On the use of buffy or whole blood for obtaining DNA or RNA of high quality and functionality: What is the best option?

Ferro P.¹, Díaz T.¹, Ortega-Pinazo J.², Gómez J.M.³, Martínez B.¹, Fernández M.I.¹, Martín I.¹, Jiménez A.¹, Acosta-Andrade C.¹ and Hortas M.L.⁴

¹Andalusian Public Health System Biobank, Instituto de Investigación Biomédica de Málaga (IBIMA), Sanitary District of Málaga, University of Málaga.

²Neurology Service, Instituto de Investigación Biomédica de Málaga (IBIMA), Sanitary District of Málaga, University of Málaga.

³Genetic Platform, Instituto de Investigación Biomédica de Málaga (IBIMA), Sanitary District of Málaga, University of Málaga.

⁴Costa del Sol Sanitary Agency, Sanitary District of Málaga.

INTRODUCTION

Human Biobanks are biorepositories of collection of biological samples and health information which allow the organization of biomedical research for upgrading the knowledge of human disorders from different diseases (cancer, allergy, rare diseases, etc) and reach real answers for diagnosis and treatment. A wide range of samples can be stored in these biorepositories as hair, nails, urine, tissue, whole blood (WB), red blood cells (RBC), buffy coat (BC), plasma, serum, DNA, RNA, etc. Among these BC and WB are very appreciated by researchers because they can obtain DNA and RNA from this type of samples. Some preliminary studies have been performed in animals to evaluate the quality and functionality of the nucleic acids obtained from these matrices (BC and WB)^{1,2}, notwithstanding deeper studies would be interesting in order to establish the best matrix for this purpose and to be able to optimize the biobanks storage conditions.

AIM

The aim of this study is to evaluate the functionality and quality of the DNA and RNA obtained from whole blood and buffy coat to determine the best storage conditions and the optimal extraction matrix.

METHODS AND MATERIAL

Whole blood was extracted from ten healthy volunteers by venipuncture blood in the arm. For each volunteer 2 EDTA tubes, 2 citrate tubes and 1 Paxgene tube were obtained. The samples were collected and managed by the Biobank of the Public Health System of Andalusia (BSSPA) following standard work protocols. Patients participating in the study provided the informed consent of Biobanco.

After extraction some samples were processed immediately and others were pre-processed and stored at -80 °C until use. DNA and RNA were extracted following the manufacturer's suppliers and quality and integrity were analysed by spectrophotometry and agarose gel. The functionality was evaluated by PCR using the primers Forward 5'-GCCTTCCAACCATTCCTTA- 3' and reverse 5'-TCACGGATTCTGTTGTGTTC- 3' which amplify a fragment of 429 base pairs of the HGH gene.

RESULTS

DNA extraction showed similar concentrations for BC and whole cells in samples extracted the day 0, but in samples stored 30 days buffy coat showed a reduction in the efficiency of 82% less when was compared with buffy coat extracted at day 0, while whole blood showed only a reduction of 55% (Table 1 and Figure 1). However, the integrity of the all samples and the functionality was not altered with the storied time (Figure 2).

Volunteer	buffy coat		whole blood	
	Time (days)			
	0	30	0	30
1	377	77	596	105
2	277	30	190	40
3	99	9	235	92
4	252	41	162	122
5	321	16	262	117
6	250	98	449	55
7	230	29	310	99
8	235	71	350	244
9	272	51	311	160
10	207	34	169	351

Table 1. Concentration of DNA (ng/μl) obtained from buffy coat and whole blood in the volunteer studied at 0 days and 30 days after stored at -80°C.

Figure 1. Comparison between the concentration obtained from buffy coat (BC) and whole blood (WB) at 0 days and stored at -80°C for 30 days. Mean values of 10 volunteer for each condition are represented. Bars show standard deviations. Levels of statistical significance compared with storied time: * 0.01 < p ≤ 0.05, ** 0.001 < p ≤ 0.01, and *** p ≤ 0.001 (t' student test).

Figure 2. Integrity and functionality of the DNA extracted from buffy coat (BC) and whole blood (WB) at 0 days and 30 days after extraction. A. Genomic DNA extracted from each volunteer in agarose gel of 1% (number 1 to 10 reference to volunteer. M- Molecular weight marker). B. PCR of the gene HGH from genomic DNA obtained from BC and WB. (C-, negative control).

On the other hand, RNA was obtained from cells purified from EDTA tubes and frozen at -196°C for 30 days. RNA from cells stored for 30 days showed a loss of efficiency of 48% when was compared with RNA extracted from cells purified at day 0 (Figure 2).

Figure 3. Comparison between the concentration obtained from RNA extracted from blood cells at 0 days and stored at -196°C for 30 days. Mean values of 10 volunteer for each condition are represented. Bars show standard deviations. Levels of statistical significance compared with storied time: * 0.01 < p ≤ 0.05, ** 0.001 < p ≤ 0.01, and *** p ≤ 0.001. (t' student test).

CONCLUSION

In samples stored at -80°C is better the use of whole blood because the mount of DNA obtained is higher when is compared with buffy coat. In samples extrated in fresh buffy coat is better because reduce the cost of the extraction and the time of the protocol.

In general, The storied affect the quantity of the nucleic acid extracted, so it is not recommended the storied of the samples for a long time.

ACKNOELEDGMENTS

This work was carried out with the financial support of the Biobank Platform PT13/0010/0006, Health Institute Carlos III (ISCIII, Madrid, Spain)

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