MILESTONE
HELPING
PATIENTS

FineFIX (Formalin Substitute)

Handbook of Protocols for: Fixation & Immunohistochemistry

MM029

Thank you for having selected our system and welcome to the ever growing world club of users for Milestone laboratory instrumentation.
We are sure that you will be completely satisfied with this new tool entering your laboratory.
We invite you to read carefully this operator manual and to keep it in reach for convenient and fast consulting.
For any possible clarification or any request for assistance please contact either our Representative in your country:

Or contact

Milestone s.r.l.
Via Fatebenefratelli, 1/5
24010 Sorisole (BG) Italy
Tel. +39.035.412 8264
Fax +39.035.575498
web site www.milestonemedsrl.com
e-mail medical@milestonesrl.com

Please read the user manual before using the device.

MM029-005 – FineFIX – Operator Manual
2015-04

SUMMARY

1. INTRODUCTION

1.1. Symbols used

An instruction accompanied by this symbol provides important information and requires more attention.

An instruction accompanied by this symbol provides a cautionary statement: failure to follow the instruction may endanger the operator or cause damage to the instrument.

Biohazard: be careful when you execute the procedure marked with this symbol: danger of biohazard contamination.

CE logo: this instrument complies to European Community directives.

IVD medical device according to 98/79/EC directive.

Manufacturer.

Production date.

Expiration date.

Storage temperature.

Lot number.

Catalog number (reference).

1.2. Intended uses

FineFIX is a patented formalin-free, water-based concentrate tissue fixative. FineFIX working solution is prepared by mixing 1 part of FineFIX concentrated to 3 parts of ethanol (99%).
The ethanol concentration in the working solution of FineFIX is approximately 70%. This concentration was found to produce good histology and to allow optimal recovery of DNA/RNA and proteins, sufficient for several downstream molecular analyses.
When diluted with ethanol, its formulation of low toxicity additives overcomes the drawbacks commonly associated with the use of pure ethanol or ethanol based fixatives, e.g., significant tissue shrinkage, vacuolization and pyknotic nuclei. FineFIX also provides optimal preservation of tissue antigens, nuclear and cytoplasmic morphology and reduced lysis of red blood cells with preservation of the cytoplasmic membranes.

DO NOT USE THE REAGENT FOR DIFFERENT USES FROM THOSE LISTED ABOVE.
In case of doubts please contact: application@milestonemedsrl.com.

1.3. Transport and storage conditions

Handle with care and store in a cool dry space using a tightly closed container.

1.4. Warning information

Use gloves before manage the reagent.

For a better biopsies preservation, especially needle biopsies, the usage of sponges is recommended avoiding the samples drying or attaching to the cassette or floating on the liquid surface.

Be sure that such sponges are well impregnated of solution in order to eliminate air bubbles which can cause the uncomfortable drying of the tissues.

The sponges, as like as thick paper filter, could affect the processing time as a barrier between the tissue and the reagents.

In course of change from formalin to FineFIX, we advise to use FineFIX as a secondary fixative to be used into the processing machine, letting the use of formalin for the primary fixation while the tissues transportation from the clinic to the histology laboratory.

Formalin is a fixative and may produce harmful vapors.
All work with formalin at any temperature must be performed in a fume hood.

Do not breathe formalin vapors especially when warmed.

Processing “mirror blocks” during the evaluation/changing period should be adopted for a better acquisition of the new fixative.

The formalin artifacts, even if sometimes they are diagnostic, are not present when using an alcoholic based fixative. Thus it means that the tissues could have a better and realistic appearance showing the architecture as it should be in reality.

1.5. Waste disposal

Disposal of FineFIX Working Reagent (exhausted): after the use, treat it as hazardous. Waste material should be disposed in an approved incinerator in compliance with all federal, provincial and local government regulations.

Disposal of expired FineFIX Concentrate (not yet used): disposal must be done according to official regulations.

2. SETTING UP

2.1. Introduction to FineFIX

Formalin creates methylenic covalent bonds and realizes the protein "cross-linking" intra and intermolecular, altering the conformation of macromolecules.
The FineFIX is a coagulant fixative which leads to a reversible denaturation of the proteins with the result that antigen retrieval, immunohistochemistry and molecular biology are better. Alcohol alone has these positive effects but altering the morphology and creating artifacts. The FineFIX instead contains other additives in low percentage, which allow the fixation of solid and smear tissues, thus replacing the formalin and achieving excellent results even from morphological point of view.
Its major advantage is the absence of carcinogenicity than the formalin.

FineFIX is a formalin-free fixative. After preparation of the working solution, read carefully the enclosed Handbook of protocols before starting to operate with FineFIX. Failure to do so will no allow satisfactory results of the fixation procedures.

For I.H.C. (Immunohistochemistry) make sure to carefully read and follow the guidelines at A Appendix – Immunohistochemistry guidelines on FineFIXED tissues.

2.2. Unpacking

FINEFIX CONCENTRATE

Code 70148 Tank of 5 litres FineFIX concentrated
Code 70149 Tank of 10 litres FineFIX concentrated

2.3. How to prepare FineFIX working reagent

The working solution must be prepared by adding 720 ml of ETHANOL reagent pure/denatured to 280 ml FineFIX Concentrate.

280 ml FineFIX Concentrate
+
720 ml Ethanol

II

1000 ml Working Solution

Never dilute this prepared solution with saline or other solution.

2.4. General rules

A. Open the bottle and take only the necessary quantity of FineFIX concentrate considering that the volume of working solution (FineFIX+Ethanol) must be at least five times that of the specimen mass.

B. Mix FineFIX concentrate with Ethanol (99%) in an intermediate container according to proportion defined on label. When the necessary quantity of FineFIX has been taken, close the bottle and store in a cool area.

C. FineFIX working solution has to be placed into the specimen container before the specimen, to avoid the possibility of tissue adhering to the wall of the container and therefore inhibit homogenous penetration of fixative. If sticking to the container wall occurs in the first instance, simple agitation of the specimen container will detach it from the container wall.
The penetration of this alcohol based fixative is relatively fast, compared to formalin fixation, producing optimal fixation in a short time. Whereas the actual fixation times are not so different to that of formalin.

D. Microwave-stimulated fixation with working solution (FineFIX+ethanol) is particularly suited for producing a dramatic reduction in fixation times, without the presence of artifacts. For example, a small biopsy may be completely fixed in 10-15 minutes at a temperature of 50°C. It is important to note that the microwave instrument quality must be laboratory grade, which allows accurate temperature control to avoid overheating induced artifacts.
Complete fixation of tissues is of paramount importance whether fixed at room temperature or with microwaves, since histoprocessing of under fixed tissues will produce processing artifacts.

E. Place the fresh specimen immediately into working solution before being sent to the surgical pathology laboratory, thus avoiding any artifacts attributed to delayed fixation. This practice is the same as that used with conventional formalin fixation following surgical excision. In cases where immediate placement of the specimen into fixative is not possible, then ensure minimal delay in transportation to the laboratory and place into fixative on arrival.

F. When dealing with the larger gross organs, as in the case with formalin fixation, the penetration of fixative to the inner aspect is rather slow, risking autolytic changes. In situations like this there are two courses of action:

The first is that the surgeon opens the specimen, allowing the fixative to penetrate more effectively. This procedure is frowned upon by pathologists as it may create artifacts in the appearance during gross description and cutting.

The second option is to put the container (specimen with working solution) in a refrigerator, reducing the risk of autolysis to the tissue, without compromising the morphology.

An added feature of this alcohol-based fixative is that it does not freeze enabling us to place the specimen in at traditional temperatures available as low as minus 20°C, further reducing risk of autolytic changes, without freeze- thaw artifacts.

G. The working solution for cytological use is an effective alternative to the present fixatives used for cytological preparations, either by immersing the slides in the fixative or using it as a spray.

H. Frozen sections will also benefit with the use of working solution with optimal results better than existing fixatives. The immersion of slides for 60 seconds in preheated (37°C) FineFIX working solution gives simultaneous: specimen fixation, dehydration and clearing.

Any bottle of FineFIX concentrate is identified by a lot number; we suggest to completely use a bottle before opening a new one identified by a different lot number with a more recent date. However it is possible to mix different quantities of FineFIX concentrate from different lots; in this case it is responsibility of the user to assure the correct management and traceability of the lot numbers used.

3. OPERATE WITH FINEFIX

At the macroscopic level, the use of FineFIX (Formalin Substitute) as a fixative for histology is not so dissimilar from that of formalin, however its action on tissue is different and is reflected in the macroscopic appearance of the specimen, i.e. the consistency of the specimen will be increased, but less than that of formalin, with preservation of tissue elasticity (rubbery consistency).
The macroscopic color produced with FineFIX will be akin to the fresh state of the specimen, as opposed to the greyish appearance of formalin-fixed material.

3.1. Gross specimen fixation with the working solution

To ensure optimized fixation with the working solution the following guidelines are recommended for tissues received, either as a biopsy or, the larger gross specimen. This will avoid the difficulties associated with inappropriate handling and fixation procedures and highlight the benefits for morphology and handling of specimens with this formalin substitute.

The following list of tissue types have been tested with the working solution, received as a biopsy and the larger gross specimen.

3.1.1. Breast

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for fresh **fine needle core** biopsies. For **core biopsies of larger dimensions** fix at least 90 minutes before processing, or use microwave-stimulated fixation.	**Nodulectomy**: if the largest diameter of the specimen doesn't exceed 2cm, put the material in the fixative without bisecting it. When dimensions are larger, one needs to ink the margins then immediately cut the specimen into slices of 5 mm thickness and pin them out onto cork board. In this way the penetration of the fixative will be optimal. **Mastectomy**: to obtain optimal fixation of the fresh specimen it is necessary to "bread loaf" cut (slice thickness about 1cm). This operation will avoid the formation of artifacts (detachment of cells from basement membrane) present in specimens left in the fixative for long periods of time, without "bread loaf" cuts. For **axillary nodes**: make some cross cuts, this allows a rapid penetration of working solution and allows for an easy detection as they appear white in color, contrasting with the yellow color of adjacent fat.
MICROWAVE ACCELERATED FIXATION	
Fresh **fine needle core** biopsies require 10 minutes of microwave treatment at 50°C. **Larger biopsies** (trucut) need an extended time of 30 minutes.	For microwave-stimulated fixation, take representative blocks from the fresh material. Minimum fixation time is around 45 minutes at 50°C, more than other parenchyma's because of the large amount of adipose tissue.

3.1.2. Bone

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Bone Marrow and Bone Biopsies: allow an adequate penetration of the fixative and then decalcify the material. The duration of decalcification depends on the type of bone and on the decalcifying solution used.	Fix before decalcification. For **large osseous fragments** is recommended to saw them beforehand (slice thickness of about 4-5 mm) to improve the penetration of fixative and decalcifying solution.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C, before decalcification.	Thoroughly fix the specimen (time duration depends from the dimensions) followed by decalcification by use of microwaves.

3.1.3. Central Nervous System

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
The dimensions are usually very small (stereotactic biopsies): use polypropylene bags to avoid the loss of material. Minimal fixation time is about 30-40 minutes at room temperature.	This covers the full range of brain and pituitary lesions and material excised from lesions of sella turcica. Large quantities are rare events (some meningiomas or gliomas) and cutting is usually not necessary.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is around 30 minutes at 50°C.

3.1.4. Esophagus

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	It's preferable to receive fresh material, and avoid delays in reaching your laboratory the day after, especially if it has both extremities closed. In that case, the penetration of the fixative will occur via the serosa and musculature, rich in connective and muscular tissue, which are particularly resistant to the penetration of fixatives. Open the esophagus longitudinally from one end to the other; if possible dissect the perivisceral fat. The open esophagus may be distended (mucosal side up) on a corkboard and fixed with pins. Place the material in a container with the fixative and fix overnight, followed by traditional processing.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, correct fixation is extremely important for avoiding the formation of processing artifacts. Time needed for fixation of the fragments: is around 30 minutes at 50°C.

3.1.5. Lymph nodes, Thymus, Spleen

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for biopsies, one hour for the small fragments.	**Lymph nodes**: cut the nodes longitudinally in two halves. If the dimensions are larger, make multiple slices (about 5mm thick). **Spleen**: the consistency of this organ makes it necessary for "bread loaf" slicing at a thickness of around 0.5-1cm to obtain optimal fixation. **Thymus**: to allow good fixation, the gland must be sliced (parallel). When a cyst is present, empty the contents and then fix.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is around 45 minutes at 50°C for spleen, 30 minutes for thymus and nodes.

3.1.6. Intestine (Jejunum, Ileum, Colon)

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes. In biopsies for celiac disease the material is usually oriented with agar. It doesn't interfere with fixative penetration.	**Polipectomy**: place the polyp into the fixative for sufficient time (fixation time is related to the dimensions of the neoplasia). Prefixation is important because the material is very delicate and when cut it tends to separate in small fragments (especially true for some villous type of lesions). FineFIX preserves quite well the structure of these tumors, with minimal loss of material. **Surgical specimens**: absolutely important to receive fresh material or material preserved in a refrigerator covered with sufficient working solution. Open the bowel longitudinally through its entire length, eliminate the lumenal content. Pin the specimen on a corkboard and fix adequately. It's preferable to dissect the bowel wall from the perivisceral fat. When the length of the specimen is great (especially for ileal resection with infarct) it is almost impossible to fix the material on a corkboard. In this case, fix the segment where representative section for histology will be taken from and immerse the remaining part of the specimen in a large container of fixative. For intestinal infarcts it is absolutely important to empty the lumenal contents of necrotic debris and bloody material as it will significantly interfere with fixative penetration. When large tumors are present, some cuts make fixation much more homogeneous. Cuts in the perivisceral fat allow a simple recognition of lymph nodes. In fact they will assume a white color in contrast with the yellow color of the fat. **Appendicectomy**: make some cross cut slices, sparing the distal third for the longitudinal section. When it is atrophic, cuts aren't necessary, because the lumen is usually filled with fibrous material.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	Material taken from fresh tissue can be fixed with microwave in around 30-40 minutes at 50°C.

3.1.7. Heart and Vessels

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Endomyocardial biopsies for transplant follow up or for intrinsic heart pathology: place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	If the dimensions of the specimen are not large (less than 2cm in largest diameter), place the material into the fixative without cutting it. For larger dimensions, "bread loaf" cut into slices approximately 1cm thick. In the case, of whole heart examination (specimen from a transplant): fresh examination followed by representative sections for histology and fixation of the organ in a large volume of fixative for further sections if necessary. **Valve replacement**: place the valve into the fixative and for heavily calcified valves allow for longer penetration of working solution before use of decalcifying agent. **Vessels**: the relative low thickness allows placing the material directly into the fixative without slicing it.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is 30 minutes at 50°C.

3.1.8. Kidney and Adrenal Gland

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature for about 30-40 minutes.	Surgical specimens (polar resection, nephrectomy): it is important to receive the material fresh because renal parenchyma (cortex structures) is particularly delicate and the frequent presence of perivisceral fat may interfere with the penetration speed of the fixative, leading to the formation of artifacts in the histological slides. Cut the kidney sagittally, open hilar vascular structures and ureter or inject them with fixative. On the two halves of the kidney make other cross cuts (thickness about 1-1.5cm) to allow a perfect penetration of the working solution. When a neoplasia of the pelvis or ureter is present, open them carefully to allow a rapid and good penetration inside the complex tumoral structures. For ureteral neoplasias, where dimensions aren't usually large, it is also possible to make some cross cuts (at a distance of about 1cm from each other) as an alternative to the above mentioned procedure. Adrenal gland is frequently present in the specimen of radical nephrectomies, in this case, a cross cut will allow optimal fixation. In cases, of intrinsic adrenal pathology (myelolipoma, neoplasms) the dimensions of the specimen may be very large; in those cases it is necessary to cut the specimen.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take the representative from the fresh material. Minimum fixation time is 30 minutes at 50°C.

3.1.9. Larynx

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	Open the larynx along the posterior midline, eventually fixing it to a corkboard and place into the fixative. When calcified areas are present, ensure thorough fixation is performed before commencing decalcification.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material, place them into cassettes and microwave fix. Placing the tissue in the cassette will avoid twisting of the specimen during fixation. Correct fixation is extremely important for avoiding the formation of processing artifacts. Time needed for fixation of fragments is around 30 minutes at 50°C. For calcified specimens, allowing fixation it is possible to accelerate decalcification by the use of microwaves.

3.1.10. Liver, Gallbladder and Pancreas

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	**Partial hepatectomy, segmental resection**: “bread loaf” slice the specimen, with the thickness of the parallel slices about 1-1.5cm. When the organ presents a high content of fibrous tissue (cirrhosis for example) it is preferable to reduce the thickness of the slices. For neoplasias of the bile ducts, open them longitudinally and fix. **Colecystectomy**: empty the lumen and place the specimen into the fixative fluid. Bile present in the lumen for a prolonged period can cause loss of epithelial layer; for this reason it is important to receive the specimen soon after excision. **Pancreatectomy**: “bread loaf” slice the parenchyma with slices about 1cm in thickness. Thick to avoid extensive autolytic changes. If a duodenal segment is present, open it longitudinally, identify the region of papilla and individuate coledocus and pancreatic duct. Ensure that the fixative accesses these structures, even if it requires filling using a syringe.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, thorough fixation is very important to avoid formation of artifacts during processing. Time needed for fixation of the fragments: is around 30 minutes at 50°C.

3.1.11. Lung and Pleura

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	**Wedge biopsies, pleural fragments**: when the largest diameter of the fragments is less than 2cm, place the material into the fixative without slicing. For larger dimensions it is preferable to do some “bread loaf” slicing. **Lobectomy, pneumonectomy**: open the main bronchi (especially in cases of large endobronchial masses) and slice the parenchyma with a sharp knife. The “spongy” structure of lung allows a rapid penetration of fixative. If there is a large peripheral tumour, cut it preserving the relationship with pleural surface. In cases of pleuro-pneumonectomy for malignant mesothelioma, cut carefully the thick pleura and the adjacent pulmonary parenchyma (remember that the rich content in connective tissue of pleural neoplasias interferes with the penetration of the fixative).
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is 30 minutes at 50°C.

3.1.12. Oral Cavity, Mandible and Maxilla

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for biopsies, one hour for the small fragments.	There is great variation in size and kind of material: from small tumors excised in the oral cavity to mandibulectomy or maxillectomy. For small fragments, made of soft tissue (not bone or teeth), place them into the fixative. For wide resections cut the material, not altering the spatial relations between organs and place it into the fixative. Take representative sections for histology and then decalcify. When mandible or maxilla are present, cut with a saw before decalcification. For teeth an adequate time of fixation is necessary, since the bone density will require the use of prolonged decalcification times.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, the correct fixation is very important to avoid the formation of processing artifacts. Time needed for fixation of the fragments: around 30 minutes at 50°C. For specimen with an osseous component, microwave-accelerated decalcification can be performed before fixation.

3.1.13. Ovary and Fallopian Tube

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Small fragments (wedge biopsy of ovary, tubal ligation): place the material immediately into the fixative and follow the same procedure as with formalin. Fixation time depends from the dimension of the specimens.	**Ovariectomy**: frequently ovarian masses have a cystic component. Empty the content of the cyst and make some cuts in the solid portion of the tumor, not altering the relationship between capsule and neoplasia and between ovary and tube, when present. **Teratomas**: eliminate the pilosebaceous material to favor the penetration of the fixative. When teeth or bone islands are present, ensure thorough fixation before decalcification. **Solid neoplasias or neoplasms with a rich fibrous component** (fibromas, thecomas and Brenner's tumours): "bread loaf" slice with at a thickness of about 1-1.5cm, to allow an adequate penetration of the working solution. **Salpingectomy** for inflammation, neoplasia or tubal pregnancy: cross section cut the entire length of the specimen. For tubal pregnancy the presence of blood clots may interfere with penetration of fixative, careful slicing will allow optimal fixation. **Cysts and vaginal fragments**: usually have small dimensions and may be placed directly into the fixative without slicing.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative from the fresh material. Minimum fixation time is about 30 minutes at 50°C. For neoplasias with a rich fibrous component extend the time to 40 minutes.

3.1.14. Prostate

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Biopsies and transurethral resection (TUR-P): place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for biopsies and about one hour and half for prostatic chips from TUR-P.	**Adenomectomy** for nodular hyperplasia: cut the fragments whenever the largest diameter exceeds 2cm. **Prostatectomy**: to perform optimal fixation the organ must be sliced with sections of about 0.5cm in thickness. This procedure is necessary because the capsule and the richness in connective and muscular tissue of prostatic parenchyma interferes with rapid penetration of the fixative. Place some absorbent paper between the sections and wrap with a rubber band to reduce irregularities on the cut surfaces after overnight fixation.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative from the fresh material. Minimum fixation time is 30 minutes at 50°C.

3.1.15. Salivary Glands

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	**Major Salivary Glands**: it is preferable to slice them to allow a rapid and homogeneous penetration of the fixative. **Radical neck or submandibular resections**: dissect the individual components of the specimen, slicing the salivary gland, the muscle and the fat; this allows a good penetration of the fluid and an easy recognition of nodes.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 10 minutes of microwave treatment at 50°C.	In microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, thorough fixation is very important avoid the formation of artifacts during processing. Time needed for fixation of the fragments: around 30 minutes at 50°C.

3.1.16. Skin

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
For small fragments or larger but without subcutaneous fat: place into the fixative without cutting. When the dimensions are large pin the specimen on a corkboard.	For large fragments without subcutis: place the representative material into the fixative, eventually pinning it out on a corkboard. For large specimen with subcutis present: the ideal procedure is to take cut sections from the fresh material.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material needs about 20 minutes (because of the large content of collagen) of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is around 45 minutes at 50°C, more than for other parenchymas because of the large content in collagen (including fat).

3.1.17. Soft Tissues

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	The dimensions may vary from a relatively small fragment to a regional excision for sarcoma. It is important to receive the material as soon as possible and take sections for histology (and staging) immediately. If fixation of the entire specimen mass is necessary, make some “bread loaf” cuts, preserving the relationship between the anatomic structures present in the specimen.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 20 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is around 30 minutes at 50° C.

3.1.18. Stomach

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	Open the specimen along the greater curvature, dissect the perigastric fat for lymph nodes, subdividing them on the basis of their location. Place the stomach on a corkboard, fixing it with pins, then into a container of fixative overnight, followed by traditional processing. Avoid the specimen from standing too long without fixative with both extremities closed as there is the possibility of marked autolysis of the mucosa due to the enzyme/acid contents of the stomach.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, correct fixation is very important to avoid the formation of artifacts during processing. Time needed for fixation of fragments: is around 30 minutes at 50°C.

3.1.19. Testis

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	**Orchiectomy**: the presence of tunica albuginea makes difficult the penetration of fixative inside the testicular parenchyma. For this reason it is very important to receive the specimen soon after excision. Cut the testis sagittally and make also some cross section cuts on the two halves, not complete, to preserve the relationship between parenchyma and tunica albuginea. The number of sections is a function of the dimension of the specimen. In cases of neoplasia with necrotic areas, reduce the thickness of the cuts to allow optimal fixation of the residual vital areas. Some cuts may be done also on the Epididymis and Spermatic Cord (cross sections).
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is 30 minutes at 50°C.

3.1.20. Thyroid and Parathyroid Glands

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes.	**Thyroid**: the large majority of these relate to non-neoplastic pathology (nodular goiter, Grave's disease, Hashimoto's thyroiditis), in such cases there is an increase in the dimensions of the gland and it is necessary to slice it. Sometimes calcified areas are present. In these cases allow thorough fixation, taking sections for histology from the soft areas, before the use of decalcifying agents. **Parathyroid**: weigh them and immerse in the fixative. If the dimensions are large, slice longitudinally to allow thorough homogeneous fixation.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material and microwave fix. In this case, optimal fixation is very important to avoid the formation of artifacts during processing. Time needed for fixation of these fragments: is around 30 minutes at 50°C. For calcified specimens, after fixation it is possible to accelerate decalcification with the use of microwaves.

3.1.21. Urinary Bladder

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Biopsies and transurethral resections (TUR-B): place the material immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for biopsies and about 1 hour for TUR-B.	**Cistectomy** with or without adjacent organs such as prostate or uterus, there are two options: the first is to fill the bladder with the fixative using a syringe, the second is to open it with scissors through the anterior wall and pin onto a corkboard. The second option is preferable when a large tumoral mass occupies the cavity or in cases of highly infiltrative tumors and it may be necessary to make several cuts. The bladder cancer frequently has a papillary appearance; for this reason it is of paramount importance to receive the specimen fresh or soon after excision. Representative sections for histology may be taken from the fresh material or after overnight fixation. The second option is preferable. In the case of a papillary tumour: the delicate structures may become slightly more resistant (firm) to cutting after fixation. When prostate or uterus are present, follow the specific instruction for each tissue type.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C.	For microwave-stimulated fixation, take representative sections from the fresh material. With papillary neoplasms it is preferable to take large fragments, fix them and then take sections for histology. Minimum fixation time is around 30 minutes at 50°C.

3.1.22. Uterus and Placenta

BIOPSIES	SURGICAL SPECIMENS
ROUTINE FIXATION	
Cervical, endometrial biopsies and endometrial curretings: are placed immediately into the fixative and follow the same procedure as with formalin. Minimum fixation time, at room temperature, is about 30-40 minutes for biopsies, about one hour for curretings. Be careful, when dealing with curretings, the presence of large quantities of blood may inhibit the penetration of fixative and affect optimal fixation time.	**Cervical conization**: fresh material is the ideal situation; cut longitudinally with scissors and pinned onto a corkboard. In this case, it is preferable to fix the specimen for 24 hours because of the delicate nature of cervical epithelium, in contrast with the cervical wall, and if the sections for histology are performed on fresh or poorly fixed material, there is the possibility of epithelial detachment. **Hysterectomy**: open the uterine cavity to allow thorough fixation of endometrium. In the presence of leiomyomas make some cuts along the longer aspect. Optimal fixation of fresh material is necessary to obtain the best results, especially in cases of malignancies involving endometrium and myometrium. **Placenta**: for optimal fixation it is recommended to wash away part of the blood present (using a small quantity of fixative) and then make multiple parallel cuts of around of around 1-1.5cm (the large quantity of blood present interferes with the penetration of fixative). For amniotic membranes there is no problem and for umbilical cord it may necessitate some cross cuts, especially if it is edematous.
MICROWAVE ACCELERATED FIXATION	
Fresh biopsy material requires about 10 minutes of microwave treatment at 50°C. For curretings where large quantities of blood clot are present it is recommended to prolong the time to 30 minutes.	For microwave-stimulated fixation, take representative sections from the fresh material. Minimum fixation time is around 30 minutes at 50°C for endometrium and 40 minutes for leiomyomas and cervical cones.

3.2. Rules For successful FineFIX application on LARGE specimens

1. Place the tissue into the fixative as soon as possible. Avoiding delays. (recommended volume ratio 1:5).

2. In dealing with large specimens it is important to open the organ allowing the FineFIX to penetrate effectively.
Injecting the solution or place the submerged specimen in fridge can be helpful where cutting the organ is not possible once.

3. Within 24 hours, stimulate the action of FineFIX by applying microwaves using MILESTONE unit where possible, to reduce fixation time without artifacts (about 30 minutes up to 50°C).
If not possible replace the solution with new FineFIX. Placing the specimen in fridge can preserve better the tissue but in the same time can affect the FineFIX action.

4. Within 24 hours complete the microwave fixation by cutting the specimen into slices of 2/3cm in thickness. If specimens are coming from the fridge, keep the pre-soaking time before applying microwaves (15 min to 90 min).
The inner part of the organ could be still soft and a microwave hardening is advised irradiating the slices for about 15 minutes up to 50°C.

5. From larger slices, representative blocks are sampled and processed starting with the fixation step (few minutes up to 50°C, depending on the block size).

A. Appendix – Immunohistochemistry guidelines on FineFIXED tissues

Epitope Retrieval (ER) procedures vary considerably when performed on formalin fixed, paraffin-embedded tissues, the main reason being the continued cross-linking action of formalin: the longer tissue remains in the fixative. This inevitably produces varying quality of immunostains for both sensitivity and intensity of staining.
Mode of action of the working solution based on FineFIX, precipitative in nature, avoids the deleterious affects associated with cross-linking fixatives, enabling ER to be performed without the requirement for aggressive retrieval methods. This enables a more standardized approach for ER techniques. The added bonus of avoiding cross linking fixatives is that the improved sensitivity of antigens to antibodies will in many cases, especially for polyclonal antibodies, necessitate an increase in antibody dilution, thus reducing the amount of antibody required and extending the number of tests per vial of antibody.
The following guidelines are recommended for the ER process as a routine on FineFIXed, paraffin processed tissues.

A.1. Epitope Retrieval (ER) Process

Points to note:

1. ER temperature NOT to exceed 85°C.
2. No change to composition of retrieval solution: use the existing retrieval buffer established as appropriate for the antigen under examination, in your laboratory.
3. Time at Temperature for ER for up to 20 minutes at 85°C.

The ER protocol described above has been evaluated as the most effective for the range of antibodies indicated in the list below. Additional antibody types and variations in clones may require additional evaluation for optimal retrieval conditions.

Antibodies Evaluated: (40).

Cytokeratin: Cam5.2	GFAP	TSH	CD20
Cytokeratin: AE1/AE3	Neurofilaments	Prolactin	CD21
Cytokeratin: Pancytokeratin	Chromogranin	Estrogen Receptor	CD31
Cytokeratin: CK7	Synaptophysin	Progesteron Receptor	CD45
Cytokeratin: CK20	S100 protein	MIB1	CD138
Cytokeratin: CK5	HMB45	CD3	Cyclin D1
Cytokeratin: CK6	ACTH	CD4	p53 (monoclonal)
Cytokeratin: CK14	GH	CD5	PSA
Vimentin	FSH	CD8	Thyroglobulin
Desmin	LH	CD15	Gastrin

B. Appendix – Guidelines for fixation

The following tables show the guidelines times and temperatures for the fixation process.

The FIXATION PROCESS is composed by two PHASES:

1. PRESOAKING
Allows penetration of fixative in the tissue before real fixation.
To avoid fixation only outside the tissue and hardening.

2. FIXATION
Prevents the progress of putrefactive processes in the biological samples and give the samples themselves adequate mechanical properties in order to enable appropriate processing.

PHASE 1: PRESOAKING

THICKNESS	PHASE 1: PRESOAKING
Transplant (less than 1mm)	15min
1mm	30min
2mm	45min
3mm	1hr
4mm	1hr 15min
5mm	1hr 30min
5mm fatty	2/3hr

PHASE 2: FIXATION, different times at different temperatures.
It is possible to perform this step at room temperature or accelerate it using heating.
It is possible to heat up the solution to 37°C or 50°C.

THICKNESS	POSSIBILITY 1 ROOM TEMPERATURE	HEATED FIXATION	
		POSSIBILITY 2 37°C	POSSIBILITY 3 50°C
Transplant	30min	10min	5min
1mm	1hr	40min	20min
2mm	1hr 30min	50min	25min
3mm	2hr	1hr	30min
4mm	4hr	2hr	50min
5mm	5hr	2hr 30min	1hr
5mm fatty	10hr	4hr 30min	2hr

All above are indicative times range for generic specimens.
Times may change related to the tissue type.

Frozen sections will also benefit with the use of working solution with optimal results better than existing fixatives. The immersion of slides for 60 seconds in preheated (37°C) FineFIX working solution gives simultaneous: specimen fixation, dehydration and clearing.

C. Appendix – Brief morphology guide

For over one hundred years, formalin has remained the tissue fixative of choice. Formalin being a major health hazard, it has spurred a move to a suitable substitute at the earliest.

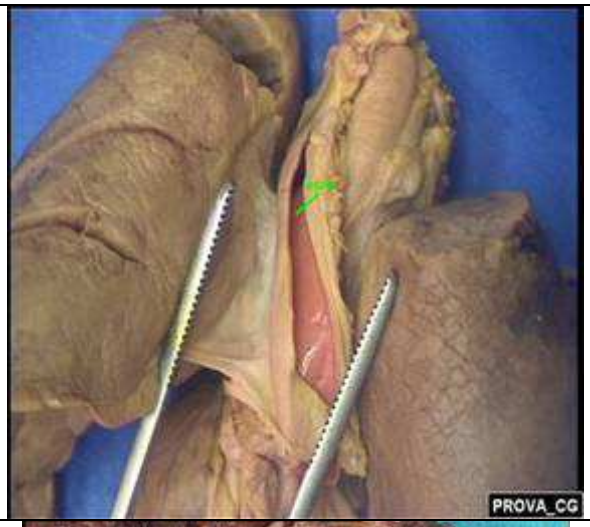
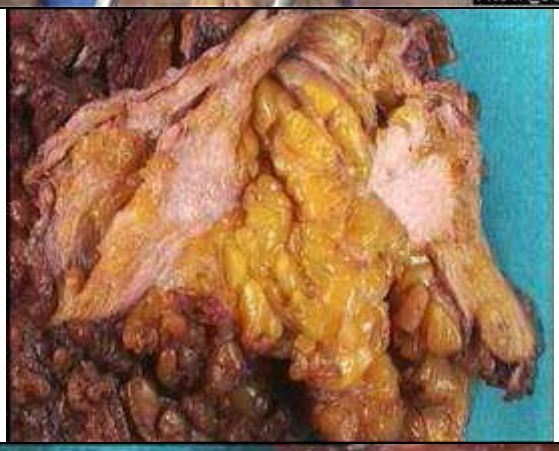
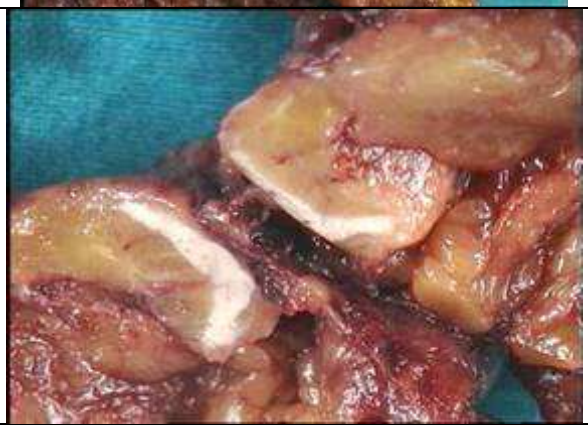
The International Agency for Research on Cancer (IARC1) has classified formaldehyde as a class 1 carcinogen. On December 2013 the EU-REACH adopted a decision to reclassify formaldehyde as a Cat. 1B carcinogen and Cat. 2 mutagen under the EU CLP Regulation. As a Cat. 1 carcinogen, formaldehyde use will be regulated by the restrictive Carcinogens Directive in EU workplaces. This new classification is encouraging health authorities, surgical staff, pathologists and histotechnicians to look for ways to eliminate the substance from work environments.

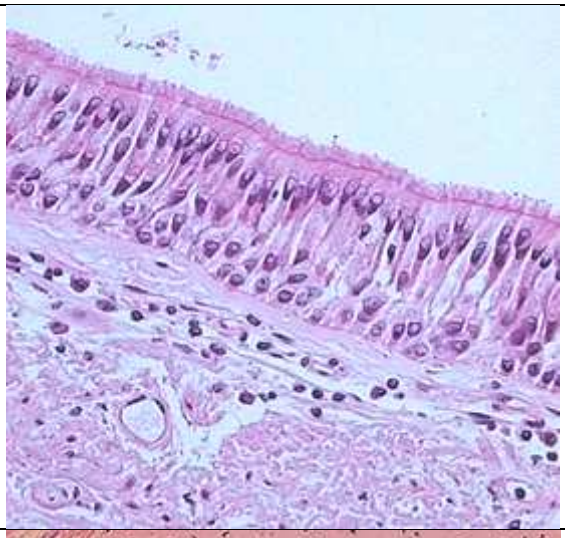
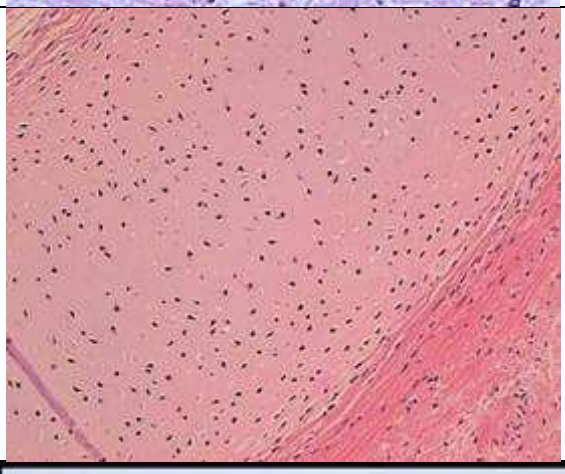
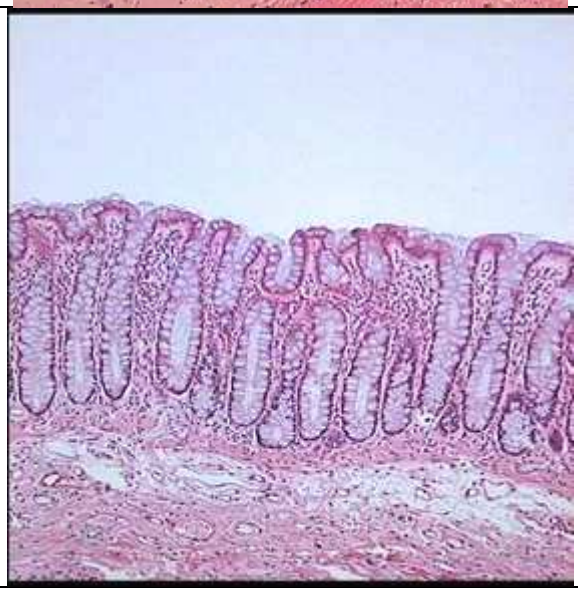
In addition, the ever growing interest for genomic and proteomic assays becomes increasingly limited with extended formalin fixation times.

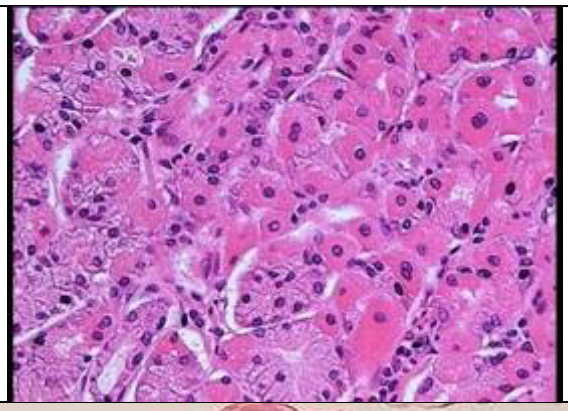
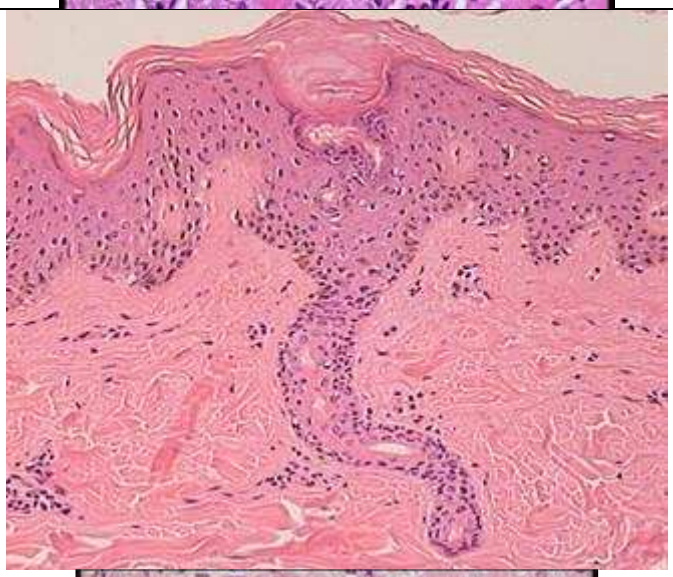
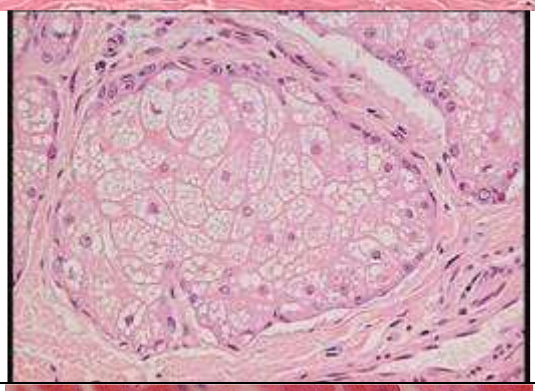
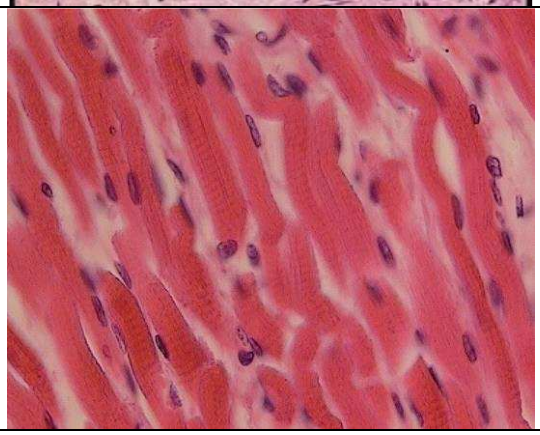
At the macroscopic level, the use of FineFIX as a fixative for histology is not so dissimilar from that of formalin, however its action on tissue is different and is reflected in the macroscopic appearance of the specimen, i.e. the consistency of the specimen will be increased, far improved from that of formalin, with preservation of tissue elasticity (rubbery consistency).
The macroscopic color produced with FineFIX will be akin to the fresh state of the specimen, as opposed to the grayish appearance of formalin-fixed material.

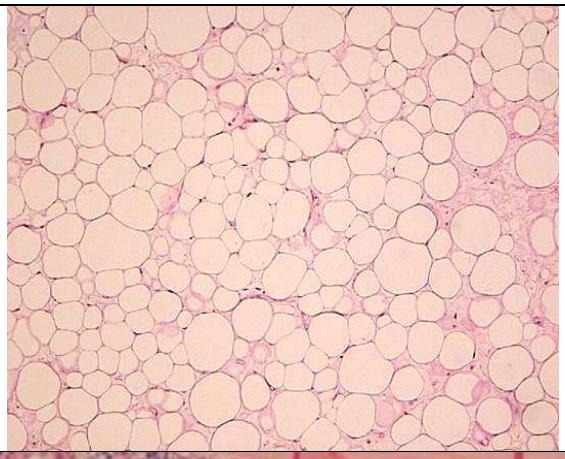
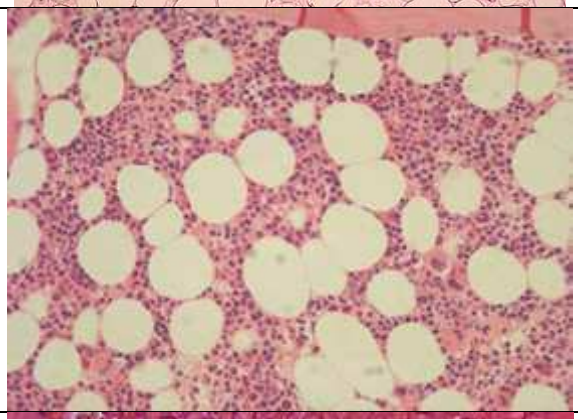
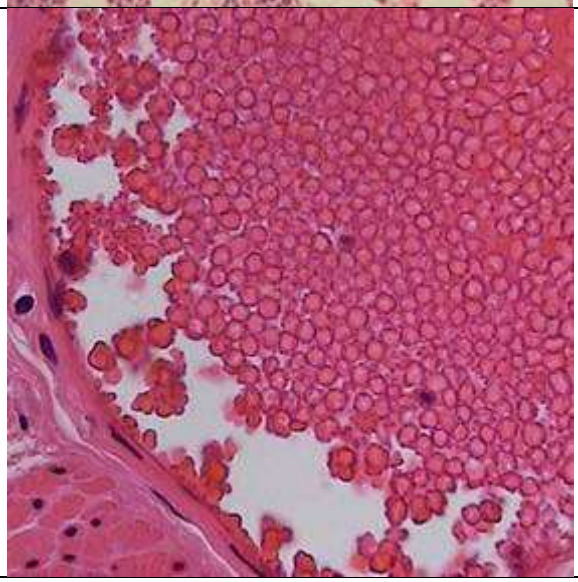
The following list of tissue types have been fixed with FineFIX.
This library of archived images' provides the intended user with a detailed overview of expectations when moving from formalin to FineFIX, a safer alternative.
Hence this guide should only be taken as an example of the macro/microscopic results that the user can obtain when employing this innovative fixative.
Together with fixed organs appearing much more fresh in appearance and with physiological consistency than that in formalin, FineFIX provides equal to or better morphology.

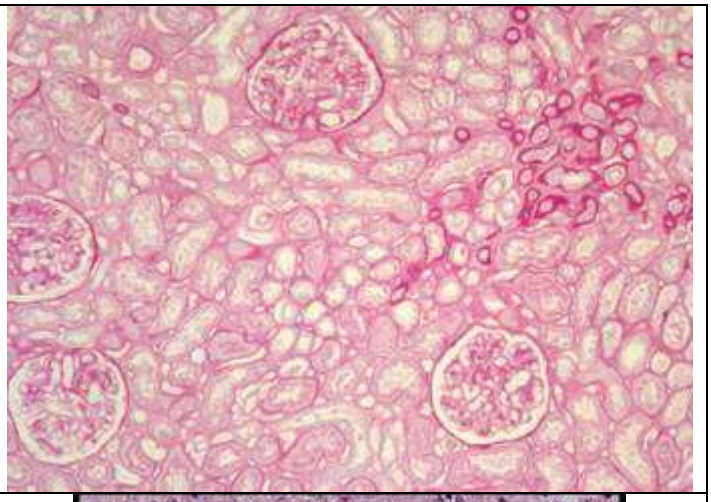
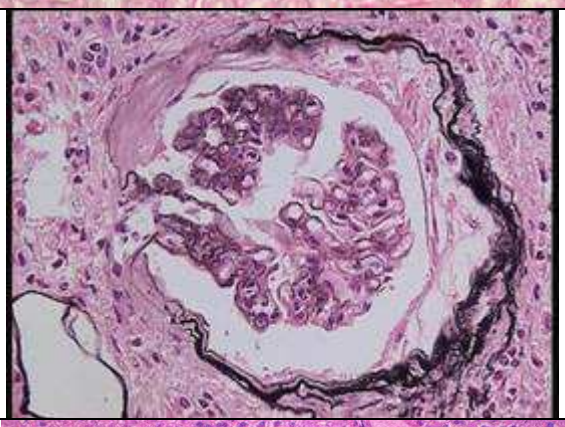
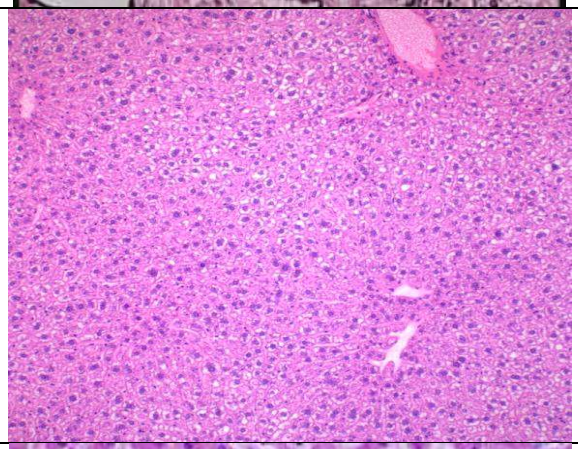
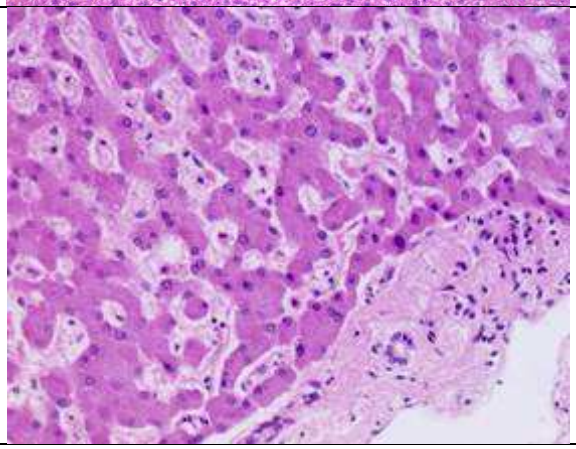
Once tissues are processed and stained the intensity of staining colors strongly depend on many variables such as dyes' manufacturer and staining time.

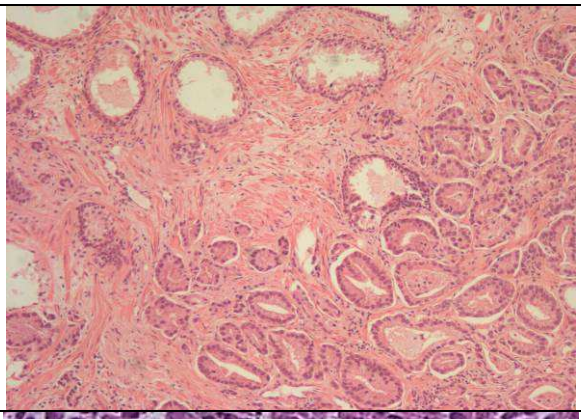
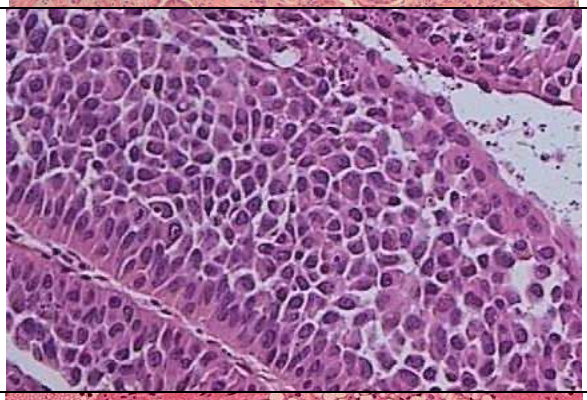
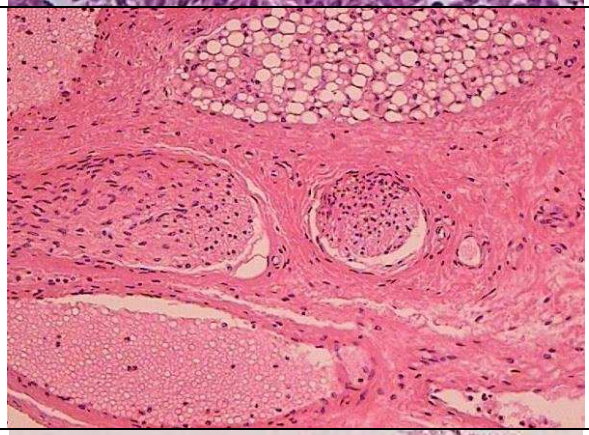
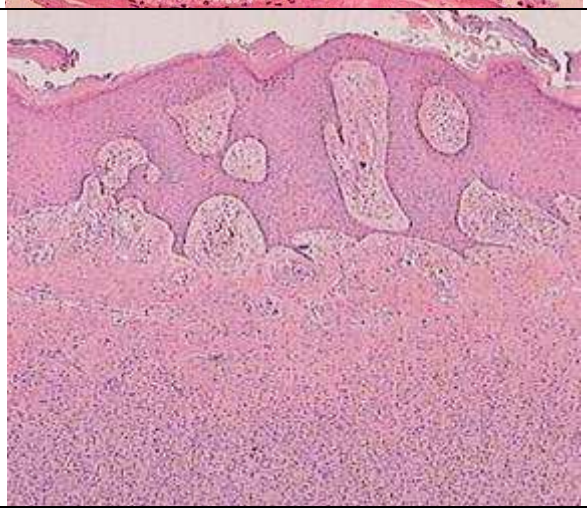
Lung and Aorta	
Breast	
Axillary tail lymph node	

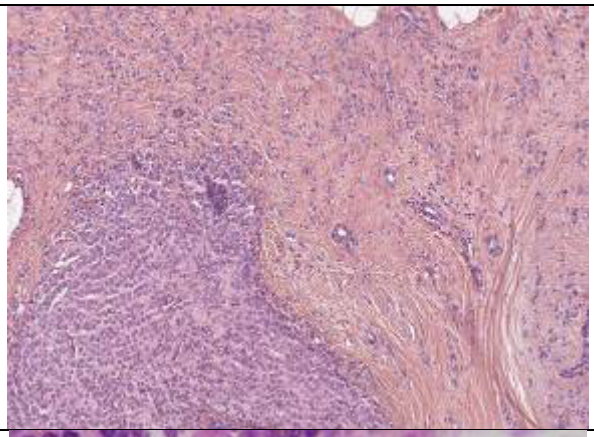
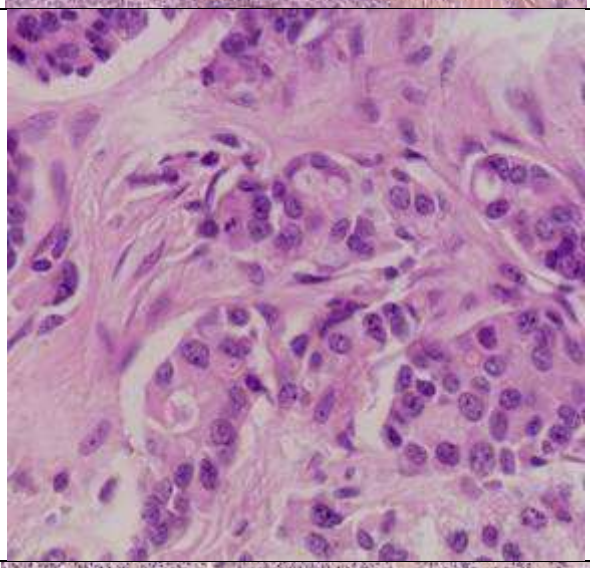
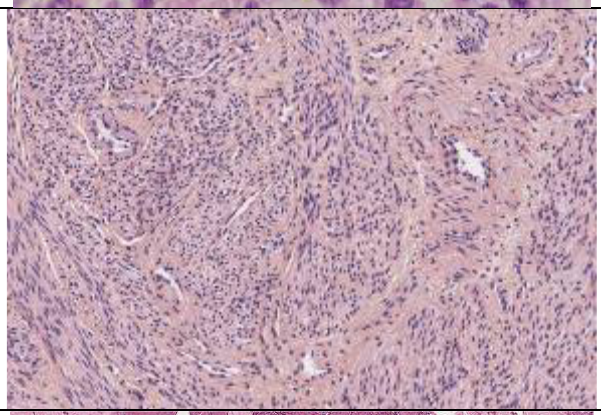
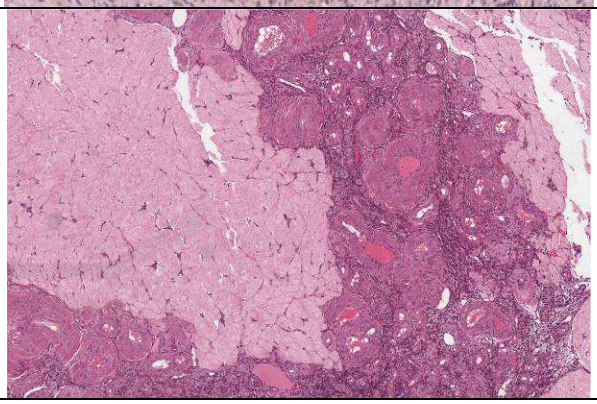
Bronchial epithelium, H&E x400	
Bronchial cartilage, H&E x200	
Large bowel mucosa, H&E x100	

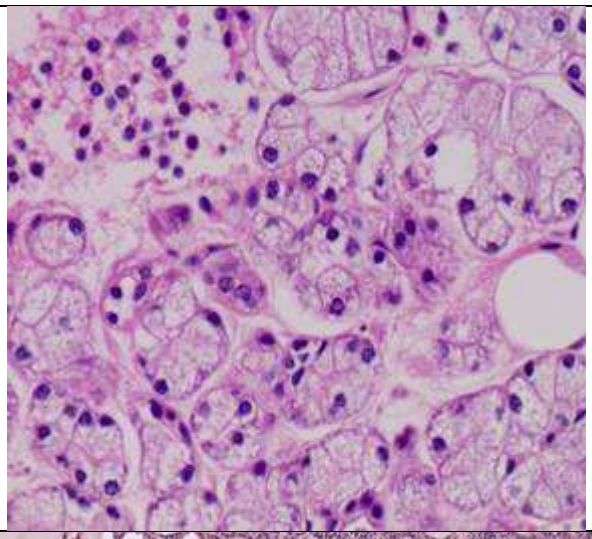
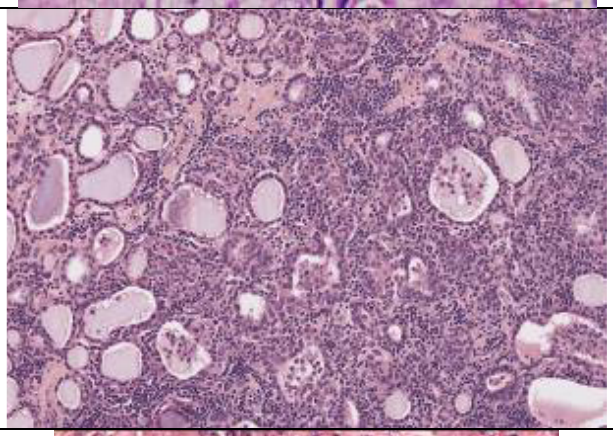
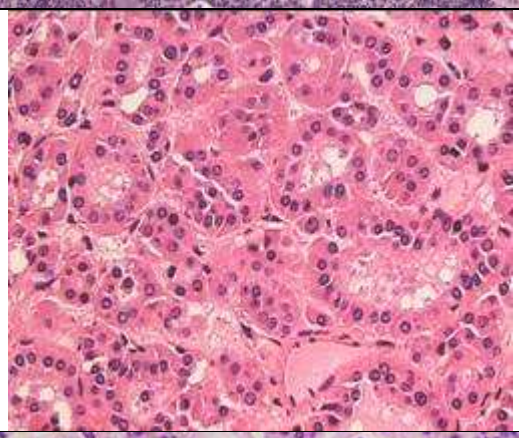
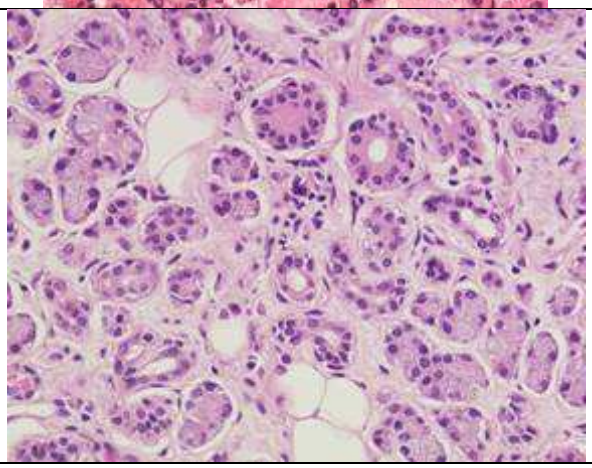
Stomach glands (Parietal and chief cells), H&E x400	
Normal skin and sebaceous gland, H&E x100 and x400	 
Skeletal Muscle, H&E x600	

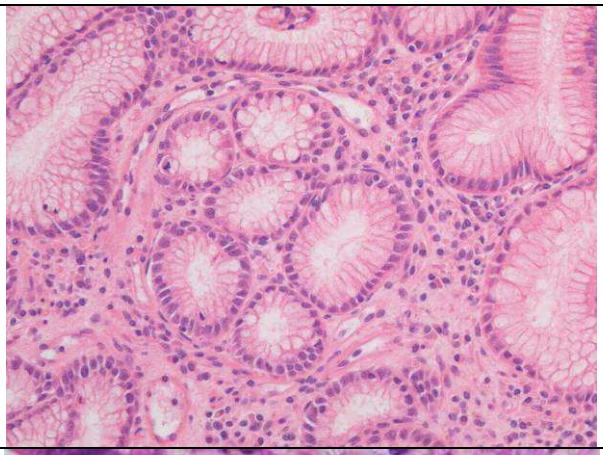
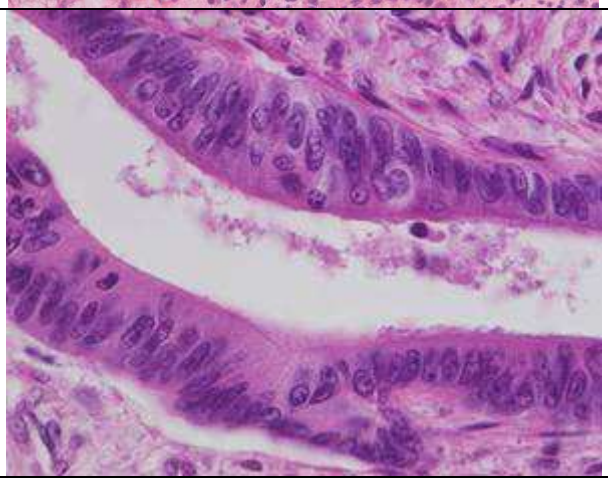
Adipose tumor, H&E x100	
Bone marrow, H&E x100	
Red blood cells	

Kidney, PAS x200	
Kidney, Jones Silver stain x400	
Mouse liver, H&E x200	
Human liver, H&E x400	

Prostate, H&E x100	
Urinary bladder carcinoma, H&E x200	
Brown fat with nervous trunk and blood vessel, H&E x200	
Fibrous histiocytoma, H&E x100	

Breast carcinoma, H&E (and safranine) x100	
Breast carcinoma, H&E x400	
Uterine myometrium, H&E (and safranine) x200	
Ovarian carcinoma infiltrating colonic wall, H&E (and safranine) x100	

Parotid gland, H&E x400	
Multinodular thyroid goiter, H&E (and safranine) x200	
Grave's desease, H&E x400	
Parathyroid, H&E x400	

Normal colon, H&E x200	
Rectal cancer, H&E x400	

CE IVD

Milestone Srl - via Fatebenefratelli 1/5 - 24010 Sorisole (BG) - Italy
Phone +39 035 4128264 - Fax +39 035 575498
e-mail: medical@milestonesrl.com - web: www.milestonemedsrl.com