

Comparison of Tris-EDTA and Trehalose on DNA Quality under Different Storage Conditions "Medico-legal View"

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Abstract

Background: DNA storage is important to ensure integrity of DNA sample and maintain its availability while investigations. The best known condition for storage of DNA samples is by using Tris-EDTA (TE); as preservative agent, stored at -80°C . A potential alternative to TE is trehalose which could stabilize any biological molecule at room temperature (RT). **Objective:** assessment of the optimal storage conditions which maintains quality of blood DNA samples with economical advantage. **Methods:** A case-control study using 8 groups of human blood DNA stored at 2 different temperatures (-80°C , RT) and preserved by using TE and trehalose. The effectiveness of storage conditions were tested at certain intervals (at set-up then after 3 & 6 months) using PCR assay of 18s ribosomal gene to evaluate DNA quality. DNA was assessed by running yield gels. **Results:** PCR success rate were 43.8% and 62.8% using TE and trehalose respectively. After 6 months, PCR success rate were 25% for TE and 62.5% for trehalose ($p < 0.05$). The relative risk (RR) of poor quality associated with using trehalose is 0.4. **Conclusions:** trehalose serves as an alternative to expensive freezer storage. It has a DNA protective effect which helps in preservation even trace DNA while judicial proceedings continue.

Keywords: DNA quality, storage conditions, trehalose, tris-EDTA.

Introduction

DNA typing has revolutionized forensic science and the ability of law enforcement to match perpetrators with crime scenes (Butler, 2005). DNA profiling is not only used to identify both victims and assailants in serious crimes such as rape and homicide but also used for paternity testing and used to establish family relationships among people claiming visas (Gunn, 2009). DNA is a precious resource in the forensic field because once gone it cannot be retrieved. The discriminating power of DNA-based evidence can be compromised by several factors that need to be taken into account. For instance, if the DNA has begun to degrade, it may not be possible to obtain a full profile that accounts for all the loci (Gunn, 2009). Fragmentation of DNA molecules may be due to improper storage conditions, isolation from stale material or bacterially contaminated conditions (Kondo and Ohshima, 2003). These conditions give rise to poor quality of DNA, leading to unsuccessful DNA profiles (Timken et al., 2005). Although DNA storage is a critical issue, there are few studies that provide definitive answers to the question of optimal storage conditions for DNA (Baust, 2008). The traditional storage condition is the use of tris-EDTA (TE) as the preservative agent stored at -80°C providing the samples is not subjected to any freeze-thaw cycles which may cause DNA breakage. TE is widely used as a solution for long-term storage of DNA due to action of EDTA to bind divalent cations that are necessary cofactors for DNA nucleases (Micklos and Freyer, 2003). Trehalose has been reported to be the most effective sugar for stabilizing proteins and biological system against damage caused by desiccation or freezing (Mathlouthi, 1999). Smith and Morin (2005) stated a new suggested storage condition in which use trehalose as a preservative agent and storage at RT. This may reduce the need of huge expensive storage facilities and may provide more

stabilization of stored DNA samples. The aim of present study was to compare the effect of different storage conditions on quality of DNA samples.

Material & methods

Blood collection

Blood was drawn from volunteers who had given informed consent and collected into a vacuoliner containing EDTA.

Study site

The present study was performed in Biotechnology Research Center, Suez Canal University.

DNA extraction

DNA was extracted by using QIAamp® DNA blood mini kit (QIAGEN Inc., Valencia CA).

Preparation & Preservation of samples

DNA samples were divided into 2 groups: First group: Each sample was diluted in 0.1X TE. Second group: Each sample was combined with disaccharide trehalose in 0.1X TE to a final concentration of 0.2M (Smith and Morin, 2005). In the present study, the number per group was 96 DNA samples. Each group was divided into 3 parts to be tested at set-up, after 3 and 6 months so that at each time of PCR, each group contained 32 DNA samples. Those 32 samples were subdivided into 4 subgroups which preserved at different temperatures [-80°C , RT] with different concentrations of DNA [5 & 10 ng/ μl] as shown in table (1). Before storage at RT, the samples had been left to dry in desiccators overnight and then stored at RT. RT was ranging between [22°C - 38°C] because

storage time began in June and ended in December. To represent the range of variation in DNA concentration typical for extracts from trace samples. The concentration of DNA was measured by NanoDrop apparatus (NanoDrop Technologies, Wilmington, DE).

Table (1): Studied groups' classification at each time of PCR.

Preservative agents	TE buffer N=32				Trehalose N=32			
	RT		-80°C		RT		-80°C	
Temperature	RT		-80°C		RT		-80°C	
DNA concentration (ng/ µl)	10	5	10	5	10	5	10	5
Group code	A	B	C	D	E	F	G	H
Total number of each group	8	8	8	8	8	8	8	8

TE: Tris-EDTA

RT: Room Temperature

PCR: Polymerase Chain Reaction

PCR amplification

A PCR assay of a 757 bp fragment of the 18s ribosomal gene (housekeeping gene) was performed to give an indication of DNA quality. The assay was performed in 20 µL reaction volumes containing 5µl of DNA sample, 1 X PCR Buffer, 4.5 mM MgCl₂, 0.8mg/mL BSA, 250 µM each of dATP, dGTP, dCTP, dTTP, 0.025 U/µl Taq polymerase and 300 nM of each primer. The following primers were used:

– *Forward primer:*

ATTCGATTGCGCCGCTAGA

– *Reverse primer:*

ACAAAGGGCAGGGACTTAATCA

Cycling was performed on Mastercycler® Gradient (Eppendorf, Netheler, Hinz GmbH). The conditions was 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 59°C for 30 seconds, then 72°C for 2 minutes (Smith and Morin, 2005). Assay was performed 3 times; at set-after 3 and 6 months.

Electrophoresis of amplified products

Electrophoresis was performed by using E865 Consort Electrophoresis apparatus (Topac Inc., Cohasset, MA). The PCR products were loaded in 1% agarose gel which was stained by ethidium bromide to detect the amplified PCR products.

Evaluation of DNA quality

Amplification products were visualized in 1% agarose gel stained with ethidium bromide. The quality of DNA was evaluated by the presence or absence of a band at the corresponding product size (i.e. at 757 bp) which was estimated by using 100 bp DNA ladder (i.e. DNA marker).

Statistical analysis

Statistical analysis was performed to determine the effects of storage condition (preservative agent/temperature combinations) on the quality of DNA. The data entry was done by Microsoft Excel Program. Chi-square test was done to test statistical significance difference between storage conditions. Relative risk (RR) was used to evaluate the effect of trehalose versus TE on DNA samples.

Ethical considerations

Written informed consent was taken from each volunteer before blood sample was drawn.

Results

DNA quality was evaluated by presence or absence of a band at the corresponding product size (i.e. at 757bp). In Figure (1), after 6 months group E; which was preserved by trehalose, stored at RT and 10ng/µl in concentration, had highest PCR success rate (100%) in comparison with other study groups.

Samples were preserved with tris-EDTA: A, B: samples were stored at room temperature (RT) & their concentrations were 10,5ng/µl respectively. **C, D:** samples were stored at -80°C & their concentrations were 10,5ng/µl respectively.

Samples were preserved with trehalose: E, F: samples were stored at RT& their concentrations were 10,5ng/µl respectively. **G, H:** samples were stored at -80°C & their concentrations were 10,5ng/µl respectively.

Table (2) shows the effect of storage conditions (preservative agent in addition to storage temperature) on DNA quality (PCR success rate) after 3, 6 months. The best DNA storage condition was preservation with trehalose either stored dried at RT or at -80°C without any statistical significance. Table (3) shows the effect of preservative agents on DNA quality (PCR success rate) after 3 & 6 months (regardless of storage temperature). The PCR success rate showed no significant difference between trehalose and TE after 3 months storage while there is significant difference between them after 6 months storage (P = 0.0275).

Table (4) shows the effect of storage temperatures on DNA quality (PCR success rate) after 3 & 6 months (regardless of preservative agent). PCR success rate showed no significant difference between -80°C and RT either after 3 or 6 months storage.

Figure (2) shows the preservation trend of each preservative agent (TE & trehalose) through time. PCR success rate with TE was 43.75% (after 3 months) then decreased to 25% (after 6 months). While PCR success rate with trehalose was 62.5% either after 3 or 6 months of storage. The relative risk (RR) of degradation (i.e.: poor quality) associated with using trehalose is 0.4.

Table (2): Effect of storage conditions (preservative agent in addition to storage temperature) on DNA quality (PCR success rate) after 3, 6 months.

Preservative Agent	PCR success rate							
	-80 ^o C				RT			
	3M		6M		3M		6M	
	N=16	%	N=16	%	N=16	%	N=16	%
Tris-EDTA	6	37.5	4	25	8	50	4	25
Trehalose	10	62.5	10	62.5	10	62.5	10	62.5

M: month

N: number of DNA samples/group

Table (3): Effect of preservative agents on DNA quality (PCR success rate) after 3 & 6 months (regardless of storage temperature).

Preservative Agent	PCR success rate			
	3 months		6months*	
	N=32	%	N=32	%
Tris-EDTA	14	43.75	8*	25
Trehalose	20	62.5	20	62.5

Chi-square test: * P value < 0.05 when compared to group preserved with trehalose (after 6 months).

Table (4): Effect of storage temperatures on DNA quality (PCR success rate) after 3 & 6 months (regardless of preservative agent).

Storage Temperature	PCR success rate			
	3 months		6 months	
	N=32	%	N=32	%
-80 ^o C	16	50	14	43.75
Room Temperature	18	56.2	14	43.75

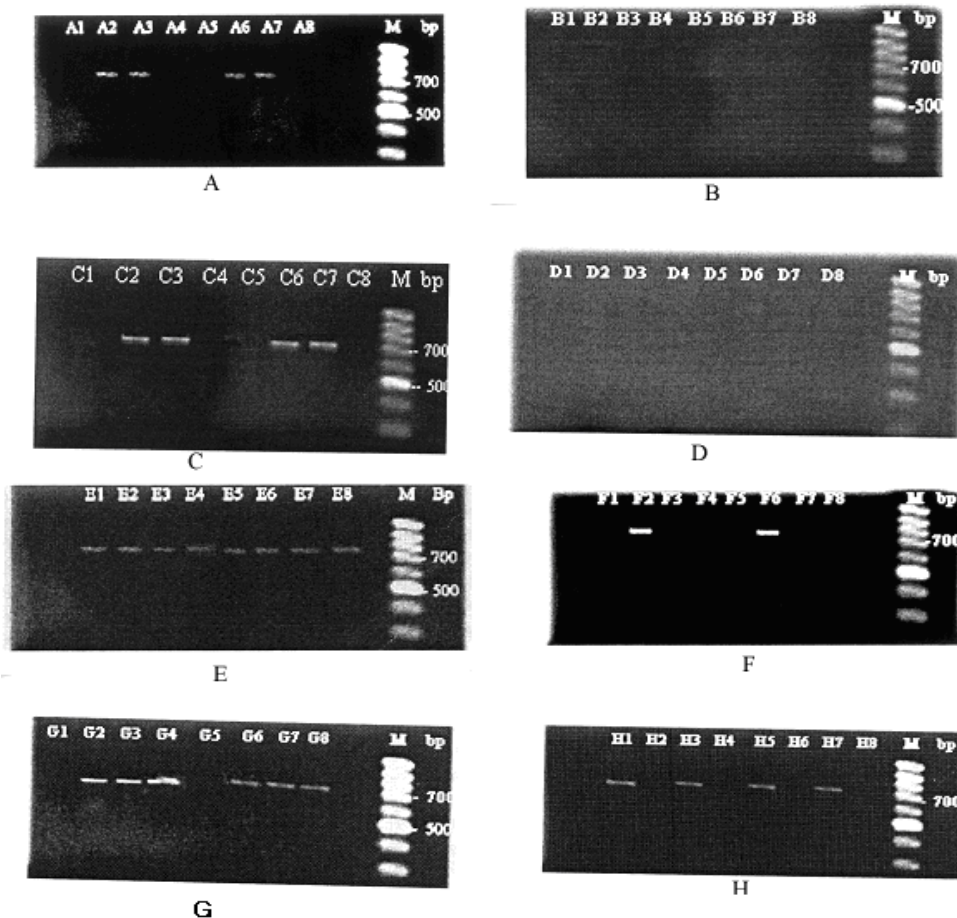


Figure (1): Gel images of PCR products for a 757 bp fragment of the 18s ribosomal gene amplified from DNA after 6 months. M: Marker or DNA ladder, bp: base pair.

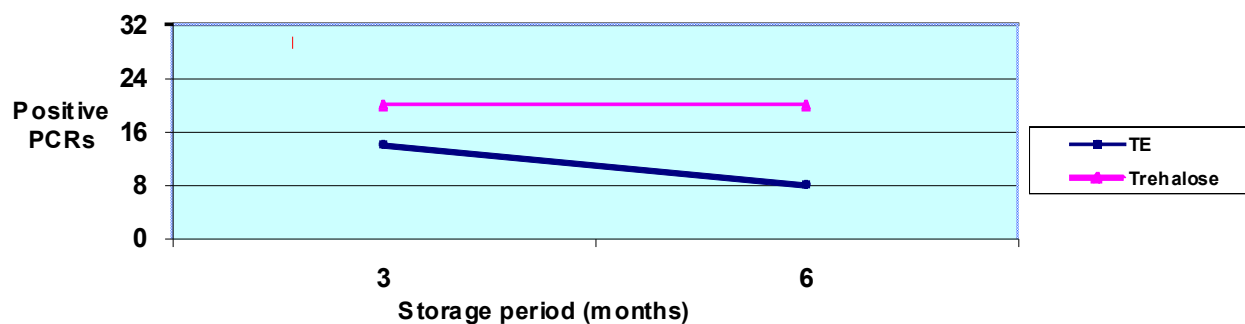


Figure (2): Preservation trend of each preservative agent (TE & trehalose) through time.

Discussion

In the present study, the best DNA storage condition (after 3 & 6 months) was preservation with trehalose either stored at -80°C or stored dried at RT. This could be attributed to trehalose can form special protective membrane above the molecules under bad conditions of hyperthermia, high-coldness and dehydration, which can protect structure of molecules from being destroyed (Mathlouthi, 1999). This can explain why trehalose either with storage at RT or -80°C were the best conditions. The result of present study is consistent with Smith and Morin (2005) who stated that the observed improvement in PCR yield with the addition of trehalose to DNA samples may be due to either its role as a biomolecule stabilizer or as a PCR enhancer. In addition, trehalose forms a glass upon drying and this process has been shown to stabilize proteins in a dry state due to the action of the glass-like trehalose on the intra-molecular folding (Colac et al., 1992). It is likely that a similar process occurs in the case of DNA with the trehalose acting to replace the water molecules in the folded chain and keep the DNA as in a hydrated state (Smith and Morin, 2005).

In the present study, DNA quality was nearly equal for samples stored at RT and -80°C . Chaturvedi et al., (1999) who stated that typing of human urinary DNA samples demonstrated that samples stored at RT produced higher PCRs success rates than that of frozen samples. In the contrary, Smith and Morin (2005) stated that after 12 months of human placental DNA storage, the samples stored at -80°C produced higher PCRs success rates than that stored at RT. This difference between results of various studies about the best storage temperature of DNA samples may be due to the difference in storage period of DNA samples in these studies.

The present study showed that the preservation trend when using trehalose have not been changed over time of preservation while the preservation trend when using TE buffer have been decreased over time of preservation. This may be explained by that trehalose has a good power of DNA preservation across time of preservation. While TE buffer led to some degree of DNA degradation across time of preservation. The results of present study is in consistent with stored samples resulted in 100% PCR success as well as it may provide a more consistent level of DNA quality preservation Smith and Morin

(2005) who stated that the addition of trehalose to dry relative to other storage methods. Higashiyama (2002) reported that trehalose protects organisms against various stresses such as dryness, freezing and osmopressure such as in the case of urrection plants, which can live in a dry state, when the water dries up, the plant dry up too. They can successfully revive when placed in water.

The present study concluded that using trehalose in DNA preservation has dual benefits; first, it has a protective effect and stabilization action on DNA in samples and second, it has an economical benefit because it will be used in addition to storage at RT thereby limiting the need for huge expensive storage facilities.

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الملخص العربي

المقارنة بين تريس إيديتا و التريهالوز في جودة حفظ الحامض النووي في ظروف التخزين المختلفة من الواجهة الطبية الشرعية

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الخلفية العلمية: إن حفظ الحامض النووي من التلف أثناء فترة التقاضي يعد شيئاً هاماً لعمل البصمة الوراثية التي تمثل أهمية قصوى في الطب الشرعي. ومن المتعارف عليه أن أفضل طرق حفظ الحامض النووي هي استخدام تريس إيديتا عند درجة حرارة -80°م. ولقد ظهر في الأونة الأخيرة خصائص مميزة لمادة التريهالوز والتي تتميز بحفظ و تثبيت العينات الحيوية عند درجة حرارة الغرفة. **هدف الدراسة:** تهدف هذه الدراسة إلى تقييم أفضل طرق التخزين التي تحافظ على عينات الحامض النووي بالدم من التلف مع اعتبار البعد الاقتصادي. **الطرق المستخدمة:** تم استخدام ثماني مجموعات تمثل عينات من الحامض النووي مستخلصة من عينات الدم ومخزنة عند درجة حرارة -80°م ودرجة حرارة الغرفة باستخدام تريس إيديتا وتريهالوز. ثم استخدام آلية تفاعل البلمرة المتسلسل للجين الريبوسومي (18s) لتقييم جودة الحامض النووي عند بداية التخزين ثم بعد 3 و6 أشهر، وذلك بجريانه على الجيل. **النتائج:** أظهرت النتائج أن معدل نجاح تفاعل البلمرة المتسلسل هو 43,8% للتريس إيديتا و 62,8% للتريهالوز. و بعد 6 أشهر أصبح 25% للتريس إيديتا و 62,5% للتريهالوز ($P < 0.05$) كما بلغ الخطر النسبي لسوء الجودة 0,4 مع استعمال التريهالوز. **الخلاصة:** تعتبر التريهالوز مادة حفظ جيدة و بديل لتخزين عينات الحامض النووي في درجة التجميد العالية التكاليف و هي تحافظ عليه و لو بكمية قليلة أثناء فترات التقاضي.

الكلمات المفتاحية: نوعية الحامض النووي – ظروف التخزين- تريهالوز – تريس إيديتا