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Tissue storage solution for preservation and transfer of forensic specimen in high ambient-temperature

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ABSTRACT

Storage of tissue samples in high ambient-temperature can affect the quality of forensic evidence. Experiments were conducted to investigate the potential use of 3 tissue storage solutions for the preservation and transfer of forensic specimen in high ambient temperature conditions, i.e., DMSO, Longmire's buffer, and trehalose solution. Results showed that DNA in tissue was best preserved in DMSO buffer. Samples preserved in Longmire's buffer gave DNA analysis results for temperatures up to 60 °C, however, amplification between replications were not reproducible. For those tissue samples preserved in trehalose solution, DNA markers larger than 300 bp were absent, and irreproducible amplification results were detected at a higher level when the storage temperature increased, and storage time was over 2 weeks. Tissue storage condition at high temperature over 1 week is not recommended. Experimental results here provided an alternative collection and preservation method for tissue samples at ambient temperature (without cold-storage) for subsequent DNA analysis. These can potentially be implemented in forensic biological evidence collection, preservation and transfer in hot climates.

1. Introduction

Biological samples are recommended to be stored cool and dry. However, things can deviate in practice, In hot climates, quality of forensic tissue sample can be easily affected by the high ambient-temperature, i.e. between 30–45 °C, during evidence transfer. Temperature of an item placed under direct sunlight can increase up to 10–15 °C from the actual ambient temperature. A number of non-cold storage tissue sample preservation methods have been reported for field applications.

Alcohol is a simple, easy-to-find, tissue preservation solution. It has bacterial and fungicidal activities, as well as causing tissue dehydration, it is applied for short-term storage. There are reports on DNA degradation after 6-month-storage and negative effect on PCR [1,2]. DMSO (dimethyl sulfoxide) solution is one of the recommended and widely used tissue preservation solution due to its efficiency and cost-effectiveness. Combinations of 20–25% DMSO, 0.25 M EDTA, and saturated NaCl (pH 7.5–8.0) solutions can prevent degradation of tissue samples for up to 6 months - 2 years in room temperature [1,3,4]. It has also been reported that full DNA profiles had been obtained from human muscle tissue preserved in DMSO solution at 35 °C after storage for one month [5]. Longmire's buffer is another widely used tissue preservation solution. Sodium dodecyl sulfate (SDS), a strong anionic detergent that solubilizes proteins and lipids in cell membranes, is the major composition. It can break down the cell membranes and expose the chromosomes [6]. In addition, other compositions in Longmire's buffer can help preserve DNA for up to 6 months. The amount of PCR-amplified products were considerably higher than from those extracted from ethanol-preserved tissue samples, but not as much as that stored in DMSO [4]. Trehalose is a biopolymer that has been used in DNA preservation. It has been showed that insect's DNA, human placenta DNA, and gorilla fecal DNA's quality was well preserved in the presence of trehalose [3,7].

Experiments were conducted to investigate the possibility of DNA analysis after preserving tissue samples in 3 different storage solutions without cold-storage for 1–4 weeks.

2. Material and methods

Fresh porcine thigh-muscle tissue was cut into pieces, each were approximately 300 mg. To investigate the tissue storage conditions, tissue samples were put into 5-ml Eppendorf tubes containing 4 ml of 3 different solutions and a 'no buffer' storage. Solutions used were DMSO buffer (0.25 M EDTA, pH8.0 and 20%(v/v) DMSO in the ratio 80:20), Longmire's buffer or lysis buffer (2 M Tris-HCl, 0.5 M EDTA, 5 M NaCl, and 20%(w/v) SDS, pH 8.0 in double-distilled water), and 15%(w/v)

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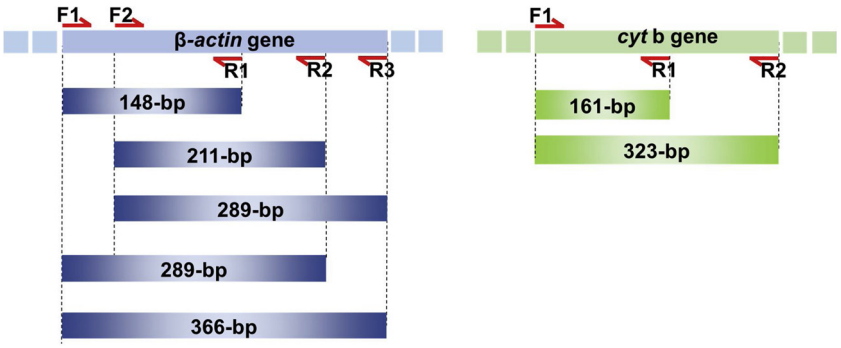

Fig. 1. Primer maps. The marker sets were modified from Phengon et al, 2008. Expected PCR product sizes amplified by the *β-actin* nuclear DNA marker set were 148, 211, 289, and 366 bp. For the mitochondrial the *cyt* BDNA marker set, expected PCR product sizes were 161 and 323 bp.

Storage time	1 week							2 weeks						
	β-actin DNA marker					*Cyt* b		*β-actin* DNA marker					*Cyt* b	
	148 bp	211 bp	289 bp	289 bp*	366 bp	161 bp	323 bp	148 bp	211 bp	289 bp	289 bp*	366 bp	161 bp	323 bp
Positive PCR control														
Negative PCR control														
D 25°C														
D 40°C														
D 60°C														
D 80°C														
L 25°C														
L 40°C														
L 60°C														
L 80°C														
T 25°C														
T 40°C														
T 60°C														
T 80°C														
N 25°C														
N 40°C														
N 60°C														
N 80°C														

Storage time	3 weeks							4 weeks						
	β-actin DNA marker					*Cyt* b		*β-actin* DNA marker					*Cyt* b	
	148 bp	211 bp	289 bp	289 bp*	366 bp	161 bp	323 bp	148 bp	211 bp	289 bp	289 bp*	366 bp	161 bp	323 bp
Positive PCR control														
Negative PCR control														
D 25°C														
D 40°C														
D 60°C														
D 80°C														
L 25°C														
L 40°C														
L 60°C														
L 80°C														
T 25°C														
T 40°C														
T 60°C														
T 80°C														
N 25°C														
N 40°C														
N 60°C														
N 80°C														

Consistent amplification of *β-actin* PCR product
Consistent amplification of *cyt* B PCR product
No PCR product
Inconsistent amplification of *β-actin* PCR product
Inconsistent amplification of *cyt* B PCR product

Fig. 2. DNA analysis results using the nuclear *β-actin* and the mitochondrial *cyt* B marker sets. Abbreviations; D: DMSO buffer, L: Longmire buffer, T: trehalose solution, N: no buffer solution.

trehalose solution [4,8–11]. Samples were incubated in 25, 40, 60, and 80 °C, for 1, 2, 3, and 4 weeks. Experiments were conducted in 4 replicas.

For DNA analysis by PCR, both nuclear and mitochondrial DNA were analysed with modifications to Phengon et al. [12]. Expected PCR products amplified by the nuclear *β-actin* marker set were 148, 211, 289, and 366 bp. For the mitochondrial *cyt* B marker set, expected PCR product sizes were 161 and 323 bp. Primer maps are showed in Fig. 1.

3. Results and discussions

DNA analysis results (Fig. 2) showed that all size range of PCR products could be reproducibly amplified from tissue samples preserved in DMSO buffer for 1 week at varying temperatures up to 80 °C. When storage time was extended to 2 weeks, DNA analysis results were reproducible for those samples preserved at temperatures up to 60 °C. Higher level of inconsistency in PCR amplification or no PCR products were observed in other methods being compared. For 3- and 4-week stored tissue samples, results showed that samples were best preserved in DMSO and Longmire's buffer for up to 60 °C. Although samples preserved in Longmire's buffer solution gave DNA analysis results for temperatures up to 60 °C, amplification results between replications were not reproducible. For those tissue samples preserved in trehalose solution, DNA markers larger than 300 bp were absent and irreproducibility of amplification results were detected at a higher level when the storage temperature increased from 25 °C to 40 °C, 60 °C, and 80 °C, and storage period was prolonged over 2 weeks. Results suggested that storage temperature at 80 °C and prolonging of sample storage over 1 week is not recommended. From all storage conditions compared, results also showed that samples preserved in buffer solutions gave better DNA analysis results than without buffer.

Composition of buffer solutions facilitates the mechanisms leading to stabilizing DNA in the cell. DMSO buffer solution inactivates nucleases and prevent DNA denaturation at room temperature because the solution has a high concentration of salt. DMSO is an amphipathic molecule, which could solubilize polar and nonpolar substances and transpose biological membranes. This enables the buffer to permeate into the cell. For Longmire's buffer, SDS plays role on cell membrane differently by disrupting the membrane (cell lysis). EDTA binds divalent cations, which are needed for nuclease activity. The Na^+ ions in the solution surrounded the negatively-charged DNA phosphate backbone, thus help preserving the double-stranded structure. Therefore, buffers help preserving the DNA structure [4,7,9]. Trehalose can stabilize DNA by the formation of H-bonds to the phosphate groups along the DNA backbone, thus leading to shielding the phosphate-phosphate repulsion. It can also help stabilize the base [13]. The addition of trehalose to tissue storage buffers may enhance the effectiveness of preserving the DNA in tissue.

In summary, DNA in tissue was best preserved in DMSO buffer at the conditions provided in this study. Both DMSO and Longmire's buffer gave DNA analysis results when samples were exposed to temperatures up to 60 °C, continuously. However, continuous exposure of tissue samples to temperature exceeding 60 °C could resulted in irreproducible DNA analysis results between replications.

4. Conclusion

An alternative collection and preservation method for tissue samples at ambient temperature (without cold-storage) for subsequent DNA analysis is reported. Tissue samples can be stored and preserved in DMSO buffer at temperature not exceeding 60 °C for up to 2 weeks. Longmire's buffer can be an alternative tissue storage solution under the same condition. However, tissue storage condition at high temperature over 1 week is not recommended. These could potentially be implemented to the preservation and transfer of forensic tissue samples in tissue storage solutions in hot-climate countries.

Declaration of Competing Interest

None.

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