JOURNAL OF FORENSIC SCIENCES

J Forensic Sci, July 2011, Vol. 56, No. 4
doi: 10.1111/j.1556-4029.2011.01771.x
Available online at: onlinelibrary.wiley.com

PAPER

CRIMINALISTICS; PATHOLOGY/BIOLOGY; ANTHROPOLOGY

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Simplified Field Preservation of Tissues for Subsequent DNA Analyses*

ABSTRACT: Successful DNA-based identification of mass disaster victims depends on acquiring tissues that are not highly degraded. In this study, multiple protocols for field preservation of tissues for later DNA analysis were tested. Skin and muscle samples were collected from decaying pig carcasses. Tissues were preserved using cold storage, desiccation, or room temperature storage in preservative solutions for up to 6 months. DNA quality was assessed through amplification of successively larger segments of nuclear DNA. Solution-based storage, including a DMSO/NaCl/EDTA mixture, alcohols, and RNA*later* preserved DNA of the highest quality, refrigeration was intermediate, and desiccation was least effective. Tissue type and extent of decomposition significantly affected stored DNA quality. Overall, the results indicate that any tissue preservation attempt is far superior to delaying or forgoing preservation efforts, and that simple, inexpensive methods can be highly effective in preserving DNA, thus should be initiated as quickly as possible.

KEYWORDS: forensic science, DNA preservation, mass disaster, tissue preservation, tissue storage, DNA quality, disaster response, insulin growth factor 1

DNA analysis has become an invaluable tool for the identification of human remains. Whether a case involves a single individual or a large-scale disaster, the ability to identify victims is a critical service that forensic science is able to provide. Today, technological advances allow for robust DNA analysis using small or degraded biological samples (1–4); however, in many instances DNA profiling is still unsuccessful. Tragedies such as 9/11, Hurricane Katrina, and the 2004 Indian Ocean tsunami illustrate that the enormity of a disaster may hinder and delay identification efforts. The number of victims from the World Trade Center attacks stands at 2749 (5). By September 11, 2005, only 1594 of those victims had been positively identified, approximately 850 of which were based solely on DNA analysis (5); four more victims were identified in April 2008 using DNA obtained from bone fragments (6). Certain advances, such as the development of mini-STRs for degraded samples (1,2), have helped with identifications, although more than 1150 World Trade Center victims still have not been identified (5).

The disappointing World Trade Center outcome largely resulted from remains/DNA that were too degraded for effective analysis, a scenario that is not uncommon in the forensic sciences. Clearly, preserving remains for subsequent DNA identification is requisite; however, prescribed procedures for maintaining biological samples vary greatly. The National Association of Medical Examiners (NAME) and Federal Bureau of Investigation solely recommend cold storage for recovered remains (7,8), which may or may not be practical given the circumstances under which remains are recovered. The DNA Commission of the International Society for Forensic Genetics also recommends cold storage for tissues, though briefly mentions placement in a preservative solution as an alternative if storage at room temperature is required (9). By contrast, the National Institute of Justice (NIJ) generated these instructions for later DNA analysis from mass fatality incidents: "Collect, place, and appropriately store samples of suitable size in separately labeled containers … Store samples without preservatives (e.g., formaldehyde)" (10), and makes no mention of cold storage or other DNA preservation techniques. The NAME disaster response manual places DNA sample collection tenth in the progression through the morgue—after radiology, photography, anthropological examinations, and other administrative procedures (7). According to NIJ recommendations, a set of remains passes through triage, admitting, radiology, photography, and cataloguing of personal effects prior to any anthropological or biological examinations (10). In both sets of recommendations, multiple procedures and identification methods are undertaken before tissue collection that could be used for DNA analysis is considered, even though such collection might itself be one of the fastest and simplest procedures performed.

Given these standard recommendations for tissue storage and the not infrequent lack of success in DNA analysis when following them, it would seem prudent to examine and develop contingency plans for simple, effective tissue/DNA preservation in the field. Multiple factors must be considered when evaluating tissue preservation protocols under sub-optimal conditions. First, cold storage may not be possible at all, requiring samples to remain at ambient

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*Presented at the 58th Annual Meeting of the American Academy of Forensic Sciences, February 20–25, 2006, in Seattle, WA; and the 59th Annual Meeting of the American Academy of Forensic Sciences, February 19–24, 2007, in San Antonio, TX.
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Received 10 Mar. 2010; and in revised form 03 June 2010; accepted 24 July 2010.

temperatures for extended periods of time. Retarding enzymatic or microbial degradation of DNA during this time is a primary concern, and methods that do this most effectively are desirable. Likewise, the portability of storage equipment or materials, the ease and cost of obtaining them, shelf-life, and toxicity or other dangers come into play. Finally, the length of time tissues must be stored and the ease of downstream DNA extraction and analysis should be considered.

Three basic categories of tissue storage and DNA preservation exist: the aforementioned cold storage, desiccation, or storage in a preservative, all of which have distinct advantages and disadvantages. Refrigeration is widely used for tissue preservation, as cooling/freezing reduces chemical or physical modifications of DNA, and subsequent DNA extractions do not require any special considerations—samples are simply thawed and processed. Storage at −20°C is the primary technique recommended following a mass disaster (8,10), although freezing at −80°C or in liquid nitrogen (−196°C) are also used (11,12). This method however, necessitates either refrigeration units that are bulky and require a power source, or ice/dry ice/liquid nitrogen that must be brought in and are transient. Desiccation, on the other hand, impedes nuclease and microbial activity simply through removal of water. Heat drying of tissue destroys bacteria and cellular enzymes that degrade DNA, and drying at 70°C is considered sufficient for sterilization (USDA food safety guidelines [13]). Placement in silica desiccant has also been used to rapidly dry and store tissues (12,14–16). Silica is safe, inert, inexpensive, and easily transported in the field, thus presents advantages over cold storage methods. Negatively, desiccation of tissue can make it brittle and more difficult to process.

Storing tissue in a liquid preservative offers a wide range of options, with a variety of advantages and disadvantages. Aldehydes and alcohols are common preservative solutions, which are effective at dehydrating and sterilizing samples. Aldehydes, such as formaldehyde, are excellent at preserving tissues, as they permeate tissue and crosslink proteins and nucleic acids; however, this makes subsequent DNA isolation and analysis potentially unsuccessful (17,18). Alcohol storage fixes tissue samples without cross-linking DNA, which is important for subsequent nucleic acid extractions (17). Ethanol has proven to be an effective long-term tissue storage method that allows DNA recovery (18–20), as have proprietary alcohol-based preservatives (21), and they have the known advantage of killing a range of bacteria and fungi (22). Additionally, ethanol and isopropanol are inexpensive and readily obtained, making them attractive candidates for field applications.

Other less common tissue storage solutions have the potential to be as easy to utilize as alcohols, while being more effective at DNA preservation. One of these, dimethyl sulfoxide (DMSO) is highly permeable in tissues, dehydrating them by displacing water. When combined with NaCl and EDTA, DMSO has been shown to be very effective at preserving DNA (19,20,23). Likewise, the commercially available product RNA*later* (Ambion, Austin, TX) is widely used by molecular biologists for tissue storage. This product is an aqueous sulfate salt solution that rapidly fixes fresh tissue and does not require subsequent refrigeration of the sample (24). Storage in RNA*later* may yield nucleic acids of a quality and quantity comparable to fresh or flash-frozen tissues (25). Finally, various detergent-based solutions have been used for successful tissue storage, including SDS, Triton, and Tween (26,27), with the solution itself sometimes being used for DNA retrieval.

The goal of the research presented here was to compare methods of field-preserving tissues for future DNA analysis, considering their effectiveness, ease, safety, and utility under suboptimal conditions. The research was conducted in two phases. The first entailed developing an assay for DNA preservation following a variety of tissue storage protocols in summer, which was followed by retesting and augmenting the most promising storage procedures during both summer and winter. Skin and muscle from euthanized pigs were collected and preserved for various time intervals, after which DNA degradation was examined. Results across preservative method, storage time, tissue type, and degradation level were considered.

Materials and Methods

Tissue Collection

Tissue samples were collected from euthanized pig carcasses obtained from the Swine Teaching and Research Center at Michigan State University. First, two pig carcasses weighing approximately 30 and 60 lbs were placed in a remote research field on the MSU campus in partial shade, while a third weighing approximately 90 lbs was placed in full sun nearby. The following summer, two pig carcasses weighing 120–160 lbs were placed in the shade or sun at the same site, which was repeated the following winter.

Skin and muscle tissue were excised within an hour of death (day 0), and again on days 3 and 5 postmortem for the original set of pigs, on days 0, 3, and 6 for the subsequent sets of pigs, and again after 3 weeks for the latter animals, if tissue was still available. Skin and muscle sections approximately 10 cm × 5 cm × 3 cm deep were excised, using a scalpel, from the anterior exposed shoulder of each pig; on subsequent days samples were taken posterior to the previous cut sites.

Tissue Preservation

Collected tissues were placed in a clean plastic bag and set on ice, then returned to the laboratory and either processed immediately or placed at −20°C and processed the following day. Hair was removed using a disposable razor. Skin and muscle samples (0.2–0.5 g, weighed to ±0.002 g) were excised and stored at room temperature (unless otherwise noted), in different formats as described below. Tissues processed immediately acted as controls.

Screw-top, polyethylene 6 mL Sampule Vials (Wheaton, Millville, NJ) were used for tissue storage. Six tissue preservation methods were selected for initial experiments: −20°C freezing, 70°C oven-drying, and storage in 4 mL 70% ethanol, 4 mL 70% isopropanol, 2.5 mL RNA*later*, or 2.5 g silica desiccant (28–200 mesh, Fisher Scientific, Pittsburgh, PA). Tissues stored in solution or silica were added to the storage medium immediately after excision, and kept at room temperature thereafter. Samples stored at −20°C were added to empty vials and placed in a non-self-defrosting freezer. Samples preserved by 70°C oven-drying were placed on waxed paper in an incubator for ca. 72 h, then transferred to empty vials and stored at room temperature.

In the second round of testing, RNA*later* storage was eliminated, as this product is not readily available (and see "Results" section). Oven-drying at 70°C was also eliminated, owing to difficulty in implementing this method in the field. The remaining storage methods were expanded upon, storage in a DMSO solution was added, and −80°C storage replaced −20°C. Alcohol storage included 4 mL of either 40%, 70%, or 100% ethanol or isopropanol. Seventy percent solutions performed as well or better than the other dilutions, so for simplicity only those results are presented below. Storage in 12.5 g versus 2.5 g of silica was also examined, again with similar results, thus only the results using 2.5 g of silica

are presented. Storage at −80°C consisted of placing samples in empty vials and storing in a standard laboratory deep freezer. DMSO samples were placed in vials containing 4 mL of DMSO salt solution (20% DMSO, 0.25 M disodium-EDTA, and NaCl to saturation, pH 7.5 [23]).

Samples remained in storage until specified time points, when tissues were removed for DNA extractions. In initial experiments, samples were stored for 2 weeks or 2 months; subsequent experiments examined storage times of 1 week, 2 months, and 6 months.

DNA Extraction

Tissues from the initial study were removed from preservative after 2 weeks and divided in half, one portion of which was processed immediately, while the other was processed following the 2 month storage period. In some instances bisecting the samples resulted in some tissue loss, therefore in subsequent experiments tissues were preserved as 0.250 g sections, which could be processed independently. Preliminary experiments also showed that tissues stored in DMSO or RNA*later* required de-salting prior to DNA extraction; these samples were removed from preservative and soaked in 2 mL TE (10 mM Tris, 1 mM EDTA) for 0.5–2 h. If softening of tissue was required before grinding, it was allowed to sit in digestion buffer prior to maceration.

Tissues were placed in 1.5 mL microfuge tubes with 500 μL of digestion buffer (20 mM Tris, pH 7.5, 100 mM EDTA, 0.1% SDS) and 5 μL of proteinase K (20 mg/mL, Roche, Indianapolis, IN). Tissues were macerated for ca. 10 s in the microfuge tube by hand, using a conical glass pestle. Digestion was completed by placing samples at 55°C for 48–72 h. Samples were vortexed for 10–15 s daily to aid in the digestion of the tissue.

DNA was extracted by adding 500 μL of phenol to the digested tissues, followed by vortexing for 10 s and centrifugation at 14,000 rpm for 5 min. The aqueous layers were removed and transferred to new 1.5 mL microfuge tubes. Five-hundred microliters of chloroform was added to the extracts, and samples were vortexed for 10 s and centrifuged at 14,000 rpm for 5 min. It was noted during the phenol and chloroform steps that the aqueous and organic layers of some RNA*later* stored samples inverted after centrifugation; those samples were monitored to ensure that the appropriate layer was transferred in subsequent steps. The aqueous layers of all samples were transferred to new 1.5 mL microfuge tubes, to which two volumes of cold 95% ethanol and 1/10 volume of 3 M sodium acetate were added and mixed by inversion. The samples were placed in −20°C for at least 24 h. DNAs were then centrifuged for 15 min at 21,000 × *g*, and supernatant was removed. DNAs were vacuum-dried for 15 min, resuspended in 50 μL of TE, and stored at −20°C. In the interest of treating each sample equally, no additional steps were taken to purify troublesome extracts (e.g., those showing incomplete tissue digestion or downstream discoloration), and all were resuspended in an equal volume of TE.

Evaluating DNA Quality

A rough estimate of DNA quality was made by electrophoresing 5 μL of each DNA sample on a 1% agarose gel. Next, amplification of successively larger nuclear DNA fragments was attempted. Three sets of primers were designed for porcine insulin growth factor 1 gene (IGF-1), using the same forward primer and increasingly distant reverse primers, resulting in amplicons of 257, 457, and 642 bp. Primers (Table 1) were designed using gene sequences obtained from the online National Center for Biotechnology database (28) and Primer 3 (29). Primers were received from Integrated DNA Technologies, Inc. (Coralville, IA) as lyophilized pellets, which were resuspended in TE to a concentration of 200 μmol. Ten microliter PCR reactions contained 1 U of Hot Master Taq Polymerase (Eppendorf, Hamburg, Germany), 1× Hot Master Taq PCR Buffer (Eppendorf), 20 μM dNTPs (Promega, Madison, WI), and 1 μM forward and reverse IGF-1 primer. PCR consisted of a 2 min denaturation at 94°C, followed by 35 cycles of 94°C for 30 s, 58°C for 1 min, 72°C for 1 min, and a final incubation at 72°C for 5 min. Results were visualized by electrophoresing 5 μL of each sample on a 1.0% agarose gel and staining with ethidium bromide.

Data Analysis

Samples were scored as either positive (amplification occurred) or negative (no amplicon was visible). One-way ANOVA was performed to compare differences in mean amplification rates among collection time points and preservation methods at each of the amplicon sizes. Differences in DNA obtained from skin and muscle were analyzed for the IGF-1 642 bp fragment only, due to limited differences among groups for the 257 and 457 bp sizes. Bonferroni tests were performed on all pair-wise comparisons within a group if ANOVA indicated a significant difference among means ($\alpha = 0.05$). Statistical analyses were performed using R (Version 2.7.2) (30).

Results

Over the course of this study, 756 tissue samples were used to evaluate 12 preservation methods and four storage times. Eight hundred and eighty-five DNA extractions were performed, as were more than 2000 PCR assays of the various sized amplicons.

Tissue Digestion and DNA Extraction

Gross differences were observed among tissues preserved using the various techniques. For instance, oven-dried and silica desiccated tissues often crumbled during processing, leading to potential sample loss. The oven-dried tissues developed a substantial odor by day 2, while the silica-stored samples required extra care during organic extraction to ensure no residual silica mesh was carried

TABLE 1—*Sequences of PCR primers used to amplify porcine DNA.*

Primer	Sequence (5′–3′)	Primer Size (nt)	Amplicon Size (bp)
IGF-1 forward	AAT CAT TTG CCC CTC AAG TG	20	n/a
IGF-1 R257	TGA CCC CCT CAT CCT AGT TG	20	257
IGF-1 R457	GGC AGG AAG ACA CAC ACA TC	20	457
IGF-1 R642	TCT CTC CCT CTT CTG GCA AA	20	642

The DNA sequences for porcine IGF-1 primers, pairing the same forward primer with various reverse primers (R) to create amplicons of increasing lengths. nt = nucleotide; bp = basepairs.

over. Precipitated DNA from silica storage had a viscous layer at the bottom of the tube, which presumably resulted from carried-over silica. Tissues preserved in ethanol and isopropanol for months, particularly at higher concentrations, hardened and began to fragment. DMSO stored samples became quite leathery. The aqueous and organic layers of RNA*later* stored tissues often inverted upon addition of phenol and chloroform, thus the upper, organic layer was discarded and the lower, aqueous layer was retained for the next step. Further, the precipitated DNA pellet showed a white interface not seen with the other storage methods.

The yield gels indicated that all extractions contained some level of DNA, although these often varied widely among storage technique, storage time, tissue type, and age of remains (exemplified in Fig. 1). As expected, tissues collected during later stages of decay typically contained more degraded DNA. Further, samples stored in solution generally appeared to have larger quantities of high molecular weight DNA than frozen or desiccated samples. This information was useful in that samples with large amounts of DNA had to be diluted for successful PCR amplification, but otherwise no obvious correlation was observed between yield gel results and DNA amplification.

Factors Influencing the Quality of Recovered DNA

Carcasses placed out in winter quickly froze, and there was little or no variability among the storage techniques in successfully generating the various sized amplicons; virtually all DNAs amplified (the sole exception was DNA from the unpreserved samples, wherein many failed to amplify). Placement of the carcasses in sun versus shade during summer, while affecting larval insect activity, also had no influence on DNA results. Given this, neither of these parameters is considered below.

The length of time a carcass remained unpreserved in summer had a substantial effect on DNA recovery, when all storage methods were considered together (Table 2). This was most notable for larger amplicons; the 457 bp target amplified significantly less often than the smallest target after 6 months of storage using two preservation methods, while the 642 bp target amplified significantly less in six instances, primarily at longer storage times, but in one case even after 2 weeks.

When the results were broken down by storage methods, all procedures helped preserve DNA, although to differing levels (Table 3). Amplification of the 257 bp target ranged from 92% (ethanol) to 81% (silica) in initial experiments, and from 100% (ethanol) to 89% (silica) in the follow-up experiments. Differences among storage methods were not statistically significant. DMSO showed the highest overall amplification rate for the 457 bp

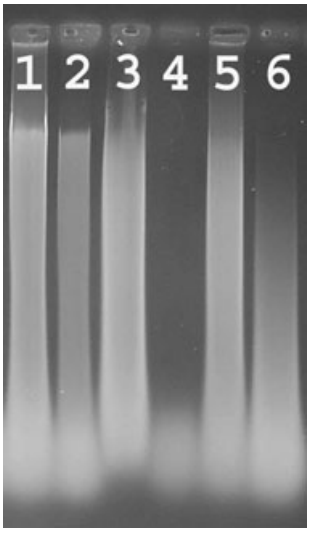

FIG. 1—*Sample 1% agarose yield gel. Shown are equal volumes of DNA from 250 mg of skin from summer day 5 remains stored for 2 months. Storage conditions were: (1) 70% ethanol; (2) 70% isopropanol; (3) RNAlater; (4) silica; (5) 70°C; (6) −20°C. Note the highly variable DNA storage success. Yields on gels varied substantially, thus these results are exemplary only. All DNA extracts were included in subsequent PCR-based testing regardless of yield.*

TABLE 2—*The effect of collection time and storage time on successful amplification of increasingly longer IGF-1 PCR targets.*

		DNA Fragment Length		
Collection Day	Storage Time	257 bp	457 bp	642 bp
(a)				
Day 1	2 wk	1.00	1.00	1.00
	2 mo	1.00	1.00	1.00
Day 3	2 wk	0.92	0.92	0.92
	2 mo	1.00	1.00	1.00
Day 5	2 wk	0.69	0.61	0.56*
	2 mo	0.75	0.61	0.42*
(b)				
Day 0	1 wk	1.00	1.00	0.95
	2 mo	1.00	0.95	0.90
	6 mo	0.95	0.65*	0.40*
Day 3	1 wk	1.00	1.00	1.00
	2 mo	0.95	0.95	0.80
	6 mo	0.95	0.75	0.45*
Day 6	1 wk	0.95	0.85	0.75
	2 mo	1.00	0.90	0.55*
	6 mo	0.80	0.25*	0.20*

Combined results for all storage techniques. Values shown are the proportions of samples that successfully amplified; significantly different means ($\alpha = 0.05$) are marked (*). In initial experiments, samples collected on day 5 were of significantly lower quality than samples from earlier time points (a) when amplifying the largest target DNA. Subsequent experiments (b) showed that samples collected on day 6 and samples stored for 6 months had significantly lower amplification success for the larger DNA targets. wk = week; mo = month; bp = base pairs.

TABLE 3—*The influence of tissue storage method on subsequent DNA amplification.*

	DNA Fragment Length		
Preservation Method	257 bp	457 bp	642 bp
(a)			
Ethanol	0.92	0.89	0.86
Isopropanol	0.89	0.89	0.81
RNA*later*	0.92	0.89	0.86
Silica	0.81	0.75	0.72
70°C drying	0.95	0.86	0.81
−20°C storage	0.89	0.86	0.83
(b)			
Ethanol	1.00	0.83	0.67
Isopropanol	0.94	0.75	0.61
DMSO	0.97	0.97	0.92*
Silica	0.89	0.72	0.53
−80°C storage	0.97	0.78	0.61

In initial experiments (a), ethanol and RNA*later* showed the best DNA preservation success. Subsequent experiments (b) showed that the salt-saturated DMSO solution best preserved DNA, followed by ethanol. Values are proportions of samples that successfully amplified; significantly different means ($\alpha = 0.05$) are marked (*). bp = base pairs.

amplicon, while ethanol, isopropanol, and RNA*later* also maintained high levels. All storage methods displayed reduced successful amplification of this larger amplicon, with the exception of DMSO and one of the isopropanol trials. Again, no significant differences among preservation methods were found. Finally, DMSO maintained the highest amplification success when examining the largest amplicon, followed by RNA*later*, and ethanol in one instance. Silica storage was the least effective in both trials. DMSO storage consistently resulted in a significantly better amplification rate than the other storage methods based on the largest amplicon.

Finally, tissue type (skin or muscle) often influenced amplification success. When considering storage method and amplification of the 642 bp target, skin resulted in equal or better quality DNA in all instances (Table 4). Likewise, skin produced higher quality DNA for the smaller amplicons, as well as when considering tissue decay time and tissue storage time.

Discussion

The goal of the experiments presented here was to determine if a simple method or methods for field preservation of tissue could be identified that would substantially improve the ability to obtain amplifiable DNA at a later time. Special consideration was given to methods that were inexpensive, required little preparation, and did not require bulky or specialized equipment. This inquiry came from the failure to identify many of the 9/11 World Trade Center victims, where greater on-site attempts to preserve tissue may have increased identification success. Multiple techniques were examined, encompassing refrigeration, desiccation, and storage in preservative solutions. Downstream ease in processing the tissue for DNA isolation was considered, as were variables such as postmortem interval, tissue storage time, and tissue type. The overarching finding from this study is that virtually any tissue preservation effort is superior to postponing tissue collection, and therefore that preserving a small amount of tissue should be done immediately upon recovery of remains, before initiating a standard processing regimen that might take hours or days to complete.

This is not to say that all tissue storage techniques are equal; the data show significant differences in each method's ability to retain amplifiable DNA. It was interesting to note that freezing, the more typical method for preserving tissue, was not necessarily better than other storage techniques, even though the latter were held at room temperature. Freezing was superior to desiccation however, which performed relatively poorly based on both oven drying and the more convenient silica-based method. Previous research has shown silica to be an effective tissue preservation medium for subsequent DNA analysis (14,17,19); however, these studies were based on comparatively fresh hair and tissue samples. The different tissue types examined in this study, and their varying levels of decay, indicate that desiccation is not as effective on decomposing tissue, most likely because it does not rapidly arrest the decay process.

TABLE 4—*The influence of tissue type on successful DNA amplification.*

	Tissue Type		
Preservation Method	Skin	Muscle	*p*-Value
Ethanol	0.94	0.78	0.16
Isopropanol	1.00	0.61	0.02*
RNA*later*	0.89	0.83	0.64
Silica	0.72	0.72	1.00
70°C drying	0.89	0.72	0.22
−20°C storage	0.89	0.78	0.39
Avg.	0.89	0.74	0.005*

Example of how tissue type affected successful PCR amplification of the largest target amplicon (642 bp). Only silica preservation produced equal PCR positive skin and muscle samples, while for all other methods DNA from skin amplified more often. Isopropanol was the only method showing a significant difference between tissue types. Numbers reported are proportions of samples that successfully amplified; significant results ($\alpha \leq 0.05$) are marked (*).

The alcohols, DMSO salt solution, and commercially available storage solution RNA*later* were also more effective than desiccation at preserving DNA, most likely because they quickly penetrated the tissue and halted decay. Among these, the most successful was storage in salt-saturated DMSO containing EDTA, which preserved DNA out to 6 months at room temperature, even from the most degraded tissue. DMSO readily permeates tissues, presumably taking the NaCl and EDTA with it. Salting is a long established method for preserving tissue, and further, high salt levels can help to precipitate DNA, making it less susceptible to degradation. EDTA, widely used for DNA storage, binds the divalent cations nucleases require for activity. The DMSO/NaCl/EDTA combination proved extremely effective for preserving DNA, although tissues stored in this way became a bit leathery so were slightly more difficult to grind than some of the others, but this was a minor inconvenience. Clearly the one real drawback of DMSO-based storage is that it is not available off the shelf; however, the solution is simple to formulate, inexpensive, and stable, so could easily be prepared in large quantities in advance. These factors, along with its effectiveness and safety (being neither toxic nor flammable), make it ideal for field utilization.

The commercial product RNA*later*, designed for tissue storage and subsequent nucleic acid isolation, was not quite as successful at preserving DNA, and had some potential major drawbacks. One of these is expense, being a proprietary product. Another is the ease of obtaining it; in a mass disaster situation, needed amounts of RNA*later* would likely be difficult to procure, or expensive to keep on hand. Both of these drawbacks are true of other proprietary products as well (e.g., [21,27]). Finally, RNA*later* storage interfered with DNA extraction, as the organic and aqueous layers inverted, a clear detriment for crime lab usage.

The alcohols, isopropanol and particularly ethanol, were very good for shorter-term storage of tissues, although by 6 months DNA often showed degradation. Small chain alcohols permeate tissues relatively easily, deter microbes, do not harm DNA, and equally important for these purposes, they are easily obtained. Isopropanol can be cheaply purchased from any drug or grocery store in large quantities, while ethanol is inexpensively available for laboratory use, or if necessary could be obtained as liquor. The percentages of alcohol tested (40%, 70%, and 100%) did not show a noticeable difference in DNA preservation potential, meaning that the percentages available commercially are suitable for tissue storage. Samples preserved in alcohols did become friable, but this did not seem to affect DNA isolation except when trying to cut the tissue into defined size pieces, which would not occur in forensic testing. Alcohols were not as useful for longer-term storage of tissues (e.g., 6 months), but from a forensic context this is probably unimportant, as 6 months of room temperature storage would rarely be a goal. For short-term storage, alcohols performed very well overall.

It is not surprising that the length of time a carcass was exposed to summer conditions influenced DNA recovery (pigs placed out in winter quickly froze and DNA was preserved in all instances). Among all preservative methods, DNA from carcasses exposed longer was more degraded. However, when this fact was incorporated into storage method success, significant differences among

methods were still seen. This indicates that the storage methods retarded advanced tissue/DNA degradation differently, another important consideration when choosing among them. The most impressive storage technique in this regard was again the DMSO solution, which had over 90% successful amplification of the largest amplicon out to 6 months of storage, while the other techniques ranged from 53% to 67% in the same set of experiments.

Lastly, a stark difference in successful amplification was seen between the two tissue types examined. DNA from skin preserved better overall, resulting in significantly higher amplification rates ($p = 0.005$). This is interesting, since skin is virtually never collected for forensic DNA typing, while "deep muscle" is considered one of the better tissues for this purpose. Indeed, the revised edition of the FBI Handbook of Forensic Services now specifically mentions "red skeletal muscle" as the preferred tissue type for DNA sample submission (8). It has been suggested that skin is naturally more resistant to decomposition, pathogens, and moisture (31), and that after death, the cellular structure of skin remains intact, resisting degradation processes. Skin might simply desiccate more quickly on a carcass, helping to slow DNA degradation, or the preservatives used in this study may penetrate skin more easily than muscle. Regardless, tissue type is certainly an important variable to be considered when collecting for subsequent DNA testing (e.g., [32]), and further research into the best tissue(s) to collect from decaying remains for DNA analysis should be carried out.

Most importantly, the results presented here clearly show that expedited tissue preservation should always be considered when DNA identification of remains may be required. Such preservation need not be an elaborate or expensive endeavor, nor require complex equipment or electricity. As was demonstrated, immersing a small amount of tissue in something as low-tech as rubbing alcohol or ethanol can result in a substantially better DNA analysis outcome than delaying preservation, and can work as well or even better than standard cold storage methods. The most successful procedure tested was storage in an easy to prepare and inexpensive salt-saturated DMSO solution with EDTA, which preserved DNA out to 6 months at room temperature with almost no measurable reduction in DNA quality. This takes no special training or expertise, it simply involves collecting a small piece of tissue, submerging it in the storage medium, and recording what was done. There seems to be little reason to postpone tissue preservation until human remains can be brought to the morgue, or until after the far more elaborate undertakings recommended for processing remains in mass disaster situations are completed. Given the outcomes of 9/11 and similar dire situations involving identification of human remains, conducting this simple procedure at the earliest opportunity could greatly augment identification success.

Acknowledgments

Thank you to the staff at the MSU Swine Teaching and Research Facility and Dr. Rich Merritt for their assistance in obtaining the pigs used in this study. Thanks to the staff of the MSU Research Technology Support Facility, as well as Aaron Tarone, for help with some of the experiments undertaken. Finally, a special thank you to Rith Yim, whose hard work in the lab and field made much of this study possible.

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