Ain Shams Journal of Forensic Medicine and Clinical Toxicology
July 2021, 37: 49-56

Comparative study of the efficiency of ethanol 95% and salt-saturated dimethyl sulfoxide preservatives on quality and quantity of DNA extracted from soft tissues

Sahar Mohamed Moustafa[1],

[1]Assistant Professor of Forensic Medicine and Clinical Toxicology Faculty of Medicine-Suez Canal University Suez, Egypt.

Abstract

Background: Preparation of the purified and high molecular weight DNA is considered as a first step in tissue molecular analysis. Optimization of the conditions at which samples are received as duration, temperature and preservative agents used is important to avoid affecting the quantity and quality of the extracted DNA. **Aim of the work:** comparing the efficiency of two DNA preservatives agents [ethanol 95% and salt-saturated dimethyl sulfoxide (SSDMSO)] on the extracted DNA from soft tissues and specifying which of the two studied organs (kidney and heart) gives better DNS yield after preservation at room temperature and at -20ºC. **Materials and methods:** The study was done on 19 autopsy cases. 300 mg of each selected soft tissues (kidney and heart) were preserved as follow: In 2 ml sterile water (Control group), at -20°C (Freezing group), Ethanol 95% (Ethanol group) and in SSDMSO (SSDMSO group). After DNA extraction, DNA quantity was measured using NanoDrop method while DNA quality was measured using agarose gel electrophoresis. **Results:** All preservatives could retain DNA up to the two months. SSDMSO gave the highest DNA concentration followed by Ethanol 95%. The superiority to heart tissues in SSDMSO preservative over kidney tissues in both Ethanol 95% and SSDMSO preservatives up to two months. **Conclusion**: It is concluded that SSDMSO is a successful preservative agent, and it is superior to ethanol 95% for preservation of DNA in soft tissues up to two months at room temperature. Heart tissue is less susceptible to degradation and hence more suitable for DNA fingerprinting than kidney tissue. **Recommendations**: It is recommended to use SSDMSO as preservative for soft tissue collected for DNA analysis and to choose the heart for sampling.

Key words DNA quality, DNA quantity, tissue preservatives.

Introduction

DNA profiling is considered a gold standard for victims' identification of mass-casualty accidents. Proper DNA preservation from the point of collection to the laboratory is very crucial to genetic studies because many genomic protocols require high quantity samples (Prinz et al., 2007; Sanchez et al., 2013).

Preparation of purified high molecular weight DNA is considered the first step in molecular analysis of tissues. Moreover, determination of genetic profiles of collected samples from deceased individuals is one of the routine tests performed in legal medicine laboratories and the most important requirement for a successful extraction is a good-quality DNA (Nagy, 2010).

After cellular death, DNA become prone to damage and degradation by both cellular nuclease effects and exogenous factors leading to base changes, strands breaks and crosslink. So, the successful sample collection and the optimal preservation of the tissue samples is irreplaceable. Optimizing the conditions of sample collection as duration, temperature and preservation methods is crucial as they can affect DNA quality and quantity (Raina et al., 2006; Graham et al., 2008; Diegoli et al., 2012).

Performance of the different preservation methods is not fully searched; however, it is approved that adding a preservative buffer prior to freezing is better than ultra-cold conditions. Although there are many alternative methods developed for tissues' samples preservation for forensic DNA analysis at room temperature, the most used tissue preservation method for DNA analysis is freezing (Fregeau et al., 2001; Nagy, 2010; Mulcahy et al., 2016).

The technological advances had permitted a high DNA profiling using the crime scenes' trace samples. While all body tissues can be used for DNA extraction, it is crucial to specify the most reliable organ for DNA extraction and to investigate the effect of post-mortem interval (PMI) on DNA degradation (Raina et al., 2006; Reza et al., 2010; Jakubowska et al., 2012;Pooniya et al., 2014).

Ethanol is the most frequently used medium for tissue preservation and it has the ability to remove

water content of tissues causing enzymes and proteins denaturation in addition to its antimicrobial effect (Julian, 2003; Nicklas and Eric, 2003).

Salt-saturated dimethyl sulfoxide (SSDMSO) is not only considered as preservative agent but also, is considered as a better enhancing vehicle for other preservatives owing to its high tissue permeability and dehydrating effect through water displacement (Kilpatrick, 2002).

Aim of the Study

This study aimed at comparison of the efficiency of two DNA preservatives **[**ethanol 95% and salt-saturated dimethyl sulfoxide (SSDMSO)**]** on the quantity and quality of extracted DNA from soft tissues. Also, it aimed at the comparison of two soft tissue organs (kidney and heart) preserved at room temperature for DNA analysis yield in terms of quantity and quality for forensic medicine applications.

Subjects and Methods

Subjects:

The current study was conducted on 19 autopsy cases referred to the mortuary of Forensic Medicine Council in Riyadh of Kingdom of Saudi Arabia. The maximum PMI of the selected cases was 48 hours to ensure tissue viability. All cases with PMI more than 48 hours were excluded for the beginning of their decomposition. The study was conducted from October 2018 to March 2019 as in cooler situations, successful recovery of DNA from soft tissues after several days can be achieved (Prinz et al., 2007).

Sample collection and preparation:

Viable heart's tissues (part of the right ventricle) and kidney's tissues (part of the upper pole) of the studied bodies were collected, cut into small pieces, weighed, and stored in sterilized plastic containers on ice at -20°C (Sorensen et al., 2016).

Samples were divided into four groups as follows:

- *Control group:* It contained 300 mg of each selected soft tissues (kidney and heart) with no preservatives in 2 ml sterile water in 10 ml-sterile test tubes. DNA was extracted at the first day of the experiment.
- *Freezing group:* It contained 300 mg of each selected soft tissues (kidney and heart) with no preservatives in 10 ml-sterile test tubes and stored on ice at -20°C to limit DNA degradation as freeze-thaw cycles accelerate the DNA breakdown (Sorensen et al., 2016). The samples were extracted at two different time intervals (one and two months).
- *Ethanol 95% group:* It contained 300 mg of each selected soft tissues (kidney and heart) with 2 ml Ethanol 95% in 10 ml-sterile test tubes. Samples were extracted at two different time intervals (one and two months).
- *SSDMSO group:* It contained 300 mg of each selected soft tissues (kidney and heart) with 2 ml salt-saturated dimethyl sulfoxide (SSDMSO) in 10 ml-sterile test tubes. The samples were extracted at two different time intervals (one and two months).

The tissues were fully submerged in the preservative for protection against degradation as preservative volume exceeding tissue volume is optimum (Graham, 2008).

Samples were sent to the laboratory and processed for DNA analysis in the following weeks. With the ability of tissues to withstand a high temperature up to 30°C, they were kept at room temperature (20-30°C) for simulating the expected mass disaster conditions in different climates (Graham et al., 2008; Aladdin et al., 2010).

Tissue preservation methods in the current study were examined for short periods (maximum two months) as forensic situations are mainly field based and focused on storage for a short-term and it may take few weeks till transporting the preserved tissues to the laboratories (Aladdin, 2010).

DNA extraction and evaluation:

Samples were processed in the Clinical Pathology Department of Elite Medical Hospital in Riyadh of Kingdom of Saudi Arabia after approval of the Medical Committee.

After DNA extraction DNA quantity of each sample was measured using NanoDrop (ND-1000) full-spectrum ultraviolet spectrophotometer at 260 nm and 280 nm wavelength (NanoDrop Technologies, Inc. Wilmington, DE USA, 2005) (Haque et al., 2003).

Estimation of DNA quality was measured using agarose gel electrophoresis (0.8%) which can be used for a reliable and sensitive analysis of post-mortem interval (PMI) studies especially with more prolonged PMI (El-Harouny et al., 2008; Kuhn et al., 2018).

Statistical analysis:

Statistical Package for Social Sciences (SPSS) version 22 was used. All numeric values were presented as range and mean ± standard deviation. Significance of differences in DNA concentration among the studied groups was determined using one-way analysis of variance (ANOVA) test followed by LSD and the calculations were considered significant if P value was less than 0.05.

Ethical considerations:

Approval of the Scientific Ethical Committee of Forensic Medicine Council in Riyadh of Kingdom of Saudi Arabia was taken prior to the study. All data were confidential as anonymized residual samples and were completely unknown to the examiner. All samples will not be used in the future.

Results

The quantity of extracted DNA from human kidney tissues after one month of preservation in ethanol 95% and SSDMSO preservative agents at room temperature revealed a significant decrease in DNA concentration in comparison to both control and freezing groups (Table 1).

The quantity of extracted DNA from human heart tissues after one month of preservation in ethanol 95% at room temperature revealed a significant decrease in DNA concentration in comparison to both control and freezing groups, while that preserved in SSDMSO showed non-significant change in

comparison with both control and freezing groups (Table 2).

The quantity of extracted DNA from kidney and heart tissues after two months of preservation in ethanol 95% at room temperature revealed a significant decrease in DNA concentration in comparison to both control and freezing groups, while that preserved in SSDMSO showed non-significant change in comparison with control and freezing groups and significant increase in comparison with ethanol 95% group (Tables 3 and 4).

The quantity of extracted DNA from heart tissues after one and two months of preservation in either ethanol 95% or SSDMSO at room temperature revealed a significant increase in DNA concentration as compared to that extracted from the kidney (Tables 5 and 6).

Regarding estimation of DNA quality, figure (1) illustrates a gel electrophoresis of extracted DNA from freezing kidney tissues at -20ºC and preserved tissues at room temperature in ethanol 95% and SSDMSO preservative agents for two months. It shows incomplete DNA degradation in kidney tissue which were preserved in Ethanol 95% preservative agents in comparison to control or freezing and SSDMSO preservatives. Figure (2) illustrates a gel electrophoresis of extracted DNA from freezing heart tissues at -20ºC and preserved tissues at room temperature in ethanol 95% and SSDMSO preservative agents for two months. It shows no DNA degradation in all samples over the studied time in comparison with control or freezing and SSDMSO preservatives.

Table (1): Statistical analysis of DNA concentrations extracted from human kidney tissues after preservation for one month in either Ethanol 95% or SSDMSO at room temperature compared to control and freezing groups using ANOVA.

Type of preservative	DNA concentration (ng/ml)		P-value
	Range	Mean ± SD	
Control group	53-55	54.29±0.75	
Freezing group	49-53	51±0.15	0.117
Ethanol 95% group	32-37	35.2±0.75*a,b	0.05
SSDMSO group	43-45	44±0.5*a,b	0.05

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group.*

Table (2): Statistical analysis of DNA concentrations extracted from human heart tissues after preservation for one month in either Ethanol 95% or SSDMSO at room temperature compared to control and freezing groups using ANOVA

Type of preservative	DNA concentration (ng/ml)		P-value
	Range	Mean ± SD	
Control group	50-52	51±0.15	
Freezing group	48-52	50.2±0.15	0.117
Ethanol 95%	37-39	38±0.08*a,b	0.05
SSDMSO	50-52	51.5±0.5	0.352

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation,
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group.*

Table (3): Statistical analysis of DNA concentrations extracted from human kidney tissues after preservation for two months in either Ethanol 95% or SSDMSO at room temperature compared to control and freezing groups using ANOVA

Type of preservative	DNA concentration (ng/ml)		P-value
	Range	Mean ± SD	
Control group	53-55	54.29±0.75	
Freezing group	45-47	47±0.5	0.107
Ethanol 95%	30-33	30.5±0.78*a,b	0.05
SSDMSO	46-48	45.2±0.68*c	0.05

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group, c in comparison to Ethanol 95% group.*

Table 4: Statistical analysis of DNA concentrations extracted from human heart tissues after preservation for two months in either Ethanol 95% or SSDMSO at room temperature compared to control and freezing groups using ANOVA

Type of preservative	DNA concentration (ng/ml)		P-value
	Range	Mean ± SD	
Control group	50-52	51±0.15	
Freezing group	47-50	47.5±0.5	0.107
Ethanol 95%	35-39	36.4±0.3*a,b	0.05
SSDMSO	48-49	48.2±0.5*c	0.05

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group, c in comparison to Ethanol 95% group*

Table 5: Statistical comparison of DNA concentration extracted from human kidney and heart tissues after preservation for one month in either Ethanol 95% or SSDMSO at room temperature using ANOVA

Type of preservative	DNA concentration (ng/ml)		P-value
	Kidney	Heart	
	Mean ± SD	Mean ± SD	
Control group	54.29±0.75	51±0.15	
Freezing group	51±0.15	50.2± 0.15	0.105
Ethanol 95%	35.2±0.75*a,b	38±0.08*a,b	0.05
SSDMSO	44±0.5*a,b	51.5±0.5	0.05

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group, c in comparison to Ethanol 95% group*

Table 6: Statistical comparison of DNA concentration extracted from human kidney and heart tissues after preservation for two months in either Ethanol 95% or SSDMSO at room temperature using ANOVA

Type of preservative	DNA concentration (ng/ml)		P-value
	Kidney	Heart	
	Mean ± SD	Mean ± SD	
Control group	54.29±0.75	51±0.15	
Freezing group	47±0.5	47.5±0.5	0.107
Ethanol 95%	30.5±0.78*a,b	36.4±0.3*a,b	0.05
SSDMSO	45.2±0.68*c	48.2±0.5*c	0.05

Number of cases 19 cases, Freezing group: -20ºC, SSDMSO: salt-saturated dimethyl sulfoxide, SD: standard deviation
** Significant P≤0.05, a in comparison to control group, b in comparison to freezing group, c in comparison to Ethanol 95% group*

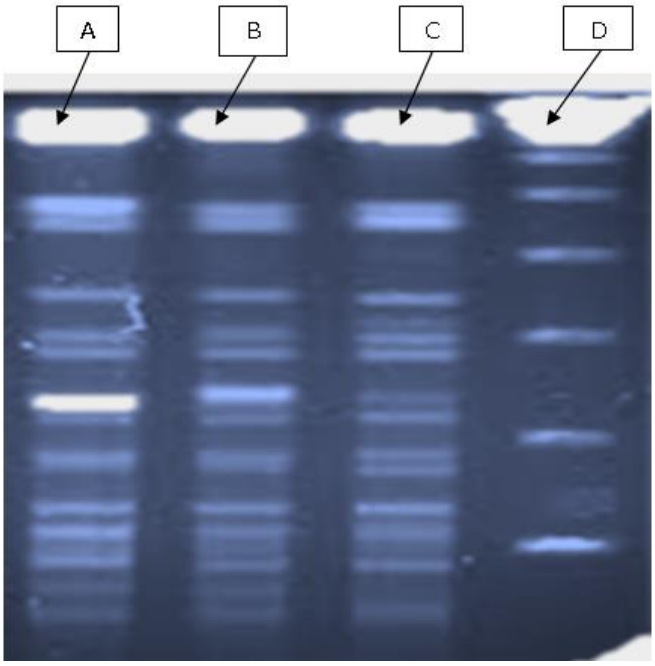

Figure (1): Gel electrophoresis of extracted DNA after two months from kidney tissues preserved at -20ºC and (Ethanol 95% and SSDMSO) in room temperature (20-30°C).

The samples from left to right as follow:
A = Control
B = Kidney tissue freezing at -20°C
C = Kidney preserved in SSDMSO (for 2 months)
D = Kidney preserved in ethanol 95% (for 2 months)

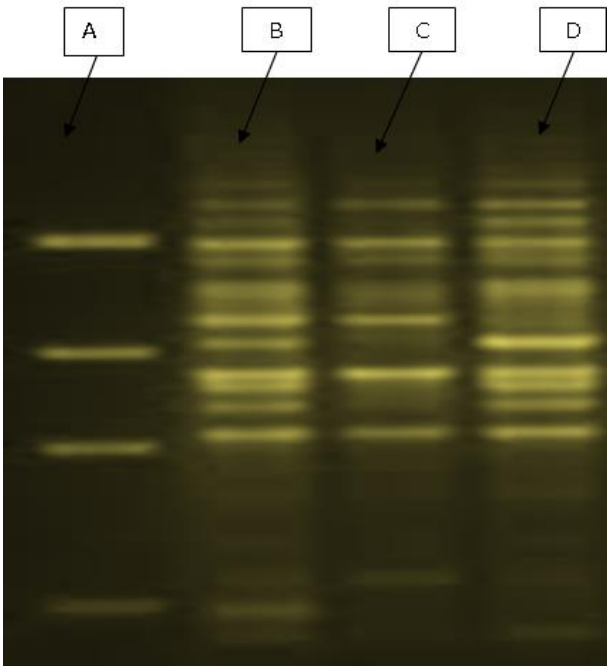

Figure (2): Gel electrophoresis of extracted DNA after two months from heart tissues preserved at -20ºC and in (Ethanol 95% and SSDMSO) at room temperature (20-30°C).

The samples from left to right as follow:
A = Control
B = Heart tissue freezing at -20°C
C = Heart tissue preserved in SSDMSO agent (for two months)
D = Heart tissue preserved in ethanol 95% agent (for two months)

Discussion

As molecular genetic analysis based on tissue samples is foreseen, many important issues must be considered prior to collection of samples. DNA Commission of the International Society for Forensic Genetics recommended that tissue samples must be preserved in a suitable preservative at room temperature. Tissue type and the storage temperature may affect DNA quality and quantity therefore, the issue of appropriate tissue collection and preservation is very crucial in all forensic analyses (Prinz et al., 2007; Nagy, 2010; Pooniya et al., 2014).

The present results concluded that there is a significant difference between ethanol 95% and SSDMSO preservatives in DNA concentrations which were extracted after one and two months at room temperature with the superiority to SSDMSO in retaining DNA concentrations along the period of study.

The results are in accordance with a study that compared the effect of six different preservative agents and concluded that dimethyl sulfoxide (DMSO) is suitable for preservation of DNA at 35°C up to one month (Allen-Hall and McNevin, 2012). Also, Ali et al. (2016) stated that DMSO is a successful method of preservation of DNA in human muscle tissue up to 4 weeks in room temperature. While the other studies used DMSO, the current study used SSDMSO agent which is not only a preservative agent but also an enhancing vehicle for other preservatives.

The present results demonstrated a significant decline in DNA concentration in samples preserved in ethanol 95% over (two months) especially in kidney tissues in comparison to SSDMSO group samples which showed a minor decline. These results indicates that SSDMSO is better than ethanol in DNA extraction and analysis.

The present study agrees with Gaither et al. (2011) who concluded that specimens preserved with SSDMSO yielded higher molecular weight DNA hence a higher quality of DNA. On the other hand, Caputo et al. (2011) concluded that there are no changes in DNA quantities after storage in ethanol up to one year.

Ethanol (100%) was highly effective for up to twenty-six days storage for molluscan tissues with adding EDTA for more effective preservation. A recent research indicated that soft tissues including kidney tissue in ethanol 100% preservation for at least 6 months was more reliable sample. Additionally, it was documented that ethanol is highly validated as a long-term DNA preservative agent for forensic autosomal short tandem repeat DNA analysis in comparison to 10% neutral buffered formalin (Williams, 2007; Alqaydi and Roy, 2016 ;Ayana et al., 2019).

The present study demonstrated that there is no degradation in DNA extracted from the two studied preservatives after two months of preservation at room temperature in heart tissues while kidney tissues showed partial degradation especially in ethanol agent, so a good quality of DNA can be achieved for subsequent forensic use in heart tissues especially in SSDMSO agent preservation.

These results are in accordance with previous results which concluded that SSDMSO provided the best protection against DNA degradation up to two years and up to six months at room temperature (Kilpatrick 2002; Michaud and Foran, 2011).

The advent of heart tissue on the kidney tissue is in accordance with the study which concluded that blood and kidney tissues considered unsuitable for DNA profiling because of rapid degradation of their DNA contents after little weeks (Pooniya et al., 2014).

Ebuehi et al. (2015) concluded that extracted DNA from heart, as compared to liver and kidney showed a slower degradation rate which in agreement with the current study results. It is worth mentioning that isolation of a high amount of DNA from the biological materials does not always guarantee the positive determination of a proper genetic profile .as While DNA degradation is an indicator of low DNA quality, the contrary is not true and the preservative role is very crucial (Fan et al., 2016; Kuhn et al., 2018).

Conclusion and recommendations

The current study concluded that SSDMSO is a successful preservative agent, and it is superior to ethanol 95% for preservation of DNA in soft tissues up to two months at room temperature. The heart tissue is more suitable for DNA fingerprinting than the kidney. It is recommended to use SSDMSO as preservative for soft tissue collected for DNA analysis and to choose the heart for sampling.

The effect of other preservation conditions (as longer duration and higher temperature than used in the present study) and testing other soft tissue organs is highly recommended for future research.

References

Aladdin R, Walsh SJ and Abbes A (2010): Forensic implications of genetic analysis from degraded DNA. Forensic Science Inter. Genetic Supplement; 4 (3): 148-157.

Ali M, Abdel-Karim, Mahmoud R, et al., (2016): Effect of different human muscle tissue preservatives on quality and quantity of DNA: Medico-legal aspect. Egypt J. Forensic Sci. Appli. Toxicol. Vol 16(1). Pp. 179-191.

Allen-Hall A and McNevin D (2012): Human tissue preservation for disaster victim identification in tropical climates. Forensic Science Inter. Genetics; 6(5): pp. 653-657.

Alqaydi M and Roy R (2016): Quantitative and qualitative study of STR DNA from ethanol and formalin fixed tissues. Forensic Science International; 7(262): pp. 18–29.

Ayana, M, Cools P, Mekonnen Z, et al., (2019): Comparison of four DNA extraction and three preservation protocols for the molecular detection and quantification of soiltransmitted helminths in stool. PLOS Neglected Tropical Diseases; 13(10).

Caputo M, Bosio LA and Corach D (2011): Long term room temperature preservation of corpse

soft tissue: an approach for tissue sample storage. Investigative Genetics; (2): pp. 2-17.

Diegoli TM, Farr M, Cromartie M et al., (2012): An optimized protocol for forensic application of the PreCR™ Repair Mix to multiplex STR amplification of UV-damaged DNA, FSI: Genet; 6 (4): pp. 498–503.

Ebuehi OA, Amode M, Balogun A, et al., (2015): Postmortem Time Affects Brain, Liver, Kidney and Heart DNA in Male Rat. American Journal of Biochemistry; 5(1): pp.1-5.

El-Harouny MA, El-Dakroory SA and Attalla SM (2008): The relationship between postmortem interval and DNA degradation in different tissues of drowned rats. Mansoura J. Forensic Med. Clin. Toxicol. Vol. XVI, No. 2, pp. 45-62.

Fan J, Khanin R, Sakamoto H, et al., (2016): Quantification of nucleic acid quality in postmortem tissues from a cancer research autopsy program. Oncotarget; 7(41): pp. 6906-6921.

Fregeau CJ, Vanstone H, Borys S, et al., (2001): AmpFlSTR Profiler Plus and AmpFlSTR COfiler analysis of tissues stored in GenoFix, a new tissue preservation solution for mass disaster DNA identification, J. Forensic Sci; (46): pp.1180–1190.

Gaither MR, Szabo Z, Crepeau MW, et al., (2011): Preservation of corals in salt-saturated DMSO buffer is superior to ethanol for PCR experiments. Coral reefs; (30): pp. 329-333.

Graham EA, Turk EE and Rutty GN (2008): Room temperature DNA preservation of soft tissue for rapid DNA extraction: an addition to the disaster victim identification investigators toolkit? FSI: Genet; (2): pp. 29–34.

Haque KA, Pfeiffer RM, Beerman MB, et al., (2003): Performance of high- DNA quantification methods. Biomedical Central Biotechnology; pp. 3-20.

Jakubowska J, Maciejewska A and Pawłowski R (2012): Comparison of three methods of DNA extraction from human bones with different degrees of degradation. Int. J Legal Med. 2012; (126): pp.173–178.

Julian CD (2003): The effects of preservation and conservation treatments on the DNA of museum invertebrate fluid preserved collections; (164): pp.33-44.

Kilpatrick C W (2002): Non–cryogenic preservation of mammalian tissue for DNA extraction: an assessment of storage methods, Biochemical Genetics; (40). Pp. 53-62.

Kuhn R, Böllmann J, Krahl K, et al., (2018): Data on DNA gel sample load, gel electrophoresis, PCR and cost analysis. Data in Brief; (16). Pp. 732–751.

Michaud L and Foran R (2011): Simplified preservation of tissues for subsequent DNA analysis. Journal of Forensic Science; 56(4): pp. 846-852.

Mulcahy DG, Macdonalld K, Brady S, et al., (2016): Greater than X kb: a quantitative assessment of preservation conditions on genomic DNA quality, and a proposed standard for genome-quality DNA. PeerJ, DOI 10.7717/peerj.2528.

Nagy ZT (2010): A hands-on overview of tissue preservation methods for molecular genetic analyses Org Divers Evol; (10). Pp. 91–105.

Nicklas AA and Eric B (2003): Quantification of DNA in forensic samples. Analytical and bio-analytical chemistry; 376(8): pp.1160-1167.

Pooniya S, Lalwani S, Raina A, et al., (2014): Quality and Quantity of Extracted Deoxyribonucleic Acid (DNA) from Preserved Soft Tissues of Putrefied Unidentifiable Human Corpse. J Lab Physicians; Jan-Jun. 6(1): pp. 31–35.

Prinz M, Carracedo A, Mayr WR, et al, (2007): DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI), FSI: Genet; (1): pp. 3–12.

Raina A, Yadav BK, Lalwani S, et al., (2006): Effect of different preservatives on the human soft tissues stored at different temperatures and intervals of time for the isolation of DNA for DNA fingerprinting. IIJFMT; 4 (2).

Reza A, Simon JW and Ali A (2010): Forensic implications of genetic analyses from degraded DNA–A review. Forensic Sci. Int. Genetics; (4): pp.148-157.

Sanchez MC, Burraco B, Mestre MI, et al., (2013): Preservation of RNA and DNA from mammal samples under field conditions Molecular Ecology Resources; 13, pp. 663–67.

Sorensen A, Rahman E, Canela A, et al., (2016): Preservation and rapid purification of DNA from decomposing human tissue samples. Forensic Science International: Genetics; (25) 182–190.

Williams ST (2007): Safe and legal shipment of tissues samples: does it affect DNA quality. Journal of Molluscan Studies; (73): pp. 416-418.

دراسة مقارنة لكفاءة كل من الإيثانول بتركيز ٩٥٪ والسائل الملحي المشبع لداي ميثيل سلفوكسيد كمواد حافظة على جودة وكمية الحمض النووي المستخرج من الأنسجة الرخوة

سحر محمد مصطفى[١]

الملخص العربى

الخلفية العلمية: يعتبر تحضير الحمض النووي النقي ذو الوزن الجزيئي العالي بمثابة الخطوة الأولى في تحليل الأنسجة الجزيئية ، يعد تحقيق الدرجة المثلى من الظروف التي يتم فيها تلقي العينات مثل المدة الزمنية ودرجة الحرارة والمواد الحافظة المستخدمة أمرًا مهمًا لتجنب التأثير على كمية وكفاءة الحمض النووي المستخلص.

الهدف من البحث: مقارنة إثنين من المواد الحافظة (إيثانول ٩٥% و السائل الملحي المشبع لداي ميثيل سلفوكسيد) على كمية و كفاءة الحمض النووي المستخلص من الأنسجة الرخوة بالإضافة إلى تحديد أي من العضوين المدروسين (الكلى والقلب) أفضل فى إعطاء مستخلص الحمض النووي بعد الحفظ في درجة حرارة الغرفة والتجميد عند -٢٠ درجة.

الطرق و المواد: تم إجراء هذه الدراسة على تسعة عشرة حالة خاضعة لتحليل الصفة التشريحية. تم حفظ ٣٠٠ مجم من كل من الأنسجة الرخوة المختارة (الكلى والقلب) على النحو التالي: في ٢ مل من الماء المعقم (المجموعة الضابطة) ، عند -٢٠ درجة مئوية (مجموعة التجميد) و الإيثانول ٩٥% (محموعة الإيثانول) والسائل الملحي المشبع لداي ميثيل سلفوكسيد (مجموعة السائل المشبع لداي ميثيل سلفوكسيد).وقد تم قياس كمية الحمض النووي المستخلص بإستخدام جهاز النانو دروب في حين تم قياس كفاءة الحمض النووي بإستخدام الفصل الكهربائي لهلام الأجاروز.

النتائج: جميع المواد الحافظة تمكنت من الإحتفاظ بالحمض النووي لمدة تصل إلى شهرين. أعطي السائل الملحي المشبع لداي ميثيل سلفوكسيد أعلى تركيزللحمض النووي يليه الإيثانول بتركيز ٩٥%. تفوق نسيج القلب المحفوظ في السائل الملحي لداي ميثيل سلفوكسيد على نسيج الكلى المحفوظة في كل من السائل الملحي لداي ميثيل سلفوكسيد والإيثانول ٩٥% في حفظ الحمض النووي لمدة تصل شهرين.

الخلاصة: قد خلصت النتائج إلى أن السائل الملحي المشبع لداي ميثيل سلفوكسيد يعتبر عامل حافظ ناجح و متفوق على الإيثانول بتركيز ٩٥% كمادة حافظة للحمض النووي في الأنسجة الرخوة لمدة تصل إلى شهرين في درجة حرارة الغرفة في حين أن أنسجة القلب كانت أقل عرضة لتحلل الحمض النووي وبالتالي فهي أكثر ملاءمة لإجراء البصمة الوراثية أكثر من أنسجة الكلى.

التوصيات: يوصى بإستخدام السائل الملحي المشبع لداي ميثيل سلفوكسيد كمادة حافظة للأنسجة الرخوة التي تم جمعها لتحليل الحمض النووي و إختيار القلب للحصول على العينات.

١. أستاذ مساعد الطب الشرعي والسموم الإكلينيكية-كلية الطب-جامعة قناة السويس