



Sarstedt S-Monovette[®] DNA Exact – A New IVD-Certified Blood Collection Tube for Collection, Transport and Stabilization of Whole Blood for Genomic DNA Analysis.

Kinitz T., Kämper M. & Flach D.

Sarstedt AG & Co., Research & Development, Nümbrecht, Germany

Abstract

To address the need for a regulatory proven and certified blood collection tube for the preservation of genomic DNA in whole blood, Sarstedt has developed the IVD-certified S-Monovette[®] DNA Exact. Whole blood can be stored within the tube for at least 14 days at room temperature, five days at 35°C, 28 days at 4°C and at least three months at -20°C (study ongoing) while maintaining DNA integrity. Furthermore, up to five freeze/thaw cycles were performed without affecting the DNA in quantity and quality for further processing. Thus, whole blood for genomic DNA analysis can be stored and transported within the S-Monovette[®] DNA Exact and DNA integrity and DNA concentration are maintained even under critical transport conditions, e.g. uncooled car transport in summer, until DNA isolation and analysis by molecular diagnostic assays are performed.

Introduction

In the past decades, molecular biological diagnostics based on the analysis of deoxyribonucleic acid (DNA) from whole blood have become standard tools in clinical diagnostics. Due to the non-invasive and contamination free sample collection by using a modern blood collection system like the Sarstedt S-Monovette[®], whole blood is the first choice sample material for clinical diagnostics based on DNA, namely for the identification of genetic disorders, paternity testing, human leucocyte antigen (HLA) typing and many more. With the rapidly increasing importance of molecular diagnostics in the clinical routine, the formation of large diagnostic laboratories, the emerging number of diagnostic methods as well as the rising number of biobanks which store whole blood for retrospective DNA analyses, the need for standardized and regulatory proven methods and products increased dramatically. Finally, this progress led to the *In Vitro Diagnostics Directive* (IVDD) [1] and the IVD certificate to identify proven products for in vitro diagnostics. For clinical diagnostics based on DNA from whole blood, the first critical points in the workflow are the blood collection (1), transport (2) and storage (3) until further processing, namely DNA isolation, polymerase chain reaction (PCR),

sequencing and many more. Up to now, the importance of these pre-analytical challenges has poorly been investigated in the context of genetic testing [2]. However, the experiences from biobanks which operate with strict internal standard operation procedures (SOPs) reveal a big impact of the choice of the respective blood collection tube and the storage conditions on quality of nucleic acid based molecular diagnostics [3, 4, 5]. The storage and transport of whole blood has been investigated in several studies [e.g. 6, 7, 8] but up to date any strict rules concerning the transport and storage of whole blood were set. The need for standardization and quality standards in this context was recently discussed within the EC founded project SPIDIA [9, 10] and within the U.S. National Cancer Institute (NCI, Maryland) [11].

To face these pre-analytical challenges (1-3) and to address the need of clinical diagnostics, biobanks and researchers for a certified blood collection tube, Sarstedt has developed the IVD certified S-Monovette[®] DNA Exact, a blood collection system containing a proprietary EDTA-based solution which anticoagulates the blood and preserves genomic DNA by inhibiting DNases. In this paper we demonstrate that the S-Monovette[®] DNA Exact preserves genomic DNA in whole blood for at least 14 days at room temperature, 28 days at 4°C, up to five days at 35°C

Correspondance address:

Sarstedt AG & Co. | Sarstedtstraße 1 | 51588 Nümbrecht | Germany

Mail: info@sarstedt.com | Phone: (+49) 0 22 93 305 0 | Fax: (+49) 0 22 93 305 24 70

For further information on our products please visit: www.sarstedt.com | © 2015



and at least three months at -20°C (study ongoing) as well as after up to five freeze/thaw cycles of the blood within the S-Monovette® DNA Exact.

Materials & Methods

Blood Collection & Storage

Blood from 20 healthy donors (7 female, 13 male) was drawn in S-Monovette® DNA Exact blood collection tubes (Cat. No. 04.1948.001, Sarstedt AG & Co., Nümbrecht, Germany) (Tab. 1). To estimate possible differences between samples from same donors, e.g. due to pipetting errors or variances within the isolation step, each sample was collected, isolated und analyzed in duplicate.

Tab.1 Number of donors and storage conditions until DNA isolation [d = days, m = months].

Number of donors	Storage conditions	Tubes per donor / total tubes
20	Room temperature (22°C) [0d, 3d, 7d, 14d]	8 / 160
20	4°C [14d, 1m]	4 / 80
20	-20°C [1m, 3m]	10 / 200
20	35°C [3d, 5d]	4 / 80
20	-20°C + freeze-thaw cycles [3x, 5x]	4 / 80

White blood cell content

The amount of white blood cells (WBC) in whole blood of each donor was determined using a pocH-100i hematology analyzer (Sysmex, Kobe, Japan) directly after blood collection according to manufacturers protocol.

DNA isolation

Stored whole blood samples in S-Monovette® DNA Exact were vortexed prior to DNA isolation and genomic DNA was isolated from $400\ \mu\text{l}$ whole blood using the innuPREP Blood DNA Mini Kit (Cat. No. 845-KS-1020250, Analytik Jena Innuscreen, Berlin, Germany) according to manufacturers protocol. DNA eluates were stored in 1.5 ml DNA LowBind Micro tubes (Cat. No. 72.706.700, Sarstedt, Nümbrecht, Germany) at 4°C until further processing as described below.

DNA quantity & purity

DNA quantity was determined by measuring absorbance at 260 nm and the quality of eluates was estimated by determining the 260 nm/280 nm ratio with a BioPhotometer plus (Eppendorf, Hamburg, Germany) according to manufacturers protocol.

Realtime PCR

To investigate genomic DNA preservation over time (Tab.1), realtime PCRs of a 500 bp GAPDH fragment (fw: $5^{\prime}\text{-ggtgaaggctcggagtcacg-3}^{\prime}$; rv: $5^{\prime}\text{-caaagttgtcatggatgacc-3}^{\prime}$, BioSpring, Frankfurt a.M., Germany) were conducted and Cq values were determined to demonstrate that template DNA concentration is stable over indicated time points. All realtime PCRs were performed with the Maxima SYBR Green/ROX qPCR Master Mix (Cat. No. #K0222, Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to manufacturer's protocol with eluates diluted 1:1 with PCR grade water on a Mastercycler ep realplex 4S (Eppendorf, Hamburg, Germany). Primers were used in a final concentration of $0.5\ \mu\text{M}$. After an initial denaturation of 10 minutes at 95°C , 40 cycles of denaturation (15 sec. at 95°C), annealing (15 sec. at 58°C) and elongation (20 sec. at 68°C) were performed.

HLA typing

Human leucocyte antigen (HLA) typing of HLA DR and HLA DQ alleles was conducted using the HISTO TYPE DR/DQB Kit (Cat. No. 710311, BAG Health Care, Lich, Germany) according to manufacturers protocol. The Kit is based on the principle of sequence specific primer (SSP) PCR. Gel electrophoresis & gel documentation were performed with standard agarose gel electrophoresis equipment (Biometra, Göttingen, Germany) as proposed in the kit handbook. Briefly, $14 \times 14\ \text{cm}$ sized, 2 % agarose gels were prepared with NEEO Ultra-Quality Agarose in 0.5x TBE buffer and ethidiumbromide (Cat. No. 2267.5, Cat. No. 3061.1 & Cat. No. 2218.1, Carl Roth, Karlsruhe, Germany) was added for DNA staining. Gels were run for 35-40 min. at 200 V.

Data analysis

Median and the 25%-75% interquartile range of Cq values were calculated to present data with respect to its biological variations (inter donor variations in white blood cell content).

Results

The amount of white blood cells varied from donor to donor. Thus, the mean DNA concentration varied as well with an average concentration of $87.3\ \text{ng}/\mu\text{l}$ eluate and an overall concentration range of 29.5 to $162.3\ \text{ng}/\mu\text{l}$ eluate (Tab.2). Manual DNA extraction with the innuPREP Blood DNA mini Kit resulted in highly pure DNA eluates. The DNA purities, determined photometrically as ratio 260 nm/280 nm, were within the manufacturers reference values of 1.7

- 2.0 with a mean of 1.83 and a very low standard deviation of 0.08 (Tab.2 & Fig.1).

Tab.2 DNA specific sample characteristics of all analyzed samples.

Sample characteristics	Measurement
Mean amount of WBC \pm SD (n = 40)	5.8 ± 1.6 [$\times 10^3$ / μ l whole blood]
Mean DNA concentration \pm SD (n = 480)	87.3 ± 23.9 [ng/ μ l eluate]
Concentration range (n = 480)	29.5 – 162.3 [ng/ μ l eluate]
Mean ratio 260 nm / 280 nm \pm SD (n = 480)	1.83 ± 0.08

The stability of DNA in whole blood stored as described (Tab.1) was determined by realtime PCR (Fig. 2).

Cq values did not increase in samples which were stored at room temperature, fridgerated at 4°C or frozen at -20° or after up to 5 freeze-thaw cycles. Incubation at 35°C led to the tendence of a slight increase of the Cq values, but were far away from statistical relevance.

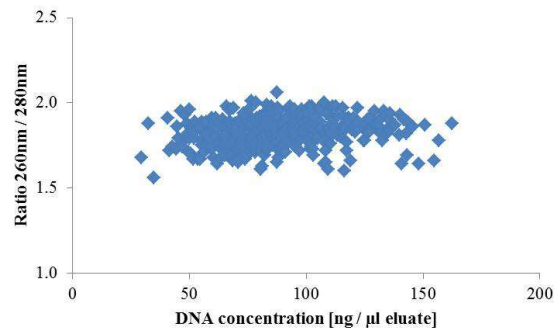
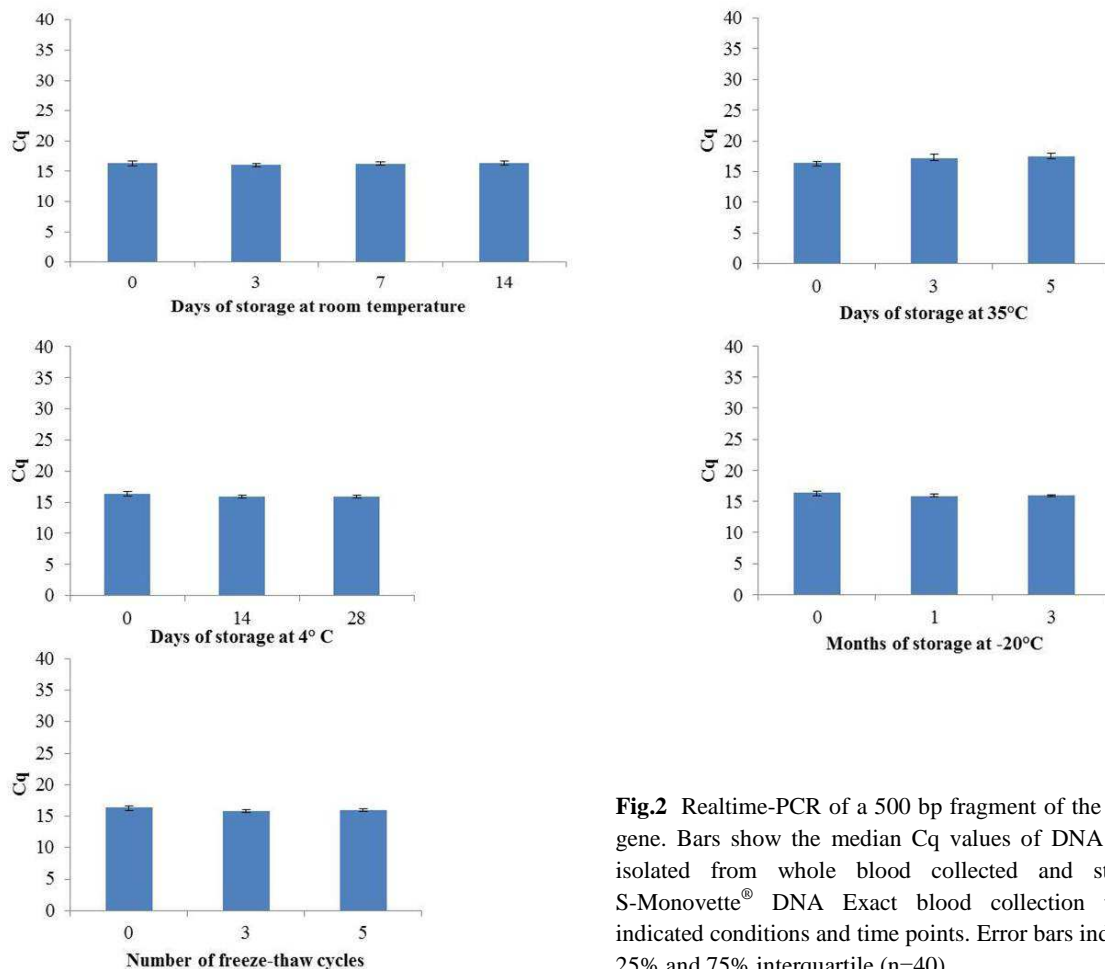


Fig.1 DNA concentration and 260 nm/280 nm ratio (n = 480).

Typing of donor specific HLA DR and HLA DQ alleles was performed with samples from different donors, with samples from the starting point (day 0) and with samples of the endpoint of the different storage conditions (14 days at room temperature, five days at 35°C; 28 days at 4°C, 5 freeze-thaw cycles and 3 months at -20°C). All analyzed samples showed clear and distinct bands in the gel electrophoresis (examples are shown in Fig.3). The control bands which indicate a positive PCR reaction were present in all lanes. All contamination controls were negative.

Fig.2 Realtime-PCR of a 500 bp fragment of the GAPDH gene. Bars show the median Cq values of DNA samples isolated from whole blood collected and stored in S-Monovette® DNA Exact blood collection tubes at indicated conditions and time points. Error bars indicate the 25% and 75% interquartile (n=40).

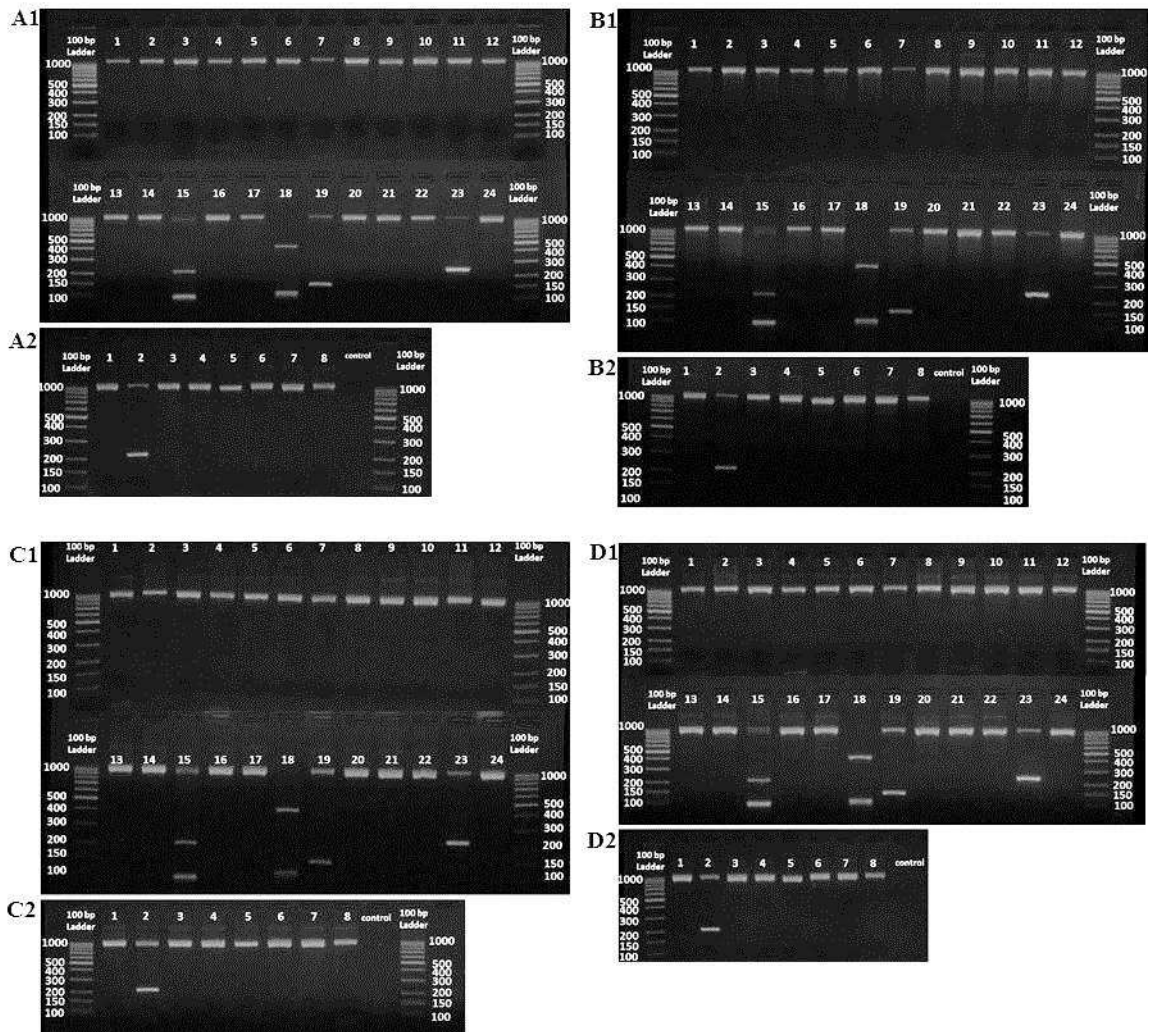


Fig.3 HLA typing of HLA alleles DR (A1-D1) and DQ (A2-D2) in samples from one donor after different storage conditions: (A) room temperature, 0d / (B) room temperature, 14d / (C) 35°C, 5d / (D) 5 x freeze-thaw cycles. The 1070 bp band serves as control for a successful PCR reaction (434 bp for lane 18).

No problems of band identification or mismatches occurred and all analyses of the different sample storage conditions led to the same donor specific band pattern.

Discussion

The identification of pre-analytical factors which affect the DNA yield and quality from whole blood samples has been discussed since the late 1980s. Gustafson et al. [12] concluded that the choice of anticoagulant, incubation time and temperature as well as the number of freeze-thaw cycles are the main pre-analytical parameters, whereas other authors mainly focused on the storage and transport conditions of whole blood prior to DNA extraction [6, 13]. Such studies on the storage conditions of whole blood came up from time to time and most of the authors concluded that blood storage at 4°C or -20°C lead to comparable DNA yield and bias-free qualities

[7, 14]. Repeated freeze-thawing as well as storage temperatures above room temperatures were found to have negative effects on the DNA yield and integrity [e.g. 5, 15, 16].

In the present study, we show that DNA from whole blood collected with the Sarstedt S-Monovette® DNA Exact is preserved at least for 14 days at room temperature, 5 days at 35°C, 28 days at 4°C and at least 3 month at -20°C (study ongoing) as well as after five freeze-thaw cycles. Although, in realtime PCR typically small amplicons up to 220 bp are analyzed, here consciously a 500 bp GAPDH fragment was chosen to improve the detection of possible DNA-fragmentation upon the storage conditions. As the probability of DNA-fragmentation increases in proportion to the DNA-strand length, we expected a more susceptible detection - displayed by failed PCR reactions - using this exceedingly long amplicon. We observed a slight increase of the Cq values in the samples which were stored at 35°C

(Fig.2). However, this increase had no impact on the HLA typing assay we performed. We therefore conclude that the DNA yield may be slightly decreased in those samples but the DNA integrity was not affected and HLA typing still worked out well. Besides the pre-analytical factors mentioned above, many other sources of pre-analytical variations were identified [17]. Nevertheless, the choice of the DNA extraction method plays the most crucial role affecting the yield and purity of DNA from whole blood samples. We show that high yields of highly pure DNA were isolated from whole blood samples collected with the S-Monovette® DNA Exact (Tab.2 & Fig.2) using a spin column based extraction kit which is probably the most common DNA extraction method. Due to the proprietary EDTA based solution, the S-Monovette® DNA Exact can be combined with any method or kit validated for EDTA anticoagulated whole blood. For further information on extraction methods for DNA from whole blood, recently published reviews give overviews [18, 19].

The choice of EDTA as anticoagulant has major advantages compared to other anticoagulants. EDTA does not inhibit Taq polymerases as shown for heparin anticoagulated blood [20,21]. Furthermore, EDTA inhibits DNA degradation by DNases effectively [22].

With the development of the IVD certified S-Monovette® DNA Exact, Sarstedt contributes to improvement of standardization in clinical diagnostics, biobanking and research.

References

- [1] Council Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices. *Official Journal of the European Union*, L331 (December 12, 1998).
- [2] Ravine D., Suthers G., (2012) Quality Standards and Samples in Genetic Testing. *Journal of Clinical Pathology*, 65, 389-393.
- [3] Palmirotta R., Ludovici G., De Marchis M.L., Savonarola A., Leone B., Spila A., De Angelis F., Della Morte D., Ferroni P., Guadagni F. (2011) Preanalytical Procedures for DNA studies: The Experience of the Interinstitutional Multidisciplinary Biobank (BioBIM). *Biopreservation and Biobanking*, 9, 34-45.
- [4] Shabihkhani M., Lucey G.M., Wei B., Mareninov S., Lou J.L., Vinters V., Singer E.J., Cloughesy T.F., Yong W.H. (2014) The Procurement, Storage, and Quality Assurance of Frozen Blood and Tissue Biospecimens in Pathology, Biorepository, and Biobank Setting. *Journal of Clinical Biochemistry*, 47, 258-266.
- [5] Charde M.S., Ingole P., Welankawir A.S., Kumar J., Chakole R.D. (2014) The Procurement, Storage and Quality Assurance of Frozen Blood and Tissue Biospecimens. *International Journal of Pharmacological Research*, 4 (2), 49-52.
- [6] Hankinson S.E., London S.J., Chute C.G., Barbieri R.L., Kaplan L.A., Sacks F.M., Stampfer M.J. (1989) Effect of Transport Conditions on the Stability of Biochemical Markers in Blood. *Clinical Chemistry*, 35 (12), 2313-2316.
- [7] Steinberg K.K., Sanderlin K.C., Ou C., Hannon W., McQuillan, G.M., Sampson E.J. (1997) DNA Banking in Epidemiologic Studies. *Epidemiologic Reviews*, 19 (1), 156-162.
- [8] Meini G., Materazzi A., Saladini F., Rosi A., Vicenti I., Mancini M., Pirazzoli A., Caudai C., Zazzi M. (2013) Stability of Unfrozen Whole Blood DNA for Remote Genotyping Analysis of HIV-1 Coreceptor Tropism. *BMC Infectious Diseases*, 13 (508).
- [9] Malentacchi F., Pazzagli M., Simi L., Orlando C., Wyrich R., Hartmann C.C., Verderio P. (2013) SPIDIA-DNA: An External Quality Assessment for the Pre-analytical Phase of Blood Samples Used for DNA based analyses. *Clinica Chimica Acta*, 424, 274-286.
- [10] Malentacchi F., Ciniselli C.M., Pazzagli M., Verderio P., Barraud L., Hartmann C.C., Pizzamiglio S. (2015) Influences of Pre-analytical Procedures on Genomic DNA Integrity in Blood Samples: The SPIDIA experience. *Clinica Chimica Acta*, 440, 205-210.
- [11] Engel K.B., Vaught J., Moore H.M. (2014) National Cancer Institute Biospecimen Evidence-Based Practices: A Novel Approach to Pre-analytical Standardization. *Biopreservation and Biobanking*, 12 (2), 148-150.
- [12] Gustafson S., Proper J.A., Bowie E.J.W., Sommer S. (1987) Parameters Affecting the Yield of DNA From Human Blood. *Analytical Biochemistry*, 165, 294-299.
- [13] Madisen L., Hoar D.I., Holroyd C.D., Crisp M., Hodes M.E. (1987) DNA Banking: The Effects of Storage of Blood and Isolated DNA on the Integrity of DNA. *American Journal of Medical Genetics*, 27, 379-390.
- [14] Richardson A.J., Narendran N., Guymer R.H., Vu H., Baird P.N. (2006) Blood Storage at 4°C – Factors Involved in DNA Yield and Quality. *Journal of Laboratory and Clinical Medicine*, 147 (6), 290-294.
- [15] Cushwa W.T., Medrano J.F. (1993) Effects of Blood Storage Time and Temperature on DNA Yield and Quality. *BioTechniques*, 14 (2), 204-207.
- [16] Lahiri D.K., Schnabel B. (1993) DNA Isolation by a Rapid Method from Human Blood Samples: Effects of MgCl₂, EDTA, Storage Time, and Temperature on DNA Yield and Quality. *Biochemical Genetics*, 31 (7/8), 321-328.
- [17] Caboux E., Lallemand C., Ferro G., Hémon B., Mendy M., Biessy C., Sims M., Wareham N., Britte A., Boland A., Hutchinson A., Siddiq A., Vineis P., Riboli E., Romieu I., Rinaldi S., Gunter M.J., Peeters P.H.M., van der Shouw Y.T., Travis R., Bueno-de-Mesquita H.M., Canzian F., Sánchez M., Skeie G., Standahl Olsen K., Lund E., Bilbao R., Sala N., Barricarte A., Palli D., Navarro C., Panico S., Redondo M.L., Polidoro S., Dossus L., Boutron-Ruault M.C., Clavel-Chapelon F., Trichopoulou A., Trichopoulos D., Lagiou P., Boeing H., Fisher E., Tumino R., Agnoli C., Hainaut P. (2012) Sources of Pre-Analytical Variations in Yield of DNA Extracted from Blood Samples: Analysis of 50,000 DNA Samples in EPIC. *PLOS ONE*, 7 (7), DOI: 10.1371/journal.pone.0039821.
- [18] Psifidi A., Dovas C.I., Bramis G., Lazou T., Russel C.L., Arsenos G., Banos G. (2015) Comparison of Eleven Methods for Genomic DNA Extraction Suitable for Large-Scale Whole-Genome



- Genotyping and Long-Term DNA Banking Using Blood Samples. *PLOS ONE*, 10 (1), DOI: 10.1371/journal.pone.0115960.
- [19] Chacon-Cortes D., Griffiths L.R. (2014) Methods for Extracting Genomic DNA from Whole Blood Samples: Current Perspectives. *Journal of Biorepository Science for Applied Medicine*, 2, 1-9.
- [20] Poli F., Cattaneo R., Crespiatico L., Nocco A., Sirchia G. (1993) A Rapid and Simple Method for Reversing the Inhibitory Effect of Heparin on PCR for HLA Class II Typing. *Genome Research*, 2, 356-358.
- [21] Garcia M.E., Blanco J.L., Caballero J., Gargallo-Viola D. (2002) Anticoagulants Interfere with PCR Used To Diagnose Invasive Aspergillosis. *Journal of Clinical Microbiology*, 40 (4), 1567-1568.
- [22] Barra G.B., Santa Rita T.H., Vasques JA., Chianca C.F., Nery L.F., Costa S. (2015) EDTA-mediated inhibition of DNases protects circulating cell-free DNA from ex-vivo degradation in blood samples. *Clinical Biochemistry*, DOI: 10.1016/j.clinbiochem.2015.02.014.