**Notes from the Cloth Makers Foundation Expert Workshop on Benchmark Standards for the Preservation on Wet Collections**

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**1.0 Overview**

The purposes of this project are (1) to review established standards for the preservation of natural science and anatomical samples stored as wet (fluid preserved) specimens; (2) to establish baseline standards for the storage of these materials; and (3) to outline a training syllabus for a best practices training course on the care of wet collections.

The unavailability of appropriate expertise, materials, and chemicals in many geographic areas should be considered when establishing baseline standards for preservation and collections care.

Best practices and baseline standards for preservation of wet collections as presented in this document will continue to evolve as more information is accumulated to help understand the range of current and future materials and techniques. The gathering of additional information for the further development of these standards is encouraged.

Bronze, silver, and gold standards for collection preservation have been established to accommodate the range of institutions which maintain wet collections. The different levels are applicable to various sizes and types of collections with differing levels of support and collection use. For many materials, we do not yet know the best methods for preserving samples, and that for some modern research techniques and associated samples, best practises have yet to be developed. In both cases research must be undertaken to understand the long term stability issues, especially for the preservation of DNA and tissue collections stored in fluids.

This project was focused on the development and understanding of terminology and processes—in particular, the chemistry of fixation and preservation and the control of deterioration mechanisms in wet collections. One of our concerns is that many of the methods recommended for preservation and fixation are intended for the preparation of specimens for ease of research rather than for long-term storage (e.g., insects in small vials with insufficient fluid volume to provide stable storage). In this report, it is recognised that the process of fluid preservation starts at the point of specimen collection in the field and it is important that field procedures and processes be fully recorded. In particular, it is essential that the time and method of fixation be recorded, and that it is understood that fixation in the field is not preservation.

**Best Practice Recommendation**: *Dry collections and wet collections should not be stored in the same room due to their different storage environment requirements, shelving configuration, health and safety requirements, and because of potential risks from chemical spills*.

**2.0 Standards and Best Practices**

Three standards have been established for maintaining fluid preserved collections. All three standards are designed to maximize the useful life of the specimens depending on how the collection is used and available collection care resources.

* ***Gold Standard***―Standards and best practises for well-resourced institutions with heavily used collections. Larger gold standard collections will have good collection storage environmental controls; sufficient collection care staff to check all specimen containers at least twice a year; uses high quality glass or stainless steel containers with good, well-sealing closures; and has the means to assess and adjust preservative quality (e.g., a digital density meter). Smaller gold standard collections might use containers and closures of lower quality materials in conjunction with a more rigorous periodic monitoring of container condition.
* ***Silver Standard***―The silver standard is designed for institutions with somewhat limited resources and moderate collection use that have sufficient collections care staff to check each container on an annual basis.
* ***Bronze Standard***―The bronze standard is designed for institutions with no dedicated collections care staff, very limited resources, and collections that receive minimal use. This standard is directed towards providing basic specimen stability over long periods of time for collections with minimal monitoring by emphasizing the quality of the container seals and a stable, dark storage environment.

Workers in institutions striving to meet gold, silver, or bronze standards should follow the relevant national and international codes of conservation practice and collection care (e.g., AIC, ICOM, SPNHC).

**Best Practice Recommendation**: *Institutions housing wet preserved collections should strive to meet gold, silver, or bronze, or standards in collections care. The appropriate standard for an institution will depend on available collection care resources and collection use.*

**3.0 Collection and Specimen Useful Life**

The useful life of specimens is defined as the period of time during which the specimen can be used for its intended purpose without significant loss of data. Specimens in well-sealed containers that are housed in stable, cool storage conditions should be expected to have a minimum useful life of several decades to one hundred or more years. There are a large number of variables that affect the useful life of fluid preserved specimens. Some specimens will have a very short useful life no matter what is done to try and preserve them due, to improper fixation or poor initial specimen condition. Much depends on what uses the specimens were prepared for and how the specimens are handled (e.g., specimen preparation may be for taxonomic research, destructive sampling, teaching, or exhibition; specimens may be accessed often, rarely, or not at all; specimens may be dehydrated when handled). It is important to establish the intervals at which specimens will need to be checked and what indicators should be monitored (e.g., changes in fluid level, colour, or turbidity may be warning signs of preservation problems). An indicator check-list has been integrated into the standard maintenance routine for fluid preserved collections presented below.

**Best Practice Recommendation**: *To prolong the useful life of wet specimens, regular checks and monitoring of collections should be part of collections care practice.*

**4.0 Specimens and Samples**

This project covers the wet preservation of biological and anatomical specimens, and will be cross-referenced with the botanical standards. This project addresses tissue standards but will not directly make recommendations concerning the care of samples in buffer solutions intended for genetic research (see ISBER Best practices for repositories, 2012).

**5.0 Alternative Storage Media**

There are several proprietary solutions on the market promoted for use as safer alternatives for fixation or as wet specimen holding solutions, most based on glycol or glyoxal (a dialdehyde). We have observed that in practice, some of these proprietary solutions are used for wet collection storage or exhibition rather than as temporary holding solutions, and several have been used for fixation or preservation despite the lack of knowledge of what chemicals they contain or how they might react with tissues in the long term. Although there is a need for viable alternatives to formaldehyde fixation (due to the safety concerns and regulatory restrictions associated with formaldehyde) and safer, particularly non-flammable long-term preservative solutions, they cannot be proprietary compounds and must be subjected to adequate independent testing.

In general, it is important to identify which chemicals cause problems with long-term preservation so they can be avoided, and which chemicals have been demonstrated to cause the least problems.

**Best Practice Recommendation**: *Do not use proprietary chemical mixtures for fixation, preservation, holding, or exhibition of wet preserved collections unless the component chemicals are known and their effects on specimens are understood.*

**6.0 Documentation and Data Sets**

It is essential that complete documentation be compiled from collectors and researchers concerning how they process their samples to help establish best practices in specimen preservation and documentation both in the field and in the lab. Because there are a large number of procedures and chemicals used to fix and preserve specimens for specific research techniques, researchers are encouraged to report their methodologies and to record them as part of their published work. Documentation should begin with specimen collection and euthanizing, and continuing through fixation and preservation.

**Best Practice Recommendation**: *A standard field collection data sheet should be designed for collectors and researchers so that all pertinent information can be simply and easily recorded.*

**7.0 Key Data**

Traditionally, fluid preserved samples have been handled on an individual basis, depending on the collector or researcher; the particular techniques and chemicals used in fixation and preservation were rarely recorded. A number of different chemicals have been used in fluid preservation practice since it began in the mid-1600s (Appendix I).

Our goal is to promote techniques that preserve samples in ways that maximise the amount of data that can be obtained from the specimens and preserve them for the maximum length of time possible. For example, as recently as 20 years ago the need to preserve specimens in ways that allowed DNA extraction was not necessarily recognised because at the time it was difficult to extract from fluid preserved specimens. However, new and ever-improving techniques mean that collections have become more valuable as DNA sources. Hence, as demands for better data increase and the original intention and usage of a collection evolves, the importance of better, standardized preservation and stabilisation techniques increases.

The type of specimen may dictate the most appropriate preserving media for preservation of gross morphology, which in turn would affect which components could later be extracted from the preserved specimens. However, the preservation of gross morphology may be at odds with the best methods of DNA preservation (e.g., fixation with formaldehyde and exposure of tissues to ultraviolet light during initial preservation degrade DNA quality). We should encourage preparation techniques that preserve the specimen as near to its original condition (of collection or entry in the institution) as possible while maximizing scientific utility of the specimen (e.g., tissues for later DNA extraction should be removed from the specimen prior to fixation and preserved separately).

**Best Practice Recommendation**: *Prepare specimens using techniques and chemicals that preserve samples to maximise the amount of data that can be obtained from the specimens and prolong their useful life.*

**8.0 Fixation Techniques―How Should We Preserve Specimens?**

The fixation and preservation of a specimen strongly influences its future use and availability of data sets, therefore it is important to determine whether the specimen is to be preserved as a research resource or for another purpose.

Because some specimens may never be used for research it is suggested that baseline standards for the storage and maintenance of collections be based in part on their intended use. As collection managers we should consider the stability of the specimen as our main interest in the long-term preservation of collections. However, it should be noted that many collections were inherited by the institutions that house them (rather than being processed by in-house workers), and as such have already gone through treatment processes which may limit their future use. Prior treatment process should be documented if known. Staff managing any wet collection storage or fixation process should ensure that they preserve as many features of the specimens as possible. In the past this has often meant a trade-off between preserving anatomical features versus preserving samples more useful in other research.

In general, specimens of value are obtained through two routes―either they are collected and preserved as research samples with the researcher’s particular interest in mind (which may range from taxonomy to anatomy to vouchers collected as part of monitoring programmes), or they are identified at a later date of having potential interest or research value. In all cases, good preservation practices and documentation will enhance the value of the collection.

There is often a discrepancy between the preservation method used and the intended purpose of the sample, which means that the decision making process must be clearly understood and recorded. For a decision making strategy please see http://www.icom-cc.org/54/document/decision-making-model/?id=268#.UptPt42Fe8w

**Best Practice Recommendation**: *Intended use of the specimen should determine the best method for fixation and preservation.*

**8.1 Storage Media**

The storage media is defined as the fluid in which the specimens are stored. A comprehensive list of storage media that have been used to preserve wet collections can be found in Appendix II. The core chemicals most often used in wet collections include:

* Monohydric alcohols (e.g., ethanol, isopropanol)
* Polyhydric alcohols (e.g., glycerine, glycol)
* Aldehydes (e.g., formaldehyde, glutarldehyde)
* Oils (e.g., mineral oil)
* Aromatic solvents (e.g., turpentine, benzoates used for anatomical transparencies)
* Acids (either as a weak acid solution or as an additive such as glacial acetic acid)
* Proprietary fixatives and preservatives (often glycol-based compounds)

**8.2 Definitions**

* Preservation v Fixation—preservation generally refers to the prevention of post-mortem changes in tissues; fixation generally refers to chemical processes employed to emphasize certain morphological or biochemical features of a specimen.
* Conservation—long-term preservation of preserved specimens and objects in stable condition to prevent deterioration or damage from physical and chemical factors
* Preservation—long-term maintenance of preserved specimens and objects
* Preservatives—chemical agents used to preserve specimens
* Fixation—defined in various ways depending on purpose of fixed specimens. See section 8.2.3 below.
* Fixatives—chemical agents used to fix specimens.
* Storage—the long-term maintenance of specimens in an environment designed to prolong their useful life.
* Fluid preservatives—see discussion in section 8.2.2 below.
* Additives—chemicals added to standard fixatives or preservatives to achieve some particular fixation or preservation goal (e.g., salts, cobalt nitrate, antifungal and antibacterial agents, etc.).
* Contaminants—chemical components not intentionally added to fixatives or preservatives that may negatively affect the quality of the fixative or preservative fluid.
* Reference numbers for standard chemical definitions may be found in Appendix I.
* Denaturing agents—chemicals added to alcohols to render them unfit for human consumption.

More precise definitions and further discussion of some of these terms are provided in the sections below.

**8.2.1 Preservation v fixation**

Preservation generally refers to the maintenance of a specimen in a stable, usable condition. Fixation generally refers to the process of preventing post mortem changes in a specimen. However, both preservation and fixation may be defined in other ways, as discussed below.

**8.2.2 Preservation**

Preservation is the conservation and maintenance of an object in a usable condition by reducing the rate of deterioration to a minimum, thereby minimizing the amount of change it undergoes, or more simply, the prevention of post-mortem changes in tissue.

**8.2.3 Fluid preservation**

Fluid preservation is the preservation of an item in a fluid medium which:

* Prevents internal enzymatic attack (autolysis).
* Prevents internal and external microbial attack.
* Achieves long-term maintenance of a specimen in as near a natural state as possible.
* Provides structural support to the specimen to preserve its three dimensional structure.
* Provides a suitable osmotic pressure for the specimen.

**8.2.4 Fixation**

* Fixation is the arrest of post-mortem changes to stabilize metastable tissues by halting the autolytic process. In other words, fixation is the stabilization of tissue to stop autolysis.
* Fixation is used to stop the internal processes of deterioration. Fixation begins the initial stage of preservation by precipitating or chemically combining cellular proteins to prevent decay autolysis or osmotic collapse.
* Fixation is the chemical or other stabilization of protein in tissue to stop the process of autolysis of the tissue matrix by the exchange of ions from formaldehyde and by electrophilic attack on the amino- and imino[[1]](#footnote-1)-radicals of proteins.
* Fixation is the conservation of biological materials histological and/or cytological study by denaturing lipids and proteins to such an extent that their structure is stabilized and enzymatic degradation (autolysis) is prevented.
* An alcohol can be considered a fixative because alcohols change the protein structure and promote dehydration of tissues. Alcohol was used as a standard fixative until the 1890s, but is now generally considered to be a pseudo-fixative (*sensu* Stoddard, who made a distinction between pseudo-fixatives and the chemical changes wrought by aldehydes).
* Although 95% ethyl alcohol does ‘fix’ DNA and is suitable for long-term storage, aldehydes in general will denature DNA.

"Fixation" of the DNA helix would be mediated with formaldehyde molecules or other aldehydes interfering with the DNA helix and forming covalent bonds, thus stabilising the secondary structure of the DNA helix and the coiling of the DNA helix (tertiary structure). Formaldehyde induces protein DNA and protein-protein cross linkage.

**8.2.4 Fixatives**

Fixatives are most typically aldehydes, but also include some heavy metal fixatives such as mercuric chloride and osmium tetroxide and pseudo-fixatives such as alcohols (see fixation chart in Appendix III).

**8.2.5 Denaturants**

Denaturants are additives that make a fluid non-consumable for humans. Common denaturants include acetone, ammonia, benzene, boric acid, camphor, coal tar, denetonum benzoate, formaldehyde, gasoline, hydrochloric acid, methanol, methyl isobutyl ketone, naptha, phenol, red mercuric iodide, salicylic acid, thymol, and toluene. Some denaturants are known to have adverse effects on either preserved specimens, containers, or both (e.g., MEK, also known as methylethyl ketone).

**Best Practice Recommendation**: *Do not use denatured alcohol for preserving specimens unless non-denatured alcohol is not available.*

**9.0 Collecting Techniques**

Clear documentation of chemicals used to capture and prepare living specimens and to treat post-mortem specimens should be compiled. Typical stages include:

* Capture
* Euthanization and relaxation
* Fixation
* Preservation
* Storage

The handling and euthanization of live animals should conform to appropriate regulations and guidelines provided in national codes of practice (e.g., AVMA Guidelines for the Euthanasia of Animals: 2013 Edition). Some standard techniques are no longer considered to be appropriate or ethical (e.g., the use of clove oil or rotenone is no longer considered ethical for organisms that can feel pain).

The standards should provide clear documentation of the processes and materials used to obtain, euthanize, and preserve specimens. Collectors should follow appropriate ethical guidelines (e.g., it would be unethical to preserve specimens in a way that would make them unsuited for scientific investigation).

It is essential that collectors and museum workers understand proper fixation techniques for the specimens they are collecting and the methods for preservation of the specimens. Specimens should be documented by collecting broad data sets. Although the chemicals used to carry out euthanasia are outside scope of this project, in some instances the chemicals used to relax and/or euthanize the specimens are also essential components of specimen preservation or may otherwise affect specimen preservation quality. In the future it will be necessary to look at collection techniques and to state guidelines for collecting materials so that researchers may follow them. Common euthanizing agents may also interfere with the preservation fluid

**Best Practice Recommendation**: *Collecting and preserving procedures should be well documented and follow appropriate ethical guidelines.*

**9.1 Ethics**

Appropriate ethical guidelines, as defined by international and national organizations, should always be followed for collecting, euthanizing, and preserving specimens. For example, it would be unethical to preserve specimens in a way that would make them useless for the purpose for which they are intended. For this reason, it is essential that collectors consider and plan what they plan to do, particularly by defining the intention and scope of their sampling and methodology before killing specimens of any animals and plants.

In general, ethical standards should set a standard of behaviour that is above the law (and as collections care workers, we have an ethical duty to work with appropriate agencies to make the laws work better on behalf of our profession).

It is essential that collectors and museum specialists understand their ethical obligation to house specimens in the best storage conditions possible.

Ethical issues to be considered include:

* Access and Benefit Sharing (ABS) (http://www.cbd.int/abs/) with particular reference to new and proprietary chemicals.
* Data Preservation and anonymity.
* Regulatory bodies (including national bodies).
* Animal Care Committee approval (USA) for collecting and euthanizing specimens.
* Repatriation
* Conservation related ethics. These include (in the USA) the American Alliance of Conservation and (worldwide) ICON, ECO, ICOM, and ECHRISO. Researchers who work outside these professional groups would not be recognised as using ethical standards.

**9.2 Mechanisms of Antibacterial Action**

* Antibacterial refers to a chemical agent that kills or inhibits the growth of bacteria.
* Definition of other terms?
* Table that describes for a certain group of fixatives and preservatives their action and their different damage to the specimen.

**9.3 Typical Storage Fluids**

* Ethanol
* IMS (Industrial methylated spirits)
* Formalin (dilute aqueous formaldehyde solution)
* Glycerol (glycerin, glycerine)
* Glycols (e.g. propylene glycol and polyethylene glycol)
* Oils

**9.4 Buffers and Stabilising Agents**

Buffers are used to control pH variation, particularly in fixatives. The most commonly used buffers are salts or dilute acids.

**9.5 Holding Fluids**

Holding fluids are used for the temporary housing of specimens when the use of standard preservatives are inappropriate or not allowed by regulations (e.g., exhibition, as regulations often preclude using alcohol or formaldehyde in exhibit situations). Typical holding fluids include:

* Glycol-based compounds (most proprietary)
* Phenoxetol mixtures
* Oils

3M Corporation’s Novec (an engineered fluid) has been promoted as a holding fluid for specimen exhibition, but due to its expense and its effect on specimens, it has been only rarely used.

1. **Fixation Methods**

A comprehensive list of fixatives is provided in Appendix III.

Standard practice for fixation is a solution of one part aqueous formaldehyde (full strength) and nine parts water. Although this is commonly referred to as “10% formalin,” in fact it is actually 4% formaldehyde in water.

Rather than fix specimens in a formaldehyde solution, they may be preserved directly in 70% ethanol or IMS (at 70%, ethanol is an effective biocide; above this percentage it will dehydrate the sample and below and will not be an effective biocide). At warmer temperatures, fixation and preservation proceed more rapidly than at cooler temperatures (as does specimen deterioration). Many workers have reported that formaldehyde and alcohol purchased in developing countries is often significantly diluted even though it is sold as full strength; when using diluted formaldehyde, more may be required for fixation.

Glutaraldehyde is a good fixative for small specimens as it works fast but it doesn’t penetrate far, but is unacceptable for tissue blocks larger than about 1 cc in volume.

**Best Practice Recommendation**: *Handle chemicals appropriately (e.g., do not leave containers open for prolonged periods of time) and use chemicals used are of appropriate quality*.

**Best Practice Recommendation**: *Stage specimens up or down a concentration ladder when moving them between fluid media of differing concentrations*.

* 1. **Selecting the Best Fixation Process**

There are a variety of fixatives available for preserving biological material. The selection of the fixative should be based on the intended use of the specimen, the efficacy of the fixative, and whether or not appropriate health and safety standards can be met in its use.

In practice, the two main types of fixatives are aldehydes and alcohols; it is important to note that the fixative actions of these two types of chemicals are significantly different. In general, different chemical fixatives vary widely in their mechanism of action, penetration rate, ability to penetrate tissues, and the resulting morphological state of the fixed specimen. Fixative action may be affected by temperature, chemical concentration and volume, and the condition of the specimen (e.g., recently euthanized or not). Aldehyde-based fixatives cause degradation of DNA.

Perfusion of the fixative into the tissues by injection through the specimens circulatory system is the most efficient method for fixation of tissues. In practice, however, perfusion is rarely used in the field due to the time and equipment needed and because it requires cutting into the specimen to access the blood vessels and the loss of blood from the specimen. In field practice, smaller organisms are submerged in fixative solutions; larger specimens are injected with fixative solutions. Historically, before the availability of reliable syringes, cuts were made through the specimens to allow the penetration of fixatives.

**Best Practice Recommendation**: *Fix and preserve specimens in a variety of ways with a variety of chemicals to maximize the usefulness of the specimens in the future.*

Require guidance on this? (E.g. How will you get from a fish to the correct fixative[[2]](#footnote-2). Mind map will come out with deterioration and processes of fixation. Concise document (with minimal chemistry) methods mind map ends up with potential issues and leads to do’s and dont’s)

Two concepts - separate chemicals and physical treatments making recommendations and defining mechanisms for the process of fixation

1. freeze
2. Preserve with alcohol, or
3. fix with aldehyde
4. **Storage Media**

Although widely used in collections worldwide, ethyl alcohol may not be the best storage fluid for some collections. Such factors as the history of the specimens, how they were fixed and preserved, and what chemicals are readily available locally are all important factors to consider. It is particularly important that historic specimens not be transferred to new preservatives without compelling reasons for doing so, as transfer destroys the historical integrity of the specimens and risks damaging them.

In general, unless there is good reason to do something with a specimen then it should be left alone, particularly if the “topping up“ history is unknown or unclear (e.g, which fluids have been used to top up the container during the lifetime of the specimen). If a change of preservative is mandatory, then the change should be documented. Some institutions have made changes in the name of health and safety that have the result of risking both specimen damage and posing greater safety issues. For example moving specimens from formaldehyde into alcohol solutions without thorough soaking in water and moving specimens through a concentration ladder will result in trace amounts of formaldehyde in the final alcohol preservative and risk severe dehydration of the specimens; moving specimens to denatured alcohol risks damaging specimens as most denaturants are unknown and many react adversely with specimens or containers.

**11.1 Storage Media in Historic Collections**

Historic collections often have unexpected research value (e.g., they may be used for stable isotope research or as sources of DNA). In certain instances, historic collections are made available for educational use, but before such a decision is made, a complete and thorough investigation of the origin and history of the specimen and the container must be made to be sure that they are not of significant historic importance or linked to a significant historic event, research project, museum, or preparatory. In assessing the value of historic collections, consult the decision-making model found at: http://www.icom-cc.org/54/document/decision-making-model/?id=268#.UptPt42Fe8w

**Best Practice Recommendation**: *When assessing historic collections, managers should whether or not the specimen still has an important purpose or function. It should also be noted that we treat specimens now in a different way as aesthetics is often essential for historic specimens.*

**12.0 Recommended Storage Media for Long-Term Preservation**

* 70% ethanol (isopropyl is not recommended because it is unstable and forms very reactive peroxides)
* IMS (Industrial methylated spirits)
* Glycerol (particularly for transparencies or cleared and stained specimens)
* 1,2-propanediol (propylene glycol)
* 5% aqueous formaldehyde

Some works believe that glycerol should be used as a replacement for formalin, particularly in human anatomy collections. However, pending a thorough review of the use of glycerol, several of the workshop participants did not agree with its use.

For preservation of DNA in tissues, it is recommended that long-term storage in ethyl alcohol be avoided (ethanol will denature proteins over time), and that tissues be frozen instead.

**Best Practice Recommendation**: *For preservation of tissue samples intended for DNA extraction, avoid prolonged storage in ethanol; freeze to -80°C.*

**12.1 Glycerine**

Glycerine is prone to mould will growth at relative humidity of 65% and above, particularly at concentrations greater than 50%, and will absorb moisture from the air, often leading to an overfilled container. Specimens in glycerine should be stored below 60% RH to avoid mould growth. Alcohol or formaldehyde may be added to glycerine to control mould and bacterial growth, or an inhibitor such as thymol , camphor, or menthol can be and the container sealed tightly.

**Best Practice Recommendation**: *Specimens stored in glycerine preservative solutions should be maintained at relative humidity of 65% or higher in tightly sealed containers with an appropriate mould inhibiting agent.*

**13.0 Health and Safety**

Personal protective equipment (PPE) should include gloves (matched to the chemicals used), safety glasses or splash goggles, and lab coats. Latex gloves should not be used with fluid fixatives and preservatives as they are permeable to most chemicals; nitrile (neoprene) gloves are generally acceptable. Safety glasses should be work when working with alcohol-based fluids, and splash goggles when working with solutions containing aldehydes or heavy metals. Appropriate local and national regulations and guidelines concerning PPE and lab procedures should always be followed. Most of the laboratory safety supply vendors furnish information to help match glove types to the chemicals used in the lab.

Good ventilation is important when working with wet collections—containers should only be opened when local exhaust (vapour collectors), bench-top vapour collectors, or in a fume cabinets (fume hood) to keep chemicals out of the breathing zone.

Relevant guidelines for the disposal of waste fixative and preservative chemicals should be followed—it is illegal in most areas to dispose of used fixatives or preservatives by pouring them down the drain.

**Best Practice Recommendation**: *When handling wet preserved specimens use appropriate personal protective equipment and vapour collectors.*

**14.0 Specimen Transfer**

Specimens fixed in formalin should be washed and rinsed when they come in from the field, prior to transfer to a preservative. After washing, the specimens should be staged through an appropriate concentration ladder when transferred to storage-strength preservative.

Measurements of pH are problematic in fluid preservatives due to the chemical composition of most preservatives and the extracted chemicals from the specimens. Electrodes become clogged easily (leading to misleading measurements) and indicator papers are difficult to read in deeply stained solutions.

**Best Practice Recommendation**: *Rinse specimens and stage through a concentration ladder when changing fluids; change fluids only when necessary to prolong the useful life of the specimen.*

**14.1 Topping Up**

Topping up is a regular practice in most institutions and is routine staff chore. If the specimen is in an unknown fixative or preservative fluid, it generally should be left alone unless there is a compelling reason to change the fluid (see section 14.0). Containers should be labelled to indicate which fluid is inside. Mitigate loss of fluid by using better containers and closures. Containers that are topped up should be marked to detect those that show excess fluid loss over time. Guidelines for determining when to top up containers and when and how to adjust preservative strength can be found in Notton (2010) and Sendall and Hughes (1996).

**14.2 Process for Specimens in Collections**

When containers have lost preservative, they should be topped up and the preservative strength adjusted if necessary. Topping up delicate specimens may cause osmotic problems. Fluid level in all containers should be checked on a regular basis (at least annually); containers that show fluid loss should be marked (e.g., with a grease pencil or china marker) and monitored to detect problematic seals and closures. If significant loss of fluid has occurred, the strength of the remaining fluid in the container should be brought up to standard preservative strength (see section 14.1).

**14.3 Stepping Up Techniques**

When transferring specimens from an aldehyde-based fixative to an alcohol-based preservative, steps of equal concentration should be used, generally steps of 20% change. Alcohol concentrations of 10% may cause osmotic problems allowing air bubbles to form in specimens, causing them to float.

**14.4 Reasons to Replace Preservative Solutions**

In the past, it was customary in many collections to change discoloured preservative for fresh preservative for cosmetic reasons, or when significant fluid loss had occurred in the container. However, based on what is now known about the extraction of components of the specimens by preservative solvents, as well as the expense of fresh preservative solutions, wholesale fluid replacement is no longer recommended. Reasons for transferring specimens to a new storage fluid might include:

* When removing the specimen from a fixative to a preservative
* When the preservative has become acidified (usually due to instable products in the preservative such as formaldehyde)
* Due to health and safety mandates
* For research purposes or other use for the specimen
* Because the preservative method has been deemed inappropriate
* If extracted lipids cause sufficient acidification to damage bone or other tissues
* If extracted lipids (particularly with oily marine species) may discolour the specimen or make it difficult to handle or use for research purposes

Changes in colour, turbidity, or opacity of a liquid may indicate that a fluid should be changed (opacity is generally a better indicator than colour, e.g., petroleum ether may cause naphtha to form in the fluid). A simple turbidity test will give a relative estimate of lipids in the preservative--pipette a small quantity of the preservative into a petri dish of clean water on as dark background and observe any formation of whitish turbidity.

The formation of paraformaldehyde precipitates or incrustations may indicate that formaldehyde preservative solutions should bechanged.

When old fluids are replaced, if possible a sample of the fluid should be retained for future analysis.

**14.5 Buffers**

Buffers are used to control the pH of fixative and preservative solutions. Buffering is particularly important for formaldehyde-based solutions because formaldehyde degrades readily to formic acid, becoming sufficient acidic to demineralise tissues and cause other specimen damage. On the other hand, solutions that are too basic may cause tissues to clear (become opaque) or some tissues to gelatinise. Most traditionally used buffers are inadequate to provide long-term protection and may cause other problems (e.g., calcium carbonate and magnesium carbonate may form crystalline deposits in muscles and other tissues). The currently recommended buffering systems (e.g., two phosphate salts or sodium beta- glycerophosphate) has a superior life, but will not necessarily provide permanent protection because phosphate salts may precipitate out of solution, particularly when solutions are topped up or fluids are changed.

The desired range for buffering is fairly narrow, minimally between ph 5 and 8, but ideally between pH 6.4 and 7.0.

**14.6 Indicators for ethanol and formaldehyde**

Mould growth and discolouration, container failure (lose seal ) at NHM occurs in a large number of invertebrate specimens stored in Steedmans solution. It was suggested that the topping up regime with water was simpler and that there were greatly reduced problems with evaporation

**14.7 Reasons for changing Steedman’s Solution**

Steedman’s solution was originally proposed as a preservative for zooplankton, but has been used for a variety of organisms. Steedman’s solution consists of 10% propylene glycol, 0.5% propylene phenoxetol, 1% concentrated (40%) formaldehyde, and 88.5% distilled water. While preservation in Steedman’s solution makes some specimens usable for staining, it may cause tissues to swell in others.

**15.0 Transportation**

Movement of an item or items in their storage containers between storage, work or exhibition locations

**15.1 Internal Movement**

It is preferable to move specimens in fluid as little as possible. When containers must be moved, the route should be checked and a risk assessment conducted. Containers should be handled carefully and slowly, avoiding bumps and jolts. Check containers immediately after moving for signs of potential problems (e.g., if greased ground glass stoppers come into contact with sloshing fluids, the containers may lose fluid due to capillary action through the seal). All collection movements and spillages should be audited and documented. Trolleys (carts) should be designed to contain spillage or containers should be placed on trolleys in plastic boxes or trays.

**15.2 Regional and International Transfer[[3]](#footnote-3)**

Specimens should be transported in 70% ethanol; do not use diluted alcohols. Regulations for transportation (on plane) to be reviewed in January 2013. Packaged by @@@@ to allow movement for moving spirit collection.

Road vehicles have to be labelled.

Packages to be shipped by air should be in a double heat-sealed bags; the entire package should contain no more than 1 litre of ethanol with sufficient absorptive material absorb all fluid in the container. If higher quantities of ethanol need to be transported, specimens should be shipped as dangerous goods, with appropriate packing and labelling, meeting IATA/ICAO certified staff packing standards. Regulations must be attached with packaged. Ethanol concentration is 70% in package and therefore goes straight back in 70% in the jar. Allow for 30mls of visible fluid. The specimen and absorbent cloth can be saturated and the fluid is on top of that. Packaging should be emission free materials etc re historic collections protocol container if valuable conservation rules apply. Ensure documentation is in place before sending complying with all necessary local and international regulations.

**16.0 Storage Furniture**

Storage furniture regulations will be dictated to a large extent by local fire regulations. In general, shelving housing containers of fluid preserved specimens should not flex when loaded and should have restraints to prevent containers from falling off when subjected to vibrations (e.g., earthquakes or sudden jolts). Many fire codes do not allow the use of wooden shelving, and may require shelving to be penetrated to allow water from sprinklers to reach all shelves. Although traditionally, wider shelving was selected to maximise the available storage space, from a management point of view a series of narrow shelves (each holding a single row of containers) reduces monitoring time and minimizes handling of specimens.

* Stainless steel shelving is recommended as it will be unaffected by spills.
* Wood shelving provides a better non-slip surface, but may warp in excessive humidity, absorb spilled fluids, and presents a greater fire hazard.
* Epoxy and powder-coated shelves are chemically resistant, but the coating may be scratched, exposing the shelf material to oxidation.
* Composite materials (e.g., Trespa, a high-pressure laminate) could be considered subject to local fire regulations.

Storage systems should allow for easy accessibility, monitoring, inventory, and collection use.

**16.2 Storage Environment**

???

**17.0 Fire Prevention and Control**

The flashpoint of absolute ethanol is 12°C. Most current recommendations for storage of spirit collections are based on this flashpoint, e.g., recommendations to store collections at 12-15°C (55-60°F). However, in this temperature range, specimens brought from storage to a laboratory would warm up and cool down each time they were moved, dissolved lipids and proteins tend to turn the fluid cloudy, trace amounts of formaldehyde may solidify to paraformaldehyde, and the temperature range is expensive to maintain. A compromise temperature range of 17-18°C (about 65°F) lowers flash point but does not cause other problems. It is important to avoid temperature fluctuations, which cause rapid failure of container seals.

Ignition sources in collection storage areas should be eliminated (e.g., use safety switches, non-sparking light fixtures, do not store other materials with the colleciton, use metal shelving, and eliminate electrical outlets).

Wet specimen storage should be equipped with both fire and smoke detectors, and may be equipped with vapour detector systems as well. A fire control system that operates with water, mist, or foam should be in place in the collection storage and laboratory areas.

**18.0 Environmental factors**

A stable, dark, cool storage environment is required to maximize the useful life of fluid preserved specimens.

**18.1 Temperature Recommendations**

Temperature in collection storage should be constant with minimal fluctuations, preferably within the range of17-18°C (about 65°F), and should be a little cooler than the laboratory area in which containers are sealed. Fluctuations in temperature may cause containers or closures to fail due to cracking or loosening of closures.

Keeping fluid collection storage a few degrees cooler than the lab in which the containers are closed will improve the seals on the containers by creating negative pressure inside the containers.

The following factors should be taken into account when establishing storage temperatures for fluid preserved collections:

* At temperatures below about 15°C, alcohols tend to layer (particularly isopropyl), formaldehyde begins to precipitate out as paraformaldehyde (indicated by a whitish cloudiness in the fluid or the formation of paraformaldehyde needles), dissolved lipids will congeal, and some compressible gaskets will lose regain and therefore permit some evaporation.
* At warmer temperatures, the processes of deterioration proceed faster (e.g., extraction of lipids and proteins in alcohol) and evaporation rates are higher.
* Lowering the temperature of collection storage below flash point is a theoretical idea, not a practical one, as there is no evidence that sub-flash point temperatures will really reduce fire danger in a museum storage situation of containers of fluid preserved specimens on shelving in a controlled storage area.  A container of fluid-preserved specimens sitting on a shelf in storage will not catch fire all by itself―an ignition sources is required.  Assuming that the ignition source is a fire in the storage area, then the temperature in the room is not likely to remain below flash point when there is a fire because fire generates heat, therefore spending a lot of money to cool the entire storage area below flash point is pointless.
* Cooler temperatures are much, much more expensive to maintain, and depend on functioning, reliable HVAC equipment.  Cooler temperatures are not a wise use of resources.
* More stable temperatures can be achieved by locating storage areas in interior rooms (with no outside walls or windows) and by setting set points in consideration of the outside air temperature, building type, and HVAC system.  Transitioning slowly between a warmer summer set point and a cooler winter set point in a temperate environment will produce a better storage environment than trying to maintain an arbitrary set point that exceeds the ability of the building, HVAC, and local climate to maintain.

**18.2 Relative Humidity Recommendations**

While not considered an important cause of deterioration, relative humidity greater than 65% will increase the incidence of mould, and may cause a change in preservative concentrations at extreme levels. High and/or fluctuating relative humidity may increase the rate of glass deterioration, particularly for historic glass containers. Non-glass containers will be subject to moisture transfer at a range of moisture levels dependant on humidity. On the other hand, higher levels of relative humidity will reduce the rate of alcohol evaporation from leaking containers. Ideally, relative humidity in the collection storage area will be within the range of 35-55%.

**18.3 Light Recommendations**

There should be no sources of ultraviolet light in collection storage areas and laboratory areas where specimens are used. Work space areas should be lighted individually rather than lighting the entire room brightly. Light levels for wet specimens on exhibit should not exceed

50 lux.

**19.0 Storage Containers**

Standardized containers and closures are more cost-effective in the long-term management of wet collections. It is noted that the availability of jar lids varies greatly from one country to another around the world. In general, lids made from Bakelite or PVC plastics are unacceptable. Flexible polypropylene lids and some polyethylene lids (depending on the thickness of the plastic) work well. Some institutions have found their bests option to be metal lids, which must be closely monitored for signs of oxidation. The best containers and lids are made of borosilicate glass, but these are prohibitively expensive for most collections. Some collections are now using PET (polyethylene tetraphthalate ) containers, but we have yet to see a review of these containers in long-term use.

Liners made of Teflon (PTFE) or polyethylene may improve the quality of the closure.

**19.1 Sealants**

Containers of wet specimens should be sealed with silicone- based greases unless absolutely necessary), and not allow transfer moisture or oxygen into the jar[[4]](#footnote-4).

Silicone is difficult to reverse and make opening of the jars difficult.

Other sealants include gelatine, Stockholm tar and lead sesquioxide (only for ethical/antique situations), paraffin wax, cold bitumen.

**20.0 Monitoring Equipment**

* Visual monitoring for container integrity, fluid level, transparency, colour, efflorescence, and precipitates on an annual basis.
* Digital density meter for strength of alcohol (gold standard).
* Alcomon Indicator System
* pH indicator strips papers (the pH obtained from indicator strips is usually about 1 step off, but indicator strips are useful for rapid, relative measures of pH).
* Data loggers for temperature, humidity, and light intensity.
* Hydrometers for fluid density, kept clean and used with temperature correction.
* Digital density meters, e.g. Mettler Toledo or Anton Paar DMA 35 (these may be damaged by exposure to MEK or other denaturants)
* Solvent mixtures and aldehyde vapours systems???

**21.0 Deterioration Factors**

* Direct physical forces
	+ Oxygen permeation (integrity of container seal or container)
	+ Acidity or alkalinity of preservative fluids
	+ Chemical degradation
	+ Container failure
* Thieves, vandals, and displacers
	+ Improper handling
	+ Failure to maintain storage array in order
* Dissociation
	+ Loss of specimen tags
	+ Loss of labels
	+ Loss of association between specimens and information
* Fire
	+ Loss due to burning
	+ Heat damage
	+ Smoke damage
* Water
	+ Dehydration
	+ Shrinkage due to dehydration
	+ Cell collapse due to osmotic damage
* Pests
	+ Bacteria
	+ Mould
	+ Pests that damage labels and documentation
* Contaminants
	+ Denaturants
	+ Particulate pollution
	+ Formation of unwanted precipitates
* Radiation
	+ Ultra violet
	+ Visible
	+ Infrared
* Inappropriate temperature
	+ Too high
	+ Too low
	+ Too much fluctuation
* Inappropriate relative humidity
	+ Too high
	+ Too low
	+ Too much fluctuation

**22.0 Labels and Labelling Systems**

Labels are a combination of substrate, ink, and printing method. Papers may be laminates, have coatings, or contain lignin that will affect their lifespan in preservatives and may cause unwanted contamination of preservatives. All new combinations should be carefully evaluated before use in the collection. A wide range of labelling systems have been used for wet specimens over the last several hundred years; each has its pros and cons. The best currently available systems include:

* Thermal transfer labels—carbon powder-based toner melted into spun-bound polyester substrate. These labels have withstood tests for lightfastness and abrasion, and are widely used in North America. However, the polyester substrate will eventually break down (its lifespan is not known, but easily exceeds 20 years) and may be sensitive to certain denaturants (e.g., MEK).
* Stainless steel or gold engraved labels
* Paper labels with perforated numbers
* Resistal paper (this product is coated with a formaldehyde compound and has been implicated in the acidification of preservatives).
* 100% cotton-rag paper with India ink or graphite pencil
* Goatskin parchment (Arjo Wiggins)
* Laser printer labels (used dry only)

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**Glossary**

|  |  |
| --- | --- |
| ***Term***  | ***Definition*** |
| Acid | Fluids with a pH less than 7.0  |
| Additive |  Chemicals added to fixatives or preservatives to improve chemical action (e.g., salts for buffering; cobalt nitrate for histological preparation) |
| Alcohol | An organic compound that contains a hydroxyl group (-OH) bound to a carbon atom |
| Aldehyde | An organic compound that contains a formyl group (R-CHO) |
| Base | Fluids with a pH greater than 7.0 |
| Biological material | Material originating from a biological source |
| Buffer | Chemicals used to moderate and/or stabilize pH |
| Buffered formaldehyde | Formaldehyde with salt added to counteract the formation f formic acid |
| Closure | The means of sealing a container (e.g., a lid or stopper) |
| Concentration ladder | Steps of increasing or decreasing concentration of a particular chemical |
| Container | Vessel to house a fluid preserved specimen such as a jar, bottle, or tank |
| Contaminant | An additive that changes the properties of a fluid in a negative (undesired) way |
| Decarbonisation |  |
| Dehydration | Loss of water |
| Denaturants (denaturing agents) | Chemicals added to a fluid to render it unpalatable or non-consumable for humans |
| Destructive sampling | Non-reversible intrusion into a preserved specimen or object, including removal of material |
| DMDMH | Clear aqueous preservative containing 55% of dimethyloldimethylhydantoin (1,3-Bis(hydroxymethyl)-5,5-dimethylimidazolidine -2,4-dione). Commericla - defakald |
| Fixation | Chemical or other stabilization of tissue to prevent autolysis |
| Fluid preservation | The preservation of an object by submerging it in a liquid chemical solution |
| Fluid Storage | Physical and environmental protection of an object in a suitable fluid and environment. |
| Formaldehyde | An organic compound with the formula HCHO that is a gas at room temperature but can be dissolved in water as H2C(OH)2 |
| Formalin | A former trade name adopted in several languages to refer to a saturated solution of formaldehyde gas in water (37.7%) or a diluted solution of formaldehyde gas in water |
| Gasket | A compressible material used between the lip of container and closure to prevent the escape of fluid or vapours from the container |
| Glutaraldehyde | An organic compound with the formula CH2(CH2CHO)2 |
| Glycerin / Glycerol | An organic polyol with the formula C3H8O3 |
| Headspace | The air above the level of fluid in a container |
| Hygroscopic | Tending to absorb moisture |
| Insert | A slightly compressible material that fits either inside the closure or inside the mouth of a container to prevent the escape of fluid or vapours |
| Kew mixture | A fluid preservative promoted by Kew Gardens for preserving botanical specimens in fluid. Several variations have been published since the formula was developed in 1930. The original Kew mixture was 53% industrial methylated spirit, 37% water, 5% formaldehyde (38% w/w) and 5% glycerol. In 1989 the formula was changed to 70% ETOH, 29% water, and 1% glycerol. |
| Liner | A slightly compressible material on the inside of a closure |
| Neutral buffered formaldehyde | A formaldehyde solution buffered to a neutral pH with salts |
| Oil | A neutral, nonpolar, hydrophobic and lipophilic chemical solution |
| Paraformaldehyde | A solid poly-acetal polymer of formaldehyde |
| Perfusion | Penetration of fluid (c.f. fixative) through tissues; the introduction of fluid fixatives or preservatives via the circulation system of a biological specimen |
| pH  | A measure of acidity or basicity of an aqueous chemical solutions, expressed as the negative logarithm of hydronium ion activity |
| Phenoxetol | 2-phenoxyethanol, also called ethylene glycol monophenyl ether |
| Preservation | Maintenance of fixed state of specimen |
| Preserving fluid | See *Fluid preservative* |
| Proprietary fixatives and preservatives | Fluids of unknown composition protected by patent or copyrigh sold by commercial firms for preservation of specimens |
| Storage | Long-term warehousing |
| Storage fluid | Fluid used for long term maintenance of preserved specimens |
| Storage media | See storage fluid |
| Topping up | Addition of storage fluid to a container to maintain fluid level |
| Transfers | Moving a specimen from one fluid to another |

**Syllabus**

* History of fluid preservation
* Basic chemistry of wet preservation
* Overview of problems in preserving and maintaining wet collections
* Fixing problems with old and historic fluid preserved specimens
* Monitoring specimens, collections, and storage environments
* Laboratory sessions
	+ Actions of fixatives and preservatives
	+ Determining alcohol concentration
	+ Determining formaldehyde concentration
	+ Sealing containers
	+ Mounting specimens
	+ Repair of specimens
	+ Rehydration
	+ Using concentration ladders
* Focus on specific problems and questions of participants

**Appendix I—Approximate Date of Introduction of Chemicals to Fluid Preservation**

|  |  |  |
| --- | --- | --- |
| ***Chemical name*** | ***Date*** | ***CAS Number*** |
| Absolute alcohol (100% ethyl alcohol) | 1899 | 64-17-5 |
| Acetic acid | 1899 | 64-19-7 |
| Acetone | 1976 | 67-64-1 |
| Acrolein (C3H5O) | 1984 | 107-02-8 |
| Alcohol (ethyl alcohol) | 1662 | 64-17-5 |
| Alum (aluminum sulfate) | 1817 | 10043-01-3 |
| Aluminum sulfate | 1831 | 10043-01-3 |
| Ammonia | 1921 | 8013-59-0 |
| Ammonium sulfate (diazonium sulfate) | 1968 | 7783-20-2 |
| Amyl chlorohydrin (amylocaine; stovaine) | 1979 | 644-6-8 |
| Arsenious acid (arsorous acid) | 1892 | 13464-58-9 |
| Baking soda (sodium bicarbonate; bicarbonate of soda) | 1968 | 144-55-8 |
| Benzamine hydrochloride | 1979 |  |
| Benzoyl benzoate | ?? | 120-51-4 |
| Beverage alcohol (aguardiente, arrack, rum, brandy, whiskey)Aguardiente and rum are made from sugar cane; arrack (arak, or rack) is made from coconut flowers or sugar cane; brandy is distilled from wine; whiskey is made from fermented grain mash | Pre-history | None; see CAS number for ethyl alcohol |
| Bicarbonate of soda (baking soda) | 1911 | 144-55-8 |
| Bichromate of potash | 1884 | 7778-50-9 |
| Bichromate of potassium | 1899 | 7778-50-9 |
| Bichloride of mercury | 1884 | 7487-94-7 |
| Borax (sodium tetraborate decahydrate) | 1944 | 1303-96-4 |
| Brine = a salt solution (usually sodium chloride) of about 3.5% to 26% | Pre-history | None; see CAS number for sodium chloride |
| Butyl hydroxytoluene (BHT; Ionol-40) | 1969 | 128-37-0 |
| Cacodylate | 1985 | 75-60-5 |
| Calcium chloride | 1884 | 10043-52-4 |
| Camphor | 1942 | 76-22-2 |
| Camphorated fluid (water, wine, creosote, chalk) | 1884 | Creosote CAS 8021-39-4 |
| Cane sugar | 1919 | 57-50-1 |
| Carbolic acid (phenol, benzenol, phenylic acid) | 1892 | 108-95-2 |
| Chloral hydrate (2,2,2-trichloroethane-1,1-diol) | 1899 | 302-17-0 |
| Chloretone | 1966 | 6001-64-5 |
| Chloride of lime | 1831 | 8031-48-9 |
| Chloride of zinc | 1884 | 7685-85-7 |
| Chloroform | 1944 | 865-49-6 |
| Chromic acid | 1899 | 7738-94-5 |
| Clove oil | 1966 | 8000-34-8 |
| Cobalt nitrate  | 1962 | 10141-05-6 |
| Coconut oil | 1892 | 8001-31-8 |
| Cocaine hydrochloride | 1944 | 53-21-4 |
| Cocaine powder | 1899 | 53-36-2 |
| Copper acetate (cupric acetate) | 1968 | 142-71-2 |
| Copper sulfate | 1899 | 7758-98-7 |
| Corrosive sublimate (mercuric chloride; mercury dichloride; sublimate) | 1852 | 7487-94-7 |
| Cupric chloride | 1985 | 7447-39-4 |
| Cyanide | 1955 | 74-90-8 |
| Dibasic sodium phosphate | 1951 | 7558-79-4 |
| Dioxane | 1964 | 123-91-1 |
| Dowicil [1-(3-chlorallyl)5,7-triaza-1-azoniaadamantane chloride] | 1979 | none |
| Epsom salts (magnesium sulfate) | 1895 | 7487-88-9 |
| Ether | 1955 | 7578-38-4 |
| Ethyl alcohol | 1662 | 64-17-5 |
| Ethyl bromide (bromoethane) | 1955 | 74-96-4 |
| Ethylene glycol | 1965 | 107-21-1 |
| Ethylene glycol monophenyl ether | 1956 | 122-99-6 |
| Eucaine (ß-eucaine hydrochloride) | 1979 | 500-34-5 |
| Formaldehyde | 1893 | 50-00-0 |
| Glacial acetic acid (= undiluted acetic acid) | 1899 | 64-19-7 |
| Gluteraldehyde | 1976 | 90045-36-6 |
| Glycerine (glycerin, glycerol) | 1883 | 56-81-5 |
| Hexamethyltetramine (hexamine) | 1953 | 100-97-0 |
| Hexamine | 1953 | 100-97-0 |
| Hydrochloric acid | 1884 | 7647-01-0 |
| Iodine | 1932 | 7553-56-2 |
| Ionol (2, 6-ditert-butyl 4-methylphenol) | 1976 | 9713-41-6 |
| Isopropyl alcohol | 1928 | 8013-70-5 |
| Kalium | 1935 | 7440-09-7 |
| Kerosene | 1964 | 8008-20-6 |
| Lactic acid | 1985 | 50-21-5 |
| Lead acetate | 1968 | 301-04-2 |
| Magnesium chloride | 1939 | 7786-39-3 |
| Magnesium sulfate (Epsom salts) | 1895 | 7487-88-9 |
| Menthol | 1955 | 89-78-1 |
| Mercuric chloride (corrosive sublimate) | 1852 | 7487-94-7 |
| Mercury acetate | 1968 | 1600-27-7 |
| Methyl alcohol (methanol, wood alcohol) | 1884 | 67-56-1 |
| Methyl benzoate | ?? | 93-58-3 |
| Methyl salicylate | ?? | 119-36-8 |
| Mineral oil | 1924 | 8042-47-5 |
| Molasses (a byproduct of sugar cane | 1829 | none |
| Monobasic sodium phosphate | 1951 | 89140-32-9 |
| MS222 (ethyl-m-aminobenzoate) | 1976 | 886-86-2 |
| Natrium bisulphate | 1935 | 7681-38-1 |
| Nembutol (pentobarbital) | 1966 | 76-74-4 |
| Nitre (potassium nitrate; saltpetre; saltpeter) | 1831 | 7757-79-1 |
| Nitric acid | 1899 | 7697-37-1 |
| Osmic acid (osmium tetroxide) | 1899 | 20816-12-0 |
| Osmium tetroxide (osmic acid) | 1976 | 20816-12-0 |
| Oxyquinoline sulfate (=hydroxyquinoline sulfate) | 1930 | 3819-18-9 |
| Para-hydroxybenzoic acid | 1956 | 99-96-7 |
| Paraffin oil | 1968 | 86742-46-7 |
| Paraformaldehyde | 1976 | 69476-52-8 |
| Phenol (carbolic acid; benzenol, phenylic acid) | 1942 | 108-95-2 |
| Picric acid | 1899 | 88-89-1 |
| Picro-sulfuric acid | 1895 | unknown |
| Platinum chloride | 1955 | 10025-65-7 |
| Potassium bichromate | 1899 | 7778-50-9 |
| Potassium chloride | 1933 | 7447-40-7 |
| Potassium dichromate | 1979 | 7778-50-9 |
| Potassium iodide | 1932 | 7681-11-0 |
| Potassium nitrate (nitre; saltpetre; saltpeter) | 1939 | 7757-79-1 |
| Propylene phenoxetol | 1968 | 770-35-4 |
| p-toluenesulfonic acid | 1976 | 104-15-4 |
| Pyroligeneous acid | 1899 | 8030-97-5 |
| Propionic acid (propanoic acid) | 1976 | 79-09-4 |
| Rock salt (see sodium chloride) | 1852 | 7647-14-5 |
| Sal armoniak (sal ammoniac, ammonium chloride) | 1665 | 12125-02-9 |
| Salicyclic acid | 1939 | 69-72-7 |
| Saltpeter (nitre; potassium nitrate) | 1884 | 7757-79-1 |
| Soda sulfate (sulfate of soda; sodium sulfate) | 1884 | 7757-82-6 |
| Sodium acetate | 1939 | 127-09-3 |
| Sodium arsenate | 1892 | 10103-60-3 |
| Sodium bicarbonate (baking soda) | 1968 | 144-55-8 |
| Sodium chloride | 1852 | 7647-14-5 |
| Sodium citrate | 1968 | 8055-55-8 |
| Sodium fluoride | 1935 | 7681-49-4 |
| sodium glycerophosphate | 1995 | 154804-51-0 |
| Sodium hydroxide | 1979 | 1310-73-2 |
| Sodium hydrosulfite (sodium dithionite) | 1942 | 7775-14-6 |
| Sodium silicate | 1985 | 6834-92-0 |
| Sodium sulfite | 1928 | 7757-83-7 |
| Sodium tetraborate (borax) | 1976 | 1303-96-4 |
| Spirit of wine (ethyl alcohol) | 1662 | 64-17-5 |
| Spirit or spirits (ethyl alcohol) | 1662 | 64-17-5 |
| Stovaine (amyl chlorohydrin; amylocaine) | 1979 | 644-6-8 |
| Strontium | 1976 | 7440-24-6 |
| Sublimate (corrosive sublimate; mercuric chloride; mercury dichloride) | 1852 | 7487-94-7 |
| Sucrose | 1984 | 57-50-1 |
| Sulfate of alumina (aluminum sulfate) |  | 10043-01-3 |
| Sulfur dioxide | 1928 | 7446-09-5 |
| Sulfuric acid | 1829 | 7664-93-9 |
| Tertiary butyl alcohol (tert-butyl alcohol) | 1968 | 75-65-0 |
| Thiourea | 1968 | 62-56-6 |
| Tincture of iodine | 1899 | None; see iodine CAS |
| Tobacco (an nicotine water) | 1899 | none |
| Trichloroacetic acid | 1976 | 76-03-9 |
| Triethanolamine | 1979 | 102-71-6 |
| Trisodium phosphate | 1951 | 7601-54-9 |
| Turpentine | 1955 | 9004-90-7 |
| Urethane | 1966 | 51-79-6 |
| Vinegar (active ingredient acetic acid) | Pre-history | None; see acetic acid CAS |
| Wood alcohol (methanol; methyl alcohol) | 1919 | 67-56-1 |
| Wood tar | 1930 | 98-91-1 |
| Xylene | 1924 | 1330-20-7 |
| Zinc chloride | 1985 | 7646-85-7 |
| Zinc sulfate | 1985 | 7733-02-0 |

**Appendix II Fixatives**

**See chart** Andries J. van Dam, 13-11-2012

**Appendix III—Historic Fixatives [[5]](#footnote-5)**

"Barbers Solution":
265 parts 95 % ethanol
(or 510 parts 50 % ethanol)
245 parts water (either tab, distilled or demineralised water, no exact recommendations in original reference)
95 parts ethyl acetate
35 parts benzene (should be replaced with toluene because of high toxicity and carcinogenicity of the benzene)

According to Joachim "Barbers solutions" (with toluene) is currently coming back to use for some purposes.

The following are relevant in historic context:

"Standfuss perservation fluid":
Add peppermint oil to a given volume of benzene until fluid starts turning cloudy. Add bit more benzene, so that fluid becomes clear again. Carefully add 2 parts carbon disulfide. Store ready mixed fluid protected from light exposure. Same applies for prepared specimens, as light causes breakup of the carbon disulfide leading to sulphur and carbon manifestations on objects. Benzene can be replaced with toluene.
(Is Standfuss a proper preservation fluid? - Maybe something historical that can be encountered in historic specimens ?)

"Krygers preservation fluid":
62,5 ml ethyl acetate
62,5 ml sublimate (aqueous solution of mercury(II) chloride or mercuric chloride / corrosive sublimate)
62,5 ml glycerol
312.5 ml 90% ethanol
500 ml distilled water

 "Pennaks fixative" (fixative after Pennak):
5g mercury(II) chloride (sublimate)
25 ml 70 % ethanol
5 ml 80 % nitric acid
220 ml water (tab, distilled or demineralised water ?)
1 ml ethyl acetate

Zenker Pot. dichromate

Helly Pot. dichromate

alcoholic Bouin or Dubosq-Bresil fixative

Also: Heidenhain’s Susa, Da Fano’s, and there are others even more obscure if required.

**Appendix IV—Fluid Preservation for DNA Extraction**

For purposes of later DNA extraction, the most successful procedure is to remove a sample of fresh tissue from the specimen immediately after it is euthanized and immediately freeze the sample to approximately -80°C in liquid nitrogen; the next best procedure is to store the tissue samples in 95% ethyl alcohol, preferably below 0°C, and lacking that capability, to use a fresh DNA buffer solution such as DMSO (see reviews in Carter 2004 and Dessauer et al. 1996).

Although it is possible to extract DNA from museum specimens, even those that were fixed in formaldehyde (e.g., Chatigny 2000, Fang et al. 2002, Klanten et al. 2003), the fixative action of the formaldehyde damages the DNA (e.g., Dubeau et al. 1986, Haselkorn and Doty 1961), as do certain other chemicals and procedures employed in specimen preparation and storage (e.g., exposure to ultraviolet radiation). Several studies have been directed at finding alternative fixative and/or storage fluids for DNA samples (e.g., Greer et al. 1991, Kuch et al. 1999, Leal-Klevezas et al. 2000). Graves and Braun (1992) have raised a caution about the use of museum specimens as DNA sources due to the damage caused by sampling techniques.

Early attempts to extract usable DNA from formaldehyde-fixed tissues showed some promise (e.g., Crisan et al. 1990, Dubeau et al. 1988, Goelz et al. 1985, Impraim et al. 1987, Jackson 1978, Nuovo and Richart 1989,Nuovo and Silverstein 1988, Rogers et al. 1990, Warford et al. 1988), but reliable techniques have been slow to be developed. In a comparative study of preservatives and temperatures on arachnid DNA, the best results were obtained by preserving specimens in a commercial product, RNA*later*™ (the ingredients of which are a trade secret; see table 7) or propylene glycol, and that tissues should be stored at -80°C. The best DNA extractions from tissues preserved in 95% ETOH were from specimens stored at -80°C (Vink et al. 2005). The study found no significant difference in DNA in specimens preserved in 95% ETOH and stored in 70% ETOH and tissues maintained in 95% ETOH (Vink et al. 2005). By contrast, Mandrioli et al. (2006) reported significantly better DNA quality and extraction yield from adult cabbage moth (*Mamestra brassicae*) specimens stored in 100% ethanol at 4°C compared to 75% ethanol, which gave poor results (however, they found the best preservation was in acetone). Dean and Ballard (2001) reported lower yields from insects euthanized in alcohol, and Houde and Braun (1988) were unable to extract usable DNA from alcohol preserved bird specimens.

Carter (2004) demonstrated that DNA preservation is better in ethyl alcohol than in IMS (probably due to the presence of methanol in IMS). Chakraborty et al. (2006) concluded that good DNA could be extracted from fish specimens frozen at -20°C or preserved in 95% ethanol for up to five years, but not for specimens fixed and preserved in 10% formalin; they were able to obtain DNA from specimens fixed for just seven days in 10% formalin buffered with phosphate and subsequently stored in ethyl alcohol. Some workers have now returned to preserving entire specimens in alcohol, without fixatives or other chemicals (Chakraborty et al. 2006). It should be noted that some attempts to extract DNA from tissues in 70% ETOH have produced variable results (e.g., Criscuolo 1992, 1994 was successful, but Seutin et al. 1991 were much less successful). In many of these cases, the authors have made the assumption that they were using specimens fixed in formaldehyde without knowing whether or not they were (e.g., Hughey et al. 2001), or the preserving fluid may have been contaminated by trace amounts of formaldehyde or other chemicals. Stuart et al. (2006) reported successful extraction of DNA from bone of both skeletons and fluid-preserved specimens, including specimens that were probably fixed in formaldehyde.

Williams (2007) investigated the possibility of shipping specimens in 24% ethanol after preservation in 100% ethanol to see if the DNA was damaged, and concluded that the yield and quality of the DNA were affected by the preservative used, the length of time the samples were in the preservative prior to exposure to 24% ethanol, and the length of time the samples were in 24% alcohol (shorter time periods yielded better quality DNA).

It has been noted that most specimens preserved directly in ethyl alcohol (without formaldehyde fixation) have white eyes (caused by the lens turning opaque), while most specimens that are formaldehyde fixed do not have white eyes. When selecting fluid preserved specimens for DNA extraction, those with white eyes will probably yield better DNA than those without (Russ et al. 1997).

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1. Imines are compounds that contain the –NH- group jointed to two other groups (imino-radicals are not linked to an acid group). [↑](#footnote-ref-1)
2. Andries van Dam knock up a draft [↑](#footnote-ref-2)
3. Bentley to supply [↑](#footnote-ref-3)
4. Sealant: Alsirol Rotationsschliff-Fett und Hahnfett circulated for trial from poellath.de [↑](#footnote-ref-4)
5. Makroskopische Präparationstechni Wirbellose. Leitfaden für das Sammeln, Präparieren und Konservieren [↑](#footnote-ref-5)