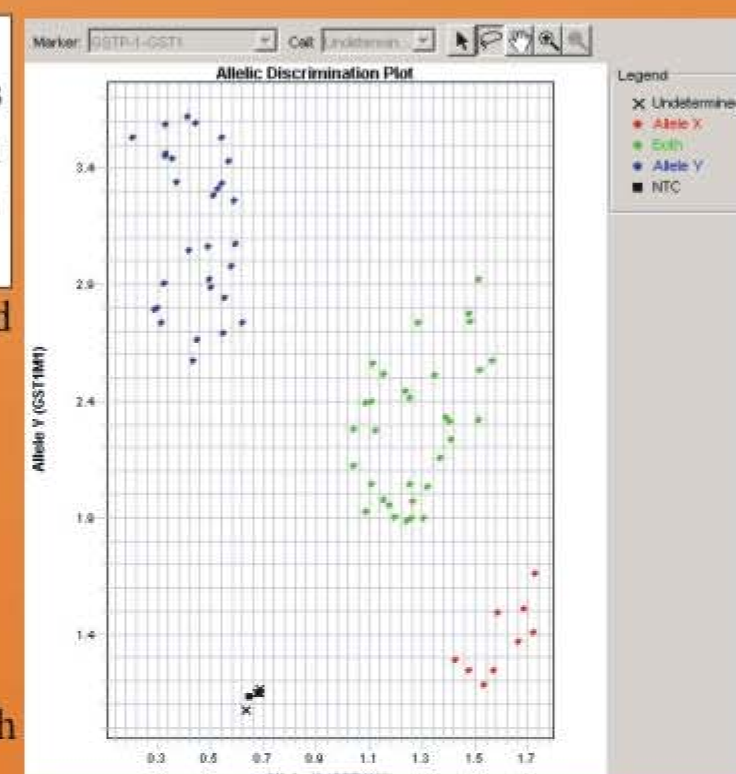


Figure 2: Allelic discrimination results with flourogenic probes in TaqMan assay