# Pathology laboratory in Formalin free system


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## Abstract

**Formalin fixation in surgical pathology is a major limiting factor to medical research and development of molecular techniques for target based medicine. Search for alternate solution of formalin fixation in the histopathology laboratory to preserve the macromolecules has led to development of various substitutes with substantial technical advantages. Though DNA and RNA could be extracted from formalin fixed archival material, the limitations have come to forefront, necessitating development of new tissue fixatives preserving the chemical nature of the proteins and antigen epitopes, so that diagnostic and biochemical characterisation is enhanced. The alcoholic fixatives preserve the tissue by causing precipitation of proteins and devoid of cross linking unlike with formalin. The application of newer commercial fixatives like Fine-Fix, universal molecular fixative (UMFIX), RCL2 have been reported to enhance the integrity of macromolecules for molecular analysis along with accurate morphological details for tissue diagnosis. Modified Methacarn fixation provides an ideal combination of maximum staining and morphological preservation of most of the antigens and is cost effective. Zinc based fixatives can avoid formalin and be prepared in the laboratory at low cost. The patented vacuum device facilitates transporting large tissues in a formalin free environment and preserves tissue integrity for diagnosis and tissue banking. Knowledge of the basic chemistry of tissue fixatives, the biochemical changes that take place in the biological materials, by cryopreservation and chemical fixation is essential while undertaking the genomic and proteomic studies on fresh and archival tissue banking.**



**Key words: Alcohol, formalin, nucleic acid extraction, tissue fixation, tissue banking, Zinc based fixatives.**

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# Introduction:

The analytic approach in histopathology underwent a significant evolution as antigenic and genetic evaluation is mandatory now for a rational and meaningful diagnosis in histopathology. This evolution is dependent on optimal tissue fixation for the molecular preservation of human specimens stored in the archives of pathology department. Many different chemicals are available for fixation and the choice depends on the purpose. The traditional method is fixation in 10% buffered formalin, a century old fixative followed by paraffin embedding. However use of formalin as a fixative and preservative for histopathological processing is facing increasing criticisms because of toxicity and environmental pollution. Moreover on long term storage in formalin, the reactive groups in peptides may be oxidised to more stable groups which are not easily removed by washing in water or alcohol and thus creating a major obstacle to medical research.[1]

Though in recent years there are technical improvements in protein and nucleic acid extraction from formalin fixed tissue, quality of phosphoproteins and nucleic acids extracted are not satisfactory for meaningful analysis. More over the analysis of mRNA and DNA from formalin fixed paraffin embedded tissue has been realised to be problematic. For gene expression analysis, the presence of intact and extractable messenger RNA in the test material is mandatory. It is essential to develop alternate methods for the better preservation of the macromolecules (DNA, RNA and proteins) that will preserve the tissue microanatomy also. In the recent years, there have been numerous attempts to find formalin substitutes that are more rational, safe and reliable.

**The main objective of this review is to present briefly the chemistry of formalin fixation with the associated problems and alternate methods of specimen collection in formalin free solutions which preserves the biomarkers without distorting the histomorphology in surgical pathology.**

***This material is available in different places on the internet and it is compiled for the convenience of the pathology residents and practicing pathologists.***

**Why do we need a formalin free fixative and environment in pathology laboratories?**

The potential problems with the use of formalin in histopathology practice are health hazards, degradation of RNA, DNA thus rendering them unsuitable for detailed molecular analysis and cross linking of proteins hampering biochemical analysis. The standard use of formalin as a fixative for histological processing has raised serious issues because of its toxicity and allergic reaction on respiratory system, eyes and skin of the laboratory personnel. As formaldehyde has been labelled as class-1 carcinogen by the International Agency for Research on Cancer, it is important to avoid/ minimise the contact with formalin substantially by the health professionals, technicians and practicing pathologists[2]. Though in most of the modern pathology laboratories the handling of formalin is carried out under the biological hoods, a critical passage still exists due to the transfer of tissues from the surgical theatre to the pathology laboratory. Small biopsies which are directly collected into pre-filled containers cause limited concerns. Problems are encountered with the immersion of large specimens in big containers with formalin, where spillage occurs during its handling freely on a dissection table, not under hood in many pathology departments in developing countries. When the containers arrive at the pathology laboratory, its opening and removal of the specimen constitutes a major cause for diffusion of formaldehyde vapours into the environment. In tropical and developing countries, histopathology work is carried out in non-air-conditioned and indifferently ventilated laboratory space, exposing the technical staff to high levels of formalin fumes emanating from the jars containing fixed tissue. The usual practice of fumigating operation theatres for weekly sterilisation is being replaced by alternate formalin free technologies. Allergic skin reaction to formalin on the hands is often neglected or treated as skin infection. The next important issue regarding the use of formalin as a fixative is the quality of extractable proteins particularly phosphoproteins, DNA and RNA for the proteomics and molecular analysis. Archival formalin fixed, paraffin

embedded tissue samples are used for the detection of protein expression by immunohistochemistry for diagnosis and retrospective research. However the detection of phosphoproteins in formalin fixed tissue is very capricious and difficult [3,4]. This may partly be due to rapid dephosphorylation of proteins in tissues that quickly become oxygen deficient during surgical procedure[5]. If the specimens are not fixed immediately, majority of the phosphoproteins are lost within 60 minutes. Protein phosphorylation is frequently used as an indicator of cellular signalling activity for targeted therapy in controlling cellular proliferation and promoting apoptosis in cancer cells. This therapeutic strategy is in clinical trial in many human cancers, particularly for breast cancers. Hyperactive signalling of growth factor receptors due to over expression or structural alterations is thought to play an important role in cancer pathogenesis[6]. Unusual signalling activities often result in elevated levels of protein phosphorylation in downstream signalling pathways and can be associated with many human cancers[7]. Targeting over expressed growth factor receptors with therapeutic monoclonal antibodies can prevent phosphorylation and activation of cellular proteins. Therefore there is an acute need to evaluate the biology of the disease process and signalling pathways. To achieve this tissue fixative utilised should permit the recovery of macromolecules without extensive biochemical modifications.

Immunohistochemistry is routinely performed in many laboratories as an ancillary method in diagnostic histopathology to detect the abnormal protein expression pattern and expression of altered genes in malignancies. Hence there is a need for efficient detection and accurate assay for biological factors of prognostic and predictive value of different oncoproteins, growth factors and hormone receptors. Additionally, immunohistochemistry is being used increasingly in characterisation of various infectious agents by using specific antibodies to viral, bacterial, parasitic or fungal antigens. The validity of immunohistochemistry in diagnostic pathology depends solely on the quality and specificity of immunostains. In addition to quality of antibodies, a number of factors have a major impact on quality of immunostaining like method and duration of tissue fixation, processing, unmasking of antigenic epitopes and the sensitivity of the detection system [8].

Among the various available fixatives, formaldehyde is the most popular because of its low cost, easy to prepare and preservation of tissue morphology with few artefacts. However formaldehyde fixation causes variable yet reversible loss of immunoreactivity by masking or damaging some of the antigen binding sites. Although such epitopes may be demasked by several epitope retrieval methods, immunohistochemical detection system must still be sensitive enough to produce a strong and non-cross reacting signal. Lastly, there is an increasing need for optimal tissue fixation for assessment of molecular factors and events for a conclusive clinical diagnosis. The molecular approach requires optimal preservation of proteins, RNA and DNA in specimens submitted for diagnosis. Progress towards clinical application of potentially useful markers is hampered by the established routine fixation methods that fail to conserve the nucleic acids and proteins in tissues and limited ability to extract sufficiently high quality RNA or protein from the fixed tissue.

**Evolution of tissue fixation and available methods:**

Biological tissue fixation is a series of complex chemical modification of macromolecules present in tissues to preserve the structural and functional components as close as possible to the living state without autolysis, bacterial and fungal decay[9,10]. Fixation by itself introduces a major artefact.

In 1886 Ferdinard Blum demonstrated that formaldehyde forms methylene compounds with amino, amide and hydroxyl groups and affects the solubility and reactivity of proteins[11]. He was the first person to use formaldehyde as a tissue fixative. To date, formaldehyde as 10% buffered formalin is the most widely used universal fixative because it could fix rapidly and permanently fairly large volume of specimens. The mechanisms by which specific fixatives act to preserve tissues and prevent the loss of macromolecules are broadly categorised into the following groups.

A) Physical methods like heating, microwaving, freeze drying and freeze substitution may be used to preserve tissues. Heat induces precipitation of proteins. Recently glyoxal-based fixatives which do not form vapours when heated at $55^0$C have been introduced as an efficient method of microwave

fixation[12]. Microwave fixation speeds fixation and reduces the time to less than 20 minutes compared to 12 hours in conventional processing.

B) Chemical methods

- Non-coagulant cross linking fixatives create cross-links between the proteins, within individual protein moieties, and nucleic acids altering their tertiary structure and between nucleic acids and proteins. The best examples are formaldehyde, glutaraldehyde and osmium tetroxide.
- Dehydrant / coagulant fixative like ethanol, methanol and acetone act by removing water from the tissues and hence precipitate and coagulate the tissues.
- Salt formation by using picric acid, acetic acid, trichloroacetic acid, mercuric chloride and zinc acetate.
- Compound fixatives – A mixture of reagents like alcoholic formalin, alcohol plus acetic acid, act by causing varied physical and chemical changes in tissues like dehydration, coagulation, salt formation and cross linking.

Each fixative has advantages and disadvantages and does not fulfil all the aims of tissue preservation. These include molecular loss in fixed tissues, swelling or shrinkage of tissues during the processing, variation in histochemical and immunohistochemical staining. Many tissue components are soluble in aqueous acidic or other liquid environments. To minimise the loss of various molecular and macromolecular components including proteins, peptides, mRNA, DNA, lipids, cytoplasmic membranes, rough endoplasmic reticulum, nuclear membranes, mitochondria etc, there is a need to develop universal ideal fixative. A good fixative should be able to destroy the infectious agents within, thereby minimizing the enzymatic degradation and prevent breakdown of tissue (autolytic cystic changes) during long term storage [13]. An ideal fixative should fix the tissue as quickly as possible because delay in fixation results in loss of mitotic index by 30-50% [14]. The fall in mitotic index may result in errors in the grading carcinomas. Lastly it should have long shelf life, compatible for wide variety of tissues, easily disposable and support long term tissue storage[15].

**Brief chemistry of formaldehyde fixation:**

Formaldehyde penetrates tissue rapidly as methylene glycol but fixes slowly as carbonyl formaldehyde which is the rate limiting step in formaldehyde fixation. The principle of formaldehyde fixation is the formation of cross-links by the reactive aldehyde group (-CHO-) between proteins, proteins and nucleic acids as well as formation of coordinate bonds with calcium ions. The cross-links and the co-ordinate bonds may be responsible for the masking of epitopes by altering the three-dimensional structure of proteins. In an aqueous solution, formaldehyde forms methylene hydrate, a highly reactive methylene glycol or methylols with uncharged amino groups. Each methylene bridge cross links an amino group to another functional group. By blocking the amino groups, formaldehyde renders the tissue more acidic thus increasing the negative charge. Only loosely bound formaldehyde is removed by washing for several hours but any bridging that has already occurred may remain. The residual formaldehyde is gradually removed when tissue are stored in water for an extended number of years.

The overall rate of formalin induced modification in DNA is dependent on the concentration, temperature and pH of the fixative. Formaldehyde fixation at room temperature results in poor preservation of high molecular weight DNA, the size of the extracted DNA being directly related to the fixation temperature. Up to 30% of nucleic acids may be lost during fixation [16]. Various studies suggest that degradation of nucleic acids are much less if the tissue is fixed in cold formalin at $4^0$C [17,18].

The average size of DNA extracted from tissues fixed in buffered formalin decreases with increasing fixation time. Attempts to extract usable DNA from formalin fixed tissues for molecular biological studies have been variably successful [19-21]. When compared to the DNA isolated from frozen tissues, formalin fixed tissue exhibits a high frequency of non reproducible sequence alteration. As a result in PCR, the Taq-DNA polymerase fails to recognise the cytosine and incorporates an adenine in the place of a guanosine, creating an artificial C-T or G-A mutation [22]. DNase in tissue is one of the important factors contributing to DNA degradation during fixation. Formaldehyde solution

containing DNase neutralising EDTA is better in preserving tissue DNA [23]. Unfortunately RNA gets fragmented, chemically altered and difficult to isolate in reasonable quantity from formalin fixed tissue [24-26].

Although it is possible to extract proteins from formalin fixed tissue, the rate of penetration of formalin is variable and hence the deeper lying tissue shows significant fluctuation in phosphoprotein analysis in large specimens. Only the in-situ protein examination is amenable by immunohistochemistry providing information about protein expression in a specific cellular population and the site of intracellular localisation. But quantitative analysis of protein analysis is not possible from formalin fixed tissues. The conditions recommended in literature for the use of formaldehyde as a tissue nucleic acid fixative are a) minimum pre-fixation time lag (< 2 hours), b) use of cold 10% buffered formalin, c) addition of EDTA to the fixative, d) adequate volume of the fixative (20 times the size of the tissue) as formaldehyde rapidly becomes hydrated to form methylene glycol and e) duration of fixation of 3-6 hours [27]. Best way is to sample multiple, small, representative bits and fix.

**Alternate methods of tissue procurement / transport:**

Tissue specimens are typically collected in dry ice or snap frozen in liquid nitrogen for the purpose of molecular diagnostic techniques and tissue banking for research. The interval from tissue collection and time to preservation may vary in a hospital/clinical setting and many a time it is not possible to preserve the procured tissue in liquid nitrogen and before freezing the tissue may remain at room temperature for variable time. The perishability of tissue molecules may profoundly be influenced by a number of factors such as 1) temperature and pH fluctuations, hypoxia and dehydration prior to fixation or freezing, 2) choice of preservative and rate of tissue penetration, 3) size of the specimen, 4) extent of handling of the specimen like squeezing or crushing to push into small containers, and 5) introduction of phasphatases, RNAases or proteinases from the environment or from dying cells. The alternate methods available in the literature can be considered in the following groups.

**A) Non formalin based fixatives**: These are non toxic and at the same time maintain the integrity of nucleic acids, proteins and histology. Some non-formalin fixatives can be made in-house from standard chemicals and are cost effective. The shelf life of the prepared fixatives may vary. Some solutions may require refrigeration and others are only stable for a short time.

**Some of the non-formalin fixatives are discussed below.**

**(a) Carnoy's fixative**: It contains six parts ethanol, three parts chloroform and one part glacial acetic acid. Carnoy's fixative is the best for optimal preservation of nucleic acids in tissues and useful for RNA stains like methyl green pyronine as well as for glycogen preservation. RNA could be easily extracted from Carnoy's fixed tissue. However the major disadvantage is the degradation of high molecular weight RNA[28]. This fixative shrinks and hardens the tissue. To counter this, thin bits of tissue 0.5x2x2cm, (stereotactic and endoscopic biopsies) can be fixed for 15-30min and processed for low melting point wax embedding. A modified preparation, also known as Clarke's solution does not include chloroform but contains three parts ethanol and one part glacial acetic acid. This solution produces good general histological results, preserves nucleic acids while lipids are extracted, thus lipoproteins can be lost.

**(b) Modified Methacarn**: Substitution of methanol for ethanol in Carnoys fixative gives methacarn which has been shown to be an excellent fixative for preserving tissue RNA[29]. In contrast to formalin fixed tissues, 300 to 700 - bp fragments of both abundant mRNA and low copy number RNA could be amplified by PCR from methacarn fixed tissues. The integrity of the freshly prepared modified methacran fixative, a mixture of methanol and glacial acetic acid is extremely gentle on tissue membranes compared to formalin and immunohistochemistry can be performed with shorter incubations, higher dilution of antibodies and little need for antigen retrieval[30]

**(c) Zinc based fixatives:** A zinc based fixative (zinc acetate, zinc chloride and calcium chloride in Tris buffer) has been documented to be superior for DNA and protein extraction analysis in a broad spectrum of tissue and does not require heat pre-treatment for antigen retrieval[31]. Recently modified zinc

based fixative, Z-7 ( Zinc trifluoroacetate, zinc chloride, zinc acetate and calcium acetate in 0.1% M Tris–HCl, pH 6.4-6.7) has been reported to be reliable, cost effective and non toxic compared to buffered formalin[32]. The integrity of DNA, RNA and protein appeared excellent, hence molecular analysis on Z-7 fixed paraffin embedded tissue samples is superior to standard formalin fixed tissues. DNA sequences up to 2.4 kb and RNA fragments up to 362 bp in length could be successfully amplified. However zinc fixation causes shrinkage of tissues and may distort the histology.

**(d) Ethanol based fixatives:** FineFIX fixative is a mixture of ethanol (65%-75% w/v), distilled water, glycerol, polyvinyl alcohol and monomeric carbohydrates. The pH of the working solution is also suitable for long term storage. Ethanol based fixatives do not create covalent bonds between proteins; they eliminate the water molecules which surrounds protein and as a consequence, protein coagulates and enzymatic functions are stopped, hence amenable to conventional proteomic techniques. One of the main problems in proteomic analysis using fresh tissues is the difficulty in obtaining homogenous population of cells because normal and pathological structures are often intermixed. In FineFIX fixed and paraffin embedded tissues the morphology is well preserved, thus facilitating mechanical or laser capture microdissection to collect a small cluster of specific cell types. As the integrity of nucleic acids is well preserved, FineFIX treated tissue allows better DNA ( Formalin upto 350 bp, FineFIX over 2400 bp) and RNA ( formalin between 100 - 200 bases, FineFIX upto 600 bases) analysis. The proteins obtained from FineFIX treated samples are comparable in quality with those obtained from fresh frozen tissue thus allowing protein extraction and conventional proteomic analysis[33]. It fixes the tissue rapidly and histological artefacts associated with alcohol based fixative are absent.

**(e)** Multipurpose ethanol based fixative containing phosphatase and kinase inhibitors preserves phosphoproteins as well as maintains tissue histo-morphology for frozen sectioning and paraffin embedding. RNA integrity is also maintained for at least 72 hours at room temperature. This multipurpose fixative also allows immunohistochemistry and proteomic analysis[34].

**(f) HOPE fixation** ( Hepes-glutamic acid buffer mediated organic solvent protection effect ) is an excellent technique for complete pathological analysis including immunohistochemistry and molecular pathology. The protection solution comprising of a mixtures of amino acids at pH 5.8 to 6.4, penetrates the tissue by diffusion. This is followed by incubation of fixed tissue in acetone at 0-4$^0$C for dehydration and subsequently the specimens are directly transferred into low melting paraffin and embedded. HOPE fixed sections have been shown to exhibit formalin like morphology and provide an excellent preservation of proteins and antigenic structures for differential analysis by immunohistochemical and/or enzyme histochemical techniques. Sufficient amount of good quality DNA and RNA can be extracted even after a period of five years from HOPE-fixed specimens[35] and suitable for molecular analysis by PCR, RT-PCR and *in-situ* hybridization[36]. Absence of cross-linking and greater yield of extractable nucleic acids suggests that HOPE fixation could be a distinct alternative method for tissue banking.

**(g) Acetone-methylebenzoate-xylene**(AMeX) fixative offers good morphology along with good quality of high molecular weight DNA[37]. This method involves overnight fixation of tissues in acetone at -20$^0$C followed by clearing in methylbenzoate and xylene before paraffin embedding. Good morphology, immunoreactivity and extraction of good quality of high molecular weight spooled DNA has been reported using this technique. In addition, RNA comparable to fresh frozen tissues could be detected by dot-blot hybridisation using RNA isolated from AMeX fixed tissues[38-40].

**(h) Universal molecular fixative** (UMFIX) is a methanol, polyethylene glycol based fixative and usually coupled with microwave assisted rapid tissue processing. UMFIX is very useful for small biopsies to amplify small ampicons by RT-PCR. This method has been recently introduced by Vincek et al to extract high molecular RNA from laser captured microdissected samples obtained from paraffin embedded blocks prepared for histologic diagnosis[41]. Tissue can be fixed in room temperature with UMFIX, Immunoreactivity of UMFIX fixed tissue is comparable to formalin fixed tissue. The quality and quantity of mRNA and DNA extracted from frozen and UMFIX fixed tissue are essentially similar.

**(h) RCL2** is a formalin free fixative containing ethanol, acetic acid and complex carbohydrate. RCL2 fixed tissue can be kept at room temperature or at $-20^0$C when an ultra high molecular quality is required. Morphology, tinctorial quality and immunoreactivity is similar to formalin fixed tissue. Quality of RNA and protein profile from RCL2 fixed paraffin embedded tissue is comparable to frozen tissues[42].

**B) Tissue transfer to histopathology laboratory under vacuum:**

Vacuum sealed device may serve as an ideal alternative to transport larger specimens from the surgical theatre to the pathology laboratory for histological evaluation. This can also be used to store and transport specimens for transplantation and tissue banking [43].

Tissues removed after surgery can be transferred either fresh or partially fixed in a tightly closed container under vacuum. The process of evacuating and sealing usually does not take more than 30 seconds. However on rare occasions under the vacuum, tissues can be kept in the refrigerator at $4^0$C up till one week. The vacuum device can be kept in the surgical theatre where it can be conveniently used by the personnel involved. The evacuation of the containers containing the tissue specimens is carried out by reducing the pressure to at least below 100 mbar in the vacuum device. Plastic bags are used as containers because of flexibility.

Advantages of vacuum sealed bags are (a) there is no chance of drying because of high vacuum conditions, (b) autolysis is slowed down due to absence of air, (c) faster cooling of tissues due to lack of insulating air all round, (d) preservation of real life colours of the specimens in the absence of the formalin, (e) preservation of morphology up to 64 hours to 9 days or more with optimum histological preservation, (f) excellent routine histology, histochemistry, immunohistochemistry and FISH technique can be achieved, (g) provides excellent material for tissue banking, (h) quality of nucleic acid is better if refrigerated under vacuum, (i) eco friendly and totally formalin free, (j) large specimens also can be easily sent by post from distant places as the containers are light compared to heavy formalin filled containers.

**C) Tissue preservation for biobanking:**

For bio-banking, transportation on ice, snap freezing by liquid nitrogen, and storage in low temperature freezers (- 80 ) are recommended. The use of RNAlater solution prevents RNA degradation in tissues and stabilizes the expression levels of selected genes. Excellent quality RNA and reliable expression patterns could be obtained even after 2 weeks from tissue stored in RNA later solution at $4^0$C. However tissue morphology is better in snap frozen compared to RNAlater preserved samples.

Following clamping the arterial supply of the tumour, within minutes the genes in the tissue start switching on and off, trying to adapt to the hypoxic/anoxic environment, yet utilising the remaining nutrients in the tissue, thus changing their gene expression pattern. The freeze thaw cycles in the presence of cryoprotectants cannot shield the genome from stress and the cells enter apoptotic cycle. DMSO, the commonly used cryoprotectant is found to have the potential of amplifying the genes related to metastatic potential thus representing the cancer banked to be a 'super cancer'. Keeping the morphology of cancer cells intact, some of the cryoprotectants can lead to over estimating the molecular quality of biospecimens, thus partly corrupting the genomic data.[44]

All the cryopreservents and tissue fixatives, have limitation, yet facilitate understanding the cellular and molecular biology of diseased tissue. The formalin free system makes the histopathology laboratory eco-friendly and integrates the routine cellular pathology with molecular pathology to understand the dynamic molecular events in health and disease.

**Some Recommendations for our Laboratories.**

In India we have not entered into the age of formalin free environment yet. Initially a pragmatic approach in handling formalin related mishaps in anatomic pathology laboratories is suggested.

(a) Avoid scrupulously the habit of sniffing the chemicals in histology laboratory.

(b) Use prefilled containers for transport of biopsies, so that the lids are opened for a short time to remove the specimens.

(c) Formalin spill in anatomical pathology laboratories fall into the category of Manageable Chemical Spill Category. Formaldehyde gas evaporates depending on the surface area but not the spilled fluid volume. Use Latex or Nitrile gloves and wear a disposable gown and goggles to minimize the contact with skin and eyes. Contain the spill by bordering the area with wet filter paper or cloth, to absorb as much as possible. The absorbing cloth is to be moved from the periphery to centre. The soaked cloth/paper should be collected in plastic bag and kept in a closed container. Enhance ventilation at the grossing station and increase humidity in the room by running a tap, to diminish formaldehyde evaporation. Mop up the spilled area with generous amount of soap water.

(d) Acute exposure to formaldehyde vapours (0.8 to 1 ppm) causes throat irritation. Drinking milk or alkaline drink is optimal or keep in mouth and gargle as emergency measure to minimize mucosal damage.

(e) Eye wash with cold water after formalin splash is obligatory. It is sufficient to wash with clean cold water for two minutes. Eye washing stations should be installed in all histopathology laboratories.

(f) Back vented histology laboratory hoods – Grossing Working Station is to be installed in the laboratories – especially those handling large specimens sent in formalin. After formalin fixation, the washing of large surgical pathology specimens like colon, breast and washing formalin fixed brain after autopsy should be carried out under a hood.

(g) Formalin warms up in the bucket with the organs by the end of the day by chemical reaction during fixation. Place the bucket in a cooler place and pour cold water before opening the lid if possible in a dedicated place in the lab.

(h) Avoid heating the formalin for fixation in non-laboratory microwave.

(i) Try to discharge used formalin in small volumes into waste water system, to reduce the damage to biology of waste water treatment plant.

(j) Introduce Passive Badge Monitors for all the lab personnel in an anatomical pathology laboratory and contain the formaldehyde level to below 0.5 ppm in the environment for the safety of the lab personnel.

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