Effectiveness of Different Preservation Techniques for Biological Specimens

Saud Saleh Alanazi [1], Mariam Ahmed Alyamani [2], Doaa Mohammed Banaama [3], Alqahtani, Hatim Ali Y [4], Abdulaziz Abdullah Alkuwaykibi [5], Mansour Mohammed Alruwaili [6], Fatimah Saud Alfuhaydi [7], Faisal Fahd Mutlaq Al-Saqri [8], Buthaina Ahmed Omar Zabrmawi [9], Almaslamani, Mohammed Mahmoud [10]

1- Specialist-Laboratory, Northern Borders Health Cluster, Medical Supply, Saudi Arabia
2- Specialist-Laboratory, King Abdulaziz Hospital, Jeddah, Saudi Arabia
3- Specialist-Laboratory, King Abdulaziz Almahjar Hospital, Jeddah, Saudi Arabia
4- Technician – Laboratory, Al-Farsha General Hospital, Asir, Saudi Arabia
5- Technician – Laboratory, Health Control Center in Haditha, Qurayyat, Saudi Arabia
6- Technician – Laboratory, Turaif General Hospital, Turaif, Saudi Arabia
7- Technician – Laboratory, Al-Nahda Health Center, Al Wajh, Saudi Arabia
8- Technician – Laboratory, King Khalid Hospital, Hail, Saudi Arabia
9- Technician – Laboratory, King Abdulaziz Hospital, Jeddah, Saudi Arabia
10- Technician – Laboratory, Ministry of Health Branch in Tabuk, - Al Wajeh, Saudi Arabia

Abstract:

The preservation of biological specimens is crucial for research, education, and medical purposes, enabling scientists to study cellular structures, genetic materials, and environmental interactions over time. Various preservation techniques, including cryopreservation, formalin fixation, and dehydration, each have distinct advantages and limitations. Cryopreservation, which involves storing specimens at extremely low temperatures, is highly effective for preserving the viability of cells and tissues for long-term studies, particularly in biotechnology and reproductive medicine. On the other hand, formalin fixation is commonly used in histopathology to preserve tissue architecture while allowing for subsequent microscopic examination. However, it often affects nucleic acid integrity, which can be problematic for molecular analyses. Dehydration, typically through lyophilization, effectively preserves specimens for long-term storage, but may lead to loss of functional properties and structural integrity. The choice of preservation technique often depends on the intended application of the specimens and the nature of the biological material. For instance, samples intended for DNA sequencing may prefer methods that retain nucleic acid quality, while those for histological examinations might benefit more from fixation techniques. Emerging techniques, such as ethanol preserving combined with specific additives, show promise in enhancing the quality of preserved specimens while mitigating the drawbacks of traditional methods. As researchers continue to weigh the effectiveness, cost, and preservation quality of these techniques, the development of new methodologies that balance these factors remains a crucial area of study in biological sciences.

Keywords: Preservation techniques, biological specimens, cryopreservation, formalin fixation, dehydration, tissue architecture, histopathology, nucleic acid integrity, lyophilization, ethanol preserving, molecular analyses.

Introduction:

Biological specimens serve as vital repositories of genetic, physiological, and morphological information, crucial for a diversity of scientific fields including taxonomy, conservation biology, and biomedical research. The integrity of these specimens is paramount, as any degradation or alteration can impede scientific findings and limit our understanding of biodiversity and evolutionary processes. Therefore, the effectiveness of various preservation techniques is of fundamental importance to both the scientific community and the broader field of life sciences. This introduction outlines the prevailing preservation methods employed for biological specimens, highlights their advantages and limitations, and underscores the significance of ongoing research into more effective and sustainable preservation strategies [1].

The preservation of biological specimens encompasses a variety of techniques, each tailored to particular types of materials, such as plant tissues, animal parts, and microbial cultures. The most commonly used methods include refrigeration, freezing, dehydration, and the use of preservatives such as formaldehyde and ethanol. Additionally, newer approaches like cryopreservation and the application of advanced techniques such as freeze-drying and chemical cross-linking are gaining traction. Each method offers distinct benefits, as well as challenges, and the choice of technique often hinges on the intended research application, the type of specimen, and logistical considerations, such as cost and ease of access to resources [2].

Refrigeration and freezing remain foundational preservation methods, especially for soft-bodied organisms or fresh tissues, where enzymatic and microbial degradation are significant concerns. These techniques are relatively straightforward and effective in maintaining cellular integrity and metabolic inactivity. However, there are limitations associated with these methods. For instance, ice crystal formation during freezing can cause physical damage to delicate cellular structures—a phenomenon that can compromise the specimen's morphological and genetic quality. Furthermore, refrigerated specimens typically have a limited lifespan, which necessitates constant monitoring and resource availability [3].

Dehydration, often employed for preserving specimens such as plants and fungi, involves removing moisture to inhibit microbial growth. Drying methods vary from air-drying to using desiccants, and the choice often reflects the intended use of the specimen—extraction of DNA or other biomolecules might favor different drying techniques. Despite its advantages, dehydration has the potential to alter the cell wall structure and biochemical composition, leading to the loss of critical information embedded in the specimen. While effective for certain organisms, it proves unsuitable for others, illustrating the need for tailored preservation strategies that consider species-specific characteristics and research goals [4].

Chemical preservation, particularly through the use of solvents like ethanol and formaldehyde, provides alternatives that can be especially effective for maintaining the morphology of specimens over extended periods. These chemicals function by cross-linking proteins, thus stabilizing cellular structures. However, the use of such preservatives is not without drawbacks. Ethical concerns regarding toxicity and biohazardous waste management arise, particularly in relation to formaldehyde, an acknowledged carcinogen. Additionally, chemical preservation may hinder subsequent molecular analyses, such as DNA sequencing, and lead to challenges in extracting nucleic acids or proteins due to residual chemicals [5].

The advent of advanced preservation techniques has opened new avenues for specimen conservation. Cryopreservation, through ultralow-temperature storage, temporarily halts biological activity and can preserve living cells and tissues for indefinite times. However, it remains an expensive approach that requires specialized equipment and technical knowledge, thus limiting its accessibility. Freeze-drying, or lyophilization, presents a viable solution for certain samples, allowing long-term preservation while maintaining structural integrity. This technique has gained traction in various fields but still requires more rigorous study to uncover its full potential and applicability across diverse biological specimens [6].

The effectiveness of preservation techniques also hinges on the specific needs of the scientific community. For instance, taxonomists often require morphological accuracy, while conservationists and bioprospectors may prioritize genetic integrity. The dynamic nature of biological research necessitates a reevaluation of existing preservation techniques to ensure they align with evolving scientific demands and ethical considerations. As the global biodiversity crisis continues to escalate, the ability to effectively preserve biological specimens can significantly influence conservation strategies and facilitate the understanding of ecological systems [7].

Overview of Preservation Techniques:

Biological specimens, including tissues, organs, and whole organisms, provide invaluable insight into a multitude of scientific fields, ranging from medicine and biology to anthropology and environmental science. The preservation of these specimens is critical, as it allows researchers to study them over extended periods, facilitating both historical analyses and the comparison of contemporary samples. However, the act of preservation presents unique challenges due to the complexity and variability of biological materials [8].

The necessity for preservation arises from the inherent nature of biological materials, which are subject to decomposition and decay due to microbial action and enzymatic processes. Without proper preservation, specimens may lose their structural integrity, molecular composition, and biological function. The implications of deterioration are particularly significant in fields such as medicine, where understanding disease progression requires access to viable tissues, and in ecology, where continuity is key to studying biodiversity and evolutionary processes. Effective preservation not only safeguards against biological degradation but also enhances the reliability of scientific research, enabling replicability and reproducibility in experimental conditions [9].

Common Preservation Techniques

The preservation of biological specimens can primarily be categorized into several techniques: freezing, drying, chemical preservation, and cryopreservation. Each of these methods has specific applications and is suited for particular types of biological samples [10].

1. Freezing

Freezing is one of the most common techniques used to preserve biological specimens, especially for tissues and cells. The principle behind freezing is to reduce the temperature to a point where biochemical processes are significantly slowed or halted altogether, thereby limiting degradation [11].

- **Method**: Biological specimens are typically cooled to below -20°C or -80°C to achieve long-term storage conditions. Flash freezing, which involves rapidly lowering temperatures through liquid nitrogen, minimizes the formation of ice crystals that can disrupt cellular integrity.
- **Applications**: Freezing is widely employed in the preservation of sperm, eggs, embryos, and even organ tissues for their future use in reproductive medicine and transplantation.
- **Advantages and Disadvantages**: The primary advantage of freezing is the relative simplicity and cost-effectiveness of the method. However, there are drawbacks, such as the potential for thermal shock and ice crystal formation, which can result in cell rupture, leading to loss of structural fidelity [12].

2. Drying

Drying, including methods such as lyophilization (freeze-drying), removes moisture from biological specimens to inhibit microbial growth and enzymatic breakdown.

- **Method**: In traditional drying, specimens are exposed to low humidity environments or direct heat to evaporate water. Freeze-drying involves freezing the specimen, and then reducing the surrounding pressure to allow sublimation, whereby ice transitions directly to vapor without becoming liquid [13].
- **Applications**: This method is particularly useful for preserving microbial cultures and certain plant specimens, where undamaged structural integrity is critical for identification and study.
- **Advantages and Disadvantages**: The main advantage of drying techniques is their effectiveness in long-term storage. Once dried, specimens can often be stored at room temperature without further deterioration. However, the process can lead to changes in the structure and function of some biological polymers, such as proteins and nucleic acids, potentially rendering them less suitable for experimental purposes [13].

3. Chemical Preservation

Chemical preservation encompasses a variety of methods that involve the use of chemical solutions to stabilize biological specimens [14].

- **Method**: Common chemicals include formalin (formaldehyde solution), ethanol, and glycerol. These media work by penetrating biological tissues and cross-linking proteins, which stabilizes the structural integrity and prevents decay.
- **Applications**: Chemical preservation is frequently used in histology, where tissue samples are fixed in formalin for microscopic examination. Ethanol is also widely utilized for the preservation of both

animal and plant specimens in biological collections [14].

- **Advantages and Disadvantages**: Chemical preservation is relatively simple and effective for many types of specimens, maintaining integrity over time. However, the use of fixatives like formalin can render some biochemical analyses impossible, as they may alter the original molecular structure of proteins and nucleic acids [14].

4. Cryopreservation

Cryopreservation is a sophisticated preservation technique designed to maintain the viability of cells and tissues at extremely low temperatures, typically around -196°C using liquid nitrogen.

- **Method**: This process usually involves the use of cryoprotectants—substances that prevent the formation of ice crystals and protect cells from damage during freezing and thawing. Glycerol and dimethyl sulfoxide (DMSO) are common cryoprotectants used in cell cultures [15].
- **Applications**: Cryopreservation is particularly valuable in reproductive technologies, such as the preservation of oocytes and sperm, as well as in the storage of stem cells and regenerative medicine therapies.
- **Advantages and Disadvantages**: Cryopreservation is highly effective in maintaining cellular viability while preserving their functional properties. Nonetheless, the technique requires specialized equipment, careful handling, and considerable expertise, which can be a barrier in some settings [15].

Cryopreservation: Principles and Applications:

Cryopreservation is a technique that has garnered considerable attention in the fields of biology, medicine, and biotechnology. It involves the cooling and storage of biological specimens at extremely low temperatures, typically in liquid nitrogen (-196°C or -321°F), to halt all physiological, metabolic, and chemical processes. This technique serves as an essential tool in the preservation of a wide variety of biological materials, ranging from cells and tissues to entire organisms, allowing researchers and medical professionals to maintain specimens over extended periods without significant degradation or loss of viability [16].

Principles of Cryopreservation

The fundamental principle of cryopreservation revolves around the avoidance of ice crystal formation within biological tissues. When cells are subjected to freezing temperatures, water within and outside the cells begins to freeze and can form ice crystals. These crystals can puncture cell membranes, disrupting cellular integrity and leading to cell death. To prevent this, cryopreservation techniques utilize various approaches and cryoprotective agents (CPAs) [17].

1. **Cryoprotectants**: These are chemicals added to biological specimens to lower the freezing point of water and inhibit ice crystal formation. Common cryoprotectants include dimethyl sulfoxide (DMSO), glycerol, and ethylene glycol. These agents work by permeating the cells and providing a protective effect during the freezing and thawing processes. The right concentration of a cryoprotectant is crucial; if the concentration is too low, ice can form, and if too high, it may become toxic to cells [18].
2. **Cooling Rate**: The rate at which cells are cooled is a crucial factor in successful cryopreservation. Too rapid cooling can result in intracellular ice formation, while too slow cooling can lead to osmotic damage as water moves out of the cells. A controlled rate of cooling, often around 1°C per minute, is typically used to optimize cell integrity during the freezing process [19].
3. **Thawing Protocols**: The process of thawing is equally important as cooling. The aim is to rapidly re-warm the samples to minimize the time spent in a state that could lead to osmotic shock or further damage. It often involves placing the frozen specimens in a warm water bath to ensure that they return to a physiological temperature as quickly as possible [19].

By adhering to these principles, cryopreservation makes it possible to maintain the viability of biological specimens over extended periods, allowing for their future use in various applications.

Applications of Cryopreservation

Cryopreservation is employed in numerous fields, yielding significant advancements across biological sciences and medicine.

1. **Stem Cell Banking**: One of the most significant applications of cryopreservation is in stem cell research and medicine. Stem cells, which have the potential to develop into different cell types, are often collected and stored for future therapies, especially in treating conditions like leukemia and other blood disorders. Cord blood stem cell banks leverage cryopreservation to store umbilical cord blood that can be used later for transplantation [20].

2. **Reproductive Medicine**: In reproductive health, cryopreservation is vital for preserving oocytes (eggs), sperm, and embryos. This is particularly important in assisted reproductive technologies (ART), such as in vitro fertilization (IVF), where embryos can be frozen and transferred in later cycles. For women undergoing treatments that may affect their fertility, such as chemotherapy or radiation, the preservation of oocytes can provide a pathway to future pregnancy [20].

3. **Tissue and Organ Preservation**: Cryopreservation allows for the storage of larger biological specimens, including tissues and organs, for research and transplantation purposes. The ability to preserve tissues for extended periods facilitates studies in developmental biology and regenerative medicine. Although organ transplantation presents more challenges due to the complex structures involved, advancements in cryopreservation techniques are being developed to improve outcomes [21].

4. **Biodiversity Conservation**: In conservation biology, cryopreservation is used to preserve genetic material from endangered or extinct species. Sperm, eggs, and embryos can be cryopreserved to assist in breeding programs aimed at revitalizing populations. Frozen genetic material can also serve to maintain genetic diversity within breeding stocks, which is critical for species conservation efforts [22].

5. **Cell Line Storage**: Cell lines utilized in biological research can be cryopreserved to prevent phenotypic drift and loss of functional characteristics over time. By freezing these cell lines, researchers can ensure consistent and reproducible results across experiments [22].

6. **Pharmaceutical Applications**: Cryopreservation plays a key role in the pharmaceutical industry as well. Live cell assays, vaccines, and therapeutic proteins can be preserved using cryopreservation techniques to maintain their functional properties throughout the production and storage processes [23].

Ethical and Technical Considerations

Despite its many advantages, cryopreservation also encounters ethical and technical challenges. In reproductive medicine, ethical concerns regarding the fate of embryos not used in procedures arise. Furthermore, regulations around stem cell usage and storage vary widely by region, complicating practices in global biomedical research [24].

Technically, the field of cryopreservation is continually evolving with improvements in IT and robotics for monitoring samples, as well as advances in CPA formulations. The development of new technologies, such as vitrification — a method of solidifying liquids into a glass-like state without forming ice — presents a promising frontier for improving cryopreservation outcomes [25].

Formalin Fixation: Benefits and Limitations:

Formalin fixation is a widely employed technique in histology and pathology used to preserve biological specimens for examination. By embedding tissues in a chemically stable environment, formalin fixation facilitates the study of cellular architecture and provides critical insights into health and disease states. Despite its prevalence, this method has its benefits and limitations, which must be understood to optimize its application in various biological fields [26].

Formalin is a saturated aqueous solution of formaldehyde, typically at a concentration of 10% (formalin is usually about 3.7% to 4% formaldehyde by volume). The process of fixation involves immersing biological specimens in formalin, which cross-links proteins and nucleic acids, thereby stabilizing the tissue's cellular structure. This chemical action preserves the integrity of the sample while preventing enzymatic degradation, autolysis, and putrefaction [27].

The fixation process has several critical steps. Initially, the tissues must be fixed promptly after removal from the body to minimize distortions. The specimen size and volume are also important considerations; the fixation solution must penetrate adequately to ensure thorough preservation. Generally, a fixation time of 6 to 24 hours is adequate for most tissues, with variations depending on the size and type of the specimen. Following fixation, samples are typically processed for embedding in paraffin wax, which further preserves the specimen for sectioning and microscopic examination [28].

Benefits of Formalin Fixation

1. **Preservation of Cellular Architecture**: One of the most significant benefits of formalin fixation is its ability to maintain the cellular morphology and tissue architecture. This preservation is crucial for diagnostic histopathology, where the visual assessment of cell structures is essential for identifying abnormalities, diseases, or tumors [28].
2. **Long-term Storage**: Fixed tissues can be stored for extended periods without significant degradation. This stability allows for retrospective studies, where previously collected specimens can be re-evaluated as new techniques or diagnostic criteria emerge.
3. **Compatibility with Staining Techniques**: Formalin-fixed specimens are compatible with various staining methods, including hematoxylin and eosin (H&E) staining, immunohistochemistry, and special stains for specific cellular components. The chemical cross-linking by formalin enhances the binding of dyes, allowing for more precise visualization of cellular elements [28].
4. **Wide Applicability**: Formalin fixation is versatile and can be applied to a broad range of tissues and biological materials, from solid organs to tumors. It is the gold standard for most routine histopathological practices and is widely accepted in clinical and research laboratories.
5. **Ease of Use**: Formalin is relatively easy to handle, store, and apply compared to other fixation agents, making it accessible for routine laboratory practices. Its effectiveness and availability have led to its widespread adoption in laboratories across the globe [28].

Limitations of Formalin Fixation

Despite its many advantages, formalin fixation has several limitations that can impact the quality of biological specimens and the outcomes of subsequent analyses [29].

1. **Artifact Formation**: The cross-linking effect of formalin can lead to the formation of artifacts—changes in tissue appearance that do not reflect actual biological conditions. These artifacts can complicate microscopic interpretation, particularly in identifying subtle pathologies.
2. **Nucleic Acid Integrity**: While formalin fixation preserves protein structures well, it can adversely affect the integrity of nucleic acids. Formaldehyde can cause fragmentation of DNA and RNA, making downstream molecular analyses, such as PCR and sequencing, challenging or even unfeasible [29].
3. **Immunohistochemistry Limitations**: Although formalin is compatible with many staining techniques, its cross-linking nature can hinder the availability of antigenic sites for antibodies in immunohistochemistry. This limitation can reduce the sensitivity and specificity of tests, thereby impacting diagnostic accuracy [29].
4. **Potential Health Risks**: The use of formaldehyde poses health risks for laboratory personnel. Formaldehyde is a known carcinogen, and its inhalation can lead to respiratory issues and skin irritation. Consequently, appropriate safety protocols

must be instituted when using formalin to mitigate exposure risks [30].

5. **Staining Challenges**: Certain types of stains, particularly those that rely on the detection of specific antigens or nucleic acids, may yield suboptimal results in formalin-fixed tissues. This necessitates careful selection of fixation protocols and potentially alternative fixatives, especially for advanced molecular biology applications [30].

Dehydration Techniques: Lyophilization and Beyond:

Dehydration plays an essential role in the preservation, storage, and transportation of biological samples. As more scientific fields seek to explore the nuances of cellular and molecular biology, the demand for innovative dehydration techniques is ever increasing. Among these, lyophilization—commonly known as freeze-drying—has emerged as a pivotal technique for maintaining the integrity of biological materials [31].

Lyophilization is a complex process that involves three primary stages: freezing, primary drying, and secondary drying. Initially, the biological sample undergoes rapid freezing to convert water into ice. This freezing process is critical since it preserves the structural and biochemical characteristics of biological materials by preventing the formation of large ice crystals that can damage cellular structures [31].

Once the sample is adequately frozen, the primary drying phase ensues. During this stage, pressure is lowered, and heat is applied to remove water through sublimation, wherein ice changes directly to vapor without passing through the liquid state. The sublimation process not only extracts most of the moisture but also preserves the sample in a more stable state. It's important to control the temperature and pressure during this phase to achieve maximum water removal while maintaining the integrity of the biological sample [31].

The final stage, known as secondary drying, involves the removal of unfrozen water molecules that may still be present in the product. This is typically introduced through a gradual increase in temperature, allowing residual moisture to evaporate away, thus enhancing the stability of the product. Following this process, the resultant lyophilized product is a porous solid, often resembling a fluffy, dry cake [32].

Lyophilization boasts several advantages that make it particularly appealing for the preservation of biological samples. One of the foremost benefits is the significant extension of shelf life: lyophilized samples can remain stable for months—or even years—compared to their non-dehydrated counterparts. This stability is particularly advantageous for expensive and sensitive biological products, such as vaccines, cells, and proteins [32].

Another significant advantage is the preservation of biological activity. Lyophilization minimizes the degradation of proteins and enzymes, which can be crucial for maintaining functionality upon rehydration. In many cases, enzymatic assays and biological experiments yield better results with lyophilized samples compared to those that have undergone traditional drying methods [33].

Moreover, lyophilization enables easier handling and shipping of biological samples. The lightweight and compact nature of lyophilized products facilitates transport, especially in regions with limited access to cold storage facilities. Additionally, the vacuum-sealed packaging of lyophilized products can protect them from exposure to oxygen and other environmental factors that could otherwise lead to deterioration [33].

Despite its many merits, lyophilization is not without its challenges. One of the primary concerns is the variability in the properties of biological samples, which can lead to inconsistent results during the lyophilization process. Components within the sample, such as salts and proteins, may respond differently to the process, complicating standardization and quality control [34].

Furthermore, the costs associated with lyophilization equipment and consumables can be prohibitive for smaller laboratories or institutions. The intricacies of the process necessitate specialized knowledge, often requiring trained personnel to oversee dehydration [34].

Another significant challenge is the risk of denaturation of sensitive biomolecules during the heating processes involved in lyophilization. While the process is designed to protect biomolecules, certain conditions, such as abrupt temperature changes, can result in irreversible modifications that affect functionality.

Once biological samples have undergone lyophilization, post-lyophilization techniques become pertinent to ensure optimal usability. One immediate post-process step involves proper storage. Lyophilized samples should ideally be kept in a desiccator or vacuum-sealed containers that limit moisture exposure. The temperature at which these samples are stored is also critical; many samples are best preserved at -20 to -80 degrees Celsius to prevent degradation [34].

Rehydration is another crucial aspect, as the method of reconstitution can significantly impact the efficacy of the lyophilized product. It is generally recommended to use buffered solutions that mirror the original hydration conditions of the sample. Proper gradual rehydration minimizes osmotic shock, mitigating risks of damage to sensitive structures.

Further, strategies such as the use of cryoprotectants can be employed during the lyophilization itself to enhance the stability of sensitive biomolecules. Cryoprotectants, such as sucrose or trehalose, can help stabilize proteins during the freezing and drying process, providing an additional layer of protection against damage and loss of activity [35].

In addition to these basic post-lyophilization techniques, advances in formulation science have led to the development of tailored excipients that can improve the stability and activity of specific biological samples. This customization not only allows for better preservation of biological activity but also enhances the rehydration properties of the product [36].

Comparative Analysis of Preservation Methods:

Preservation plays a crucial role in maintaining the integrity of various materials, whether they be food, art, historical artifacts, or biological specimens. The methods used for preservation differ significantly depending on the medium and the desired lifespan of the material in question [37].

Food Preservation Methods

Food preservation is an essential aspect of food science aimed at preventing spoilage and extending shelf life. The primary preservation methods include refrigeration, freezing, canning, drying, and fermentation [37].

1. **Refrigeration and Freezing**:

- **Mechanism**: Refrigeration slows down the growth of microbes by maintaining temperatures typically between 0°C and 5°C. Freezing, on the other hand, halts microbial activity by bringing temperatures below 0°C.
- **Effectiveness**: These methods are highly effective for short- to medium-term storage of perishable items like meats, dairy, and vegetables. However, freezing can alter texture and flavor in some foods.
- **Advantages**: Minimal nutritional loss and retention of sensory qualities. They are also convenient and require little upfront investment.
- **Limitations**: Dependence on electricity may pose risks in areas prone to power outages. Additionally, they do not prevent the growth of microorganisms indefinitely [37].

2. **Canning**:

- **Mechanism**: Canning involves sealing food in airtight containers and heating them to kill microorganisms and enzymes that cause spoilage [38].
- **Effectiveness**: It is highly effective for long-term storage and can preserve food for years when done properly.
- **Advantages**: Retains most nutritional value and offers a wide variety of products.
- **Limitations**: The canning process can be labor-intensive and requires proper technique to avoid risks of botulism. It may also alter the texture of certain foods [38].

3. **Drying**:

- **Mechanism**: Drying removes moisture from foods, thereby inhibiting the growth of bacteria and fungi [39].
- **Effectiveness**: Very effective for fruits, vegetables, and meats, drying can extend shelf life significantly.

- **Advantages**: Lightweight and space-saving, dried foods are easy to transport and store. Nutrient preservation is relatively high if done correctly.
- **Limitations**: The drying process may change textures and requires careful monitoring to avoid spoilage [39].

4. **Fermentation**:

- **Mechanism**: Fermentation uses microorganisms to convert sugars and starches into acids or alcohol. This not only preserves food but also enhances flavors [40].
- **Effectiveness**: Highly effective for products like yogurt, sauerkraut, and kimchi.
- **Advantages**: Provides unique flavors and health benefits, such as probiotics.
- **Limitations**: The process requires specific conditions and careful management to prevent spoilage [40].

Cultural Heritage Preservation Methods

Cultural heritage preservation focuses on maintaining the integrity of artifacts, sites, and artistic works. The preservation methods here include climate-controlled storage, restoration techniques, and digital preservation [41].

1. **Climate-Controlled Storage**:

- **Mechanism**: This method involves maintaining specific temperature and humidity levels to protect artifacts from deterioration.
- **Effectiveness**: Highly effective in preventing mold growth and material degradation, particularly for textiles, paintings, and paper-based items.
- **Advantages**: Long-term preservation with minimal intervention.
- **Limitations**: High operational costs associated with maintaining controlled environments can be a barrier [41].

2. **Restoration Techniques**:

- **Mechanism**: Restoration involves repairing and rehabilitating damaged items using specialized techniques and materials [42].
- **Effectiveness**: Can restore functionality and aesthetic value to artifacts, but the original integrity may be compromised.
- **Advantages**: Enables the public to experience historical artifacts in their former glory.
- **Limitations**: Ethical concerns arise regarding alterations and the potential for over-interpretation of historical significance [42].

3. **Digital Preservation**:

- **Mechanism**: Involves creating digital copies of artifacts, which can be stored and accessed electronically [43].
- **Effectiveness**: Useful for preserving intricate details and making items accessible to a broader audience.
- **Advantages**: Minimally invasive and allows for easy sharing and dissemination of cultural knowledge.
- **Limitations**: Potential risks of digital decay and the need for continual updates to technology to ensure long-term access [43].

Biological Specimen Preservation Methods

In biological and medical research, the preservation of specimens is crucial for future study. Common preservation methods include cryopreservation, formaldehyde fixation, and paraffin embedding [44].

1. **Cryopreservation**:

- **Mechanism**: This technique involves cooling biological samples to sub-zero temperatures to halt all cellular activity.
- **Effectiveness**: Highly effective for preserving cells, tissues, and even whole organisms, enabling the long-term storage of genetic material.
- **Advantages**: Long-term viability can be maintained; suitable for a variety of specimen types.
- **Limitations**: Requires sophisticated and costly equipment. Additionally, not all cell types survive the freezing and thawing processes [44].

2. **Formaldehyde Fixation**:

- **Mechanism**: Involves exposing biological samples to formaldehyde to cross-link proteins and prevent decay [45].
- **Effectiveness**: Excellent for histological studies, allowing for long-term storage of tissue samples.
- **Advantages**: Simple to perform and highly effective in preserving cellular structure for microscopic examination.
- **Limitations**: Potentially hazardous exposure to formaldehyde and can alter protein structures, impacting downstream analyses [45].

3. **Paraffin Embedding**:

- **Mechanism**: Biological tissues are dehydrated and embedded in paraffin wax, allowing for thin sectioning and easy handling [46].
- **Effectiveness**: Ideal for histological studies and long-term specimen storage.
- **Advantages**: Allows for high-quality sections suitable for microscopic investigations.
- **Limitations**: The embedding process can impact antigenicity and may hinder some types of analyses [46].

Impact on Specimen Integrity and Viability:

Specimen integrity and viability are critical factors that influence various fields, including biology, environmental science, medicine, and forensic studies. Specimen integrity refers to the maintenance of the structural and compositional aspects of a sample without degradation or alteration, while viability pertains to the ability of living specimens, such as cells or tissues, to survive, reproduce, or function appropriately under specified conditions. Together, these attributes are essential for accurate research outcomes, diagnostics, and conservation efforts. The impact on specimen integrity and viability can be attributed to multiple factors, including environmental conditions, handling procedures, storage practices, and the nature of the specimens themselves [47].

One of the primary environmental factors influencing specimen integrity and viability is temperature. For many biological specimens, such as microbial samples, plant tissues, or animal cells, temperature fluctuations can lead to cellular stress, metabolic disruption, or outright death. For instance, biological samples typically require refrigeration or freezing to slow metabolic rates and enzyme activity. If not adequately controlled, higher temperatures may lead to accelerated degradation of proteins, nucleic acids, and cellular membranes, ultimately affecting the viability of the specimen for subsequent analysis [48].

Another critical environmental factor is pH. The acidity or alkalinity of the surrounding environment plays a significant role in maintaining the integrity of various cellular components. Most biological specimens thrive within a narrow pH range; deviations can lead to denaturation of proteins and destabilization of nucleic acids. For example, the viability of microbial cultures can be substantially reduced when subjected to extreme pH levels, impacting their use in research, industry, or environmental monitoring [49].

Oxygen levels can also affect specimen integrity and viability, particularly for anaerobic organisms or certain types of cells that require specific atmospheric conditions. High oxygen levels can lead to oxidative stress, damaging cellular components like lipids, proteins, and DNA. This is particularly relevant in microbial ecology, where the introduction of oxygen into anaerobic environments can cause significant changes in community structure and function [50].

The process of specimen handling and preparation is vital for maintaining specimen integrity and viability. From the moment a specimen is collected, it is subjected to a range of physical and chemical factors that can influence its condition. For biological samples, aseptic techniques are essential to prevent contamination, which can compromise viability and hinder accurate analysis. Contamination can introduce competing organisms or degradation enzymes, skewing results and leading to misinterpretation of data [51].

In addition to sterile handling, the methods of transportation can greatly affect specimen viability. For example, biological samples like blood, which are sensitive to temperature changes and physical agitation, must be transported in insulated containers that maintain appropriate temperature ranges while minimizing movement. Improper transport can lead to hemolysis, loss of cellular integrity, or even complete sample failure, ultimately affecting diagnostic results and research accuracy [52].

Storage conditions are another key determinant of specimen integrity and viability. Biological specimens have varying requirements for storage temperature, humidity, and light exposure. For instance, tissues must often be stored at low temperatures to preserve their cellular integrity, while others, such as dried herbarium specimens, require controlled humidity to prevent infestation and deterioration [53].

Cryopreservation is a common method employed to maintain the viability of living cells, particularly in the fields of reproductive biology and cellular therapies. This process involves cooling cells to sub-zero temperatures, utilizing cryoprotectants to prevent ice crystal formation that can damage cellular structures. However, the success of cryopreservation is dependent on multiple factors, including the type of cells, cooling rate, and thawing method. Improperly executed cryopreservation can lead to reduced viability and loss of functional activity [53].

Similarly, long-term storage of DNA, RNA, and proteins requires careful consideration of temperature and conditions. For instance, while DNA can be relatively stable in a desiccated state, RNA is more prone to degradation and requires conditions that prevent ribonuclease activity. Effective storage protocols are necessary to ensure that specimens remain viable for future research or analysis [54].

The impact of specimen integrity and viability extends beyond the immediate confines of laboratory settings; it carries significant implications for broader scientific research, medical diagnostics, and environmental conservation efforts. In medical research, issues concerning specimen integrity can lead to inaccurate conclusions regarding disease mechanisms, treatment efficacy, or the development of vaccines. When biological samples such as blood, tissue, or pathogens are compromised, the risk of erroneous results can undermine public health initiatives and therapeutic advancements [54].

In environmental science, the integrity and viability of specimens play a crucial role in biodiversity assessments, conservation strategies, and ecological research. For instance, when investigating aquatic ecosystems, the viability of water samples is essential for assessing microbial diversity and ecological health. Environmental stressors such as pollution or climate change can significantly impact not only the viability of species but also the integrity of collected specimens, influencing conservation decisions and policies [55].

Moreover, forensic studies rely heavily on the integrity of biological evidence, such as blood, saliva, or tissue samples, to establish facts in legal cases. The mishandling or degradation of these specimens can have dire consequences, leading to wrongful convictions or exonerations. Ensuring the proper collection, handling, storage, and analysis of forensic specimens is thus paramount for upholding justice and the rule of law [56].

Future Directions and Innovations in Preservation Techniques:

The preservation of cultural heritage, historical artifacts, and natural resources is a crucial aspect of ensuring that future generations can experience and learn from the past. As society evolves and technology advances, the methods and techniques utilized in preservation are also transforming. With the advent of new materials, digital technologies, sustainable practices, and interdisciplinary approaches, the future directions in preservation techniques promise to enhance, protect, and extend the life of our world's invaluable resources [57].

One of the most significant advancements in preservation techniques is the use of digital technology. Digital preservation encompasses a range of techniques aimed at maintaining digital content over time, ensuring that valuable data is not lost to technological obsolescence. The rise of 3D scanning, for instance, has revolutionized the way museums and archives document and preserve artifacts. This non-invasive technique allows for high-resolution, three-dimensional replicas of cultural objects to be created, enabling broader public access and virtual exhibitions [58].

Additionally, advancements in photographic technology contribute to the preservation of artworks and historical documents. High-dynamic-range photography (HDR) and multispectral imaging allow researchers to capture details invisible to the naked eye and record the state of an object at multiple stages. Combined with artificial intelligence (AI) algorithms, these imaging techniques can also enhance the analysis of deterioration patterns in artworks, facilitating preventive conservation measures before visible damage occurs [59].

The concept of the "digital twin"—a virtual replica of a physical object or environment—offers revolutionary potential in preservation. By creating these precise digital representations of artifacts, it becomes possible to conduct simulations on climate impact, restoration techniques, and conservation strategies without risking damage to the original object. As museums increasingly adopt digital preservation strategies, the potential for immersive experiences through augmented and virtual reality further extends public engagement with history and art [59].

As the world faces pressing environmental challenges, the integration of sustainability into preservation practices has become paramount. Future preservation techniques prioritize eco-friendly materials, processes, and methods to minimize the ecological footprint of conservation work. Sustainable conservation practices incorporate recycled, renewable, and biodegradable materials in the restoration of artworks and artifacts. For instance, cellulose and plant-based adhesives are gaining popularity due to their eco-friendly properties and lower toxicity compared to traditional synthetic options [60].

Furthermore, innovative techniques such as bioremediation are being explored to combat the deterioration of cultural heritage artifacts. This method employs microorganisms to degrade pollutants or unwanted materials in conservation contexts, reducing toxic waste and promoting sustainable outcomes. For example, microbial treatments can be used to clean metal artifacts while also preserving their structural integrity [61].

The preservation of natural resources also follows the sustainability trend, particularly in the context of biodiversity conservation. The use of drones equipped with remote sensing technology to monitor ecosystems, assess wildlife populations, and detect changes in habitat has significantly improved conservation efforts. This innovative approach fosters a proactive stance towards preserving biodiversity, enabling timely reactions to emerging threats like habitat destruction and climate change [61].

The future of preservation techniques is marked by a shift towards interdisciplinary collaboration. Engaging experts across various fields, including the sciences, humanities, engineering, and information technology, enables more holistic and innovative approaches to preservation. The convergence of disciplines fosters a milieu of ideas and methodologies, enhancing problem-solving capabilities [62].

For example, the collaboration between conservators and materials scientists is enabling groundbreaking developments in the analysis and restoration of artworks. By utilizing sophisticated analytical techniques—such as X-ray fluorescence (XRF) and infrared spectroscopy—scientists can identify the original materials used by artists, leading to more accurate restorations. Similarly, interdisciplinary teams are addressing urgent environmental concerns by linking ecologists with cultural heritage experts to develop integrated management plans that safeguard both biodiversity and cultural landscapes [63].

Community engagement and participatory methods also play an essential role in preservation. Local communities often hold valuable knowledge about their cultural heritage that can enhance preservation strategies. By embracing shared stewardship models that involve the public in preservation efforts, organizations can gain insights into traditional practices, foster a sense of ownership, and cultivate a new generation of advocates for cultural and natural preservation [64].

Conclusion:

In conclusion, the effectiveness of preservation techniques for biological specimens is paramount in ensuring the reliability and longevity of research findings across various scientific disciplines. Each method—be it cryopreservation, formalin fixation, or dehydration—offers unique advantages that align with specific research goals and specimen types. While cryopreservation is optimal for maintaining cellular viability and functionality, formalin fixation excels in preserving tissue architecture for histological analysis. Dehydration techniques, though effective for long-term storage, often compromise certain biological properties. As ongoing research evaluates and optimizes these techniques, it is essential to consider a method’s suitability based on the intended application, balancing preservation quality with practical constraints. Future advancements in preservation science hold the potential to mitigate the limitations of existing methods, paving the way for breakthroughs in biological research, diagnostics, and biobanking. Ultimately, a nuanced understanding of these preservation techniques will enable scientists to make informed decisions, enhancing the quality and applicability of biological specimens in their work.

References:

1. Uenishi G, Fujita S, Ohashi G, Kato A, Yamauchi S, Matsuzawa T, et al. Molecular analyses of the intestinal microbiota of chimpanzees in the wild and in captivity. Am J Primatol. 2007;69:367–76. doi: 10.1002/ajp.20351
2. Crawley JAH, Chapman SN, Lummaa V, Lynsdale CL. Testing storage methods of faecal samples for subsequent measurement of helminth egg numbers in the domestic horse. Vet Parasitol. 2016;221:130–3. doi: 10.1016/j.vetpar.2016.03.012
3. Fischer TK, Steinsland H, Valentiner-Branth P. Rotavirus Particles Can Survive Storage in Ambient Tropical Temperatures for More than 2 Months. J Clin Microbiol. 2002;40:4763–4. doi: 10.1128/JCM.40.12.4763-4764.2002
4. Wilke H, Robertson LJ. Preservation of Giardia cysts in stool samples for subsequent PCR analysis. J Microbiol Methods. 2009;78:292–6. doi: 10.1016/j.mimet.2009.06.018
5. Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, et al. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for Schistosoma mansoni and soil-transmitted helminths. PLoS Negl Trop Dis. 2010;4:e754 doi: 10.1371/journal.pntd.0000754
6. Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J, et al. Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of Colorectal Cancer. Clin Cancer Res Off J Am Assoc Cancer Res. 2016. doi: 10.1158/1078-0432.CCR-16-1599
7. Song SJ, Amir A, Metcalf JL, Amato KR, Xu ZZ, Humphrey G, et al. Preservation Methods Differ in Fecal Microbiome Stability, Affecting Suitability for Field Studies. MSystems. 2016;1 doi: 10.1128/mSystems.00021-16
8. Madinga J, Polman K, Kanobana K, van Lieshout L, Brienen E, Praet N, et al. Epidemiology of polyparasitism with Taenia solium, schistosomes and soil-transmitted helminths in the co-endemic village of Malanga, Democratic Republic of Congo. Acta Trop. 2017;171:186–93. doi: 10.1016/j.actatropica.2017.03.019
9. Higgins D, Rohrlach AB, Kaidonis J, Townsend G, Austin JJ. Differential Nuclear and Mitochondrial DNA Preservation in Post-Mortem Teeth with Implications for Forensic and Ancient DNA Studies. PLoS ONE. 2015;10 doi: 10.1371/journal.pone.0126935
10. Papaiakovou M, Pilotte N, Grant JR, Traub RJ, Llewellyn S, McCarthy JS, et al. A novel, species-specific, real-time PCR assay for the detection of the emerging zoonotic parasite Ancylostoma ceylanicum in human stool. PLoS Negl Trop Dis. 2017;11:e0005734 doi: 10.1371/journal.pntd.0005734
11. Utzinger J, Rinaldi L, Lohourignon LK, Rohner F, Zimmermann MB, Tschannen AB, et al. FLOTAC: a new sensitive technique for the diagnosis of hookworm infections in humans. Trans R Soc Trop Med Hyg. 2008;102:84–90. doi: 10.1016/j.trstmh.2007.09.009
12. Kongmuang U, Luk JM, Lindberg AA. Comparison of three stool-processing methods for detection of Salmonella serogroups B, C2, and D by PCR. J Clin Microbiol. 1994;32:3072–4.
13. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of Soil-Transmitted Helminths in the Era of Preventive Chemotherapy: Effect of Multiple Stool Sampling and Use of Different Diagnostic Techniques. PLoS Negl Trop Dis. 2008;2:e331 doi: 10.1371/journal.pntd.0000331.
14. Montgomery TL, Künstner A, Kennedy JJ, Fang Q, Asarian L, Culp-Hill R, D'Alessandro A, Teuscher C, Busch H, Krementsov DN. 2020. Interactions between host genetics and gut microbiota determine susceptibility to CNS autoimmunity. Proc Natl Acad Sci USA 117:27516–27527. doi: 10.1073/pnas.2002817117
15. Wang Y, Zhou Y, Xiao X, Zheng J, Zhou H. 2020. Metaproteomics: a strategy to study the taxonomy and functionality of the gut microbiota. J Proteomics 219:103737. doi: 10.1016/j.jprot.2020.103737
16. Rogers GB, Keating DJ, Young RL, Wong M-L, Licinio J, Wesselingh S. 2016. From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways. Mol Psychiatry 21:738–748. doi: 10.1038/mp.2016.50

17. Theriot CM, Koenigsknecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, Huffnagle GB, Z Li J, Young VB. 2014. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to Clostridium difficile infection. Nat Commun 5:3114. doi: 10.1038/ncomms4114
18. Gentile CL, Weir TL. 2018. The gut microbiota at the intersection of diet and human health. Science 362:776–780. doi: 10.1126/science.aau5812
19. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. 2005. Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 102:11070–11075. doi: 10.1073/pnas.0504978102
20. Somme F, Bäckhed F. 2013. The gut microbiota — masters of host development and physiology. Nat Rev Microbiol 11:227–238. doi: 10.1038/nrmicro2974
21. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. 2014. Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563. doi: 10.1038/nature12820
22. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G. 2009. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci USA 106:3698–3703. doi: 10.1073/pnas.0812874106
23. Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, Noble NA, Unser AB, Daita K, Fisher AR, Sikaroodi M, Gillevet PM. 2014. Altered profile of human gut microbiome is associated with cirrhosis and its complications. J Hepatol 60:940–947. doi: 10.1016/j.jhep.2013.12.019
24. Hooper LV, Littman DR, Macpherson AJ. 2012. Interactions between the microbiota and the immune system. Science 336:1268–1273. doi: 10.1126/science.1223490
25. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031. doi: 10.1038/nature05414
26. Chevalier G, Siopi E, Guenin-Macé L, Pascal M, Laval T, Rifflet A, Boneca IG, Demangel C, Colsch B, Pruvost A, Chu-Van E, Messager A, Leulier F, Lepousez G, Eberl G, Lledo P-M. 2020. Effect of gut microbiota on depressive-like behaviors in mice is mediated by the endocannabinoid system. Nat Commun 11:6363. doi: 10.1038/s41467-020-19931-2
27. Blakeley-Ruiz JA, Erickson AR, Cantarel BL, Xiong W, Adams R, Jansson JK, Fraser CM, Hettich RL. 2019. Metaproteomics reveals persistent and phylum-redundant metabolic functional stability in adult human gut microbiomes of Crohn's remission patients despite temporal variations in microbial taxa, genomes, and proteomes. Microbiome 7:18. doi: 10.1186/s40168
28. Nechvatal JM, Ram JL, Basson MD, Namprachan P, Niec SR, Badsha KZ, et al. Fecal collection, ambient preservation, and DNA extraction for PCR amplification of bacterial and human markers from human feces. J Microbiol Methods. 2008;72:124–32. doi: 10.1016/j.mimet.2007.11.007
29. Gorzelak MA, Gill SK, Tasnim N, Ahmadi-Vand Z, Jay M, Gibson DL. Methods for Improving Human Gut Microbiome Data by Reducing Variability through Sample Processing and Storage of Stool. PLoS ONE. 2015;10. doi: 10.1371/journal.pone.0134802
30. Deer DM, Lampel KA, González-Escalona N. A versatile internal control for use as DNA in real-time PCR and as RNA in real-time reverse transcription PCR assays. Lett Appl Microbiol. 2010;50:366–72. doi: 10.1111/j.1472-765X.2010.02804.x
31. Meurs L, Polderman AM, Vinkeles Melchers NVS, Brienen EAT, Verweij JJ, Groosjohan B, et al. Diagnosing Polyparasitism in a High-Prevalence Setting in Beira, Mozambique: Detection of Intestinal Parasites in Fecal Samples by Microscopy and Real-Time PCR. PLoS Negl Trop Dis. 2017;11:e0005310. doi: 10.1371/journal.pntd.0005310
32. King JR, Porter SD. Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae). Insectes Sociaux. 2004;51:197–202. doi: 10.1007/s00040-003-0709-x
33. Choo JM, Leong LEX, Rogers GB. Sample storage conditions significantly influence faecal microbiome profiles. Sci Rep. 2015;5:16350. doi: 10.1038/srep16350
34. Ricalde RHS, Lean LJ. The effect of tropical ambient temperature on productive performance and grazing behavior of sows

kept in an outdoor system. Livest Res Rural Dev. 2000;12.
35. Verweij JJ, Mulder B, Poell B, van Middelkoop D, Brienen EAT, van Lieshout L. Real-time PCR for the detection of Dientamoeba fragilis in fecal samples. Mol Cell Probes. 2007;21:400–4. doi: 10.1016/j.mcp.2007.05.006
36. Nakamura N, Amato KR, Garber P, Estrada A, Mackie RI, Gaskins HR. Analysis of the hydrogenotrophic microbiota of wild and captive black howler monkeys (Alouatta pigra) in palenque national park, Mexico. Am J Primatol. 2011;73:909–19. doi: 10.1002/ajp.20961
37. Kaisar MMM, Brienen EAT, Djuardi Y, Sartono E, Yazdanbakhsh M, Verweij JJ, et al. Improved diagnosis of Trichuris trichiura by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites. Parasitology. 2017;144:965–74. doi: 10.1017/S0031182017000129
38. Drag M, Höglund J, Nejsum P, Thamsborg SM, Enemark HL. The level of embryonation influences detection of Ostertagia ostertagi eggs by semi-quantitative PCR. Parasit Vectors. 2016;9. doi: 10.1186/s13071-016-1657-4
39. Brooker S, Kabatereine NB, Tukahebwa EM, Kazibwe F. Spatial analysis of the distribution of intestinal nematode infections in Uganda. Epidemiol Infect. 2004;132:1065–71. doi: 10.1017/S0950268804001691
40. Wickham H. ggplot2—Elegant Graphics for Data Analysis. Springer-Verlag, New York; 2009.
41. Faraway JJ. Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models. CRC Press; 2016.
42. Verweij JJ, Brienen EAT, Ziem J, Yelifari L, Polderman AM, Van Lieshout L. Simultaneous detection and quantification of Ancylostoma duodenale, Necator americanus, and Oesophagostomum bifurcum in fecal samples using multiplex real-time PCR. Am J Trop Med Hyg. 2007;77:685–90.
43. Reck M, Tomasch J, Deng Z, Jarek M, Husemann P, Wagner-Döbler I. Stool metatranscriptomics: A technical guideline for mRNA stabilisation and isolation. BMC Genomics. 2015;16. doi: 10.1186/s12864-015-1694-y
44. Yu YJ, Majumdar APN, Nechvatal JM, Ram JL, Basson MD, Heilbrun LK, et al. Exfoliated cells in stool: a source for reverse transcription-PCR-based analysis of biomarkers of gastrointestinal cancer. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2008;17:455–8. doi: 10.1158/1055-9965.EPI-07-2515
45. Nsubuga AM, Robbins MM, Roeder AD, Morin PA, Boesch C, Vigilant L. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Mol Ecol. 2004;13:2089–94. doi: 10.1111/j.1365-294X.2004.02207.x
46. Kaisar MMM, Brienen EAT, Djuardi Y, Sartono E, Yazdanbakhsh M, Verweij JJ, et al. Improved diagnosis of Trichuris trichiura by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites. Parasitology.
47. Gouveia D, Pible O, Culotta K, Jouffret V, Geffard O, Chaumot A, Degli-Esposti D, Armengaud J. 2020. Combining proteogenomics and metaproteomics for deep taxonomic and functional characterization of microbiomes from a non-sequenced host. NPJ Biofilms Microbiomes 6:23. doi: 10.1038/s41522-020-0133-2.
48. Zhang X, Chen W, Ning Z, Mayne J, Mack D, Stintzi A, Tian R, Figeys D. 2017. Deep metaproteomics approach for the study of human microbiomes. Anal Chem 89:9407–9415. doi: 10.1021/acs.analchem.7b02224.
49. Salvato F, Hettich RL, Kleiner M. 2021. Five key aspects of metaproteomics as a tool to understand functional interactions in host-associated microbiomes. PLoS Pathog 17:e1009245. doi: 10.1371/journal.ppat.1009245.
50. Long S, Yang Y, Shen C, Wang Y, Deng A, Qin Q, Qiao L. 2020. Metaproteomics characterizes human gut microbiome function in colorectal cancer. NPJ Biofilms Microbiomes 6:14. doi: 10.1038/s41522-020-0123-4.
51. Kleiner M, Dong X, Hinzke T, Wippler J, Thorson E, Mayer B, Strous M. 2018. Metaproteomics method to determine carbon sources and assimilation pathways

of species in microbial communities. Proc Natl Acad Sci USA 115:E5576–E5584. doi: 10.1073/pnas.1722325115.
52. Tanca A, Palomba A, Fraumene C, Pagnozzi D, Manghina V, Deligios M, Muth T, Rapp E, Martens L, Addis MF, Uzzau S. 2016. The impact of sequence database choice on metaproteomic results in gut microbiota studies. Microbiome 4:51. doi: 10.1186/s40168-016-0196-8.
53. Zhang X, Deeke SA, Ning Z, Starr AE, Butcher J, Li J, Mayne J, Cheng K, Liao B, Li L, Singleton R, Mack D, Stintzi A, Figeys D. 2018. Metaproteomics reveals associations between microbiome and intestinal extracellular vesicle proteins in pediatric inflammatory bowel disease. Nat Commun 9:2873. doi: 10.1038/s41467-018-05357-4.
54. Hettich RL, Pan C, Chourey K, Giannone RJ. 2013. Metaproteomics: harnessing the power of high performance mass spectrometry to identify the suite of proteins that control metabolic activities in microbial communities. Anal Chem 85:4203–4214. doi: 10.1021/ac303053e.
55. Justice NB, Li Z, Wang Y, Spaudling SE, Mosier AC, Hettich RL, Pan C, Banfield JF. 2014. 15N- and 2H-proteomic stable isotope probing links nitrogen flow to archaeal heterotrophic activity. Environ Microbiol 16:3224–3237. doi: 10.1111/1462-2920.12488.
56. Pible O, Allain F, Jouffret V, Culotta K, Miotello G, Armengaud J. 2020. Estimating relative biomasses of organisms in microbiota using “phylopeptidomics.” Microbiome 8:30. doi: 10.1186/s40168-020-00797-x.
57. Kleiner M, Kouris A, Jensen M, Liu Y, McCalder J, Strous M. 2021. Ultra-sensitive protein-SIP to quantify activity and substrate uptake in microbiomes with stable isotopes. bioRxiv. doi: 10.1101/2021.03.29.437612.
58. Starr AE, Deeke SA, Li L, Zhang X, Daoud R, Ryan J, Ning Z, Cheng K, Nguyen LVH, Abou-Samra E, Lavallée-Adam M, Figeys D. 2018. Proteomic and metaproteomic approaches to understand host-microbe interactions. Anal Chem 90:86–109. doi: 10.1021/acs.analchem.7b04340.
59. Hale VL, Tan CL, Knight R, Amato KR. 2015. Effect of preservation method on spider monkey (Ateles geoffroyi) fecal microbiota over 8 weeks. J Microbiol Methods 113:16–26. doi: 10.1016/j.mimet.2015.03.021.
60. Tap J, Cools-Portier S, Pavan S, Druesne A, Öhman L, Törnblom H, Simren M, Derrien M. 2019. Effects of the long-term storage of human fecal microbiota samples collected in RNAlater. Sci Rep 9:601. doi: 10.1038/s41598-018-36953-5.
61. Carruthers LV, Moses A, Adriko M, Faust CL, Tukahebwa EM, Hall LJ, Ranford-Cartwright LC, Lamberton PHL. 2019. The impact of storage conditions on human stool 16S rRNA microbiome composition and diversity. PeerJ 7:e8133. doi: 10.7717/peerj.8133.
62. Kim D, Hofstaedter CE, Zhao C, Mattei L, Tanes C, Clarke E, Lauder A, Sherrill-Mix S, Chehoud C, Kelsen J, Conrad M, Collman RG, Baldassano R, Bushman FD, Bittinger K. 2017. Optimizing methods and dodging pitfalls in microbiome research. Microbiome 5:52. doi: 10.1186/s40168-017-0267-5.
63. Blekhman R, Tang K, Archie EA, Barreiro LB, Johnson ZP, Wilson ME, Kohn J, Yuan ML, Gesquiere L, Grieneisen LE, Tung J. 2016. Common methods for fecal sample storage in field studies yield consistent signatures of individual identity in microbiome sequencing data. Sci Rep 6:31519. doi: 10.1038/srep31519.
64. Jordan S, Baker B, Dunn A, Edwards S, Ferranti E, Mutic AD, Yang I, Rodriguez J. 2017. Maternal–child microbiome: specimen collection, storage and implications for research and practice. Nurs Res 66:175–183. doi: 10.1097/NNR.0000000000000201.