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Preservation of biological tissue samples and their effective DNA extraction

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ABSTRACT

The quality and quantity of a biological tissue sample is a major concern in tissue preservation and DNA extraction. It affects the success of DNA analysis and the overall quality of the final DNA profiling. DNA studies are of utmost importance in biological sciences. A tissue sample has to be properly fixed and preserved for its successful DNA analysis. Fixation of tissue is an initial and important step in tissue processing in histological examination. A fixative basically forms the cross-linking between the proteins present in the sample to help in retaining its appearance for life and hence it fixes the sample, whereas, a preservative is used to keep and store the sample for a long time without getting degraded. Historically, formalin and ethyl alcohol (ethanol) has been the most commonly used fixative and preservative for biological specimens respectively. There are different methods of tissue preservation which have been used earlier which includes natural as well as artificial methods. Little is known about natural substitutes of preservation. Less is known about the most suitable method for DNA extraction and yield from them, in the past researches. This review article is aimed to present an understanding of tissue preservation methods. Moreover, because of the carcinogenic nature and toxicity of formalin and considering it as a poor conserver of nucleic acids, a quest for its substitute has already begun. In addition, the parameters of different preservation methods with respect to DNA extraction are discussed here.

Keywords: Tissue preservation, tissue fixation, DNA extraction, DNA quantification.

INTRODUCTION

DNA studies carries overwhelming importance in biological sciences. Although there is plenty of publications and reviews on DNA isolation protocols from various preserved tissue types (Nishiguchi *et al.* 2002), the essential prerequisite for successful extraction is the presence of (ideally good-quality) DNA. Hence, appropriate preservation of the tissue samples is indispensable. In this field, there is still a certain lack of studies

summarizing applicable methods for specific tissue storage. Almost all research institutions and laboratories working with biological material are faced with this issue, either no special attention is given to that and long-established methods are used irrespective of specific needs or different types of biological materials, or specific preservation methods are actually being tested and optimized without publication of experiences and results (Kumar *et al.* 2018). An ideal fixative is the one which is little to non-toxic, give morphologically clear and understandable results, good quality of staining can be performed and most importantly allows to preserve nucleic acids at affordable rate. An optimal preservative should be non-toxic and allow for detailed morphologic analysis, high-quality special histochemical and immunohistochemical staining, and good preservation of DNA and RNA at a reasonable price. Due to the non-availability of such a fixative/ preservative, it is needed to find the pros and cons of the existing and upcoming fixatives (Moelans, 2012). Here we present an overview of available preservation techniques with regards of different tissue types, duration difference and different temperatures. In the current paper, we present an update by citing some further techniques and a couple of recent developments, while focusing on the possible (dis) advantages, practical considerations and feasibility issues for preserving the tissue samples.

Fixatives

The basic function of a fixative is to fix a sample so that it will maintain its composition and morphological appearance over time. This is done by stabilizing the proteins within a tissue. It also help to enhance staining of the sample. Formalin is one of the most widely used fixatives since ages. It is generally used in the concentration of 10% which is prepared by adding water to formaldehyde solution (commercially available) in the ratio of 9:1. Bases such as sodium hydroxide, sodium tetraborate can be added if one needs to neutralize the acidic effect of the formaldehyde solution (Martin, 2004).

A tissue should not be left in a fixative for long time as this will make the tissue fragile and susceptible to damage. The tissue should be cleaned in distilled water prior transferring it to a preservative which will maintain the tissue for a long time (Martin, 2004).

Formalin leads to degeneration of DNA so it interrupts in extraction of nucleic acids from a tissue (Bucklin and Allen, 2003). It has been reported in the past researches that DNA extraction has been possible even from specimens which were decades old. It has been recommended in the literature that fixation step is not suggested if any work related to DNA has to be done on a sample. (France and Kochler, 1996; Bucklin and Allen, 2003, Martin, 2004).

Preservatives

A preservative aims to provide a bacterial and infestations free environment to the tissue for long-term storage. Historically, ethanol is the most widely used preservative. It is generally used in the concentration of 70% -75%. It has been reported in the past literature that ethyl alcohol, being the primary component of liquor can be used in different concentrations for preservation of tissue Additionally, hard liquors can also be used for the same purpose (Martin, 2004).

Fixation, Preservation & Conservation

Tissue fixation is an essential step for the proper assessment and preservation of biological samples. Fixation aids in short term storage of tissue (Martin, 2004; Bhattacharyya *et al.* 2018; Gunasekaran *et al.* 2019). Preservation is an act or process of preserving, or keeping safe; the state of being preserved, or long term protection from deterioration whereas Conservation is a method of carefully preserving and protecting a specimen, and embalmment is a process of treating a dead body with specific chemicals in order to prevent the dead body from decay. These phrases define show that the words 'preservation' and 'conservation' may be replaceable, there are favored by different languages differently. Whereas the countries which uses German language rely more often on the word 'conservation' of a human body, the term 'preservation' is used in English. However, both the terms involves more than the only process of embalming, which uses specific chemicals on a body (Brenner, 2014).

It has been seen that DNA profile cannot be generated in many cases of sexual assault where pregnancy is terminated and foetus tissue is not properly preserved due to which the accused escape conviction (Kumar *et al.* 2018). Moreover, in cases of Disaster victim identification (DVI) which often occurs in remote locations with extremes of temperatures and humidities. Access to mortuary facilities and refrigeration are not always available. An effective and robust DNA sampling and preservation procedure would increase the probability of successful DNA

profiling and allow faster repatriation of bodies and body parts (Connell *et al.* 2018).

There are different preservatives which are used to preserve a tissue or a specimen in the process of preservation. There has been tremendous research in order to replace formalin with other fixatives having lesser side-effects, but the outcomes have not been upto the mark, leading to changes in morphology of cells and the ability to be specifically recognized by the antibodies generated as a result of the immune response to the given substance (Paavilainen *et al.* 2010). It is very much required to find an alternative to formalin fixation, which can offer better performance technically and is lesser toxic for health workers (Buesa, 2008). Nevertheless, the quality of any sample can be affected by various variables which include factors like sample acquisition, mishandling of sample, improper storage, and inadequate processing. In this process, proper long-term storage plays a key role in the preservation of tissue quality (Anton *et al.* 2015; Dawst *et al.* 2015). In this review article, the commonly used preservatives which include formaldehyde, non-formaldehyde, ethanol, saline, DMSO-salt solution, Glutaraledhyde and natural substitutes to formalin will be discussed.

Tissue preservation can be done either by a natural mean or an artificial one. Natural mean include removing moisture content of a sample by dry cold or by dry heat or by freezing them. Liquid nitrogen is considered as a gold standard in tissue preservation (Mirabet *et al.* 2008; Mercuri *et al.* 2013). It has been reported that ultralow-temperature freezers can be a substitute to liquid nitrogen for long-term storage (Andreasson *et al.* 2013; Auer *et al.* 2014). Due to the limited accessibility of both the above-mentioned methods, it is required to find substitutes that are required to be assured about the tissue quality after collection. In contrast, artificial means of preservation involves use of simple heat or cold, powders, arterial and cavity injections during embalming and use of preservatives such as formalin, ethanol (ethyl alcohol), normal saline etc. (Brenner, 2014).

Preservatives commonly used in the literature

Formalin

It is made of a saturated water solution which contain 39–40% of formaldehyde. It was discovered in the year 1869. Formaldehyde is usually in the form of a white hydrated solid polymer consisting of 80–100 methanal units (polyoxymethylene) called paraform or paraformaldehyde. It is used in the manufacture of adhesives, in animal nutrition and agriculture, cosmetics, deodorants, detergents, dyes, explosives, and many more which makes it a substance easy to come in contact with different concentration levels and environments. Formalin has been regarded as the "gold standard" in fixation of tissue and found to be an outstanding preservative (Shian *et al.* 2016; Bhattacharyya *et al.* 2018). It is also used as an embalming fluid. It is comparatively lower in price than other preservatives available in the market. It is well applied on the tissues and has anti-bacterial and anti-fungal properties which helps in retaining the tissue in a life-like stage. It maintains morphology of a tissue for long time, allows histologic stains and hence aids in immunochemistry results (Moelans *et al.* 2011). The volume of formalin needed to fix a specimen is not generally agreed. Different values of formalin have been argued upon (Buesa and Peshkov, 2012).

Since the use of formalin can be hazardous to its workers, the International Agency for Research on Cancer has categorized formaldehyde to (group 1) "carcinogenic to humans" (Cogliano *et al.* 2005). The yield and quantity of nucleic acids is affected due to the cross-linking agents in formalin. These agents not only have adverse effect on immunohistochemical testing but they also denature the DNA (Shibata, 1994; Lewis *et al.* 2001; Jones, 2007).

Duval K *et al.* 2010 demonstrated in his study that buffered formalin or the alcohol-based fixative GenoFix can be used for preservation to extract DNA from cells or tissues competently.

Formalin is used in the form of formalin fixed, paraffin-embedded (FFPE) blocks in clinical and research laboratories. In this process, tissue fixed in formalin underwent a short process which produce a FFPE block (Grizzle, 2009; Bonin *et al.* 2010; Sadeghipour and Babaheidarian, 2019). In tissue preservation, formalin fixed, paraffin-embedded (FFPE) blocks have a great role in maintaining the tissue for years. These blocks have been reported to preserve tissues at room temperature without any degradation. They allow for extraction of nucleic acids along with their histopathological analysis (Moelans *et al.* 2011; Ludgya *et al.* 2012; Patel *et al.* 2016; Carlsson *et al.* 2018; Nikolaou *et al.* 2013; Suhovskih *et al.* 2019).

The severe smell of the formaldehyde has a negative impact on health workers (Campbell and Margrave, 1998; Shi, Shao and Yin, 2012; Bhat *et al.* 2019). Formalin as a preservative lead to health issues, leads to discolorations, remove the moisture from tissues, and has an unpleasant odor (Shian, *et al.* 2016). It is a highly toxic systemic poison that is absorbed well by inhalation. The vapour is a severe respiratory tract and skin irritant and may cause dizziness or suffocation. Contact with formaldehyde solution may cause severe burns to the eyes and skin (Lu *et al.* 2010; Gunasekaran *et al.* 2019). Different systems of human body has been found to be adversely affected with the use of formalin (Patil *et al.* 2013; Bhattacharyya *et al.* 2018).

Bajracharya described in the paper his first experience in Anatomy dissection classes in which they had used the embalming solution containing chemicals namely Phenol, Borax, Sodium citrate, Formalin, Methanol, Glycerin and Water (Bajracharya and Magar, 2010; Balta *et al.* 2018). Another study which was carried out for the duration of five years in the Goa Medical College Bambolim (India) used total hundred cadavers which were embalmed with formalin, water, methyl alcohol, glycerin, cetrimide, eosin, eucalyptus Oil. Results showed that no fungus was found in intact bodies which were kept for five years but minimal growth of fungus could be found after a period of twelve months in the dissected cadavers which were kept separately containing 10% formalin (Natekar and DeSouza, 2012).

Kalanjati *et al.* (2012) in his study used low formalin (5-7.5% formaldehyde) technique which comprised of 7.5% formalin as the active fixative, glycerin, phenol powder and tap water, which resulted in paling of cadaver with more as a whole and easier to dissect specimen (which were drier and still moist with absence of any fungal growth).

Considering the negative toxic effects of formaldehyde-based fixatives and the past researches that indicates that it hamper the recovery of nucleic acids, an urge to seek alternatives for formaldehyde based solutions has started (Buesa, 2008; Bhattacharya *et al.* 2018).

Non-formaldehyde Solution

In literature, tissue fixative efficacies of non-formalin fixative agents such as jaggery, ethanol have been compared with formalin. They have found to give good results depending on the time variability of their preservation (Lam-ubol *et al.* 2018).

The recent publication by Hauptmann *et al.* in the Journal provided clear evidence for increased mortality from cancer induced by exposure to formalin, as outlined by The Final Report on Carcinogens Background Document for Formaldehyde of the National Toxicology Program, 2010.

A study demonstrated the use of non-formaldehyde solution to preserve a specimen so as to avoid the toxic effects of formalin. This study on rabbit cadavers was conducted using improved or non- formaldehyde preservative which used a mixture of acid along with a buffer solution (85%) and an agent i.e. Tetrakis hydroxymethyl phosphonium salt (THP salt) (15%) which will cross-link and act as mildew preventive, stabilizer and fixative respectively. The results obtained by this method in terms of preservation and fixation were not only comparable to that of formaldehyde. Additionally, it had low volatility and smell and protected the specimen form bacterial infestations. Considering, its advantages over formaldehyde solution, this solution is recommended for future usage in tissue preservation (Shi *et al.* 2012).

Ethanol

Fixatives which are alcohol-based are regarded as one of the most promising substitutes to potentially carcinogenic formalin in tissue preservation. 95% Alcohol is a widely used preservative in Fine Needle Aspiration Cytology (FNAC) which although readily available, has a number of limitations for use. It is an easy to use but an expensive fixative and hence subject to pilferage. Also, it is a volatile liquid with an irritant smell and is said to be carcinogenic (Ishaq *et al.* 2017). Literature confirms that the fixation by alcohol has been found to be superior than that by formalin, if DNA and RNA extraction has to be performed from paraffin blocks, even after a long duration of fixation by alcohol (Perry *et al.* 2016; Chung *et al.* 2017; Panzacchi *et al.* 2019).

Different concentrations of alcohol to be used as a preservative have been debated in the literature. 70% by volume in water act as an effective biocide (Waller and Strang, 1996). However, with 70% ethanol, a high degree of degradation has also been observed, even after one year. An alternative is 70% ethanol diluted

with 1xTE buffer (instead of water), which yields good results (B. S. Hedges pers. comm.) According to plenty of test, the optimal concentration is about 95– 99% (King and Porter 2004, who also compared with isopropanol). EDTA (ethylenediaminetetraacetic acid) can be added to 95% ethanol (Dessauer *et al.* 1996). 70–80% ethanol with 1–3% glycerine keeps the specimens moist if the alcohol evaporates (Bechmann, 2011). However, in the latter case similar quality loss can be expected as with slightly diluted ethanol. Absolute ethanol may not be the optimal medium as it is the most expensive and can contain traces of benzene that seriously affect DNA preservation.

In the recent Journal of Histochemistry & Cytochemistry (JHC) article by Perry et al., a fixative mixture designated "buffered ethanol 70%" (BE70) was judged best for preserving structural integrity, providing satisfactory immunostaining with three commonly used antibodies, and for allowing optimal extraction and amplification of nucleic acids (Kiernan, 2017). Recently, Weigners fixative (WF), a nitrite pickling salt solution composed of ethanol and Pluriol, has been introduced as an alternative to formalin for embalming of cadavers (Janczyk *et al.* 2011; Klopfleisch *et al.* 2012).

Past studies have proposed less toxic cross-linking fixatives which are alcohol-based and non–cross-linking fixatives as NBF alternatives. These fixatives have found to perform fixation at a good rate, eliminates the risk of cancer by eliminating the carcinogen vapors, better nucleic acids preservation and allows good staining for histological examination. There are few disadvantages also associated with it which include variance in staining of tissue, RBC breakage and high volatility etc. (Boestik *et al.* 1994).
Various researches on ethanol as a preservative has demonstrated that health risk issues associated with it are lesser.

Shian *et al.* (2016) conducted a study which demonstrated that ethanol's preservative formula can be used as a substitute in place of formalin as presservative formula in terms of color, odor and consistency.

Another study which compared embalming fluids to check the quality of preservation in terms of histological features in human cadavers revealed that the clearest morphology was observed in the skin and skeletal muscle sections, and in tissues embalmed with fluids which do not contain phenol (Nicholson *et al.* 2005).

Phenoxyethanol

Phenoxyethanol has been proven to be a viable replacement of formaldehyde. It's efficacy as a preservative and fixative is supposed to be better than formalin. In addition to avoid the well-known hazardous effects of formaldehyde, the use of Phenoxyethanol also prevents the pungent and irritating smell associated with formalin and other local and systemic manifestations of its toxicity (Chhabra, 2020).

So in order to get rid of irritating and potentially hazardous chemicals, phenoxyethanol has been proven to be a good alternative for preserving human biological tissues. This is relatively less toxic and cost effective to ethanol. In 1989, Wineski & English, conducted a study on 138 human cadavers which suggested that phenoxyethanol is an excellent, easily manageable alternative preservative to standard formaldehyde/ phenol-based fluids. Results were comparable with those described by Frolich *et al.* in the year 1984.

Natural Substitutes

Literature search reveals very sparse studies on natural substitutes for formalin. The health concerns with formalin can be avoided by using alternative fixation methods and fixatives. Natural substitutes such as honey which is a natural organic product, is odourless and not known to cause any disease. Moreover, it is relatively cheaper and not prone to be mishandled, making it an efficient contender for use as a fixative (Ishaq *et al.* 2017). Studies have shown that natural substitutes such as honey, sugar and jaggery are capable of tissue preservation which is comparable to formalin and helps in immunohistochemical staining (Sabarinath *et al.* 2014; Patil *et al.* 2015).

Studies have been conducted on the fixative characters of natural substances such as honey, sugar syrup, jaggery syrup, Khandsari etc. to check if an alternative to formalin can be adopted (Patil *et al.* 2015). They have been found to be dehydrative and protective in nature and results obtained were similar to formalin in efficiency (Emsen, 2007; Ozkan *et al.* 2012; Sabarinath *et al.* 2014). A study has reported that honey can be used in successful transportation for direct immuno-fluorescence (DIF) for skin biopsy (Rao, 2017).

Gunasekaran *et al.* (2019) conducted a study on thirty tissue samples obtained from normal gingiva patients using two natural fixatives i.e. Apple cider vinegar and Honey Lemon Ginger Tea extract. It was done to eliminate the toxic effects of harsh chemicals and to determine the best fixation among the two. Tissues were analyzed for histological features and results were compared statistically. Out of the two, apple cider vinegar was found to give good results than honey ginger lemon tea after comparing with formalin.

Another study included jaggery as it is also one of the natural sweetener derived from sugarcane. Study concluded that honey and jaggery in particular amount is able to fix the oral smears efficiently in a mechanism similar to alcohol by coagulating and denaturing proteins (Pandiar *et al.* 2017).

A study conducted on goat tissue recommended jaggery syrup as an equally effective formalin substitute which had compared fresh tongue fixed in honey, sugar syrup and jaggery syrup (Bhattacharyya *et al.* 2018). Studies have shown that histological features such as overall nuclear detail, cytoplasmic details and nuclear staining quality of the tissue was found to be better in jaggery syrup than conventional method of formalin fixation (Patil *et al.* 2013; Patil *et al.* 2015; Sinha *et al.* 2017).

Studies have found that honey has always been considered as wonder component as it has antimicrobial agents which are capable of preserving any specimen without having any toxic effects on its workers. Honey has anti-oxidative and antibacterial effects as its pH concentration is low and osmolarity is high. Moreover, studies have demonstrated that honey has acidic and dehydrative properties. Various studies have recommended honey as a natural substitute to formalin in conventional histochemical and immunohistochemical staining methods.

Honey cannot be used in large scale due to its unavailability universally and the fact that it is expensive, sugar and jaggery have started being chosen as a substitute for formalin (White, 1978; Maaini and Bryant, 2006).

Normal Saline

Saline is in the crystalloid family of medications. It is most commonly used as a sterile 9 g of salt per litre (0.9%) solution, known as normal saline.

Its efficiency and reliability as a soft tissue preservation method leading to recovery of DNA samples suitable for polymorphic loci analysis has been reported (Caputo *et al.* 2011).

Kumar *et al.* (2018) conducted a study in which they had taken three types of samples from foetus i.e. One was toe, limb (leg) in normal saline and rest upper major part of the foetus in formalin. Phenol-Chloroform Extraction method was applied to isolate DNA. This study concluded that normal saline gave better DNA quality, quantity and DNA profiles compared to formalin. Al-Hubaity and Al-Saraj had carried out work on saturated table salt and suggested that it could be used in place of formalin to preserve bodies (Al-Hubaity, 2003). Al-Saraj worked on saturated sodium chloride solution and concluded that it can be used as a fixative in histopathological procedures. The results obtained were same as that obtained by using conventional formaldehyde. An added advantage of saturated sodium chloride was that it did not cause any distortion of the cell.

It has been noted in an observation that often operating surgeons, oral surgeons and dentists do not have the required fixatives available to them in their surgery, and a common practice followed is to initially keep the removed tissue in normal saline, before transferring it to a fixative later. The tissues kept in normal saline before fixation underwent various changes. In no way should normal saline be used, even for a short time, to store and transport excised tissue; it is imperative that such tissues be placed immediately in a proper fixative like 10 % formalin (Sengupta *et al.* 2014).

Dimethyl Sulphoxide (Freezing)

The discovery that glycerol acts as a protective substance to prevent damage to cells by freezing has allowed the development of reliable methods for the routine culture cells at low temperature. An attempt has been made in which chick embryo fibroblasts were prepared from embryos to estimate the cell viability by using Dimethyl Sulphoxide. It permitted the recovery of a very high proportion of the frozen cells with little or no impairment of their ability to grow in vitro (Dougherty, 1964).

Ice-free vitrification

Ice-free vitrification of biospecimens is an alternative cryopreservation strategy to conventional preserva-

tion by freezing. Vitrification is the amorphous solidification of a supercooled liquid. This state is achievable by adjusting the cryoprotectant concentration and cooling rate to minimize nucleation and growth of ice crystals. The cooled liquid is then converted to a glassy state, notice. Without ice crystal formation, the biospecimens' extracellular matrix and cell viability is often better preserved. Ice-free vitrification has major advantages for preservation of ovaries, heart valves, articular cartilage, and both natural and tissue engineered blood vessels, protecting the extracellular matrix and cells. In the extreme case of articular cartilage freezing results in less than 20% cell viability in contrast with ≥80% after ice-free vitrification. (Brockbank *et al.* 2014).

Hanks and Wallace, 1949 made an inquiry into conditions which may influence the viability of 1x1 cm areas of biopsied rabbit skin during refrigeration at 0°C and 68°C. He used Mineral oil and rabbit diluted serum as preservative. The results were approx. 50% of the cells remained alive during first week. No cells survived after preservation in oil for two weeks which indicates that 10% serum is superior to mineral oil as a refrigeration menstruum because it provides nutrient and dilutes or buffers the acids which result from metabolism.

DMSO Salt Solution

William Kilpatrick, 2002 conducted a study which aimed to compare the abilities of three chemical methods of tissue storage to prevent degradation of DNA in the absence of refrigeration or freezing. In this study liver, a soft tissue typically used for DNA extraction, was collected from three white-footedmice. Minced liver was stored at room temperature in 20 mL polyethylene scintillation vials in one of three chemical preservatives; 95% ethanol (Sibley and Ahlquist, 1981), DMSO-salt solution (Seutin *et al.* 1991), or lysis (Longmire) buffer (Longmire *et al.* 1997). The results showed that greatest DNA extracted from DMSO salt solution preserved tissues compared to lysis buffer or ethanol.

Glutaraledhyde

Glutaraledhyde successfully used by Harries and Tank in 1908 exhibited properties that were many ways superior to formaldehyde in fixation of proteins in electron microscopy. It is a 1, 5-pentanedial; 1, 5-pentanedione; glutaric dialdehyde, pungent colorless liquid marketed as Cidex, Sonacide, Sporicidin, Hospex and Omnicide used as a chemical preservative. It kills the cells quickly by crosslinking their proteins. It is frequently used as a disinfectant and sterilizing agent as bacterial, sporicidal, fungicidal and viricidal against bacteria and viruses, an embalming fluid and tissue fixative, a component of leather tanning solutions and an intermediate in the production of certain sealants, resins, dyes and electric products (Natekar and DeSouza, 2014).

A study performed in Goa Medical College (India) reported that Glutaraledhyde with other chemical compositions such as Methyl alcohol, Glycerin, Cetrimide, Eosin and Eucalyptus oil was proved to be very effective in preservation of cadavers as it prevented bacterial and fungal growth and the deterioration for upto four years. The cadaver remained hygienically safe, very supple, shows life like appearance with no rigidity and hardness (Natekar and DeSouza, 2014).

RNALater

RNAlater Stabilization Solution (Ambion, Darmstadt, Germany) is an aqueous ammonium sulfate tissue storage reagent. It is a stabilizer, which was originally developed for RNA preservation in fresh tissues and is important for collection and transportation (Wang *et al.* 2018). Histological and immunohistochemical (IHC) analysis of any tissue involves formalin fixation of biological material with subsequent production of paraffin blocks.

RNALater comes to rescue in cases where it is not possible to use formalin as a fixative. It is known to preserve a tissue for molecular studies as well as biobanking (Wolfe *et al.* 2014; Bennike *et al.* 2015). It certain the stability of RNA and DNA for testing (Grotzer *et al.* 2000; Mutter *et al.* 2004; Wolfe *et al.* 2014). According to the specification, RNALater-preserved tissue samples are suitable for protein and histological studies as well and can be processed like fresh tissue. It has been reported that RNALater is able preserve protein content in human colon mucosal biopsies (Bennike *et al.* 2015) and postmortem lacrimal and submandibular gland tissues (Hawley *et al.* 2016). It efficiently preserves the sample for as long as seven years (Drakulovski *et al.* 2013); However, the efficiency of RNALater preservation depends on the starting biological material/tissues and downstream application.

Suhovskih *et al.* (2019) investigated in their study whether the medium-term preservation of different tissues with RNALater is suitable for their histological and immunohistochemical analyses.

However, RNALater lacks a comprehensive and systematic evaluation of its preservative effect on different mammalian tissues under consistent experimental conditions (Wang *et al.* 2018).

DNA yield

DNA can be isolated from a variety of human sample sources including anti-coagulant whole blood, bloodstains, hairs, tissue samples and buccal epithelial cells (Gari *et al.* 2006). In the literature, DNA Extraction has been performed using different extraction methods. Some previous studies have shown the possibility of extracting amplifiable DNA and RNA from archival air-dried unstained bone marrow slides (Fey *et al.* 1987; Grünewald *et al.* 1991; Pabst *et al.* 1996), as well as from archival Giemsa-stained peripheral blood smears (Kimura *et al.* 1995; Yokota *et al.* 1995; Schoch *et al.* 1996), saliva, virginal and postcoital smears (DinoSimonin *et al.* 1997), smears of tissue fluid and inflammatory exudates (Alger *et al.* 1996), archival cytogenetic slides (Sago *et al.* 1996) and archival Giemsa-stained bone marrow slides (Vince *et al.* 1998).

In literature, quantity and quality of nucleic acids extracted from archival formalin fixed paraffin embedded prostate biopsies has been evaluated using different DNA extraction kits (Staff *et al.* 2014; Carlsson *et al.* 2018). The alcohol-based non-crosslinking fixatives is found to perform better than crosslinking fixatives with in terms of DNA and RNA yield and quality (Moelans *et al.* 2011).

DNA extraction using Organic extraction method for enzymatic digestion of proteins and nonnucleic acid cellular components has been reported. It uses phenol: chloroform: isoamyl alcohol (25:24:1) to promote the partitioning of lipids and cellular debris into the organic phase, leaving isolated DNA in the aqueous phase. Following centrifugation, the aqueous phase containing the purified DNA can be transferred to a clean tube for analysis. DNA can then be recovered from aqueous phase using centrifugation method. Organic extraction gives double stranded DNA which is prerquisite for restriction fragment length polymorphism. It is considered as a trusted and effective method. It has few dis-advantage that it takes time to produce the result and it involve use of harmful chemicals. Additionally, it require more labor and multiple use of tubes which might lead to misinterpretation of the final results (Köchl *et al.* 2005; McKiernan & Danielson, 2017).

Another rapid method of extraction of good molecular weight of DNA yield has been explored which uses the laundry detergent in powder as well as liquid form to isolate DNA (A. Bahl and M. Pfenninger, 1996).

One more study evaluated five different DNA extraction methods on decomposed human liver tissue specimens obtained from forensic autopsy cases. It included the phenol–chloroform, the silica based, the InstaGene MatrixE (BioTest), the glass fiber filter, and the Chelex based methods. It was reported that Chelex method is the fastest and affordable among them whereas the phenol–chlorophorm and silica extractions was proven to be the most time taking and resource demanding ones (Olsen *et al.* 1999).

CONCLUSION

Proper tissue preservation is important for the qualitative and quantitative analysis of DNA. Tissue preservation for forensic purposes is most often associated with disaster victim identification which aims at identification of victims of mass disaster and recovery of the deceased body parts. Forensic DNA analysis along with fingerprint and dental analysis has been recommended by the International Criminal Police Organisation (INTERPOL) as the primary methods of identification (INTERPOL, 2009), and can therefore play a crucial role for identifying victims. Skeletal muscle tissue is typically sampled for DNA analysis (Prinz *et al.* 2007) and requires preservation, from the time of collection to the point at which it can be transported to a laboratory refrigerator/freezer. INTERPOL guidelines state that formalin or formaldehyde solution should not be used as a preservative because it degrades DNA.

Literature reveals that the preservatives are responsible for arresting DNA degradation. Sodium chloride and silica beads is being used since ages as a common preservative. NaCl in solid state removes the moisture from the sample and reduce any microbial growth but in aqueous state, it disintegrate the proteins (Grassberger, 2005; Nagy, 2010). EDTA, being

a chelating agent cease the nuclease activity on DNA by binding to metal ions (Seutin, 1991). Alcohol dehydrates the sample by denaturing the proteins and nucleases. Moreover, it is also an antimicrobial agent and will provide a shield against bacterial degradation (Seutin, 1991). Preservatives such as Dimethyl sulphoxide (DMSO) does not directly preserve DNA of a sample. It is easily absorb by the outer layer of the skin when combined (Elfbaum, 1968) across biological membranes and into the cell (Kilpatrick, 2002).

Nucleic acids of high quality are vital for downstream molecular applications, however fixation and preservation of tissues causes modifications of biomolecules such as cross-linkage of nucleic acids with proteins, covalent modifications of both DNA and RNA, and fragmentation of RNA, making it challenging to extract nucleic acids of high quality from preserved tissues. As well as protecting DNA for subsequent analysis, tissue preservation methods ideally should be safe, readily available, and easy to transport at relatively low cost.

The better the quality of a preservative, the better are the chances it will preserve the specimen well. It is needed to find an alternative to carcinogenic preservatives and to study the DNA extraction and yield from them which has not been worked on, in previous researches. It is required to study various tissue preservation methods for effective extraction and quantification of DNA from various biological tissue samples and also, to analyze the effective preservation method with respect to temperature and duration of preservation.

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REFERENCES

Alger J, Acosta MC, Velasques C and Labrada LA (1996) Stained smears as a source of DNA. *Memorias do Instituto Oswaldo Cruz*, 91: 589- 591.

Al-Hubaity A and Al-Saraj A (2003) The use of saturated table salt solution as an embalming agent. *ZJMS,* 7: 37–*40.*

Andreasson A, Kiss NB, Juhlin CC, *et al.* (2013). Long-term storage of endocrine tissues at -80 degrees C does not adversely affect RNA quality or overall histomorphology. *Biopreserv Biobank,* 11: 366–370.

Anton G, Wilson R, Yu ZH, et al (2015) Pre-analytical sample quality: Metabolite ratios as an intrinsic marker for prolonged room temperature exposure of serum samples. *PLoS One, 10*(3), e0121495.

Auer H, Mobley JA, Ayers LW *et al.* (2014) The effects of frozen tissue storage conditions on the integrity of RNA and protein. *Biotech Histochem,* 89(7): 518–528.

Bahl A and M Pfenninger (1996) A rapid method of isolation using laundry detergent. *Nucleic Acids Research,* 24(8): 1587-88.

Bajracharya S and Magar A (2006) Embalming: An art of preserving human body. *Kathmandu University Medical Journal,* 4(16): 554-557.

Balta, JY *et al.* (2018) Comparison of embalming fluids on the structures and properties of tissue in human cadavers. *Anat Histol Embryol*, 4: 64–73.

Bennike TB, Kastaniegaard K, Padurariu S, Gaihede M, Birkelund S, Andersen V, Stensballe A (2015) Comparing the proteome of snap frozen, RNAlater preserved, and formalin-fixed paraffinembedded human tissue samples. *EuPA Open Proteom*, *10:* 9–18.

Bhat D, Chittoor H, Murugesh P, Basavanna PN and Doddaiah SK (2019) Estimation of occupational formaldehyde exposure in cadaver dissection laboratory and its implications. *Anatomy and Cell Biology,* 52: 419-425.

Bhattacharyya A, Gupta B, Singh SK and Gupta V (2018) Probing natural substitute for formalin: Comparing honey, sugar, and jaggery syrup as fixatives. *National Journal of Maxillofacial Surgery,* 9(1): 14–21.

Bonin *et al.* (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arch,* 457: 309–317.

Bostwick DG, al Annouf N and Choi C (1994) Establishment of the formalin-free surgical pathology laboratory: utility of an alcohol-based fixative. *Archives of Pathology and Laboratory Medicine*, 118: 298- 302.

Brenner, E (2014) Human body preservation – old and new techniques. *Journal of Anatomy,* 224*:* 316-344.

Brockbank KGM, Chen Z, Greene ED and Campbell LH (2014) Ice-free cryopreservation by vitrification. *MOJ Cell Sci Report,* 1(2): 27-31.

Bucklin A and LD Allen (2003) MtDNA sequencing from zooplankton after long-term preservation in buffered formalin. *Molecular Phylogenetics and Evolution*, 30: 879-882.

Buesa RJ (2008) Histology without formalin. *Annals of Diagnostic Pathology,* 12(6): 387-96.

Buesa RJ and Peshkov MV (2012) How much formalin is enough to fix tissues? *Annals of Diagnostic Pathology*, 16: 202-209.

Campbell JW and Margrave JL (1998) United States Patent. 5,827,511.

Caputo M, Bosio LA and Corach D (2011) Long-term room temperature preservation of corpse soft tissue: an approach for tissue sample storage. *Investigative Genetics*, 2(17): 1-6.

Carlsson *et al.* (2018). Quantity and quality of nucleic acids extracted from archival formalin fixed paraffin

embedded prostate biopsies. *BMC Medical Research Methodology,* 18, 161.

Chhabra N (2020) Long term preservation of cadavers: Substitution of formalin with phenoxyethanol: Need of the hour. *International Journal of Anatomy and Research*, 8(2.3):7557-63. (ISSN 2321-4287).

Chung JY, Song JS, Ylaya K, Sears JD, Choi LCH, Rosenberg AZ and Hewitt SM (2017) Histomorphological and Molecular Assessments of the Fixation Times Comparing Formalin and Ethanol-Based Fixatives. *Journal of Histochemistry & Cytochemistry:*1–15.

Cogliano VJ, Grosse Y, Baan RA, et al (2005) Meeting report: summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tert-butoxy-2-propanol. *Environ Health Perspect*, 113: 1205-1208.

Connell J, Chaselinga J, Pageb M and Wright K (2018) Tissue preservation in extreme temperatures for rapid response to military deaths. *Forensic Science International: Genetics,* 36:86-94.

Dapson RW (2007) Glyoxal fixation: how it works and why it only occasionally needs antigen retrieval. *Biotechnic Histochemistry*, 82: 161-166.

Dapson RW (2007) Macromolecular changes caused by formalin fixation and antigen retrieval. *Biotech Histochemistry*; 82: 133-140.

Dessauer HC, Cole CJ and Hafner MS (1996) Collection and storage of tissues. In D. M. Hillis & C. Moritz (Eds.), Molecular systematics (2nd ed., pp. 29–47).

Dougherty RM (1962) Preservation of Cells in Tissue culture: Use of Dimethyl Sulphoxide for preservation of tissue cell culture by freezing. *Nature,* 193: 550-553.

Dowst H, Pew B, Watkins C, McOwiti A, Barney J, Qu S and Becnel LB (2015) Acquire: An open-source comprehensive cancer biobanking system. *Bioinformatics, 31(*10): 1655–1662.

Drakulovski P, Locatelli S, Butel C, Pion S, Krasteva D, MougdiPole E, Delaporte E, Peeters M and Mallié M (2013) Use of RNAlater as a preservation method for parasitic coprology studies in wild-living chimpanzees. *Exp Parasitol,* 135(2): 257–261.

Duval K, Aubin RA, Elliott J, Gorn-Hondermann I, Birnboim HC, Jonker D, Fourney RM and Frégeau CJ (2010) Optimized manual and automated recovery of amplifiable DNA from tissues preserved in buffered formalin and alcohol-based fixative. *Forensic Science International: Genetics,* 4(2), 80-8.

Elfbaum SG and Laden K (1968) The effect of dimethyl sulfoxide on percutaneous absorption: a mechanistic study, part I. *Journal of the Society of Cosmetic Chemists*, 19: 119–127.

Emsen IM (2007) A different and safe method of split thickness skin graft fixation: Medical honey application. *Burns*, 33(6): 782–787.

Fey MF, Pilkington SP, Summers C and Wainscoat JC (1987) Molecular diagnosis of haematological disorders using DNA from stored bone marrow sides. *British Journal of Haematology,* 67: 489-492.

Frolich KR, Anderson LM, Knutsen A and Flood PR (1984) Phenoxyethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration of purposes, *Anatomical Record,* 208: 271-278.

Gari MA, Abuzenadah AM, Chaudhary AG, Al-Qahtani MH, Al-Says FM and Dmanhouri G (2006) Full Length Research Paper - Pilot study of DNA extraction from archival unstained bone marrow slides: comparison of three rapid methods. *African Journal of Biotechnology,* 5(6): 532-535.

Grassberger M, Stein C, Hanslik S et al (2005) Evaluation of a novel tagging and tissue preservation system for potential use in forensic sample collection. *Forensic Science International*, 151(2–3): 233–237.

Grizzle WE (2009) Special symposium: fixation and tissue processing models. *Biotechnic & Histochemistry,* 84(5): 185–19.

Grotzer MA, Patti R, Geoerger B, Eggert A, Chou TT and Phillips PC (2000) Biological stability of RNA isolated from RNAlater treated brain tumor and neuroblastoma xenografts. *Medical and Pediatric Oncology,* 34(6): 438–442.

Grünewald K, Lyons J, Hansen-Hagge TE, Janssen JWG, Feichtinger H and Bartram CR (1991) Molecular genetic analysis of DNA obtained from fixed, air dried or paraffin embedded sources. *Annu. Hematology,* 62: 108-114.

Gunasekaran S, Jayant VS, Krishnakumar, K and Ajay, A (2019) Evaluation of Tissue Fixation using Organic Substitutes – A Comparative Study. *International Journal of Scientific Research,* 8(3): 37-38.

Hauptmann M, Stewart PA, Lubin JH, *et al.* (2019) Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde. *Journal of National Cancer Institute,* 101(24): 1696–1708.

Hawley D, Aluri H, Armaos H, Kim G, Kublin C, Zoukhri D (2016) Human postmortem lacrimal and submandibular glands stored in RNAlater are suitable for molecular, biochemical, and cell biological studies. *Molecular Vision,* 22: 1221–122.

INTERPOL (2009) Disaster victim identification guide. INTERPOL, Lyon.

Ishaq R, Azmat H, Omair M, Sheikh AK and Tanwani AK (2017) Comparison of Honey With Alcohol as a Fixative in Fine Needle Aspiration Cytology. *International Journal of Pathology,*15(1):15-18.

Janczyk P, Weigner J, Luebke-Becker A, *et al.* (2011). Nitrite pickling salt as an alternative to formaldehyde for embalming in veterinary anatomy: a study based on histo- and microbiological analyses. *Annals of Anatomy*, 193: 71–75.

Jones ML (2007) How formalin affects the outcome of routine and special stains. *Biotech Histochemistry,* 82: 155-159.

Kalanjati VP, Prasetiowati L and Alimsardjono H (2012) The use of lower formalin-containing embalming solution

for anatomy cadaver preparation. *Low formalin embalming technique,* 21(4):203-207.

Kiernan J (2017). Buffered Ethanol Fixative. *Journal of Histochemistry & Cytochemistry, 65(4)*:251–252.

Kimura M, Kaneko O, Inoue A, Ishil A and Tanabe K (1995) Amplification by polymerase chain reaction of Plasmodium falciparum DNA from Giemsa-stained thin blood smears. *Molecular and Biochemical Parasitology,* 70: 193- 197.

King JR and Porter SD (2004) Recommendations on the use of alcohols for preservation of ant specimens (Hymenoptera, Formicidae). *Insectes Sociaux,* 51: 197–202

Klopfleisch R, Deetzen M, Weiss AT, Weigner J, Weigner F, Plendl J and Gruber AD (2012) Weigners Fixative–An Alternative to Formalin Fixation for Histology With Improved Preservation of Nucleic Acids. *Veterinary Pathology 50(1),* 191-199.

Koziej KP, Hołda J, Koziej M, Tyrak K, Jasińska KA, Bonczar A, Walocha JA and Hołda MK (2019) Fixative properties of honey solutions as a formaldehyde substitute in cardiac tissue preservation. *Folia Medica Cracoviensia,* LIX, 1, 101–114. (ISSN 0015-5616).

Kumar N, Chauhan A, Sharma D and Shukla SK (2019) DNA profiling from drown dead bodies. *International Journal of Molecular Biology,* 4(3): 90–94.

Kumar N, Maitray A, Gupta R and Shukla SK (2018) Effects of preservative on foetus tissues and DNA profiling in forensic cases. *International Journal of Molecular Biology, 3*(4): 16-167.

Lam-ubol A, Kitrueangphatchara K, Putthanuparp T, Arayakhun R, Kwanthong R and Choonhawarakorn K (2018) Non-formalin Fixative Agents: A Comparative Study of Fixative Efficacy and Histomorphology. *International Journal of Surgical Pathology*: 1–6.

Lewis F, Maughan NJ, Smith V, *et al.* (2001) Unlocking the archive: gene expression in paraffin-embedded tissue. *Journal of Pathology;* 195: 66-71.

Lu K, Collins LB, Ru H, Bermudez E and Swenberg JA (2010) Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasalcarcinoma but not leukemia. *Toxicological Sciences,* 116(2): 441–451.

Ludyga N, Grünwald B, Azimzadeh O, Englert S, Höfler H, Tapio H and Aubele M (2012) Nucleic acids from long-term preserved FFPE tissues are suitable for downstream analyses. *Virchows Arch,* 460: 131–140.

Maaini R and Bryant P (2006) The Effectiveness of Honey as a Substitute for Formalin in the Histological Fixation of Tissue. *The Journal of Histotechnology*, 29, 3: 173-176.

Mcdowell EM and Trump BF (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. *Archives of pathology & laboratory medicine*, 100(8): 405-14.

McKiernan HE and Danielson PB (2017) Molecular Diagnostic Applications in Forensic Science. *Molecular Diagnostics (Third Edition)*.

Mercuri A, Turchi S, Borghini A, *et al.* (2013) Nitrogen biobank for cardiovascular research. *Curr Cardiol Rev, 9*(3): 253– 259.

Mirabet V, Carda C, Solves P, *et al.* (2008) Long-term storage in liquid nitrogen does not affect cell viability in cardiac valve allografts. *Cryobiology,* 57(2): 113–121.

Moelans CB, Hoeve N, Ginkel JW, Kate FJ and Diest PJ (2012) Analysis of Macroscopy, Morphologic Analysis, and Immunohistochemical Analysis. *American Journal of Clinical Pathology,* 136: 548-556.

Moelans CB, Oostenrijk D, Moons MJ, Diest PJV (2011) Formaldehyde substitute fixatives: effects on nucleic acid preservation. *Journal of Clinical Pathology,* 64: 960-967.

Muralidharan K and Wemmer C (1994) Transporting and storing field-collected specimens for DNA without refrigeration for subsequent DNA extraction and analysis*. Biotechniques* 17(3): 420, 422.

Mutter GL, Zahrieh D, Liu C, Neuberg D, Finkelstein D, Baker HE and Warrington JA (2004) Comparison of frozen and RNALater solid tissue storage methods for use in RNA expression microarrays. *BMC Genom,* 5, *88.*

Nagy ZT (2010) A hands-on overview of tissue preservation methods for molecular genetic analyses. *Organisms and Diversity Evolution*, 10(1): 91–105.

Natekar PE and DeSouza FM (2012) A New Embalming Fluid for Preserving Cadavers. *Journal of Krishna Institute of Medical Sciences University,* 1(2): 76-80.

Natekar PE and DeSouza FM (2014) Glutaraledhyde: A New Embalming Chemical Composition for Preservation of Cadavers for Innovative Surgical Procedures. *Indian Journal of Anatomy, 3*(1.s).

National Toxicology Program. Final report on carcinogens background document for formaldehyde. Rep Carcinog Backgr Doc. 2010 Jan; (10-5981): i-512. PMID: 20737003.

Nicholson HD, Samalia, L., Gould, M, Hurst, PR and Woodroffe MA (2005) Comparison of different embalming fluids on the quality of histological preservation in human cadavers. *European Journal of Morphology*; 42(4/5): 178–184.

Nikolaou P, Papoutsis L, Dona A, Spiliopoulou C, Athanaselis S (2013) Toxicological analysis of formalin-fixed or embalmed tissues: a review. *Forensic Science International*, 233(1-3): 312-319.

Nishiguchi MK, Doukakis P, Egan M, Kizirian D, Phillips A, Prendini L, *et al.* (2002) DNA isolation procedures. Methods and tools in biosciences and medicine. In R. DeSalle, G. Giribet, & W. Wheeler (Eds.), Techniques in molecular systematics and evolution (pp. 249–287). Basle: Birkhäuser.

Olsen PF, Mevag B, Staalstrom E, Hovde B, Egeland T and Olaisen B (1999) Extraction of DNA from decomposed human tissue. An evaluation of five extraction methods for short tandem repeat typing. *Forensic Science International*, 105(3): 171-83.

Özkan N, Şalva E, Çakalağaoğlu F, Tüzüner B (2012) Honey as a substitute for formalin? *Biotechnic and Histochemistry,* 87 (2): 148–153.

Paavilainen L, Edvinsson A, Asplund A, Hober S, Kampf C, Pontén F, *et al.* (2010) The impact of tissue fixatives on morphology and antibody-based protein profiling in tissues and cells. *Journal of Histochemistry and Cytochemistry*, 58: 237-46.

Pabst T, Schwaller J, Tobler A and Fey MF (1996) Detection of microsatellite markers in leukemia using DNA from archival bone marrow smears. British *Journal of Haematology*, 95: 135-137.

Pandiar D, Baranwal HC, Kumar S, Ganesan V, Sonkar PK and Chattopadhyay K (2017) Use of jaggery and honey as adjunctive cytological fixatives to ethanol for oral smears. *Journal of Oral Maxillofac Pathology*,21(2):317.

Panzacchia S, Gnudia F, Mandriolia D, Montellaa R, Strolloa V, Merrickb BA, Belpoggia F and Tibaldia E (2019) Effects of short and long-term alcohol-based fixation on Sprague-Dawley rat tissue morphology, protein and nucleic acid preservation. *Acta Histochem,* 121(6): 750–760.

Patel PG, Selvarajah S, Boursalie S, How NE, Ejdelman J, Guerard KP, Bartlett JM, Lapointe J, Park, PC, Okello, JB and Berman DM (2016) Preparation of Formalin-fixed Paraffin-embedded Tissue Cores for both RNA and DNA Extraction. *J. Vis. Exp.* (114), e54299.

Patil S, Premalatha BR, Rao RS and Ganavi BS (2013) Revelation in the Field of Tissue Preservation – A Preliminary Study on Natural Formalin Substitutes. *Journal of International Oral Health,* 5(1): 31–38.

Patil S, Rao RS, Agarwal A and Raj T (2015) Instant Transport Media for Biopsied Soft Tissue Specimens: A Comparative Study. *Hindawi Publishing Corporation Scientifica,* Volume 2015, Article ID 876531, 5 pages.

Patil S, Rao RS, Ganavi BS and Majumdar B (2015) Natural sweeteners as fixatives in histopathology: A longitudinal study. *J Nat Sci Biological Med*; 6: 67-70.

Perry C, Chung JY, Ylaya K, Choi CH, Simpson A, Matsumoto KT, Smith WA and Hewitt SM (2016) A Buffered Alcohol-Based Fixative for Histomorphological and Molecular Applications. *Journal of Histochemistry & Cytochemistry:* 1–16.

Pham PH, Sokeechand BSH, Garver KA, Jones G, Lumsden JS, Bols NC (2018) Fish viruses stored in RNAlater can remain infectious and even be temporarily protected from inactivation by heat or by tissue homogenates. *Journal of Virology Methods,* 253: *31–37*.

Prinz M, Carracedo A, Mayr WR et al (2007) DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic Sci Int, Genet Suppl Series,* 1(1):3–12.

Rao, R (2017) Direct immunofluorescence microscopy of skin biopsy samples preserved in honey. *Journal of American Academy of Dermatology*, 76 (4).

Sabarinath B, Sivapathasundharam B, Sathyakumar M (2017) Fixative properties of honey in comparison with formalin. *Journal of Histotechnol*ogy, 37(1): 21–25.

Sadeghipour A and Babaheidarian P (2019) Making Formalin-Fixed, Paraffin Embedded Blocks. Biobanking: Methods and Protocols, Methods in Molecular Biology, 1897, © Springer Science+Business Media, LLC, part of Springer Nature 2019.

Sago H, Goldberg JD and Lebo RV (1996) Point mutation analysis of archived cytogenetic slide DNA. *Cytogenetic and Genome Research,* 73: 343-346.

Schander C and Halanych KM (2003) DNA, PCR and formalinized animal tissue -- a short review and protocols. *Organisms, Diversity & Evolution,* 3(3): 195-205.

Schoch R, Jenisch S, Haferlach T, Müller-Kuchholtz W, Gassman W and Löffler H (1996) Glass slide smears are a suitable for RT-PCR-based analysis of chromosomal aberrations in leukemias. *British Journal of Haematology,* 92: 140-142.

Sengupta S, Prabhat K, Gupta V, Vij H, Vij R and Sharma V (2014) Artefacts Produced by Normal Saline When Used as a Holding Solution for Biopsy Tissues in Transit. *J. Maxillofac. Oral Surg;* 13(2): 148–151.

Shi K, Shao S and Yin W (2012) An improved non-formaldehyde tissue preservative. *Advanced Materials Research,* 356-360, 360-363.

Shian W, Soenggono A and Pramod SV (2016) Comparison of Tissue Preservation using Formalin and Ethanol as Preservative Formula. *Althea Medical Journal, 3*(3).

Shibata D (1994) Extraction of DNA from paraffin-embedded tissue for analysis by polymerase chain reaction: new tricks from an old friend. *Human Pathology*, 25: 561-563.

Sibley CG and Ahlquist JE (1981) Instructions for specimen preservation for DNA extraction: A valuable source of data for systematics. *Assoc. Syst. Collections Newsletter*, 9: 44–45.

Sinha N, Nayak MT, Sunitha JD, Dawar G, Rallan N and Gupta S (2017) Comparitive efficacies of a natural fixative with a conventional fixative. *Journal of Oral Maxillofacial Pathology,*21:458.

Staff S, Kujala P, Karhu R, Rökman A, Ilvesaro J, Kares S and Isola J (2014) Preservation of nucleic acids and tissue morphology in paraffin-embedded clinical samples: comparison of five molecular fixatives. *Journal of Clinical Pathology*, 66: 807–810.

Stoughton RB and Fritsch W (1964) Influence of dimethylsulfoxide (DMSO) on human percutaneous absorption. *Archives of Dermatology*, 90: 512.

Suhovskih AV, Kazanskaya GM, Volkov AM, Tsidulko AY, Svetlana V, Aidagulova SV and Grigorieva EV (2019) Suitability of RNALater solution as a tissue-preserving reagent for immunohistochemical analysis. *Histochemistry and Cell Biology*; 152:239-247.

Sunderland: Sinauer. France SC and Kocher TD (1996) DNA sequencing of formalin-fixed crustaceans from archival

research collections. *Molecular Marine Biology and Biotechnology*, 5(4): 304-313.

van Eijsden RG, Stassen C, Daenen L, Van Mulders SE, Bapat PM, Siewers V, Goossens KV, Nielsen J, Delvaux FR, Van Hummelen P, Devreese B and Willaert RG (2013) A universal fixation method based on quaternary ammonium salts (RNAlater) for omics-technologies: Saccharomyces cerevisiae as a case study. *Biotechnology Letters,* 35(6): 891–900.

Waller RR and TJK Strang (1996) Physical Chemical Properties of Preservative Solutions— I. Ethanol-Water Solutions. *Collection Forum,* 12 (2): 70–85.

Wang M, Ji X, Wang B, Li Q and Zhou J (2018) Simultaneous Evaluation of the Preservative Effect of RNAlater on Different Tissues by Biomolecular and Histological Analysis. *BiopreservationandBiobanking*,16:6.

White JW (1978) Honey. *Advances in Food Research,* 24: 287-374.

Wille M, Yin H, Lundkvist Å, Xu J, Muradrasoli S, Järhult JD (2018) RNAlater® is a viable storage option for avian influenza sampling in logistically challenging conditions. *Journal of Virology Methods,*252:32–36.

William HY (2019) Biobanking: Methods and Protocols, Methods in Molecular Biology, 1897, © Springer Science Business Media, LLC, part of Springer Nature.

William Kilpatrick (2002) Noncryogenic preservation of Mammalian Tissues for DNA Extraction: An Assessment of Storage Methods. *Biochemical Genetics, 40* (½).

Wineski LE and English AW (1989) Phenoxyethanol as a Nontoxic Preservative in the Dissection Laboratory. *Acta Anatomica,* 136: 155-158.

Wolfe LM, Thiagarajan RD, Boscolo F, Taché V, Coleman RL, Kim J, Kwan WK, Loring JF, Parast M, Laurent LC (2014) Banking placental tissue: an optimized collection procedure for genomewide analysis of nucleic acids. *Placenta,* 35(8): 645–654.

Yokota M, Tatsumi N, Tsuda I and Yano I (1995) DNA extraction and amplification from Giemsa-stained blood smears. *Journal of Clinical Laboratory Analysis*, 9: 387-391.