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Autopsy Biobanking: Biospecimen Procurement, Integrity, Storage, and Utilization

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Summary

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An autopsy is a specialized surgical procedure consisting of external and internal examination of a deceased individual for the purposes of documenting abnormalities and determining or confirming medical diagnoses that may have contributed to their death. One of the benefits of an autopsy is the opportunity to collect and store biospecimens for the purposes of biobanking. This chapter outlines the procedures necessary to procure, store, and utilize biospecimens obtained during an autopsy. With the emergence of molecular diagnostics, this chapter will also discuss factors that influence the integrity of autopsy biospecimens prior to procurement. These include the postmortem interval, as well as premortem factors such as the patient's agonal state, biospecimen temperature, and pH.

Keywords

Autopsy; Biobank; Biospecimen; Integrity; Postmortem interval; Agonal state; Temperature; pH

1. Introduction

An autopsy, or postmortem examination, is a specialized surgical procedure, usually performed by a pathologist, which consists of an external and internal examination of a deceased individual. This chapter will focus exclusively on medical (clinical) autopsies that are conducted in a hospital setting and may be utilized for biobanking. Medico-legal autopsies fall under the jurisdiction of the county or state coroner or medical examiner and hence will not be discussed. The purpose of a medical autopsy is to document both external and internal abnormalities, with the aim of determining or confirming medical diagnoses that may have contributed to an individual's death. Once the patient's complete medical

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history is reviewed, including available radiographs and laboratory results, then the examination may begin. During the process of documenting external and internal findings, whole tissue samples are collected and examined by light microscopy. Additional studies may also be requested, such as microbiology cultures, and occasionally, if an autopsy is performed with a short postmortem interval (PMI, usually one to six hours), some tissues may be amenable to ultrastructural and/or biochemical analysis. A final autopsy report is then synthesized, and the cause(s) and manner of death are derived from the available clinical and anatomic information.

The rate of medical autopsies has been in decline for several years (1, 2), which is an unfortunate trend as one of their major benefits is the opportunity to collect and store tissue samples for the purposes of biobanking (i.e., building a repository of specimens that may later be utilized in research protocols). The collection of autopsy biospecimens may be performed provided that a consent form has been signed by the next of kin to allow such samples to be procured for research objectives. The tissues that may be sampled are generally limited by autopsy restrictions specifically documented on the consent form, so it is imperative that this information be known prior to the start of the postmortem examination. The patient should be correctly identified once the consent form has been reviewed. Dissection and evisceration techniques, as well as the biohazards of performing a postmortem examination, are discussed in detail elsewhere (3, 4) and are beyond the scope of this chapter.

1.1 Postmortem interval (PMI)

When procuring biospecimens during an autopsy, both pre- and postmortem conditions may influence the ability to yield accurate results from clinical and research studies. During the postmortem period, biospecimens go through reactive changes that begin with oxidative, hypoxic, and metabolic stresses, and these culminate in apoptosis and necrosis. While tissue architecture, proteins, and nucleic acids are reasonably stable in the immediate postmortem period, reactive changes have the potential to create marked intra- and intercase variability, making diagnoses and comparisons between case-groups difficult (5). To surmount this, one of the first variables to consider is the time interval between death and collection of the biospecimens, referred to as the PMI. Ideally, tissues should be procured as soon as possible after death (i.e., with a minimal PMI) in order to limit reactive changes and degradation. The different effects that the PMI has on biospecimen integrity are dependent on the nature of the sample and subsequent studies that will be performed (5, 6). Nevertheless, the PMI should always be reported, and comparable PMIs are preferred when studying different populations, e.g., disease versus control patients (7). While bodies of decedents are typically refrigerated awaiting an autopsy in an attempt to slow reactive changes, the PMI can still vary widely from 1 hour to greater than 24 hours. In our experience, the postmortem interval should be under 24 hours, but intervals of up to 72 hours may still yield satisfactory biospecimens, depending on purpose of the sample. Tissue architecture, DNA, and protein concentration levels are most stable in the postmortem period (7, 8). However, while general immunohistochemical staining profiles for many proteins remain unchanged up to a PMI of 50 hours, distinctive western blot degradation patterns may be observed at that time (7), with protein phosphorylation states being the most labile (9). Furthermore, molecular profiling

techniques have determined that, compared to DNA, prolonged PMIs may dramatically affect the integrity of phosphoproteins and RNA (6–10). RNA molecules, in particular, are rapidly degraded by ubiquitous, highly reactive ribonucleases, also referred to as RNases (11, 12).

1.2 Pre-mortem and agonal factors

Studies of the PMI have found that within 3–5 hours, postmortem variations in biospecimen integrity are both tissue- and donor-dependent (6), even within the same decedent procured under identical conditions (7). This suggests that the initial state of the biospecimen is even more critical than the PMI (5) and is dependent on numerous premortem factors, such as age, gender, body mass, disease state, cause of death, medications, and other medical interventions (5, 7, 13). The condition of the patient prior to death (i.e., periods of hypoxemia, hypoglycemia, hyperpyrexia, or coma) is referred to as the agonal state, the duration of which is suggested to have the greatest premortem influence on the integrity of biospecimens, particularly RNA (5, 10, 13–17). While attempts should be made to control premortem factors, they are often too numerous or varied. Therefore, assay dependent quantifiable measurements, standards, and markers of stability must be developed and utilized to assess for biospecimen integrity.

1.3 Temperature and pH

Enzymatic kinetics are highly dependent upon both temperature and pH. As such, numerous premortem factors that affect these variables have direct effects on the initial state of biospecimens and subsequent reactive changes that occur during the PMI. To begin with, there is a temperature gradient between body core and shell (18) that may influence the integrity of the biospecimen. Changes in this gradient are dependent on body mass and agonal state (i.e., pyrexia versus hypothermia), as well as the nature and location of the biospecimen. In addition, the PMI may be subdivided into warm ischemia time (i.e., room temperature) and cold ischemia time (i.e., the time of period during which the body is refrigerated). Analogous to the PMI, warm ischemia time may restrict which biospecimens may be used for projects studying phosphorylated proteins (9) and RNA (5), but it is less likely to impact studies involving DNA (8). The pH is also known to have significant effects upon RNA and protein integrity and should be measured in studies of postmortem tissue (5, 9, 13, 14). A prolonged state of hypoxia increases tissue lactate concentration, which subsequently lowers pH, and current studies with microarray-based gene expression profiling suggest that the duration of agonal state with acidosis may be the most critical factor accounting for RNA variation across biospecimens (15, 16). This is likely true for phosphorylation states of proteins, as well. Currently, the best indicator of overall sample quality is the RNA integrity number (RIN), which is a numerical scale derived from electrophoretic measurements of whole RNA, wherein a low number indicates substantial degradation (19). Not surprisingly, there is a direct correlation between RIN and pH (16, 17). With respect to protein integrity, in addition to measuring pH, surrogate markers of stability, such as proteins from specific classes or cellular compartments, may be utilized to qualify the state of the biospecimen (9). In summary, in order to use postmortem biospecimens for research and comparison between individuals, all available clinical and quantifiable

information must be accounted for, especially the duration and extent of changes in temperature and pH.

1.4 Precautions

As with all tissue and bodily fluids collected from human sources, appropriate universal precautions must be strictly observed in order to minimize the possibility of exposure of the prospector to infectious agents. This is especially true in patients with confirmed or suspected infections due to blood-borne pathogens, including hepatitis B, hepatitis C, and human immunodeficiency virus (HIV). The same precautions apply to confirmed or suspected cases of Creutzfeldt-Jakob disease (CJD) and other human prion disorders (transmissible spongiform encephalopathies), which are a group of rare, untreatable, and invariably fatal neurodegenerative diseases (20). Direct inoculation is the main risk when handling tissues and bodily fluids from these patients. As such, contamination of mucosal surfaces and the eyes must be avoided (21).

2. Materials

1. Research consent form
2. If relevant, research protocol detailing a researcher's specific request
2. Personal protective equipment (gown, mask, safety glasses, face shield)
3. Latex or nitrile gloves; cut resistant gloves
4. Scalpel, scalpel blades, forceps, scissors, ruler
5. Specimen jars or specimen tray, cryovials
6. Formalin or other fixative
7. Aluminum foil
8. RNase inhibitor solution
9. Cleaning solution or spray
10. Paper and/or cloth towels
11. Digital camera
12. Printed labels or blank label and marker pen
13. Lab notebook or logbook and pen
14. 70% ethanol
15. 10% neutral buffered formalin

3. Methods

3.1 Preparation and tissue procurement

The preparation and procurement of biospecimens is performed by a pathologist or under the supervision of a pathologist (22). The pathologist will then provide the biobank technician with a biospecimen sample. Typically, the biobank technician will not be procuring specimens directly from the decedent. Description of the pathologist's actions are to help provide context for the biobank technician involved in procurement.

1. The biobank technician should review the research consent form in order to thoroughly familiarize themselves with any restrictions on tissue sampling, if present. Make sure that the consent form has been properly signed by the appropriate next of kin. Depending on the local laws and the Institutional Review Board (IRB) protocol, de-identified autopsy samples may be collected without requiring a specific research consent.

2. The pathologist performing the autopsy will have reviewed the autopsy consent and determined whether there are any limitations on the autopsy (whole body vs. heart only or brain only etc.) and therefore limitations on what might be available to release to the biobank technician.

3. The pathologist will identify the patient by comparing the full name and medical record number present on the consent form with the information printed on the decedent identification tag, which is usually attached to the first digit of the foot, ankle, or wrist.

4. Tissue samples may be collected only after the pathologist has examined each particular specimen source in detail, recorded its weight (if the specimen source is a visceral organ), documented its characteristics and any abnormalities, took representative portions for histologic evaluation (22), and saved at least one additional piece in formalin, should additional studies be necessary in the future.

5. When the pathologist provides the biospecimen to the biobank technician, the technician should be ready with an opened container (*see* Note 1). If provided unlabeled, the technician should be ready to write the tissue origin (e.g. Lung, right lung, brain) as well as any abnormality, if relevant (e.g. type of cancer or inflammatory disorder). If patient consent has been given on an approved protocol, a patient identifier may be provided to the biobank technician.

5. Put the specimen jar on wet ice to keep it cold till the biospecimen can be processed. Minimize this time on ice by processing the biospecimen as rapidly as possible.

6. Take the biospecimens to a biosafety hood or appropriate area for dissection of tissues to aliquot them into cryovials for freezing or formalin for fixation. In some cases, the biobank technician may be releasing fresh tissue directly to researchers.

7. Quantify the biospecimen by weighing with a scale and/or measuring the specimen with a ruler. An empty specimen jar or cryovial can be used to tare the scale. Weigh the specimen

while in the specimen jar or cryovial. Document the weight and dimensions of the biospecimen.

8. Photograph the specimen with a digital camera particularly if there is a large specimen or whole organ like brain. In the photo of the specimen, include a ruler and a label that specifies research identifier, specimen type and laterality (e.g. left kidney tumor). Photos can be taken before and during dissection of the organ.

9. Use clean forceps and scalpels to cut aliquots that fit into cryovials or the appropriate specimen container. Use sterile forceps, scalpels and containers if specific microbial pathogens are implicated in the disease process or cell culture is to be attempted. RNase free instruments may be required for specific molecular techniques.

10. Tare the scale with an empty cryovial. Weigh the cryovials to establish how much tissue is in each cryovial. Record the weights into the biobank information system or a logbook.

11. Place the desired amount of tissue into formalin for fixation. Remove after 12 to 16 hours and place into 70% ethanol till the specimen can be submitted for tissue processing to make a paraffin block.

12. Thoroughly clean the workstation and rinse the reusable surgical instruments. Disposable scalpel blades must be placed within a designated sharps container.

13. Remove all personal protective equipment and dispose off in biohazard receptacles.

14. All involved personnel must wash their hands, wrists, and forearms with warm water and disinfectant soap.

3.2 Blood procurement

Blood is one of the most easily accessible and widely used biospecimen types available, and its collection and storage is not complicated (22).

1. Collection must take place as soon as possible after access is gained to the internal organs in order to minimize contamination that may occur as a result of manipulation of the viscera.

2. Blood samples are obtained with a new syringe and a short (approximately 2–4 cm), large bore needle in an effort to prevent possible contamination that may result from the use of longer needles (23). Blood may be taken from the femoral or subclavian vessels (23) and from the cardiac chambers.

3. Once collected, blood samples should be fractionated into plasma, serum, buffy coat, and red blood cells, each of which should be stored separately. Blood must be placed into different anticoagulant-coated collection tubes (e.g., citrate, ethylenediaminetetraacetic acid [EDTA], or heparin) based upon their projected utilization in research studies (22). A collection tube containing a clot accelerator such as silica or thrombin is used for collecting serum (22).

3.3 Urine procurement

Urine is another easily accessible biospecimen and, in addition to standard chemical analysis, may be utilized in studies involving proteins, nucleic acids, or cells (22).

1. As with the collection of blood, urine procurement is performed upon the initial exposure of the internal organs, and in order to minimize contamination requires the use of a new syringe and needle (23).
2. The length of the needle is not as important in urine collection as it is in blood collection because, generally, the urinary bladder is thick-walled, which reduces the likelihood of contamination that may occur with the needle passing through adjacent structures. However, the needle should be long enough to completely transverse the muscular wall of the bladder.
3. Once obtained, urine should be aliquoted and frozen as whole urine as soon as possible.
4. Alternatively, aliquots may be centrifuged, after which the pellet and the supernatant are stored separately (22).

3.4 Aliquoting

Aliquoting is an important step in the process of biobanking of both solid tissue and liquid samples. Splitting a large sample into multiple smaller portions and storing each smaller sample separately distributes the risk of specimen degradation that occurs with repeated freezing and thawing cycles (22, 24). Repeated freezing and thawing may disrupt lysosomal membranes and reduce biospecimen integrity; RNA, in particular, is very sensitive to these cyclical changes in temperature (5). Thus, all suspected or confirmed freeze/thaw cycles must be logged. Tubes manufactured with polypropylene are recommended for the purposes of aliquoting and storage of biological samples due to its low protein binding potential (24). Additives should not be placed within these containers, and the collection tubes should have screw top caps to ensure a secure seal and to prevent unintentional sample loss (22, 24).

3.5 Specimen storage

Attempts should be made to minimize the pre-processing length of time during which a sample is kept at room temperature before it is eventually frozen and stored. Once the autopsy biospecimen is procured, it should be kept on ice. Long-term storage of blood and urine requires temperatures at or below -80°C (8, 22), as do all biospecimens that require a high degree of nucleic acid or protein integrity (22). The storage container(s) must be labeled appropriately, preferably with a barcoding system (22), and labels should be water and frost resistant and designed to withstand the conditions of the extremely low temperatures present within a -80°C freezer or associated with liquid nitrogen. If additional samples are available, storage in liquid nitrogen also may be performed to serve as a backup once the samples in the -80°C freezer have been exhausted (22). Information specific to each sample, including sample identifying information, sample type, patient demographics, and clinical data, and well as freezer location, freezer identification, and sample location within freezer(s), must all be recorded and kept on a secure, centralized computer-based, password-protected database system (22, 24). This information should be backed up regularly and frequently.

3.6 Histopathologic and other analyses

Basic tissue analysis includes routine diagnostic procedures, such as evaluation for the presence of a variety of disease processes, including neoplastic, inflammatory, and infectious conditions. Much of this is possible by routine light microscopic evaluation of the specimen.

Immunohistochemistry is also often performed on autopsy tissues. A formal pathology report of the autopsy findings (deidentified if appropriate for the IRB-approved protocol) may be available to the biobank. More specific testing may also be performed to detect biomarkers for disease diagnosis and research studies (22).

3.7 Sample shipment

In the event that a frozen autopsy sample must be transported off-site, it must be done so on dry ice and preferably initiated on a Monday so that the sample arrives at its destination during the same week (24). Arrivals at the destination late in the week or on the weekend are to be avoided given the possibilities of delays or no one to receive the samples. The amount of dry ice utilized must be adequate to ensure that the specimen remains at an appropriate temperature for at least three days (22). Formalin-fixed paraffin embedded (FFPE) tissue specimens can be shipped at room temperature. While generally not needed, FFPE paraffin blocks are sometimes shipped in a plastic bag in a container with ice packs to prevent the block from melting in areas of high summer temperatures like Nevada and parts of the Southwestern United States.

4. Notes

1. If there are multiple specimens being rapidly handed off by the pathologist, a quick way to collect the multiple samples prior to aliquoting is to collect them on sheet of aluminum foil. One can write the designations with a permanent marker on the aluminum foil. There should be enough space between each section so as to ensure that tissue fragments do not touch each other. Later, when there is more time, the biobank technician can place them into appropriately labeled individual containers.
2. There is no criteria for the ideal quantity of tissue that should be collected. While it might be tempting to take as much tissue as possible and store entire organs in all cases, storage capacity particularly for freezers is limited. The quantity to collect depends on the anticipated demand from researchers and the specific needs for your program. If uncertain, a piece of tissue approximately 2–3 cm³ may suffice. For freezing, cut samples into sizes that fit in cryovials. If received fragmented, the aggregate dimensions of the tissue sample might be approximately 2 × 2 × 2 cm whenever possible. Storage of often requested and used samples is valuable. Storage of never requested samples is a waste.
3. For formalin fixation, thin slices of 0.2 to 0.3 cm thick are desirable for superior fixation. Formalin penetrates tissues slowly, often quoted at 1mm per hour, but the actual rate is variable. Regardless, relatively thin slices of tissues will fix quickly.

4. Avoid excessive tissue desiccation by working quickly to collect all of the necessary samples within the shortest possible amount of time. To reemphasize, keep samples cold on ice till they can be frozen, or formalin fixed.
5. The procurement of biospecimens for molecular techniques that utilize RNA requires very stringent methods and preparation to avoid RNase mediated degradation (11, 12). This entails working in a strictly RNase-free environment, where contact between samples and RNases via contaminated surfaces, tubes, glassware, or pipette-tips is avoided. Since RNases show extreme stability, they are not destroyed by conventional surface cleaning and disinfection, for example, using detergents and alcohol, or even by autoclaving. Thus, extensive preparation is necessary to avoid RNase contamination and to destroy or inactivate RNases that are already present in consumables, buffers, or within the sample itself. The creation of an RNase-free workspace is required and includes an extra set of pipettes, racks, tubes, and pipette-tips. Thorough cleaning of surfaces in the RNA handling area and RNase inactivation is also necessary. The most common method for RNase inactivation of water and buffers is treatment with diethylpyrocarbonate (DEPC), which is added to solution and incubated overnight before it is autoclaved. A simple method to clean surfaces, as well as plastic instruments and glassware, is the use of RNase inactivation solutions that may be purchased commercially.
6. The two most important aspects of biobanking postmortem specimens obtained from a medical autopsy are safety and biospecimen integrity. Both require ample preparation and expertise. Given the numerous potential variables that may confound studies that utilize biospecimens procured from an autopsy, extensive documentation of all pre- and postmortem factors is necessary, and this includes those involved in biospecimen processing and storage.

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