What an Endocrine Surgeon Should Know about Sample Collection and Tissue Preservation

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Abstract

One of the challenges that Endocrine Surgeons face is the collection and preservation of tissue samples from patients with endocrine disorders. Different tissue samples are the key essential for the diagnosis and treatment of various endocrine diseases, such as thyroid cancer, adrenal tumors, and parathyroid disorders. However, collecting and preserving tissue samples is a challenge and may hamper the concluding prognosis, onset of the disease, and another parameter of the disease which may mislead the treatment plan of the patients. So, it requires careful planning, preparation, and execution to ensure that the samples are representative of the disease state and suitable for analysis. In this article, we will discuss some of the factors that affect the quality and integrity of tissue samples and provide some tips and best practices for sample collection and preservation.

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The first factor to consider is the type of tissue sample that is needed for analysis. Depending on the endocrine disorder and the research question, different types of tissue samples may be required. For example, some endocrine diseases may require a biopsy, which is a small sample of tissue taken from a specific site using a needle or a surgical instrument. Other endocrine diseases may require a resection, which is the removal of a larger portion or the entire organ affected by the disease. The type of tissue sample determines the amount of tissue available for analysis, the level of invasiveness of the procedure, and the potential complications and risks for the patient.

The second factor to consider is the timing of tissue collection. Tissue collection should be done as soon as possible after the diagnosis or surgery to minimize the degradation of tissue quality and integrity. Degradation can occur due to various factors, such as enzymatic activity, bacterial contamination, oxidation, and temperature changes. Degradation can affect the morphology, viability, and molecular characteristics of the tissue sample, which can compromise the accuracy and reliability of the analysis. Therefore, tissue collection should be done on time and with proper care.

The third factor to consider is the method of tissue preservation. Tissue preservation is crucial for maintaining the quality and integrity of tissue samples until they are ready for analysis. There are different methods of tissue preservation, such as freezing, formalin fixation, paraffin embedding, and cryopreservation. Each method has its advantages and disadvantages depending on the type of tissue sample and the type of analysis that will be performed. For example, freezing preserves the molecular characteristics of the tissue sample but may cause ice crystal formation that damages the morphology. Formalin fixation preserves the morphology but may alter the molecular characteristics. Paraffin embedding preserves both morphology and molecular characteristics but requires additional steps for processing and sectioning. Cryopreservation preserves both morphology and viability but requires specialized equipment and protocols. The choice of preservation method should be based on the research question, the availability of resources, and the compatibility with downstream analysis techniques.

The fourth factor to consider is the storage and transportation of tissue samples. Tissue samples should be stored and transported in appropriate conditions to prevent degradation and contamination.


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The storage and transportation conditions depend on the type of tissue sample and the preservation method used. For example, frozen tissue samples should be stored at –80°C or lower and transported in dry ice or liquid nitrogen containers. Formalin-fixed or paraffin-embedded tissue samples should be stored at room temperature or 4°C and transported in sealed containers or bags. Cryopreserved tissue samples should be stored in liquid nitrogen tanks or freezers and transported in liquid nitrogen containers or dewars. The storage and transportation conditions should be monitored and recorded to ensure that they are within acceptable ranges.

The fifth factor to consider is the quality control and quality assurance of tissue samples. Quality control and quality assurance are important for ensuring that tissue samples are of high quality and suitable for analysis. Quality control involves checking the quality and integrity of tissue samples before, during, and after collection, preservation, storage, transportation, and analysis. Quality control can be done by using various methods, such as visual inspection, histological examination, immunohistochemistry, molecular assays, or functional assays. Quality assurance involves establishing standard operating procedures (SOPs), guidelines, protocols, criteria, documentation systems, and training programs for all aspects of sample collection and preservation. Quality assurance can help to reduce errors, variations, and inconsistencies in sample collection and preservation, and improve the reproducibility, and comparability of results.

The sixth factor to consider is compliance with regulatory requirements. Regulatory requirements are important for the collection, preservation, and analysis of tissue samples. This includes obtaining informed consent from patients, following ethical guidelines, adhering to safety protocols, maintaining confidentiality and privacy, and documenting all procedures and results.

As an Endocrine Surgeon, understanding sample collection and tissue preservation is crucial for accurate diagnosis, prognostication, and management of endocrine disorders. Here are some important things you should know:

- **Sample collection techniques:** The technique used to collect samples varies depending on the type of tissue being collected. For example, fine needle aspiration biopsy is used to collect thyroid nodules while core needle biopsy is used for adrenal gland tumors. It is important to be familiar with the appropriate collection techniques for each tissue. Some factors to consider when choosing a collection technique are the size and location of the lesion, the risk of complications, and the amount of tissue needed for analysis.

- **Handling of the specimen:** Proper handling of the specimen is essential for accurate results. It is important to ensure that the specimen is handled delicately, without damaging the tissue and is transported to the laboratory on time to avoid degradation. The specimen should be placed in a sterile container with a suitable fixative or preservative solution and labeled with the patient’s name, identification number, date and time of collection, and type of tissue.

- **Preservation techniques:** Different preservation techniques are used for different types of tissue samples. For example, formalin fixation and paraffin embedding are commonly used for histological analysis of surgical specimens, while snap-freezing and liquid nitrogen preservation are used for molecular studies. The choice of preservation technique depends on the type of analysis to be performed and the availability of resources. Preservation techniques aim to maintain the tissue’s morphology, structure, and molecular integrity.

- **Storage conditions:** The storage conditions for tissue samples are critical to preserving their integrity. Tissues should be stored at the appropriate temperature, usually between –80°C and –196°C, depending on the preservation technique used. It is also important to ensure that the tissue samples are properly labeled and tracked to avoid confusion. Storage conditions should be monitored regularly, and any deviations should be reported and corrected.

- **Quality control:** Quality control is important to ensure that the tissue samples are of high quality and suitable for analysis. This includes checking the tissue’s viability, confirming the presence of the target tissue, and assessing the adequacy of the sample for the intended analysis. Quality control methods may include visual inspection, histological examination, immunohistochemistry, molecular testing, or other techniques depending on the type of analysis.

- **Regulatory requirements:** Compliance with regulatory requirements is important for the collection, preservation, and analysis of tissue samples. This includes obtaining informed consent from patients, following ethical guidelines, adhering to safety protocols, maintaining confidentiality and privacy, and documenting all procedures and results. Regulatory requirements may vary depending on the institution, country, or region where the samples are collected and analyzed. Regulatory requirements may vary depending on the institution, country, or region where the samples are collected and analyzed. Therefore, it is essential to be aware of and comply with the relevant regulations.

In summary, as an Endocrine Surgeon, it is important to have a good understanding of sample collection and tissue preservation techniques to ensure that tissue samples are of high quality and suitable for analysis. Working closely with a pathology team and adhering to institutional and regulatory guidelines is key to achieving an accurate diagnosis, prognostication, and management of endocrine disorders.

**DNA Isolation**

Proper tissue storage is essential for preserving the DNA in the sample after surgery. The DNA can be isolated from the tissue and used for various purposes, such as diagnosis, research, or forensic analysis. However, if the tissue is not stored correctly, the DNA can degrade and become unusable.

The first step in storing the tissue is to put it in a sterile container or bag. This will prevent contamination from bacteria, fungi, or other sources that can affect the quality of the DNA. The container or bag should be sealed tightly and labeled with the patient’s information, date and time of collection, type of tissue, and any relevant medical information. This will help to identify the sample and avoid confusion.

The next step is to keep the tissue at a low temperature. If the tissue will be processed within 24 hours, it can be stored on ice or in a refrigerator at 4°C. This will slow down the enzymatic activity that can break down the DNA. However, if the tissue will not be processed within 24 hours, it should be frozen at –80°C or in liquid nitrogen. This will stop the enzymatic activity completely and preserve the DNA for a longer period. It is important to avoid freeze-thaw cycles as this can cause DNA fragmentation.
By following these steps, the tissue sample can be stored properly and the DNA can be isolated successfully. Proper tissue storage is crucial for obtaining reliable and accurate results from DNA analysis.

RNA Isolation

Tissue storage after surgery for RNA isolation is an important procedure that requires careful attention to avoid RNA degradation. RNA is a sensitive molecule that can be easily damaged by enzymes, temperature changes, or contaminants. Therefore, the tissue must be handled and stored properly to preserve the integrity and quality of RNA. The following steps summarize the general protocol for tissue storage after surgery for RNA isolation:

• **Rapid removal of tissue:** The first step is to remove the tissue from the patient as soon as possible after surgery. This minimizes the exposure of the tissue to RNases, which are enzymes that break down RNA. RNases are present in the environment and biological fluids, such as blood and saliva. The longer the tissue is exposed to RNases, the more likely it is that the RNA will degrade.

• **Snap freezing:** The second step is to snap-freeze the tissue in a very cold medium, such as liquid nitrogen or dry ice. Snap freezing stops all biological activity and preserves the RNA in its original state. To snap-freeze the tissue, it should be quickly immersed in the cold medium and then placed in a labeled container. The container should be sealed and insulated to prevent thawing.

• **Storage at –80°C:** The third step is to store the tissue at –80°C until it is ready for RNA isolation. About –80°C is a very low temperature that prevents RNA degradation and maintains RNA stability. The tissue should not be thawed and refrozen multiple times, as this can damage the RNA. The tissue should also be stored in a freezer that has a backup power supply and an alarm system to ensure a constant temperature.

• **RNA extraction:** The fourth step is to extract the RNA from the tissue using a suitable method. The tissue should be thawed on ice and then homogenized using a device that can disrupt the cell membranes and release the RNA. There are different methods for RNA extraction, such as TRIzol, which uses a chemical solution to separate RNA from other molecules; RNA isolation kits, which use columns or filters to purify RNA; and magnetic bead-based systems, which use magnetic particles to capture RNA. A fifth step is to check the quantity and quality of the extracted RNA using a device that can measure its concentration and purity. A spectrophotometer can measure the absorbance of light by RNA at different wavelengths, while a fluorometer can measure the fluorescence of RNA when bound by a dye. The quantity and quality of RNA can affect the performance and accuracy of downstream applications, such as gene expression analysis or RNA sequencing.

In conclusion, tissue storage after surgery for RNA isolation is a vital process that requires proper handling and storage of tissue samples to ensure high-quality RNA for downstream applications. By following these steps, researchers can obtain reliable and reproducible results from their RNA studies.

Protein Isolation

Protein isolation is a process of extracting and purifying proteins from biological samples, such as tissues. It is an essential step for many applications, such as proteomics, immunoblotting, enzyme assays, and antibody production. However, protein isolation can be challenging as proteins are sensitive to degradation by various factors, such as temperature, pH, proteases, and oxidation. Therefore, it is important to handle the tissues carefully and store them appropriately after surgery to preserve the proteins for isolation.

The best storage method for tissues depends on several factors, such as the type of tissue, the amount of tissue, the purpose of protein isolation, and the time available for processing. There is no single optimal method that works for all tissues and all applications. However, some general guidelines can be followed to choose the most suitable method for each situation.

One of the most common methods used to store tissues for protein isolation is snap freezing. This method involves quickly freezing the tissue in liquid nitrogen or dry ice within seconds of collection. This prevents protein degradation by slowing down enzymatic and chemical reactions. The frozen tissue can then be stored at –80°C until protein isolation. Snap freezing is suitable for most types of tissues and most applications that require intact proteins.

Another method that can be used to store tissues for protein isolation is liquid nitrogen immersion. This method involves submerging the tissue in liquid nitrogen to freeze it rapidly. This method is similar to snap freezing but does not require a container or a freezer. However, this method has some disadvantages, such as potential tissue damage by ice crystals and difficulty in retrieving the tissue from liquid nitrogen. Therefore, this method is not recommended for long-term storage or delicate tissues.

A third method that can be used to store tissues for protein isolation is formalin fixation. This method involves immersing the tissue in a solution of formaldehyde to preserve its structure and morphology. This method is widely used for histological analysis but is not suitable for protein isolation as it cross-links the proteins, making them difficult to extract and denature. Therefore, this method should be avoided if protein isolation is the main goal.

A fourth method that can be used to store tissues for protein isolation is fresh tissue storage. This method involves placing the tissue in a buffer solution on ice to prevent degradation until processing. This method is ideal if protein isolation is to be performed immediately after surgery or within a few hours. However, this method is not feasible for long-term storage or large amounts of tissue as it requires constant refrigeration and may result in bacterial contamination.

A fifth method that can be used to store tissues for protein isolation is RNA later storage. This method involves placing the tissue in a reagent called RNA later that stabilizes both RNA and protein in the tissue. This method is useful if RNA isolation is also required along with protein isolation as it preserves both molecules without affecting their quality or quantity. The tissue can be stored at –80°C in RNA later until protein and RNA isolation.

In conclusion, tissues should be stored appropriately after surgery to preserve the proteins for isolation. The recommended storage method depends on the type of tissue and the time between tissue collection and protein isolation. Some of the common methods are snap freezing, liquid nitrogen immersion, formalin fixation, fresh tissue storage, and RNA later storage. Each method has its advantages and disadvantages and should be chosen based on the specific needs and goals of each experiment.
Table 1: Tissue and their storage for molecular biology technique

<table>
<thead>
<tr>
<th>Organ/gland type</th>
<th>Storage duration</th>
<th>Storage medium/tube</th>
<th>Source (DNA/RNA/tissue)</th>
<th>FNA</th>
<th>Temperature</th>
<th>Assay type</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>Long-term</td>
<td>Formalin-fixed paraffin-embedded (FFPE) blocks</td>
<td>Tissue</td>
<td>No</td>
<td>Room temperature</td>
<td>IHC</td>
<td>Histopathological examination</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>Long-term</td>
<td>FFPE blocks</td>
<td>Tissue</td>
<td>No</td>
<td>Room temperature</td>
<td>IHC</td>
<td>Histopathological examination</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Long-term</td>
<td>FFPE blocks</td>
<td>Tissue</td>
<td>No</td>
<td>Room temperature</td>
<td>IHC</td>
<td>Histopathological examination</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>Long-term</td>
<td>FFPE blocks</td>
<td>Tissue</td>
<td>No</td>
<td>Room temperature</td>
<td>IHC</td>
<td>Histopathological examination</td>
</tr>
<tr>
<td>Blood</td>
<td>Short-term</td>
<td>EDTA tubes</td>
<td>DNA/RNA</td>
<td>No</td>
<td>4°C</td>
<td>Genetic sequencing</td>
<td>Genomic profiling/genotyping</td>
</tr>
<tr>
<td>FNA samples</td>
<td>Short-term</td>
<td>Cytopathology fixative</td>
<td>Tissue</td>
<td>Yes</td>
<td>Room temperature</td>
<td>Histopathological examination</td>
<td>Tumor diagnosis/staging</td>
</tr>
<tr>
<td>Frozen tissue</td>
<td>Long-term</td>
<td>Cryopreservation medium</td>
<td>Tissue</td>
<td>No</td>
<td>−80°C</td>
<td>Molecular assays</td>
<td>Gene expression analysis/proteomics</td>
</tr>
<tr>
<td>FNA for RNA extraction</td>
<td>Short-term</td>
<td>RNA later or RNA later solution</td>
<td>RNA</td>
<td>Yes</td>
<td>−20°C</td>
<td>Gene expression analysis</td>
<td>Gene expression analysis</td>
</tr>
</tbody>
</table>

FNA, fine-needle aspiration; IHC, immunohistochemistry

**Immunohistochemistry Staining**

Tissue storage after surgery for immunohistochemistry staining depends on various factors such as the type of tissue, fixation method, and the desired staining method. Generally, the tissue should be stored in a fixative solution immediately after surgery to prevent any degradation or changes in the tissue structure. The most commonly used fixative solution is formalin, which is used to preserve the tissue structure and prevent the loss of antigens.

The duration of tissue storage in the fixative solution varies depending on the type of tissue and the desired staining method. Typically, tissues can be stored in the fixative solution for up to 24–48 hours before being processed for staining. After fixation, the tissue can be embedded in paraffin wax or frozen for long-term storage.

It is important to note that prolonged storage of tissue samples in the fixative solution can cause changes in the tissue structure and affect the staining results. Therefore, it is recommended to process the tissue samples for staining as soon as possible after fixation.

Some factors that can influence the staining results of immunohistochemistry are:

- The type and concentration of the primary antibody used to detect the antigen of interest.
- The type and concentration of the secondary antibody are used to amplify the signal of the primary antibody.
- The type and concentration of the chromogen or fluorophore are used to visualize the signal of the secondary antibody.
- The type and duration of antigen retrieval methods used to enhance the accessibility of antigens in fixed tissues.
- The type and duration of blocking methods used to reduce nonspecific binding of antibodies to tissues.
- The type and duration of washing methods used to remove excess antibodies and unbound chromogens or fluorophores from tissues.

These factors should be optimized for each tissue type and staining method to achieve optimal staining results. Additionally, proper controls should be included in each staining experiment to validate the specificity and sensitivity of the staining method.

**Immunofluorescence Staining**

After surgery, it is important to properly store tissue samples to ensure their integrity for subsequent immunofluorescence staining. Here are some general guidelines for tissue storage:

- Immediately after surgery, the tissue should be placed in a sterile container with a small amount of the appropriate fixative. This will help preserve the tissue structure and prevent the degradation of the proteins of interest.
- The tissue should then be stored in the fixative at the appropriate temperature and for the appropriate amount of time. The specific fixative and storage conditions will depend on the tissue type and the target antigens.
- After fixation, the tissue can be stored in 70% ethanol or other appropriate storage solution. The tissue should be kept at the appropriate temperature and protected from light.
- If the tissue needs to be transported, it should be shipped in a sealed container with appropriate temperature control.
- Before immunofluorescence staining, the tissue should be rehydrated by placing it in a series of graded ethanol solutions and then in water.

It is important to note that the specific storage conditions may vary depending on the tissue type and the staining protocol being used. Consultation with a qualified pathologist or laboratory technician is recommended to ensure proper tissue handling and storage are mentioned in Table 1.

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