Quality Assurance After a Natural Disaster: Lessons from Hurricane Sandy

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Biospecimen quality can vary depending on many pre- and post-collection variables. In this study, we consider a natural disaster as a post-collection variable that may have compromised the quality of frozen tissue specimens. To investigate this possible link, we compared the quality of nucleic acids, the level of antigenicity, and the preservation of histology from frozen specimens collected before and after the power outage caused by Hurricane Sandy. To analyze nucleic acid quality, we extracted both DNA and RNA and performed capillary electrophoresis to compare the quality and concentrations of the nucleic acids. To compare antigenicity, frozen sections were cut and immunostained for thyroid transcription factor 1 (TTF-1), a nuclear transcription protein commonly used as a diagnostic biomarker for multiple cancer types, including thyroid and lung cancers. Positive expression of TTF-1, as noted by homogenous nuclear staining, would demonstrate that the TTF-1 proteins could still bind antibodies and, therefore, that these proteins were not significantly degraded. Furthermore, representative frozen sections stained with hematoxylin and eosin were also assessed qualitatively by a trained pathologist to examine any possible histologic aberrations. Due to the similar quality of the tissue samples collected before and after the storm, Hurricane Sandy had no discernable effect on the quality of frozen specimens, and these specimens exposed to the natural disaster are still valuable research tools.

Keywords: tissue banking, quality assurance, DNA extraction, RNA extraction

Introduction

BIOBANKS HAVE AN INCREASINGLY important role in translational research today.^{1,2} As the requirements for downstream analyses grow, standard operating procedures and quality control tests are needed to ensure that banked biospecimens accurately represent the genome, transcriptome, and proteome for optimum downstream analysis.^{1,2} Many conditions can adversely affect molecular quality, including tissue type,³ warm and cold ischemia time,^{4,5} storage duration,^{6,7} and successive freeze–thaw cycles.⁸ In this case, we consider the effect that a natural disaster and a subsequent power outage may have had on the quality of frozen human tissue samples in a biobank.

Hurricane Sandy made landfall in New York on October 29, 2012, causing widespread power outages and interruption of local healthcare, transportation, and emergency services.⁹ After lower Manhattan lost power on the evening of October

29, NYU Langone Medical Center had to rely on its backup generators. NYU Langone is located on the banks of the East River and the fuel pumps that supply fuel to its backup generators were located on the ground floor.⁹ With the arrival of a 14 ft storm surge at high tide later that evening, the ground floor and basement flooded, cutting off the supply of fuel to the backup generators.⁹ Without fuel, the backup generators failed, and the resulting power outage forced the evacuation of >200 patients from the hospital, which would remain without power for days after the storm.^{9,10}

The Center for Biospecimen Research and Development (CBRD) was one of many laboratories to be affected by this power outage.⁹ The CBRD runs NYU Langone's centralized biobank. Due to its intermediary role in coordinating the collection and storage for dozens of protocols, Hurricane Sandy put thousands of valuable research specimens at risk. The majority of these samples were frozen and kept in –80°C freezers that depended on electricity to maintain

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temperature and to alert personnel when the temperature inside was rising. With no power, the temperature in these freezers rose without any way to track the temperature, possibly exposing samples to ambient temperatures that can cause molecular degradation.⁸ Although many samples were moved to liquid nitrogen tanks and others kept in dry ice temporarily, there was no absolute record of how frozen samples were stored during the duration of the power outage nor how long specimens may have been exposed to ambient temperatures.

To determine the effect that the hurricane and its corresponding power outage had on the quality of these frozen tissue samples, we compared multiple molecular markers between tissue samples collected in the months before the storm in 2012 with samples collected once the hospital reopened in 2013. DNA and RNA were extracted from samples from both groups and compared qualitatively. Sections were immunostained for thyroid transcription factor 1 (TTF-1), a 38-kDa nuclear transcription factor expressed in the follicular cells of the thyroid, to see whether compromised specimens maintained antigenicity.¹¹ Sections were also stained with hematoxylin and eosin (H&E) for histological examination by a trained pathologist (A.L.M.). If the power outage compromised the quality of these frozen samples, we would expect specimens exposed to the elevated temperatures to be of lower quality as indicated by nucleic acid degradation, changes in immunostaining, or histologic abnormalities.

Material and Methods

Twenty normal thyroid samples collected before the storm in 2012 and 20 normal thyroid samples collected after the storm in 2013 were used in this study. These samples came from patients with signed, written consent to have their specimens stored for research purposes under an IRB-approved study. The samples collected in 2012 had been stored in the -80°C freezers that lost power during the storm, and thus may have been exposed to ambient temperatures. Normal thyroid samples were chosen due to the abundance of these specimens in the CBRD biobank that could be used without compromising future utilization of biobank resources. The collection date of the specimens collected before the storm in 2012 ranges from August 1 to October 9; the collection date of the specimens collected after the storm in 2013 ranges from January 3 to May 7. Specimens were chosen as close temporally as possible to minimize the effect of differing storage duration on the tissue quality. However, it is impossible to completely eliminate this effect due to the nature of this inquiry. During collection, these samples were embedded in Tissue-Tek Optimum Cutting Temperature formulation (Sakura Finetek USA, Inc., Torrance, CA), frozen, and have since been stored at either -80°C or -180°C .

DNA and RNA

From each sample, five sections were cut at $5\mu\text{m}$ using standard procedure in a cryostat twice, once for DNA extraction and again for RNA extraction, and placed in 2 mL cryovials. The sections for RNA extraction were immersed in RNAlater-ICE (Ambion Life Technologies, Foster City, CA) at -20°C overnight before RNA was extracted ac-

cording to the RNAlater-ICE frozen tissue transition solution protocol. DNA was extracted using the QIAasympphony SP and QIAasympphony DNA Mini Kit, and RNA was extracted using the QIAasympphony SP and the QIAasympphony RNA Kit and associated protocols (Qiagen, Hilden, Germany). Extracted DNA and RNA were run through capillary electrophoresis on the Advanced Analytical Fragment Analyzer (Advanced Analytical Technologies, Inc., Ankeny, IA) using the Genomic DNA 50 Kb Analysis Kit and High Sensitivity RNA Analysis Kit, respectively. RNA and DNA quality were calculated in PROSize 2.0 (Advanced Analytical Technologies, Inc.) postseparation using the Fragment Analyzer's RNA Quality Number (RQN) for RNA and General Quality Number (GQN) for DNA. RQN has been shown to be comparable with the more widely used RNA Integrity Number.¹² RQN and GQN are determined by an algorithm that scores molecular quality from 1 to 10, with 10 indicating perfectly intact molecules. The 28S/18S ratio of ribosomal RNA (rRNA) was also determined through PROSize as another measure of RNA integrity.¹³

Histology and immunohistochemistry

As a secondary analysis, an additional $5\mu\text{m}$ section was cut and stained with H&E for histologic examination. Histopathology was examined by a trained pathologist (A.L.M.) to look for artifact, autolysis, and any other histologic abnormalities that might suggest the specimen is unsuitable for pathological diagnosis. In addition, five samples from each experimental group were also used to compare immunohistochemistry. A $5\mu\text{m}$ section was cut from each block following standard protocol. Each slide was stained with TTF-1 antibodies (clone 8G7G3/1 from Ventana Medical Systems, Inc., Tucson, AZ). A positive expression of TTF-1 was noted by a trained pathologist (A.L.M.) as homogeneous nuclear staining in any of the cells.

Results

DNA and RNA

The majority of samples had sufficiently high DNA and RNA for downstream applications ($\text{GQN/RQN} > 7$).¹³ Only three samples (15%) collected before Hurricane Sandy and five samples (25%) collected after Hurricane Sandy had either GQN or RQN < 7 . Specimens collected before Hurricane Sandy and after Hurricane Sandy did not differ significantly in any measure of DNA or RNA quality or concentration. Using an unpaired *t*-test, the GQN of DNA ($p=0.49$), the RQN ($p=0.22$), and the 28S/18S ratio of rRNA ($p=0.37$) were not significantly different, indicating that the quality of the specimens collected before and after the hurricane was comparable (Table 1). Integrity of DNA (Fig. 1a) and RNA (Fig. 1b) was verified by capillary electrophoresis as high molecular weight bands.

Histology and immunohistochemistry

The follicular structure of the thyroid tissue was identifiable in all specimens and without significant alterations. There were no histologic abnormalities between sections from specimens collected before and after Hurricane Sandy. There was no increased frozen artifact or autolysis that would compromise the histologic examination of the sections. In

TABLE 1. QUALITY AND CONCENTRATIONS OF DNA AND RNA FROM 2012 AND 2013 SAMPLES

	DNA concentration (ng/ μ L)	DNA GQN (1–10)	RNA concentration (ng/ μ L)	RQN (1–10)	28S:18S ratio
2012	54.621 $\pm$ 58.984	8.03 $\pm$ 2.15	2.63 $\pm$ 2.515	8.39 $\pm$ 0.98	1.83 $\pm$ 0.35
2013	47.824 $\pm$ 39.240	8.02 $\pm$ 1.53	2.47 $\pm$ 3.261	8.12 $\pm$ 0.99	1.87 $\pm$ 0.45

GQN, General Quality Number; RQN, RNA Quality Number.

addition, cells from both experimental groups positively expressed TTF-1. Homogeneous nuclear staining was evident in the follicular cells of samples from both before and after the storm (Fig. 2).

Discussion

Biospecimen quality can vary greatly depending on the pre- and post-collection conditions the specimen is exposed to.^{4–8,14} In this case, a power outage was considered as a post-collection variable that may have affected the quality of frozen tissue samples. To test this, we evaluated several parameters of tissue quality. DNA and RNA from samples collected before and after the power outage were extracted

and compared qualitatively. The majority of specimens from both experimental groups did not show significant nucleic acid degradation in measures of DNA or RNA quality. Not only was there no significant difference between the sample groups in nucleic acid quality, the quality itself of both groups overall was high. The majority of the specimens had GQNs and RQNs sufficient for downstream analyses.¹³ In addition, the 28S/18S ratio of rRNA also approached the benchmark 2:1 ratio of intact rRNA.¹⁵ The similarity in the quality of DNA and RNA between experimental groups demonstrated that samples exposed to the power outage did not suffer molecular degradation of their nucleic acids. Although there was a high variance in the concentration of extracted nucleic acids, this was present in both experimental groups and not

FIG. 1. (a) Results of capillary electrophoresis of extracted DNA from select 2012 and 2013 specimens compared with a ladder of known nucleotide lengths and two control DNA samples with known high fidelity. (b) Results of capillary electrophoresis of extracted RNA from select 2012 and 2013 specimens compared against a ladder of known nucleotide lengths. The bands at $\sim$ 4 and $\sim$ 2 kb mark the 28S and 18S subunits of ribosomal RNA, respectively.

FIG. 2. (a) A frozen section of a sample collected before Hurricane Sandy stained with antibodies for TTF1 and magnified to 7 $\times$. (b) A frozen section of a sample collected after Hurricane Sandy stained with antibodies for TTF1 and magnified to 7 $\times$. TTF1, thyroid transcription factor 1.

related to whether the samples were collected before or after the storm. Furthermore, the amount of genomic material required for next-generation sequencing varies upon the platform used, which can range from only a few nanograms of input DNA to several hundred nanograms.¹⁶ Considering that only a few sections of each specimen were used for DNA and RNA extraction, the low concentration of genomic material for some specimens does not detract from the utility of these specimens. Additional sectioning should allow sufficient material for NGS or other downstream applications from all samples of both experimental groups. Since there is additional tissue available, the molecular quality of this tissue is more important than the quantity of extracted DNA or RNA.

Samples were also examined histologically and immunohistochemically to note any histologic or immunorepression changes in the tissue. Sections from both experimental groups demonstrated high-quality histopathology and preservation of antigenicity for the nuclear transcription factor TTF1. This preservation of antigenicity demonstrated that the TTF1 protein had not significantly degraded as it was able to bind to its selective antibody, and that these samples may be used for research and clinical trials that requires immunohistochemistry.

Unfortunately, there is no extensive timetable of what happened to these samples during the storm. There are no records of the temperature of the building, the temperature of the freezers, or how much time passed before samples were moved. Similarly, no record was kept of which samples were moved to dry ice or liquid nitrogen tanks, nor how long they remained there until power was restored. The samples collected before the storm were in the -80°C freezers at the time the power was lost, but we do not know what happened to these samples between the power being lost and the power being restored. Due to the lack of record keeping during the storm, it is difficult to isolate any independent variable that may have caused molecular degradation. However, as a measure of quality assurance, this study shows that the molecular quality of frozen samples may not have been compromised due to the power outage from Hurricane Sandy. Samples from both groups did not differ qualitatively, and the majority of samples exposed to the power outage had acceptable DNA and RNA quality for downstream analyses. Similarly, these samples still retained immunorepression for a common clinical biomarker and had acceptable histology for pathology review. Because of the similar quality of specimens collected before and after the storm, specimens from both groups are still valuable resources for research requiring human specimens.^{16,17}

To extend the analysis, future studies could also look into whether the proteome or metabolome is similarly preserved or could include further downstream analyses as more extensive validations. Other tissue types with different pathologies along with matched normal samples could be included to examine whether different tissue types respond similarly. Additional immunostains could also be used to investigate whether other proteins are similarly preserved. In addition, the power of this study could be increased with a greater sample size.

This quality assurance study demonstrates that biospecimens may be preserved through a natural disaster, and that these specimens in particular may still be used for research and clinical downstream analyses. Although these specimens have been spared throughout this disaster, this experience highlights the importance of emergency preparedness throughout the medical and science community. Each institution must prepare for the worst to preserve the valuable specimens that advance the biomedical field.

Acknowledgments

We would like to thank all the members of the CBRD as well as the Experimental Pathology Research Laboratory and the Endocrine Surgery Associates of NYU. This study was supported through the NYU Cancer Institute Center Support Grant “NIH/NCI 5P30CA016087-33.”

Author Disclosure Statement

No conflicting financial interests exist.

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