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Evaluation of novel forensic DNA storage methodologies

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ABSTRACT

An issue in forensic sciences is the secure storage of extracted DNA. Most of the time, DNA is frozen at -20°C or -80°C . Recently, new room temperature DNA storage technologies have been developed based on anhydrobiosis.

Two products use this technology: Qiasafe (Qiagen) and Gentegra (Genvault). In this study we focused on the recent Gentegra product and initiated a comparison versus -20°C and Qiasafe storage. We compared the quantity and quality of DNA stored using anhydrobiosis technology against DNA stored at -20°C , by performing STR profiling after short term storage. Furthermore, we studied the quantity and integrity of DNA after long term storage. Our results prove the high potential of this technology but it seems to be extraction dependent. Phenol/chloroform extracted DNA could be stored using the Gentegra matrix for more than 6 months without any obvious degradation. However, DNA extracted using magnetic beads could not be safely stored over the same period. Adaptations are therefore required to store this kind of samples.

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1. Introduction

Success of forensic genetics has led to a considerable DNA storage issue. The most common storage method is freezing at -20°C or -80°C . With the increasing numbers of samples, freezing appears to be costly and not without risk of failure. To avoid these disadvantages, new room temperature DNA storage technologies have been developed. These new technologies are based on anhydrobiosis, a biological process employed by some multicellular organisms that are able to survive more or less complete dehydration [1]. In this system, the DNA solution is air-dried in a chemical medium to ensure its preservation over long periods of time. Chemicals form a protective and stable barrier to protect DNA against degradation and oxidation. DNA recovery is simply done by rehydration.

In an effort to find a methodology to store DNA at room temperature, Smith and Morin have tested trehalose [2]. Trehalose is one of the major compounds accumulated during anhydrobiosis [3,4]. Trehalose, with other compounds, replaces water and interacts with macromolecules during dehydration. Previous work had shown that trehalose retains activities of dry enzymes for days [5]. The work of Smith and Morin indicated that drying DNA in presence of trehalose is a good alternative to freezing. DNA could be stored without loss of amplifiable DNA for up to 1 year [2].

Two companies, Qiagen and Genvault, have developed DNA storage systems, respectively Qiasafe and Gentegra, using anhydrobiosis. The Qiagen product is based on a synthetic polymer that mimics the anhydrobiosis process. The Genvault product is an inert mineral medium which creates a water-free environment protecting DNA samples from hydrolysis. When air-dried in Gentegra medium, DNA becomes more stable at room temperature and less sensitive to UV light [6]. The aim of these products is to provide a sure and easy way to preserve DNA.

Both companies have performed internal studies using accelerating ageing by heating DNA sample. Heating of the DNA sample is well known to induce DNA damage [7]. This allows virtual years ageing in few weeks only. Qiasafe and Gentegra can protect DNA for, respectively, 30 and 10 accelerated years [8]. But this ageing does not mimic perfectly natural ageing.

A first study has underlined the high potential of this technology using the Qiagen and Gentegra conservation matrixes. These authors stored DNA for up to 3 weeks at room temperature without obvious degradation or loss [8]. However, this study presents some limitations. The authors did not document the ability of these conservation matrixes to undergo multiple cycles of hydration/drying nor did they test long term storage [8].

Here, we assessed the Gentegra conservation matrix using as references the -20°C storage and the Qiasafe matrix. The first considered criterion was the DNA recovery. We treated several quantities of human DNA with the two conservation matrixes over multiple hydration cycles and assessed DNA quality by STR profiling. The second criterion was the integrity and quantity of DNA after storage at room temperature for 6 months.

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2. Materials and methods

2.1. DNA extraction

Blood samples were extracted using a standard organic extraction with phenol/chloroform followed by ethanol precipitation.

Buccal swabs DNA extraction was carried out using the Kingfisher robot (Thermo Electron, The Netherlands) using the NucleoMag96Trace kit from Macherey-Nagel (Germany) according to manufacturer's protocol.

2.2. DNA quantification

Quantification was performed using the Quantifiler system (Applied Biosystems, USA) and an Applied Biosystems 7500 Real-Time PCR system according to manufacturer's specifications. The quantification was done in duplicate. The average was considered for the results and the following experiments.

2.3. PCR amplification with Identifiler™ kit

The Identifiler™ kit (Applied Biosystems, USA) was used following manufacturer's recommendations. Twenty-eight cycles of reaction were performed. One ng of sample per reaction (according to Quantifiler quantification results) in a total reaction volume of 25 μ l was amplified on a C1000 thermocycler (Bio-Rad laboratories, Belgium). Samples were analyzed on a 3130 XL Genetic Analyzer (Applied Biosystems, USA) using a 36 cm capillary array (Applied Biosystems, USA), POP-4 polymer (Applied Biosystems, USA), 1 \times genetic analysis buffer with EDTA (Applied Biosystems, USA), a 18 s injection at 1.2 kV and 15 kV electrophoresis. Two μ l of PCR product were loaded with 10 μ l of formamide (Applied Biosystems, USA). Genemapper ID v3.2 software (Applied Biosystems, USA) was used for analysis.

2.4. DNA application on the conservation matrix

The Gentegra (Genvault) and Qiasafe (Qiagen) conservation matrixes were used following manufacturer's specifications. Twenty μ l of diluted sample was applied to the matrix, mixed gently by pipetting and dried overnight (16 h) under a chemical flow hood with a constant air humidity of 18%. The sealed tubes were store at 20 °C under a constant humidity of 18% in the dark. The humidity was measured using a hygrometer THG 312 (Oregon Scientific, USA). The quantities of DNA used for each experiment are reported in Section 3. Hydration of the dried samples mixed

with the matrix was done with 20 μ l of MilliQ water (Qiasafe) or the buffer purchased with the kit (Gentegra).

2.5. Hydration/drying cycles–freezing/thawing cycles

In the multiple hydration/drying cycles experiments, DNA mixed with the matrix was hydrated with 20 μ l of MilliQ water (Qiasafe) or the buffer purchased with the kit (Gentegra) and then dried overnight as described above. Twenty-four hours were let between two cycles. Reference samples frozen at –20 °C were subjected to the same freezing/thawing cycles. Thawed samples were let at room temperature for 30 min and then frozen again at –20 °C.

2.6. Statistics

Comparison between conditions was done using a TTEST.

3. Results

3.1. DNA recovery

We first determined the efficiency of the DNA recovery after storage in the conservation matrixes. Per tube, 1 μ g of DNA in 20 μ l was loaded on the conservation matrixes and dried as indicated in Section 2. Twenty-four hours later, the matrix was hydrated in 20 μ l, two aliquots were taken, one for quantification, one for STR profiling and stored until analyses at –20 °C. These steps were reproduced two more times (one cycle per 24 h). All samples were quantified twice, at the same moment, with frozen references to avoid variations between quantifications. Regardless of the precision of the real-time quantification, Fig. 1 shows a high recovery of the DNA for both Gentegra and Qiasafe matrixes. No significant change of DNA quantities was detected after the three cycles of hydration/drying except for the second cycle with the Qiasafe matrix.

The quality of the stored DNA was also investigated by STR profiling. To avoid experimental variations, all samples from the first and the last cycle of hydration/drying were freshly diluted to obtain 1 ng in 10 μ l of MilliQ water and amplified simultaneously with the –20 °C DNA references. The matrix DNA samples and the corresponding –20 °C references were electrophoresed simultaneously.

With the three conservation methods (Gentegra, Qiasafe or –20 °C freezing), the STR profiles were complete and above a threshold fixed to 50 RFU. The intensity of the peak for each allele was compared to the intensity obtained for the same allele

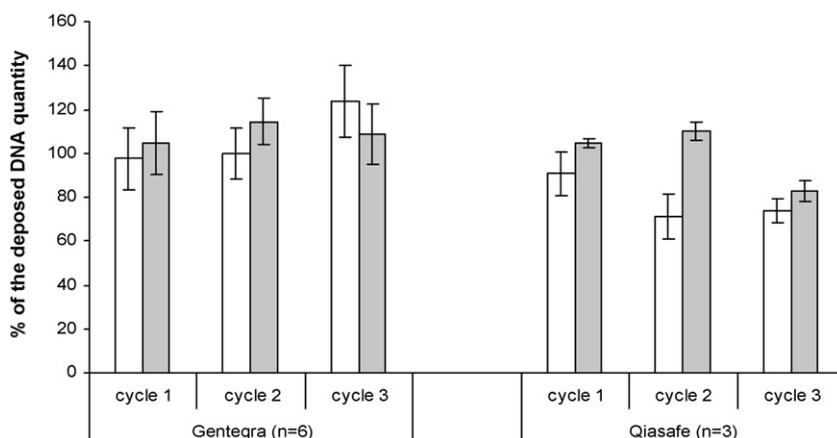


Fig. 1. Percentage of the original quantity of DNA stored using the Gentegra or the Qiasafe matrixes after one, two or three cycles of hydration/drying. Reference samples stored at –20 °C were submitted to three cycles of thawing/freezing of 30 min, one cycle per day. White columns: matrix storage; gray columns: –20 °C freezing.

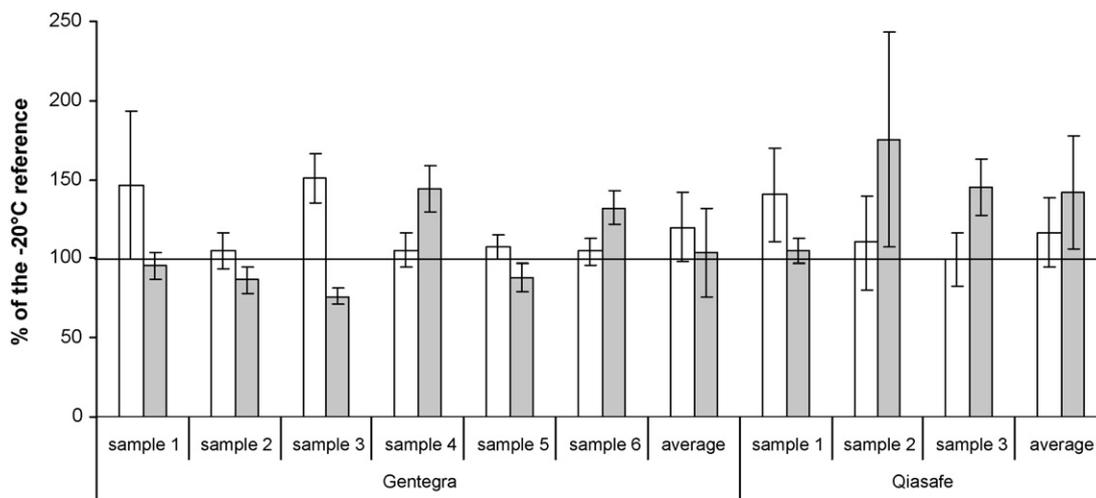


Fig. 2. Intensity of the signal obtained after STR profiling and electrophoresis of samples submitted to one or three cycles of hydration/drying. The reference samples stored at -20°C were submitted to three cycles of thawing/freezing of 30 min (one cycle per day). Results are expressed as the percentage of the respective samples stored at -20°C . White columns represent the first cycle. The third cycle is represented by gray columns.

provided by the reference DNA that was frozen. This was done for all alleles and an average was established sample per sample. Fig. 2 shows no significant difference of intensity between the Gentegra stored DNA and the frozen samples. Similar results were obtained using the Qiasafe matrix. Moreover, no significant variation of signal intensity was observed between the first and the third cycle of hydration demonstrating the absence of DNA degradation following these steps (Fig. 3). This was calculated by comparing the intensities obtained after electrophoresis for each allele using the samples submitted to one or three cycles of rehydration/drying. An average of the obtained percentages was calculated sample per sample (see Fig. 3). Similarly, no degradation and decrease of intensity was observed for the samples following three cycles of thawing/freezing.

The last quality criteria checked was the heterozygote balance. Using the sample of the third cycle of thawing/freezing–hydration/drying, we compared the intensities of the first and second alleles. The first allele represents the 100% reference value. For each of the six samples, storage and PCR were done in triplicate. STR per STR the second allele was compared to the first and an average of the

triplicate was established for each STR. Fig. 4A shows the average values obtained for each STR. We obtained the average values of $94.95 \pm 6.73\%$ and $95.58 \pm 5.06\%$, respectively, for the storage with the Gentegra technology and the -20°C freezing without any significant difference ($p > 0.05$). Similar results were obtained using Qiasafe (see Fig. 4B). We obtained an average of $94.77 \pm 6.37\%$ and $94.34 \pm 6.92\%$ for respectively Qiasafe and -20°C freezing.

3.2. Storage of low concentrations of DNA and high volumes

The Gentegra storage system is optimized for a volume of $20\ \mu\text{l}$. In many forensic samples the concentration of DNA is low and the volume is sometimes close to or above the $20\ \mu\text{l}$ limit. In a first set of experiments, the storage of $60\ \mu\text{l}$ of DNA solution was tested.

Sixty μl of six different DNA solutions were placed in a microtube with or without storage matrix and dried in a speed-vac for 90 min. Then, samples were let overnight in a flow hood for complete drying. Afterwards, samples were rehydrated in $60\ \mu\text{l}$ and quantified simultaneously with the corresponding frozen references. Results are presented in Table 1. We obtained average

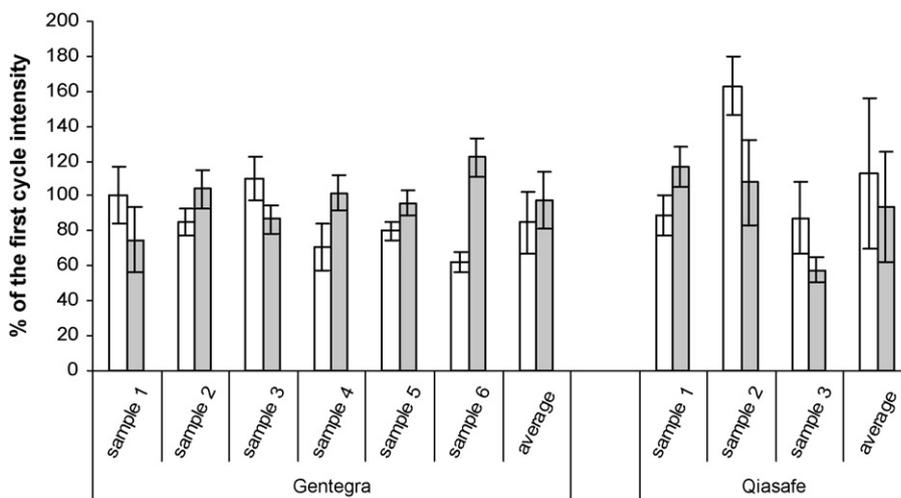


Fig. 3. Comparison of the intensity of the STR profiling following one or three cycles of hydration/drying. Reference samples were also submitted to three cycles of thawing/freezing. Results are expressed as the average of the intensity obtained for the first cycle, system per system. The white and gray columns represent, respectively, the matrix and the -20°C storages.

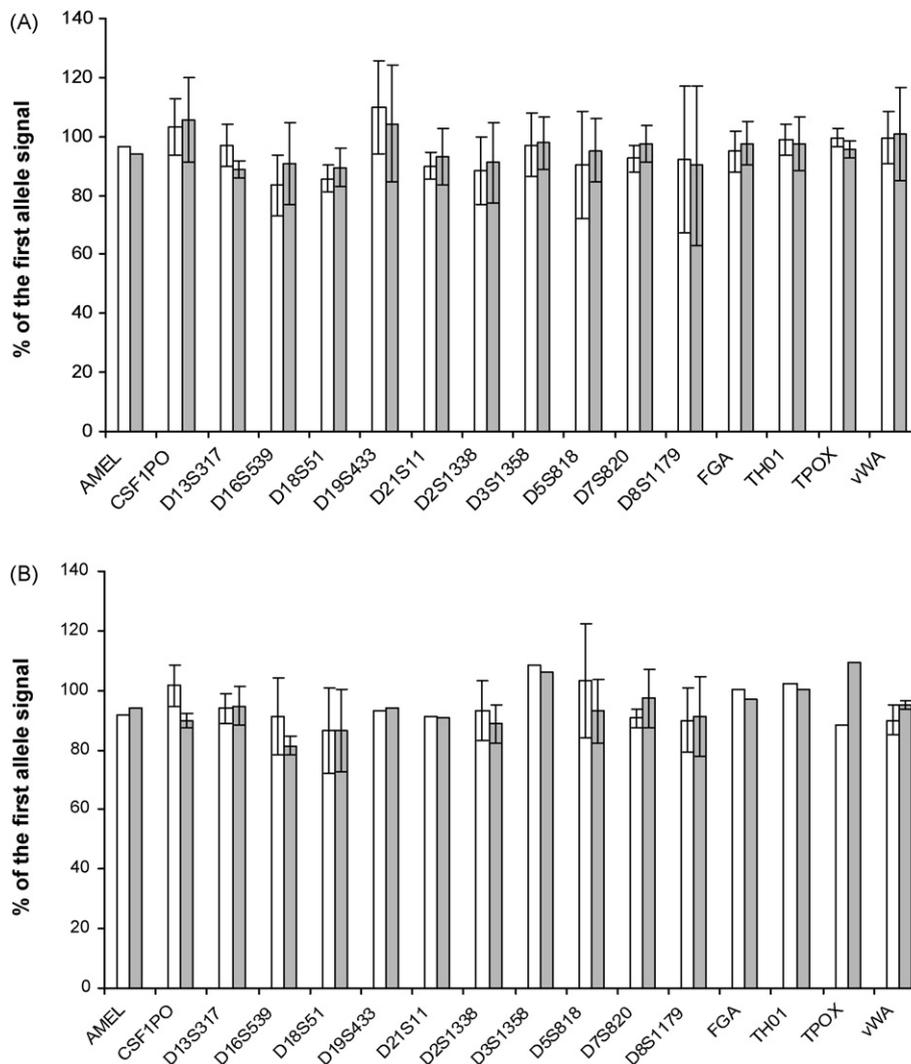


Fig. 4. Heterozygote balance for each STR amplified from matrix and -20°C stored DNA. Results are expressed as the percentage of the first allele. White columns: matrix storage. Gray columns: -20°C storage. Panel A: Gentegra ($n = 6$). Panel B: Qiasafe ($n = 3$).

DNA recoveries of $99.22 \pm 12.86\%$ and $104.30 \pm 23.33\%$ of the frozen reference samples when we used, respectively, the Gentegra and the Qiasafe systems.

In a second round of experiments, we investigated the storage of low DNA quantities. Twenty μl of a DNA solution at $0.150 \text{ ng}/\mu\text{l}$ were stored using both matrixes and the quantity and the quality of the recovered DNA were analyzed. Fig. 5 demonstrates that there is no loss of DNA after storage in the Gentegra or Qiasafe storage matrixes.

Allele per allele, we compared the low DNA quantity samples (matrix versus -20°C) and an average percentage for all the alleles was established for each sample. We observed that the intensity of

the PCR signal obtained with the Gentegra stored DNA is not closely related to the one obtained with DNA stored at -20°C . The average signal over four samples tested with the Qiasafe stored DNA reached $90.71 \pm 3.97\%$ of the reference DNA stored at -20°C instead of $70.24 \pm 15.82\%$ for Gentegra. This difference is not significant using a TTEST. The lowest performance of the Gentegra matrix is due to sample 4 as shown in Fig. 6.

3.3. Six months storage

Twenty μl of DNA from saliva extracted as described in Section 2 were stored on Qiasafe and Gentegra for a period of 6 months at

Table 1
 Recovery of DNA for high volume DNA samples. Samples were loaded on matrixes or frozen. After rehydration, matrix samples and frozen samples were quantified. Results are expressed in $\text{ng}/\mu\text{l}$ and as a percentage of frozen references.

	-20°C ($\text{ng}/\mu\text{l}$)	Gentegra ($\text{ng}/\mu\text{l}$)	Qiasafe ($\text{ng}/\mu\text{l}$)	Gentegra/ -20°C (%)	Qiasafe/ -20°C (%)
Sample 1	2.92	2.48	4.03	84.93	138.01
Sample 2	19.81	19.35	17.77	97.67	89.70
Sample 3	8.64	9.10	8.50	105.32	98.37
Sample 4	9.92	10.86	12.63	109.47	127.31
Sample 5	19.82	20.50	18.82	103.43	94.95
Sample 6	27.15	20.78	21.03	76.53	77.45
Average %				$99.22 \pm 12.86\%$	$104.30 \pm 23.33\%$

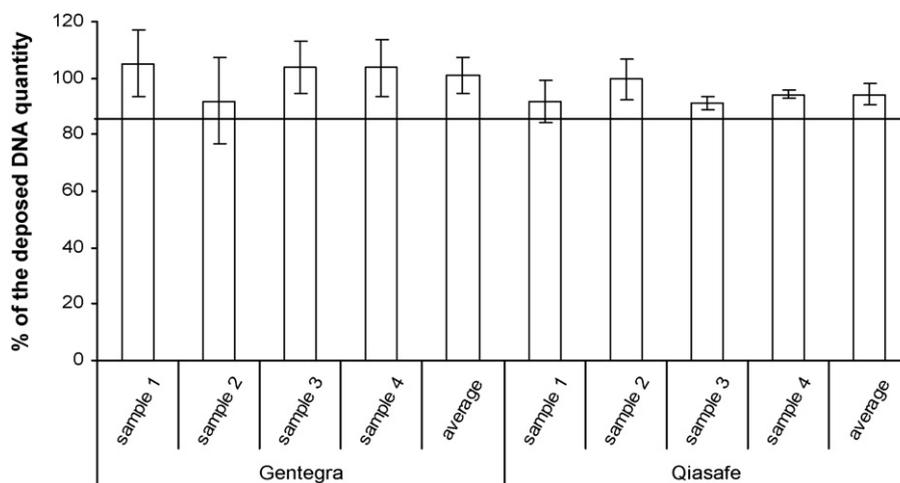


Fig. 5. Low amounts DNA storage. Percentage of the original quantity of DNA (0.150 ng/μl) stored using the Gentegra or the Qiasafe matrixes after one cycle of hydration/drying.

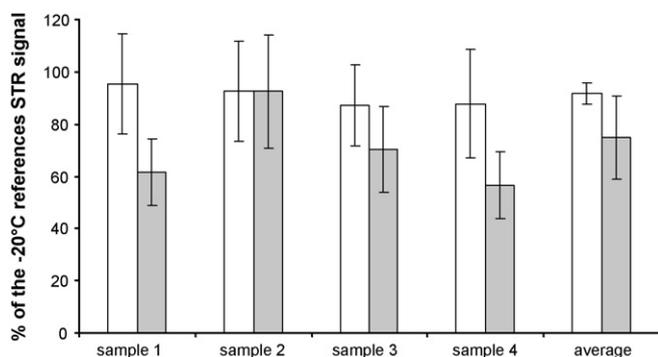


Fig. 6. Intensity of the signal obtained after STR profiling and electrophoresis of the samples containing 0.150 ng/μl of DNA. Results are expressed as a percentage of the respective samples stored at -20 °C. Gray columns represent DNA stored in the Gentegra matrix. The DNA stored using Qiasafe is represented by white columns.

high recovery of the DNA after this long term storage. We obtained $111.09 \pm 23.65\%$ and $78.58 \pm 10.38\%$ of recovery, respectively, with the Gentegra and Qiasafe systems. Table 2 presents the results obtained with these DNA. A TTEST indicated that the Gentegra and Qiasafe recoveries are significantly different in favor of the Gentegra conservation system.

The STR profile was established for 15 samples. After electrophoresis, we observed a strong decrease of the signal intensity for DNA stored using Gentegra or Qiasafe versus frozen DNA. This decrease is similar for all amplified STR's showing that this low signal intensity is not due to a typical degradation because in that case the highest molecular weights should be more affected by the signal decrease. Another hypothesis that could explain this observation is a potential inhibition of the PCR reaction. Inhibition of the PCR reaction implies typically a delay in the Internal Positive Control (IPC) Ct values during the real-time quantification. However, we observed that the IPC Ct values of frozen samples and of samples stored in both matrixes were indistinguishable, which suggests this phenomenon is not due to PCR inhibition. In the other hand, this also proves that the matrixes are not PCR enhancers. Fig. 7 shows the comparison of the obtained patterns.

20 °C under a constant humidity of 18%. After recovery, the DNA was quantified simultaneously with the corresponding frozen references and the STR profile established. Our results showed a

Table 2

Long term storage of DNA from saliva. Samples recovered from matrixes and the corresponding frozen references were quantified simultaneously. Results are expressed in ng/μl and as a percentage of frozen references.

	-20 °C (ng/μl)	Gentegra (ng/μl)	Gentegra/-20 °C (%)		-20 °C (ng/μl)	Qiasafe (ng/μl)	Qiasafe/-20 °C (%)
Sample 1	2.81	3.21	114.23	Sample 21	2.02	1.52	75.24
Sample 2	5.68	6.30	110.91	Sample 22	0.93	0.89	95.90
Sample 3	5.41	5.31	98.15	Sample 23	3.56	2.81	78.93
Sample 4	0.58	0.46	80.31	Sample 24	3.66	2.57	70.21
Sample 5	6.39	6.97	109.07	Sample 25	3.27	2.32	70.94
Sample 6	3.57	4.05	113.44	Sample 26	3.25	2.46	75.69
Sample 7	3.51	4.07	115.95	Sample 27	0.70	0.54	77.20
Sample 8	1.64	2.05	125.00	Sample 28	3.48	2.78	79.88
Sample 9	0.22	0.17	78.12	Sample 29	1.49	0.82	55.43
Sample 10	2.28	2.06	90.35	Sample 30	2.73	2.76	101.09
Sample 11	1.76	1.76	100.00	Sample 31	4.31	3.43	79.58
Sample 12	5.37	6.23	116.01	Sample 32	5.60	5.39	96.25
Sample 13	0.95	1.09	114.73	Sample 33	1.01	0.75	74.65
Sample 14	3.89	5.22	134.19	Sample 34	1.62	1.20	74.07
Sample 15	7.38	8.39	113.68	Sample 35	1.56	1.11	71.15
Sample 16	2.92	3.25	111.30	Sample 36	0.61	0.46	75.45
Sample 17	2.81	4.96	176.51	Sample 37	3.07	2.54	82.73
Sample 18	3.07	3.63	118.24	Sample 38	3.67	3.19	86.92
Sample 19	1.31	0.86	65.34	Sample 39	5.13	3.66	71.34
Sample 20	0.52	0.71	136.27	Sample 40	2.14	1.69	78.97
Average %			111.09 ± 23.65%	Average %			78.58 ± 10.38%

The quantifications of the profile intensities give, respectively, $59.66 \pm 19.53\%$ and $51.50 \pm 14.95\%$ of the signal obtained with the -20°C references when the Gentegra and Qiasafe system are used.

To further investigate this phenomenon, we have stored three different DNA samples in triplicate ($1\ \mu\text{g}$ in $20\ \mu\text{l}$) from whole blood extracted using phenol/chloroform in the Gentegra matrix for up to 6 months. The recovery rate of the DNA was $110.57 \pm 23.10\%$ confirming our previous results. As above, the STR profiles were done and the intensity of the signal for each STR was compared to the -20°C reference. In this case, we observed a signal identical to the one observed with the frozen references. No difference can distinguish the two conservation methodologies with an average signal for the Gentegra representing $95.11 \pm 17.28\%$ of the -20°C reference signal. The heterozygote balance was also very good since it reached $92.79 \pm 2.75\%$ ($\text{signal allele2}/(\text{signal allele1}/100)$) in comparison to the $97.15 \pm 5.88\%$ obtained with the frozen references.

4. Discussion

In this study, we evaluated the Gentegra room temperature storage technology in comparison to Qiasafe, the other room temperature storage technique and freezing at -20°C . One recent paper compared Gentegra, Qiasafe and freezing but this study suffers of several limitations. The authors did not study the ability of these conservation matrixes to undergo multiple cycles of hydration/drying nor did they test long term storage. Their study is limited to 3 weeks and they did not test the recovery of DNA at low concentrations [8].

Our work answers to all these questions thereby complementing the study of Wan et al. [8]. Moreover, we tested the influence of the extraction protocol on DNA conservation.

Our results prove that the DNA/matrix (Gentegra or Qiasafe) mixture can be rehydrated at least three times without affecting the recovery and the quality of the DNA. Samples stored in both matrixes and submitted to three cycles of dehydration/hydration produced STR intensities similar to the one of samples frozen at -20°C and submitted to three cycles of freezing/thawing. Moreover, no significant variation of signal intensity was observed between the first and the third cycle of hydration demonstrating the absence of DNA degradation following these steps. Three cycles of thawing/freezing had also no influence on the quality of the DNA stored at -20°C .

Because low amounts of DNA are often obtained in forensics, we have also proven that such samples can be stored using this technology. In these experiments storage of $20\ \mu\text{l}$ of DNA at a concentration of $0.150\ \text{ng}/\mu\text{l}$ using both kits of conservation gave similar results. No significant loss of DNA and decreased intensities after STR profiling were observed. We also observed that multiple rehydration/drying cycles had no influence on the quality of the DNA as proved by STR profiling.

Concerning long term storage, we showed that the protocol used to extract DNA has an impact on conservation. Indeed, when DNA was extracted using the classical phenol/chloroform extraction protocol and stored using the Gentegra matrix, the recovery after more than 6 months was high and the DNA was well preserved from degradation. However, DNA extracted from saliva

using the magnetic beads system was not optimally preserved. Indeed, in that case, we observed a global decrease of the signal suggesting an oxidation of the DNA. One possible explanation is the presence of magnetic particles in samples leading to a disruption of the matrix protection. Another possible explanation is a possible presence of iron coming from the magnetic particles catalyzing oxidation. The conservation of these samples should therefore be optimized using, for example, a drying box containing silica gels.

Because the forensics community has also interests in RNA analyses [9–11] one of the prospect of anhydrobiosis is RNA storage. This technology seems to be adapted for this purpose. Indeed, Hernandez et al. have stored RNA at room temperature during 4 weeks and did not observe any difference between frozen and stored dry in specific matrix samples [12].

In conclusion, our work demonstrates that both Gentegra and Qiasafe can preserve DNA for long periods of time at room temperature but the protocol should be optimized depending on the extraction methods. Furthermore, these results indicate that samples can be used directly after rehydration and without removing the matrix from the sample. This kind of storage reduces dependency on space, energy consumption of freezer and avoids multiple freezing/thawing cycles. It remains to be determined if these systems allow DNA storage during several years. Both manufacturers claim that it is the case but this should be independently tested.

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References

- [1] J.H. Crowe, J.F. Carpenter, L.M. Crowe, The role of vitrification in anhydrobiosis, *Annu. Rev. Physiol.* 60 (1998) 73–103.
- [2] S. Smith, P.A. Morin, Optimal storage conditions for highly dilute DNA samples: a role for trehalose as a preserving agent, *J. Forensic Sci.* 50 (2005) 1101–1108.
- [3] D.A. Wharton, C.J. Marshall, How do terrestrial Antarctic organisms survive in their harsh environment? *J. Biol.* 8 (2009) 39.
- [4] M. Sakurai, T. Furuki, K.-I. Akao, D. Tanaka, Y. Nakara, T. Kikawada, M. Watanabe, T. Okuda, Vitrification is essential for anhydrobiosis in an African chiromid, *poly-pedilum vanderplanki*, *Proc. Natl. Acad. Sci. U.S.A.* 105 (2008) 5093–5098.
- [5] M. Uritani, M. Takai, K. Yoshinaga, Protective effect of disaccharides on restriction endonucleases during drying under vacuum, *J. Biochem.* 117 (1995) 774–779.
- [6] A.A. Lamola, J.P. Mittal, Solution photochemistry of thymine and uracil, *Science* 154 (1966) 1560–1561.
- [7] V.I. Bruskov, L.V. Malakhova, Z.K. Masalinov, A.V. Chernikov, Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA, *Nucl. Acids Res.* 30 (2002) 1354–1363.
- [8] E. Wan, M. Akana, J. Pons, J. Chen, S. Musone, P.-Y. Kwok, W. Liao, Green technologies for room temperature nucleic acid storage, *Curr. Issues Mol. Biol.* 12 (2009) 135–142.
- [9] R.I. Fleming, S. Harbison, The development of a mRNA multiplex RT-PCR assay for the definitive identification of body fluids, *Forensic Sci. Int. Genet.* 4 (2010) 244–256.
- [10] C. Haas, B. Klessner, C. Maake, W. Bär, A. Kratzer, mRNA profiling for body fluid identification by reverse transcription endpoint PCR and realtime PCR, *Forensic Sci. Int. Genet.* 3 (2009) 80–88.
- [11] M. Bauer, RNA in forensic science, *Forensic Sci. Int. Genet.* 1 (2007) 69–74.
- [12] G.E. Hernandez, T.S. Mondala, S.R. Head, Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis, *Bio-Techniques* 47 (2009) 667–670.