Preservation and High Throughput Methods for Human Tissue Samples in Tropical Climates; An Improved DVI Approach

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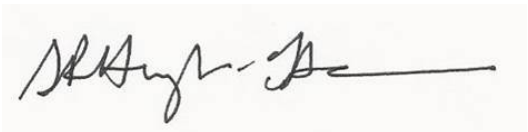
Signature of Submitting Official	 Sheree Hughes-Stamm, Ph.D.

ABSTRACT

One of the key features to be considered in a mass disaster is victim identification. However, the recovery and identification of human remains are sometimes complicated by harsh environmental conditions, limited facilities, loss of electricity and lack of refrigeration. If human remains cannot be collected, stored or identified immediately, bodies decompose and DNA degrades making genotyping more difficult and ultimately decreasing DNA profiling success. In order to prevent further DNA damage and degradation after collection, tissue preservatives may be used.

The goal of this study was to evaluate three customized (modified TENT, DESS, LST) and two commercial DNA preservatives (RNA*later* and DNAgard®) on fresh and decomposed human skin and muscle samples stored in hot (35˚C) and humid (60–70% relative humidity) conditions for up to three months. Skin and muscle samples were harvested from the thigh of three human cadavers placed outdoors every second day for two weeks. In addition, the possibility of purifying DNA directly from the preservative solutions ("free DNA") was investigated in order to eliminate lengthy tissue digestion processes and increase throughput.

The efficiency of each preservative was evaluated based on the quantity of DNA recovered from both the "free DNA" in solution and the tissue sample. The quality of DNA was measured in terms of the number of alleles recovered during downstream STR typing. As expected, DNA quantity and quality decreased with time of decomposition. However, a marked decrease in DNA quantity and quality was observed in all samples after the bodies entered the bloat stage (approximately six days of decomposition in this study). Similar amounts of DNA were retrieved from skin and muscle samples over time, but more complete STR profiles were obtained from muscle. Although higher amounts of DNA were recovered from tissue samples than from the surrounding preservative, the average number of reportable alleles from the "free DNA" was comparable.

Overall, DNAgard® and the modified TENT buffer were the most successful tissue preservatives tested in this study based on consistently more complete STR profiles from both tissue samples and "free DNA" when decomposing tissues were stored for up to three months in hot, humid conditions. In addition, adequate amounts of high quality DNA for successfully genotyping were recovered from the preservative solutions after one hour of storage, and the fastest (20 min) and most efficient method of purifying that "free DNA" in solution was found to be the QIAquick PCR Purification kit (Qiagen).

PURPOSE

The overall purpose of this study was to provide possible solutions to the problem of processing large numbers of decomposing human tissue samples for DNA-typing after a mass disaster. We aimed to investigate simple, enhanced solutions that could be used to quickly preserve tissue samples from the overwhelming number of victims that may be encountered following a mass casualty event. The time period considered relevant for sampling bodies after a mass fatality event in a tropical environment was the first two weeks. Therefore samples were harvested from three cadavers for up to two weeks after placement in the field. The liquid preservatives would maintain the quantity and quality of DNA retrieved from decomposing cadaveric samples when stored without refrigeration for one, two and three months prior to genotyping. The solutions would ideally partially lyse the tissue to release "free" DNA into solution, and also preserve that DNA from further degradation.

This project had three specific goals:

1. To investigate which preservatives would leach the most DNA from skin and muscle samples into solution and protect that "free DNA" from further degradation when stored in hot and humid conditions for up to three months.

2. To investigate the minimum time period required for adequate amounts of DNA to leach into solution and generate complete STR profiles.

3. To investigate quick DNA clean-up methods that more rapidly extract DNA from single source tissue samples by specifically targeting the "free DNA" in the preservative solution thereby avoiding a time consuming tissue digestion step.

This new combined approach may preserve decomposing tissue samples at room temperature if needed, whilst also significantly reducing the overall processing time compared to standard STR-typing procedures.

Study 1: Room temperature preservative solutions on fresh and decomposed tissues

Skin and muscle samples were collected from three human cadavers provided by Southeast Texas Applied Forensic Science Facility (STAFS) at Sam Houston State University, Huntsville, TX. The cadavers were caged and left outdoors for two weeks in October 2013. Skin samples were taken from the left thigh and muscle tissue was removed from the left quadriceps muscle group (directly under the skin sample) of each cadaver at day 0, 2, 4, 6, 8, 10, and 12.

Skin or muscle tissue (30 mg) was added to each of the five preservative solutions (300 µL) tested in this study (Table 1).

Table 1. Chemical Preservatives

Preservatives	Constituents
RNA*later* RNA Stabilization Reagent	Proprietary (Qiagen)
Modified TENT	10mM Tris, 10mM EDTA, 2M NaCl, 2% Tween 20 (120mL)

[a] as described in Graham et al. 2008 [2]
[b] as described in Allen-Hall et al. 2012 [5]

Control samples (no preservative) consisted of tissue (30 mg) in 20µL of distilled water to maintain humid conditions. Samples were stored at 35°C with relative humidity of 60-70% in a Forced Air Lab oven for one, two, and three months. Control tissue samples (no storage or preservation) were also collected from each cadaver every second day and processed immediately.

DNA Extraction

DNA extraction was performed after one, two, and three months of storage. DNA was purified directly from the tissue and liquid preservative for all three cadavers using the QIAamp DNA Investigator Kit (Qiagen) on the QIAcube robotic station (Qiagen) using the forensic casework samples protocol B (purification step only).

Skin and muscle samples were removed from the preservative solution and placed into a new tube. The DNAgard® tissues were washed with ultra-pure sterile water as per manufacturer's instructions before being placed into a new tube [11]. Tissue samples were digested with 30µL of Proteinase K (20mg/mL) and 270µL of ATL Buffer (Qiagen) and incubated at 56°C overnight. If the tissue was not completely digested, another 10µL of Proteinase K was added and incubated for an average of 4 - 6 hours until complete digestion was achieved. An aliquot (100 µL) of the preservative solution was removed from each storage tube and placed into a new tube with 200µL of PB buffer (Qiagen) prior to loading on the QIAcube.

DNA Quantification

DNA quantification was performed on a StepOne™ Real-Time PCR System (ThermoFisher Scientific, Carlsbad, CA USA). Each reaction contained 2µL of DNA, 10µL 2X SYBR® Green PCR Master Mix (ThermoFisher Scientific), 2µL hTERT primers (10µM), and 6µL diH_2O. To generate a standard curve, a 1:2 dilution series (9

standards — 50ng/µL to 0.0977ng/µL) was prepared using K526 control DNA (Promega, Madison, WI, USA). The cycling conditions were as follows: 10min at 95°C, and 40 cycles of 15s at 95°C then 1min at 60°C. Data were considered reliable if the R^2 value of the standard curve was 0.99 or greater.

STR Genotyping

Genotyping was performed using the AmpFlSTR® Identifiler® Plus PCR Amplification Kit (ThermoFisher Scientific) according to manufacturer's instructions with the modification of using a half reaction volume (12.5µL). The target amount of DNA template was 0.8ng. For low quantity DNA samples (less than 0.16ng/µL), 5µL of neat DNA extract was added to the PCR reaction. K526 control DNA was used as the positive control, and sterile water was used as the no template control. PCR was performed on a GeneAmp® PCR System 9700 (ThermoFisher Scientific) using the recommended cycling parameters.

Separation and detection of PCR products was performed using a 3500 Genetic Analyzer (ThermoFisher Scientific) with a 50cm capillary array and POP7 polymer with an injection time of 8 seconds at 1.6 kV. The reaction was prepared by adding 1µL of the amplified product or allelic ladder to a mix of 9µL Hi-Di™ Formamide and 1µL of LIZ 500 (ThermoFisher Scientific). The samples were denatured using the GeneAmp® PCR system 9700 at 95°C for 3min. Data were analyzed with GeneMapper™ software v4.1 (ThermoFisher Scientific). An analytical threshold of 100 relative fluorescence units (RFUs) was applied.

Statistical Analysis

Data were tested for statistical significance by Factorial ANOVA analysis with Fisher LSD post-hoc comparisons, using the software Statistica 12.5 (StatSoft Inc., Tulsa, OK). $P<0.05$ was accepted as the level of significance.

Results & Discussion

DNA Quantity

Controls

DNA from skin and muscle tissue was extracted immediately after collection on days 0, 4, 6, 8, 10 and 12 to determine the quantity and quality of DNA before storage or chemical preservation (no storage controls). As expected, the amount of DNA recovered from the decomposing tissue declined with time (Fig. 1). Interestingly, a rapid decrease in the amount of DNA recovered was observed in each cadaver after the bodies had initiated the "bloat" stage of decomposition at day 6 (Fig. 1). Similar amounts of DNA were recovered from skin and muscle samples (Fig. 1). However, amplifiable DNA was detected for longer during the decomposition process with skin compared to muscle (Days 12 and 10 respectively) (Fig. 1). These data suggest that skin may be a more resilient tissue to decomposition than muscle tissue.

Tissue samples were also stored under hot and humid conditions for up to three months without chemical preservative (no preservative controls). As expected, the DNA in skin and muscle decomposed more rapidly in the absence of a chemical preservative (Fig. 1). Only fresh tissue (Day 0) for all three months of storage yielded amplifiable DNA (45, 10, and 28ng/μL for one, two, and three months storage, respectively for skin, and 27, 13, and 18ng/μL for one, two, and three months storage, respectively for muscle) (Fig. 1). Tissue samples that were already decomposing when collected (Day 4 to Day 12) did not yield amplifiable DNA when stored without any chemical preservative. Not surprisingly, these data indicate that decomposing tissues stored in hot and humid conditions without any chemical preservative degrade rapidly over time.

Tissue

Results of average DNA concentrations extracted from skin and muscle samples from three cadavers stored for up to three months in various preservatives are shown in Figure 1. In general, common trends were observed in DNA quantity from skin and muscle samples for each preservative. DNA yields from skin and muscle samples over time of decomposition and storage were similar, and a decrease in DNA quantity was observed with each month of storage in all preservatives (Fig. 1). All preservatives (except for LST) effectively preserved adequate amounts of DNA for STR typing (>0.2ng) in fresh and decomposed skin (up to Day 10) for up to three months of storage (Fig. 1). The LST buffer failed to preserve the DNA in skin after Day 6 and in muscle after day 4 for all three months of storage (Fig. 1).

Compared to the other four preservatives tested, RNA*later* yielded the highest DNA concentrations (up to 270 ng/μL) in skin and muscle for up to three months of storage. However, these results differ from those previously reported by Allen-Hall et al. [5], which found RNA*later* to be relatively poor at preserving DNA for STR typing in fresh muscle tissue. This dissimilarity may be attributed to differing DNA extraction methods, tissue amounts, and preservative volumes used in both studies.

Factorial ANOVA showed that tissue type ($F_{6,67}$ = 7.8, $P<0.05$), choice of preservative ($F_{30,270}$ = 5.2, $P<0.05$), and time of storage ($F_{12,1347}$ = 4.2, $P<0.05$) all had statistically significant effects on the amount of DNA recovered from preserved tissues.

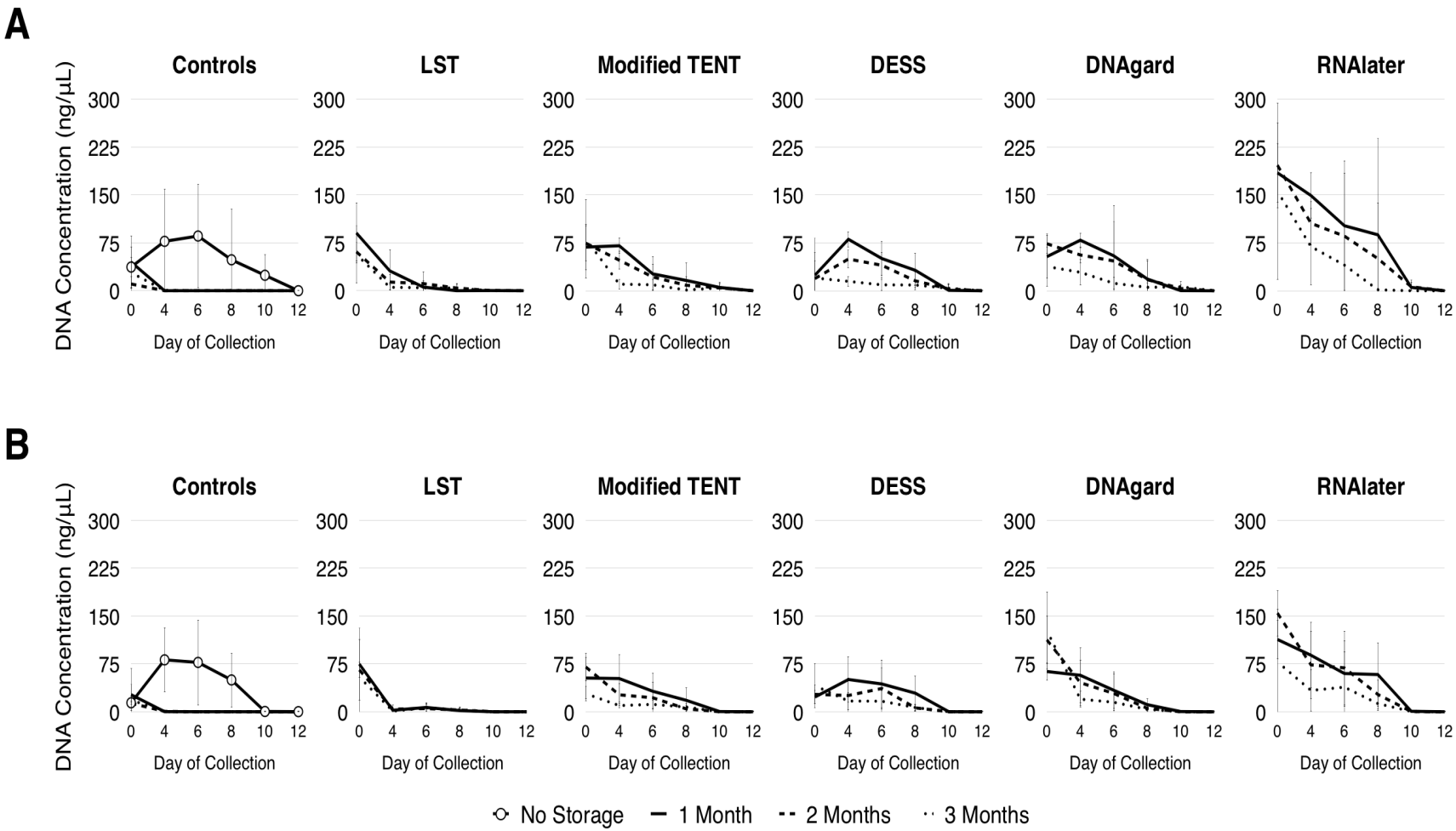


Figure 1. Average DNA concentration (ng/µL) (± SD) of the DNA from skin (A) and muscle (B) samples stored for up to three months at 35°C and 60-70% humidity in the various preservatives.

Liquid Preservative

DNA was extracted directly from each preservative solution for skin and muscle samples stored for up to three months (Fig. 2). Consistently higher amounts of DNA were obtained from the tissues preserved in RNA*later* compared to the other preservatives tested (Fig. 1). However, no "free DNA" was detected in the RNA*later* solution (data not shown). This observation was consistent with a previous report by Allen-Hall et al., 2012 [5].

DNA was consistently detected in adequate amounts for STR typing in all other preservatives (except for DESS skin samples) for up to 8 days of decomposition stored for up to three months (Fig. 2). The amount of purified DNA from preservative solution was consistently lower than that extracted from tissue. The amount of "free DNA" recovered from DESS was much higher for muscle when compared to skin (Fig. 2). These data suggest that DESS may better promote lysis and release of DNA into solution from softer tissue than from more resistant tissues such as skin. The fact that higher amounts of DNA were recovered from skin tissue than from muscle in RNA*later,* and for longer in the control samples, also confirms that skin is a more resilient tissue (Fig. 1).

Higher amounts of "free DNA" were retrieved from modified TENT and DNAgard® across time (Day 0 to Day 10) and storage (one, two, and three months) compared to the other preservatives tested (Fig. 2). DNA yields from skin and muscle stored in these preservative solutions were relatively stable for up to three months, with little decrease in the amount of DNA recovered with longer storage (in fact, slight increases were observed in some cases; Fig. 2). In addition, the "free DNA" recovered directly from DNAgard® skin samples did not notably decrease in quantity over the three month storage time (Fig. 2).

High amounts of DNA were recovered from the LST buffer after one month of storage. However, the DNA concentration in the LST buffer substantially decreased with two and three months of storage. This observation suggests that although LST promotes efficient release of DNA into solution, the buffer is unable to prevent further DNA damage and degradation over time.

Factorial ANOVA analysis showed that tissue type ($F_{6,55} = 4.0$, $P<0.05$) and choice of preservative ($F_{24,1937} = 3.4$, $P<0.05$) had significant effects on the amount of DNA recovered directly from liquid preservatives. In addition, the interaction between tissue and preservative was found to be significant ($F_{24,1937} = 1.9$, $P<0.05$). Overall, the amount of DNA retrieved from all samples (tissue and "free DNA") decreased over time (days of decomposition) regardless of tissue type (Fig. 1 & 2). Although higher amounts of DNA were recovered from tissue samples when compared to "free DNA", the amount of DNA recovered from skin and muscle samples over time in both cases (tissue and "free DNA") were similar.

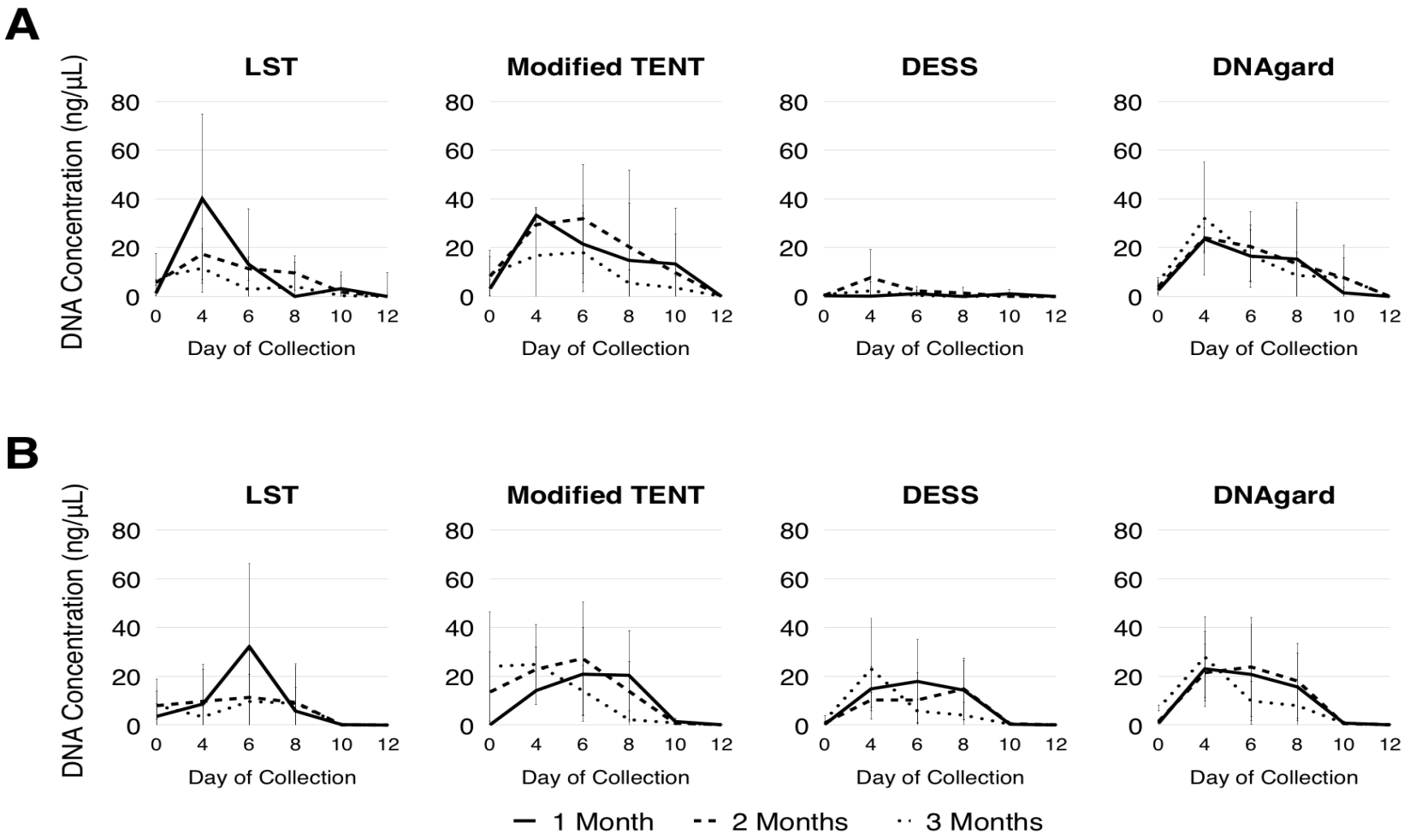


Figure 2. Average DNA concentration (ng/µL) (± SD) of the "free DNA" in the preservative solutions surrounding (A) skin and (B) muscle samples stored for up to three months at 35°C and 60-70% humidity. The data for RNAlater is not shown because it did not facilitate release of DNA into preservative.

DNA Quality

Controls

When processed immediately after collection from decomposing cadavers, complete Identifiler® Plus profiles were generated from muscle tissue for up to 6 days of decomposition, and for skin up to 8 days (Fig. 3 - No Storage). However, when tissues were stored in hot and humid conditions for up to three months without a preservative, DNA quality and therefore STR success markedly decreased (Fig. 3 - No Preservative). In most cases, complete STR profiles were obtained for fresh tissues (Day 0) stored for up to three months (without a preservative), but the DNA in all decomposed tissues (Day 4 to Day 12) degraded quickly, resulting in partial or no STR profiles being obtained (Fig. 3 - No Preservative).

Tissue

The skin and muscle samples stored in all preservatives, except for LST produced complete Identifiler® Plus profiles for up to 4 days of decomposition over the span of three months storage at 35°C and 60-70% humidity (Fig. 3). In general, the quality of DNA declined as decomposition progressed and time of storage lengthened. By day 6 of decomposition (time of bloat), partial profiles (<98% alleles) were generated in 50% of the samples. Overall, the quality of STR profiles from skin samples declined more rapidly than profiles from muscle samples (Fig. 3). DNA quality did not necessarily correlate with DNA quantity. Comparable (or slightly higher) amounts of DNA were observed for skin compared to muscle samples, but on average muscle samples generated more complete STR profiles. These results may suggest that although more DNA was detected from skin samples over time, the DNA was better preserved in the muscle tissue. This finding differs from the results of Michaud et al., 2011 [9] and Clare et al., 2015 [13] that compared DNA preservation in skin and muscle from porcine and equine tissues respectively. Both studies found that DNA from skin was better preserved than DNA from muscle [9,13]. It has been suggested that the cellular structure of skin remains intact while resisting degradative processes for longer periods of time compared to muscle, especially when the skin desiccates [9,13]. However, deeper muscle tissue may be protected by this desiccated skin and insulated from harsh environmental conditions. Of note, the FBI lists red skeletal muscle as the tissue of choice if submitting soft tissue for forensic DNA testing of unidentified remains [FBI Handbook of Forensic Services, 2013, DOJ].

As mentioned previously, the quantity of DNA was not always a reliable indicator of STR success. Similar observations have been reported in the literature [14]. Our quantification method (63bp target) may have overestimated the amount of amplifiable DNA in these degraded samples. We would expect that using a system such as Quantifiler® Trio with longer targets (>200 bases) would better correlate the amount of DNA detected with downstream STR results [14].

The number of reportable alleles from samples stored in LST buffer rapidly decreased over time (Fig. 3). Substantially fewer LST samples generated full profiles compared to the other preservatives (18% versus 43-56% respectively). The preservation efficiencies of modified TENT, DESS, DNAgard®, and RNA*later* solutions were comparable. Although RNA*later* recovered the highest DNA concentrations from fresh and decomposing tissues for up to three months of storage (Fig. 1), STR success was comparable to the other preservatives (44% samples with partial STR profiles compared to 58%, 56% and 44% of modified TENT, DNAgard®, and DESS samples respectively) (Fig. 3). Factorial ANOVA analysis showed that tissue type ($F_{5,31} = 4.8$, $P<0.05$) and choice of preservative ($F_{25,116} = 3.3$, $P<0.05$) had significant effects on the DNA quality recovered from preserved tissues.

Liquid Preservative

Similar trends in STR success rates were observed with "free DNA" in preservative solution (Fig. 3) compared DNA from tissues with the exception of RNA*later*, which did not facilitate the release of DNA into solution (data not shown). The quality of STR profiles of "free DNA" from preservatives of skin samples declined more rapidly than those generated from muscle samples (Fig. 3). The same phenomenon was observed in DNA extracted from preserved tissue. Indeed, DNA purified directly from the LST buffer also produced the least successful results. Complete profiles were only generated from LST surrounding fresh tissue (Day 0) (Fig. 3). The LST buffer generated the highest percentage of samples with incomplete profiles (81% compared to 56% for both modified TENT and DESS buffers, and 50% with DNAgard®) (Fig. 3). Modified TENT, DESS, and DNAgard® produced comparable results although DESS performed better with highly decomposing tissues (Fig. 3).

Factorial ANOVA analysis showed that tissue type ($F_{6,19} = 6.5$, $P<0.05$) and choice of preservative ($F_{18,54} = 2.8$, $P<0.05$) had significant effects on the quality of STR profiles generated from DNA recovered directly from liquid preservatives. In addition, the interaction between tissue and preservative was found to be significant ($F_{18,54} = 1.9$, $P<0.05$).

Overall, STR success rates obtained from all samples (tissue and "free DNA" in solution) decreased over days of decomposition regardless of tissue type. The average number of STR alleles recovered from "free DNA" in solution was similar to that obtained from the tissues themselves (Fig. 3). This demonstrates that DNA can be preserved and extracted directly from the preservative solution eliminating the tissue digestion step and, in this way, decreasing the extraction process time and increasing the throughput, especially when many samples need to be processed in short periods of time such as the case of DVI. In summary, muscle samples yielded more complete profiles from both tissue and "free DNA" for longer days of decomposition (Fig. 3).

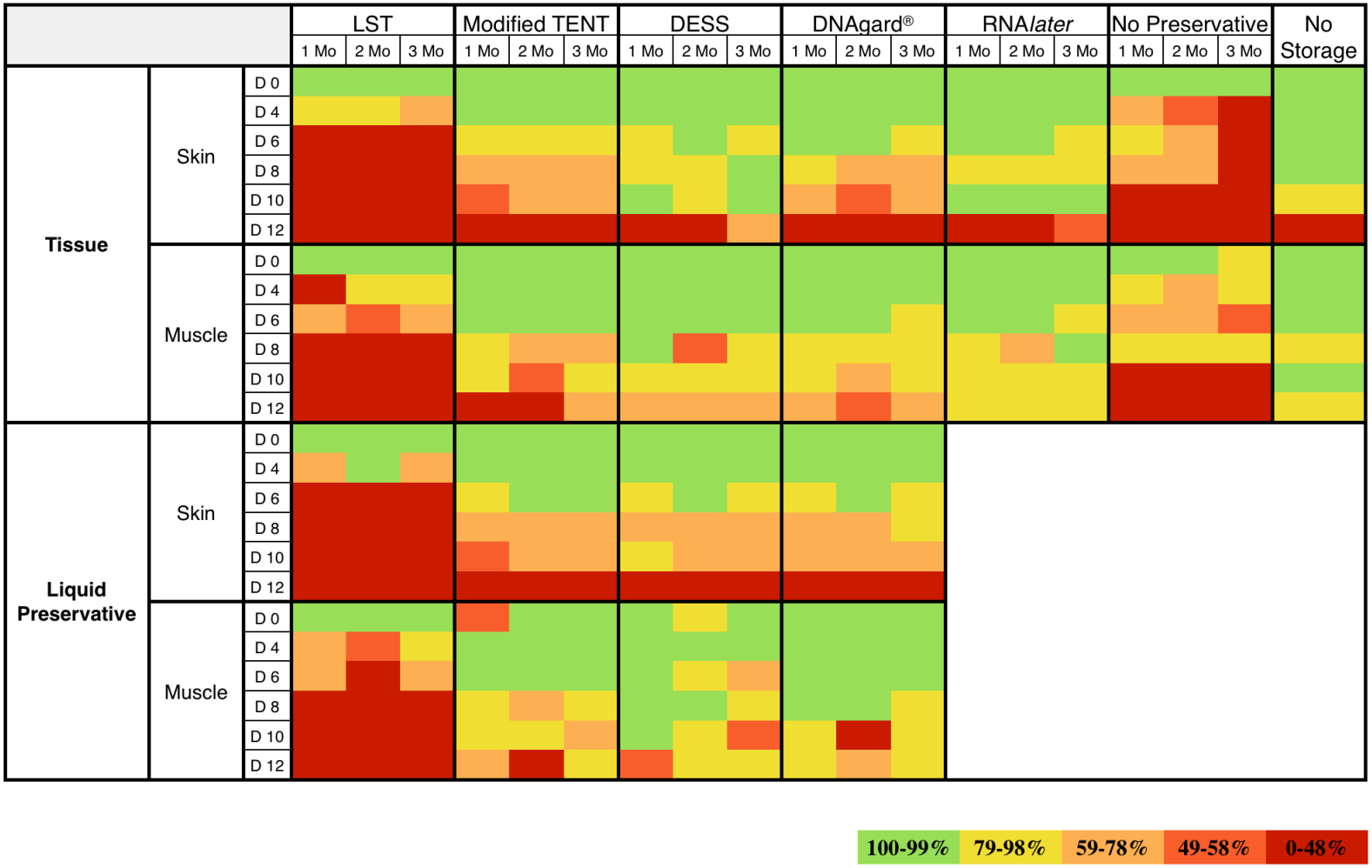


Figure 3. Average STR results of all controls and experimental samples. Percentage of alleles correct (average of three cadavers) presented as a heat map. RNA*later* results for the "free DNA" in preservative solution are not shown due to the lack of DNA released into solution. (For example; D 0 = 0 days of decomposition, so on; 1 Mo = 1 month of storage)

Conclusions

Our conclusions support those of previous authors [2, 5, 9], suggesting that the use of preservative solutions can be very beneficial in DNA-based DVI operations. We have demonstrated that all solutions preserved DNA in fresh (Day 0) and decomposed (Days 4 to 12) skin and muscle for successful STR typing after storage for up to three months. As expected, there was a general decrease in the amount of amplifiable DNA as decomposition progressed. However, we observed that DNA quantity and quality markedly decreased after the body entered the bloat stage (Day 6 in this study). We also observed that similar quantities of DNA were extracted from skin and muscle for both tissue and "free DNA" with increasing decomposition time, but the average percentage of reportable alleles was higher for muscle samples.

RNA*later* was found to be the best preserving solution of DNA in tissues; however it prevented the release of DNA into solution. On the other hand, LST, modified TENT, DESS, and DNAgard® favored the release of DNA into preservative solution allowing extraction without the lengthy digestion step. However, the LST buffer failed to preserve the "free DNA" from decomposing tissues and in extended periods of storage. While DESS, DNAgard® , and the modified TENT buffers all adequately preserved the "free DNA" in solution over time, modified TENT and DNAgard® most consistently yielded DNA of higher quantity and better quality from both the tissues and the liquid preservatives stored for up to three months at 35°C with 60-70% humidity.

Although commercial products (such as DNAgard®) may be available for a mass disaster operation, they often have short shelf-lives (6–12 months) and would require regular re-stocking. Therefore, the development of a simple in-house DNA preservative that can preserve DNA in human tissue samples would be of great benefit for DVI operations. For the ease of use, availability, cost-effectiveness, and maximum performance, we suggest that the modified TENT buffer may be the best candidate out of the five preservatives tested in this study for DNA-based DVI operations.

Study 2: Minimum time study

In addition to investigating whether DNA within tissue samples would leach into the surrounding preservative solution and be protected from further degradation and damage for one, two or three months of storage at room temperature, we were also interested in what (realistically) minimum time period is required for adequate amounts of DNA to leach into solution and generate complete STR profiles.

Fresh (Day 0) and decomposed (Day 8) skin and muscle samples were taken from cadavers 1A & 3B (N= 192). The tissue samples (30 mg) were incubated in 300 µL of four of the preservative solutions (LST, modified TENT, DESS, DNAgard®).

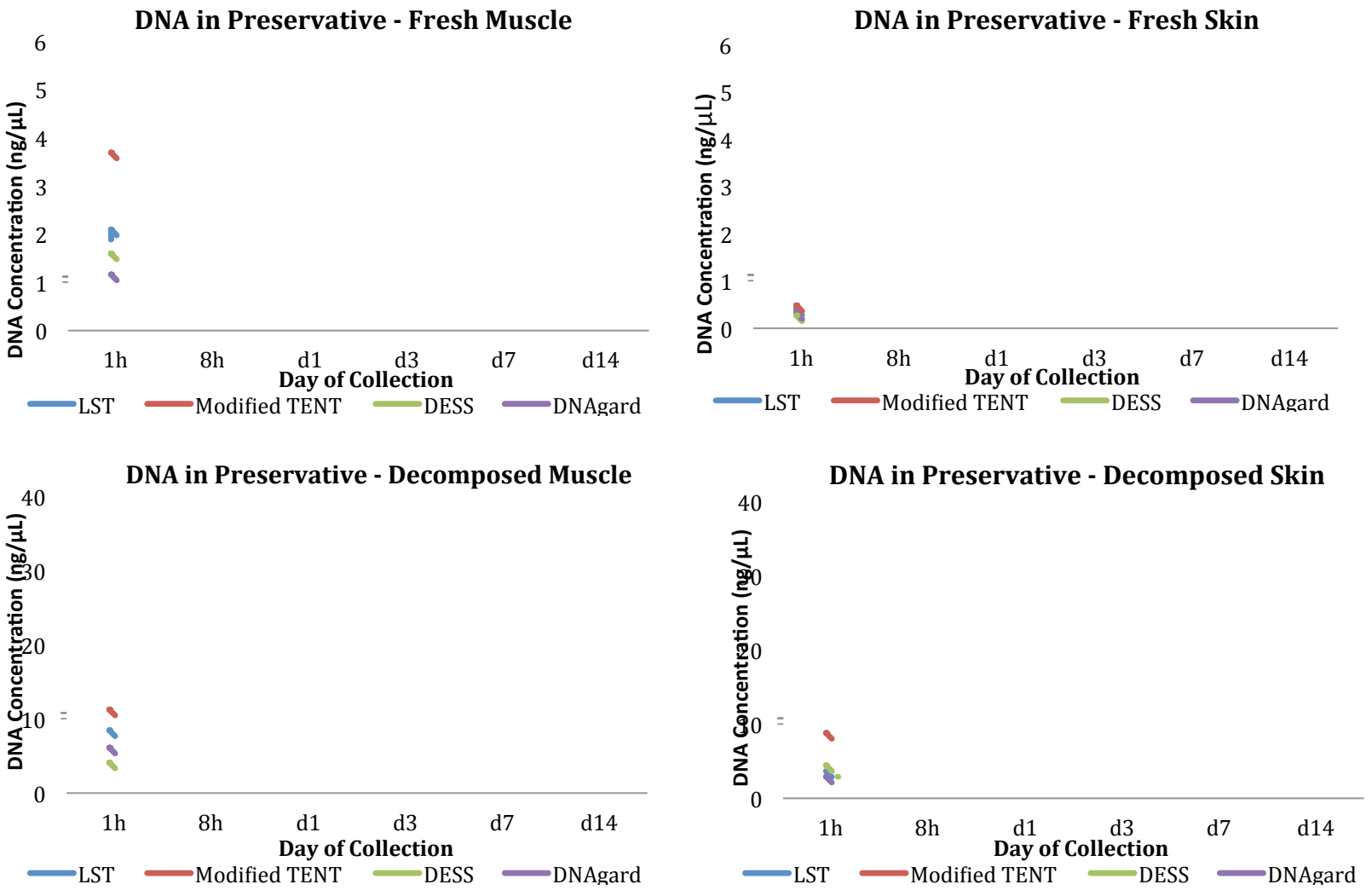


Figure 4. Concentration of DNA retrieved from "free DNA" in solution surrounding fresh (Day 0) and decomposed skin (Day 8) and muscle samples stored at 35°C for 1hr to 14 days. (Note the difference of scale in the Y axes with fresh and decomposed skin.)

Samples were stored in an incubator for 1 hr, 8 hrs, 1, 3, 7 and 14 days at 35°C with relative humidity of 60-70%. RNAlater was omitted from this study due to very low (<0.002 ng) or no DNA leaching in to solution (as observed in Study 1). The "free" DNA was extracted directly from the preservative solution of all samples using the DNA Investigator kit (Qiagen) on the QIAcube (QIAGEN) using the purification steps only (no lysis).

Results & Conclusions

In general the amount of DNA leaching into solution increased over time (from 1hr to 14 days) (Fig. 4). All samples (tissue and "free DNA") generated 100% STR profiles regardless of storage time (except DESS) (data not shown). In conclusion, we have shown that adequate amounts of DNA for successful STR-typing can be retrieved from all liquid preservatives tested (except DESS) after one hour of incubation at 35 °C.

Study 3: Test various quick DNA cleanup methods to purify the "free DNA" directly from solution

Fresh (Day 0) and decomposed (Day 10) skin and muscle sample were harvested from the right thigh of three cadavers (N = 250). Five tubes were prepared for each sample (each with 30mg tissue in 300µL preservative solution) and stored for 7 days at 35°C with relative humidity of 60-70%. Four of the five preservatives were tested (LST, modified TENT, DESS, DNAgard®). RNAlater was also omitted from this study due to very low (< 0.002ng) or no DNA leaching in to solution (as previously mentioned). After incubation, the preservative solution from the five tubes for each sample was pooled (approx. 1.2 mL) to ensure the lysate was homogenous. Aliquots (100µL) of each preservative solution were subjected to six DNA purification methods:

1. DNA Investigator kit (Qiagen) on the QIAcube (QIAGEN) using the purification steps only - Control treatment (same method used in Study 1 to extract the "free DNA" is solution). Time required = 75 min
2. QIAquick PCR Purification kit (Qiagen) on the QIAcube (as per recommended protocol). Time required = 20 min
3. Fingerprint DNA Finder (FDF) kit (Nexttec). As per recommended protocol. Time required = 15 min
4. Agencourt AMPure XP system (Beckman Coulter). Time required = 20 min
5. Modified organic precipitation. Ethanol precipitation with clean up and concentration using Microcon filters. Time required = 50 min
6. Direct Amplification (after 1:5 dilution in TE) Time required = 15 min

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Results & Conclusions

For "free DNA" extracted from the preservative surrounding fresh tissues, we performed a factorial ANOVA with two independent variables (tissue type and preservative type). There was a significant effect of tissue ($F_{6,11}$=3.8, P<0.05) only. No interactions between independent variables were detected. The type of tissue (skin or muscle) was shown to have a significant effect on the amount of DNA yielded into solution, with significantly more DNA yielded from skin samples compared to muscle tissue (data not shown). For decomposed tissues, we also performed a factorial ANOVA with two independent variables (tissue type and preservative type) for effects on DNA quantity. However, no significant effects were found.

Regarding the STR success rates from "free DNA" purified from the various preservatives surrounding fresh and decomposed tissue samples, no significant effects were found regarding the tissue, method of purification or preservative. However, full STR profiles were only obtained from fresh tissues (skin and muscle) using the DNA Investigator kit and Qiaquick methods (data not shown). In decomposed tissues, these same two methods of purification (Investigator and Qiaquick) also generated more complete STR profiles on average compared to the other methods tested (data not shown). Therefore the QIAquick (Qiagen) method is recommended as a means to more rapidly purify "free" DNA from liquid preservatives (20 min versus 75 min with the DNA Investigator kit).

PROJECT SUMMARY

Our study has shown that the use of chemical preservatives can be an effective way to preserve DNA in decomposing human cadavers for up to three months in hot (35˚C) and humid (60–70% relative humidity) conditions. In addition to preserving DNA within skin and muscle samples, our study has shown that adequate amounts of DNA for successful STR typing also leach into the surrounding preservative after one hour of storage and can be stable for up to three months of storage. By purifying this "free DNA" directly from the preservative solution, the lengthy tissue digestion step can be eliminated and throughput increased. This study found that the fastest (20 min) and most efficient method of purifying that "free DNA" in solution was the QIAquick PCR Purification kit.

As expected, DNA quantity and quality decreased with time of decomposition. However, a marked decrease in DNA quantity and quality was observed in all samples after the bodies entered the bloat stage (approximately six days of decomposition in this study). Similar amounts of DNA were retrieved from skin and muscle samples

over time, but more complete STR profiles were obtained from muscle. Although higher amounts of DNA were recovered from tissue samples than from the surrounding preservative, the average number of reportable alleles from the "free DNA" was comparable.

Overall, the modified TENT and DNAgard® buffers were the most successful tissue preservatives tested in this study, based on consistently high STR success rates with both tissue samples and "free DNA" when decomposing tissues were stored for up to three months in hot and humid conditions.

Based on the results of this study, we would recommend in situations such as mass disasters, that muscle tissue be harvested from decomposing human bodies and stored in DNAgard® or our modified TENT buffer prior to processing. In order to avoid the timely digestion step required for dense tissues and maximize sample throughput, we would also recommend isolating "free DNA" in the preservative solution using the QIAquick PCR Purification kit.

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