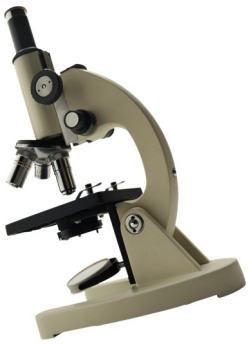
Ministry of Health

Higher Health Council

Section I

Guidelines
Tracking, Collection, Transport, Preservation and Storage of cells and tissues for diagnostic investigations of PATHOLOGICAL ANATOMY



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OVERVIEW

The anatomic pathologist or pathologist is the clinician specialized and responsible for the diagnosis of disease on cells and tissues. The role of pathologist is central to the diagnostic and therapeutic pathway as it ends up with a medical act, i.e. the diagnosis, a sequence of cognitive-technical procedures devoted to the examination of organs or organ samples (cells and/or tissues).

The diagnosis in pathological anatomy, which has to be integrated with the clinical data of the patient, is therefore the result of the interpretation of the morphological characteristics (macroscopic, microscopic) of the biological sample under examination, possibly supplemented by the analysis of specific molecular features by the pathologist; this cognitive process may benefit of quantitative data generated by analytical tools but is based almost exclusively on the expertise of the doctor.

The diagnostic activity of the anatomic pathologist covers all fields of pathology.
In the *oncological field*, it defines the nature of the lesion and identifies factors that are prognostic as well as predictive of response to therapy by using immunophenotyping and molecular analyses, and thereby provides fundamental and essential elements for the choice of targeted therapeutic regimens. In the context of cancer prevention, the pathologist is the clinician responsible for the cyto-histological diagnosis of screening programs and produces a diagnosis that affects subsequent clinical therapeutic actions.

In the context of *organ transplants* the pathologist plays a role in the assessment of the suitability of the tissue and then in the monitoring of the possible pathology of rejection and associated diseases.

In the context of *infectious diseases,* the pathologist, through morphology, immunophenotyping and molecular techniques, contributes to the identification of the pathogens, in particular in opportunistic infections of immunocompromised individuals.

No less significant is the role of pathologists in the diagnostic definition of a wide range of *degenerative, dysmetabolic, immune, malformative and inflammatory* diseases.

The pathologist is also involved in the identification and characterization of the so-called “rare diseases”.

Among the diagnostic investigations conducted by pathologists we must not forget the *autopsy*, which has allowed the birth of modern medicine and has been the cornerstone of clinical

diagnostic findings when instrumental investigations *in vivo* were not yet available. With the evolution of instrumental diagnostics, the need of the autopsy to identify pathological changes of organs is gradually reducing. However, the role of the autopsy as a control of clinical diagnosis, in terms of explanation of clinical and scientific questions and of the anatomo-clinical epicrisis as well as its role in the clinical risk management to ensure patient safety has not failed. Further important implementation of the autopsy is the study of the pathologies of foetal development and simultaneously the investigation of the causes of perinatal pathology, with the dual purpose of documenting and confirming any developmental abnormalities.

The diagnostic work carried out in the service of pathological anatomy is exercised through the production of a report that will be communicated to the clinician or the patient via an act written and signed by the anatomic pathologist. The elements that represent the basis of the quality of the report are accuracy, completeness and timeliness.

Higher Health Council of Italy

(Chair: Prof. Roberta Siliquini)

Section I

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Working Group

"Guidelines Tracking, Collection, Transport, Preservation and Storage of cells and tissues for diagnostic investigations of PATHOLOGICAL ANATOMY"

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1. INTRODUCTION

Cells and tissues derived from collection of specimens obtained from neoplastic, inflammatory, degenerative and other diseases represent biological samples often not reproducible, on which the pathologist is required to provide a diagnosis based on morphological and/or molecular characteristics. In recent years, adhering to the demands of both clinicians and patients, the pathological anatomy evolved from a branch of medicine devoted to the study and classification of diseases exclusively from a morphological point of view, into molecular pathology with the aim to provide data on gene alterations or on biomarkers present in pathological tissues, which may be essential for the accurate setting of therapies directed at molecular target (target therapy). To provide an accurate and complete diagnosis, the tissue under examination should be stored in an optimal way. However, from the moment of excision of the sample from the patient to the moment in which the sample is adequately treated with "fixing or freezing" methods, both the architecture of the tissue (histology) and the morphological characteristics of the cells (cytology), as well as the biological properties (nucleic acids and proteins) of its cells can undergo *degradation processes* and alterations. These degradation processes if not properly controlled can limit or prevent the diagnosis.

The preservation of biopsy, surgical and cytological samples becomes therefore a priority in order to ensure a correct and complete diagnosis, but an equal priority becomes their custody during time to fulfil any requests of the patient in case of need of samples for further analyses or for other clinical needs. To this end, it is important also to ensure the *tracking* of the sample from the time of removal and during the working cycle until archiving, to prevent identification errors and loss, and the *traceability, preservation* and *custody* of the archive material (slides, blocks of tissue in paraffin). In addition, the archives of pathological anatomy may represent one of the greatest bio-banks of tissue for testing new biomarkers and for the development and validation of diagnostic tests. The critical points in this process of preservation and custody essentially concern:

1. *the collection and transport of samples*
2. *the tracking of samples*
3. *the archive of samples*.

The Working Group, characterized by experts with different cultural and scientific background, has addressed the issue as a whole, taking into account the absence of specific regulatory aspects of the sector, a part from the Community regulation (Reg. EU 895/2014) concerning the carcinogenicity of formaldehyde, and has proposed a methodological approach innovative for the diagnostic investigation of Pathological Anatomy.

In particular, the Guidelines represent a document addressing towards a technical development of a medical branch that plays a key role in many scientific and economic activities of the public institutions, by proposing a regulation of the sector in all its functional components, both in the public setting and in their relations with the private healthcare sector.

The main purpose was, in fact, to produce a document intended to regulate all health issues related to the quality assurance of the material to be examined by anatomic pathologist and, consequently, the protection of patients' health and of professionals involved in the diagnostic and therapeutic process.

The guidelines derive from review of the results of scientific evidence and regulations and national and/or international guidelines specific for Pathological Anatomy.

The Italian Society of Pathological Anatomy and Cytology (SIAPEC Protocols) is expected to provide detailed procedures for the implementation of these guidelines to be adopted at the level of individual healthcare organizations/hospitals.

2. DEFINITIONS

TISSUE SAMPLES for HISTOPATHOLOGICAL EXAMINATION

The **histological examination** is needed to define the disease with the aim to provide a diagnosis and a treatment. To perform histological analysis requires a minimum standard of times to obtain the final product (histological slide) and may be necessary to use immunohistochemistry or molecular biology techniques to reach the final diagnosis or to integrate the same in order to provide all of the parameters necessary for the treatment of the disease.

The **material** that is subjected to histological examination consists of fragments of tissue (biopsies) from organs or their parts removed through a surgical intervention (resection). ***Biopsies*** can be distinguished as follow: *incisional*, in which a portion of the lesion is removed only to diagnostic purposes; *excisional* in which the totality of a lesion (for example, a skin cancer) is removed; *endoscopic*, carried out on hollow organs (e.g., bronchial tube, stomach, intestine) and finally *needle biopsies or core biopsies*, carried out under instrumental guide (e.g. ultrasound or radiological CT or MRI) on solid lesions (e.g. liver, prostate, breast, thyroid etc.).

The *critical features* of the diagnostic process may vary according to the type of biopsy. For *needle biopsies,* the number and the size of the collected specimens are important, since they may affect the representativeness of the lesion and, therefore, the diagnostic value of histopathological examination carried out on this material. For *endoscopic biopsies* crucial features are correct orientation of the specimen, which must allow an accurate assessment of the architecture of the tissue, especially in clinical situations in which it is required to perform specific morphometric evaluations (for example examination of height and morphology of the intestinal villi in the diagnosis of celiac disease). The *excisional biopsies* with therapeutic purposes must be carried out by removing the lesion *in toto* and so as not to detract from the histopathological evaluation of adequacy of exeresis, which requires a comprehensive examination of the margins of the lesion. The ***surgical resections*** can be divided into *partial*, *total,* or *widened* resections, covering part or all of one organ or involve more organs. In contrast to histopathological examination on biopsies, in which the totality of the collected material must be examined under the microscope, on surgical resections the pathologist must select the area to be examined under the microscope by performing sampling (the so-called grossing or reduction

of surgical specimen) according to specific protocols and guidelines that allow to define the nature and extent of the lesions detected, as well as the adequacy of the exeresis (examination of the margins of resection) and, in the case of cancer, the staging of the disease. For these processes, which are part of the "macroscopic examination", the pathologist can use methods of acquisition of images that allow to document more clearly the morphology and the characteristics of the surgical specimen sampled for the final histopathological diagnosis. These samples can vary in number and type depending on the characteristics of the removed material and related clinical questions. To provide an accurate diagnosis it is mandatory that the excised tissue is collected and sent in an appropriate manner (intact, and if necessary oriented with metal clips or suture points).

SAMPLING FOR INTRAOPERATIVE CONSULTING

The *intraoperative consulting* commonly called "extemporaneous" is an exam in urgency and as such should be requested only if the result is going to affect surgery and if the question cannot be resolved prior to the intervention. The intraoperative advice may be required to define the nature of a pathological tissue, the extension of a lesion, the staging of a tumour, the adequacy of the exeresis, etc.

The tissue to be examined must be sent fresh i.e. not fixed in formalin. In response to questions posed by the surgeon, the pathologist can count on macroscopic examination of the removed material, integrated by performing histological cryostat sections, cytological imprint, immunohistochemistry or molecular tests if necessary and validated. As far as possible, the intraoperative examination must not compromise the "final diagnosis" that will be performed on the same tissue or on the residual tissue after the standard procedures for fixing and embedding in paraffin.

SAMPLING FOR CYTOLOGICAL EXAMINATION

The cytological examination is aimed at defining the nature of the cells for the purpose of diagnosis and treatment. The material subjected to cytological examination consists of exfoliated cells, needle-aspirate or cells removed by abrasion or brushing from tissues of organs or cavities. The cytological examinations can be performed on:

cells from biological liquid i.e. cells exfoliated spontaneously in any liquid contained in preformed cavities (e.g. pleural and peritoneal effusions, urine etc.) or in liquids accumulated in newly formed cavity (e.g. cysts etc.);

cells obtained from needle-aspiration i.e. drawn with thin needle under suction by organ or tissue;

cells from brushing collected with manoeuvres of gentle abrasion using the so-called cytobrush (e.g. airways brushed, cervico-vaginal brushed);

cells from washing liquid collected from exfoliation produced with washing preformed or newly-formed cavities. The transfer of cells or slides on which the cells are smeared or imprinted must take place with methods that ensure the adequate preservation (e.g., in fixative, dry) and tracking.

OTHER EXAMS

Ultrastructural, immunohistochemical, and molecular flow cytometry exams are applied on the samples described above, and can be an integral part of the diagnostic –histological pathway or represent specific examinations needed to sort out a diagnostic doubt or be required for the diagnosis and treatment of diseases, after defining the cyto-morphological or histo-morphological suitability of the sample.

The ***ultrastructural examination*** or electron microscopy is indicated for specific pathologies (e.g. kidney diseases such as glomerulonephritis) where it may be necessary the morphological examination of fine structures inside cells or tissues.

The ***immunohistochemical or immunocytochemical examination*** consists of a series of procedures that, through antigen/antibody reaction, allows to locate at the microscope the topography of expression (presence in specific cells or tissues), the over-expression (excess) or the absence of expression of tissue and/or cellular antigens (biomarkers). It may be necessary for diagnostic purposes for any type of pathology to define the origin or for differential diagnosis and prognostic and/or therapeutic purposes especially in the oncology field.

Flow-cytometry is a technique applied to the cells in suspension and allows to evaluate the quantity of proteins present on the surface or inside the cell, allowing an accurate identification of the cell type under consideration. It is widely used for haemo-pathological diagnoses.

Molecular tests applied to cells and/or tissues enable to assess alterations of nucleic acids (DNA and RNA) (amplifications and deletions, mutations etc.) that may have an impact on diagnosis, prognosis, and treatment of a disease.

FIXATION AND EMBEDDING IN PARAFFIN

This refers to the processes that allow to obtain tissue sections suitably stained for microscopic examination and to maintain the histological cytological and biological characteristics of the tissue stored in the long term (years).

3. IDENTIFICATION AND TRACKING OF THE SAMPLE FOR PATHOLOGICAL DIAGNOSIS

3.1 IDENTIFICATION OF THE SAMPLE

3.1.1 REGULATION AND LITERATURE

Several works report the possibility of errors during the phase of identification of the sample in the centre that runs the collection or in the operating room (1). In a study conducted by the department of Surgery of the Johns Hopkins University in Baltimore (2) it has been shown that errors in identification in the operating room have a frequency of 4.3/1000 surgical samples. The guidelines of the College of American Pathologist (CAP) and the National Society for Histotechnology (NSH) list the procedures to be followed for the correct identification of surgical samples and biopsies (3, 4).

The Italian Ministry of Health and Social Policies has produced in 2009, the "*Safety Manual in the operating room: Recommendations and Checklist"* where, in Objective 3, (Correctly identify the surgical samples), it is stressed that "The incorrect identification of surgical samples can cause serious consequences to the patients and the prevention of such errors is critical to the safety of the patients" (5).

For samples obtained from non-surgical biopsies or fine-needle aspirations (FNABs) performed in outpatient room (endoscopy, day-surgery, etc.) do not exist, at present, specific recommendations.

The recommendations produced by the Ministry for the operating theatres with the additions of the update of this document must also be applied for the day hospital in which bioptic and/or cytological sampling are performed.

The ministerial check-list *Manual for Safety in the operating room* (5), in order to avoid errors of identification of the sample, requires that "*each surgical specimen (or multiple samples of the same patient) must be accompanied by a special request for examination"*. The operating team must check for each request, the correct compilation of the request, and the correct labelling (on the walls and not on the cover) of the bio-box containing the specimen with the following information to carry on both:

1. *Patient identification* (name, surname, date of birth, sex)

2. *Identification of the applicant* (operating unit, first name, last name and signature of the applicant).
3. *Identification of the material*, by specifying date of the surgical excision, the type of excision, location, topography and laterality of the specimen (e.g. prostate right lobe, right kidney, skin right arm, upper lobe of the left lung, etc.)
4. …. *omissis*…
5. *Number of bio-boxes*; in the case of multiple specimens from the same patient, Arabic numbers must identify the sample, corresponding to what is reported on the request; on the container it must be reported the possibility of biological risk in the case of materials from patients with relevant infectious diseases.

In the literature, **one of the most common errors reported** for point 5 is **the indication of the sole name of the patient or the identification only by number rather than by both name and number** (6).

Equally essential are three additional items, not present in the checklist, in order to allow a correct pathological diagnosis and the effectiveness of the analytical result.
These are:

(i) ***Clinical information*** useful for the diagnosis (e.g., past pathologies that can be correlated to the intervention or sampling, past histo-pathological diagnosis of cancer or related to pathology in progress, results of testing of imaging, in the ongoing or previous treatment). The absence of clinical news represents 40% of omissions observed on exam requests in pathology, as demonstrated by an analysis on 5594 cases. In 6.1% of the cases, the subsequent integration with the clinical information has led to a revision of the previous diagnosis (7);

(ii) **The time of surgical procedure** known as "time of warm ischemia" can affect the preservation of the integrity of molecules (8, 9) and of the metabolic profile (10) through processes of acidosis and enzymatic degradation. The guidelines of the Association of Clinical Oncologist (ASCO) and the CAP (11) indicate the need to monitor the time of intervention for a better preservation of tissue antigens. Guidelines on quality in pathological anatomy (3, 12) recommend to enter in the request sent for histological examination of the surgical specimen: the time of administration of anaesthesia, of

ligature of the larger blood vessels, of removing of the surgical piece from patient. Similarly, it is recommended to indicate the time of execution of biopsy sampling.

(iii) The length of time that elapses between the excision and fixation of the specimen is called “time of cold ischemia” and it is reported to exert deleterious effects on the preservation of antigens and nucleic acids. The guidelines of the American CAP/NSH (3) as well as the ASCO/CAP guidelines list the time of cold ischemia as a required field in the checklist of request for immunocytochemical tests with predictive purpose in breast carcinoma (11). Recent European guidelines for molecular investigations on tissues emphasize the importance of the cold ischemia time on the outcome of the analysis (13).

3.1.2 REGULATIONS ON INFORMED CONSENT

Each individual institution has to include the informed consent for analyses of pathological anatomy into the paths of diagnosis and treatment. The International Joint Commission on Accreditation of Healthcare Organizations (JCAHO) in theme to the rights of the patient and his family explicits: "The organization shall draw up a list of categories or types of treatments and procedures that require specific informed consent". *The hospital must draw up a list of these procedures and treatments and should educate the staff in order to ensure the uniformity of the acquisition process of informed consent. The list should* **include the procedures and treatments delivered both to outpatient and in hospital patients.**

Exceptions to the detection of informed consent are regulated by "Permit n. 9/2013 - General authorisation to the processing of personal data for purposes of scientific research" (Italian Official Journal no. 302, 27 December 2013).

3.1.3 GUIDELINES: IDENTIFICATION OF THE PATIENT AND SAMPLES

The request for analyses of pathological anatomy must be performed with online procedure. Paper copy of the same must accompany the surgical/biopsy/cytological samples.

On the request for histo-cytological examination, it must be reported:

1. *Patient identification* (name, surname, date of birth, sex, or use **systems of identification by means of a bar code or similar in the respect of the law relating to the protection of data privacy**)
2. *Identification of the applicant* (operating unit, first name, last name and signature of the applicant)
3. *Identification of the material*, by specifying date of the withdrawal, the type of specimen, topography and laterality of the sampling (e.g. prostate right lobe, right kidney, skin right arm, upper lobe of the left lung, etc.)
4. *Number of bio-boxes*; in case of multiple and different sampling of tissues and cells, Arabic number must identify each sample, specifying as in 3.
5. **Relevant clinical data:**
   - previous pathologies that can be correlated to the intervention or sampling,
   - past histo-pathological diagnosis of neoplasms or pathology which can be correlated to the pathology in act,
   - results of imaging examination,
   - ongoing or previous treatment
   - others
6. ***Notification of biological risk:*** infectious diseases for which there is a recall of specific regulatory.
7. ***Intervention time*** from skin incision to surgical excision (start/end intervention**).**
8. **Time of insertion of the tissue sample in the system of preservation/transportation.**

On the container, it must be reported:

1. *Patient identification* (name, surname, date of birth, **or use of systems of identification by means of a bar code or similar in the respect of the law relating to the protection of data privacy**)
2. *In the case of multiple retrieval of cells or tissues* it must be reported:
   - the patient's first and last names, date of birth **or identification systems using bar code or similar in the respect of the law relating to the protection of data privacy)**
   - identification of the sample with Arabic numbers, corresponding to those reported on the request **or identification systems by means of a bar code or similar;**
   - type of removal/excision,
   - topographical location and laterality of the sampling
3. *Notification of biological risk:* infectious diseases for which there is a specific regulatory recall.

The procedures described, both with reference to the request and to the container, should be indicated as mandatory for the operators in the health care facilities in order to ensure a correct identification of the origin of the sample and to minimize risks of loss of the same. The use of tracking systems must be made mandatory in order to correctly identify the sample from the time of removal.

The **errors of identification can result in serious problems for the patient**, who can be assigned a diagnosis that is not compatible with his pathology and a cure that is not suitable.

Most of the samples sent for histo-cytopathological examinations are **irreproducible** and, therefore, it is necessary to activate all of the procedures, which allow preservation of the tissue and the subsequent diagnosis for the safeguard of the patient.

To proceed to the examination of the tissue, **in the event of an error of identification, it is required**:

i) to generate written procedures for notification of errors or inconsistencies (non-compliance) between the data reported on the request for examination and those reported on the sample;

ii) to assess the risk of a correction to be done on request or a new labelling of the material, if it is decided to proceed in this sense, it should be specified the cause on the report to be delivered to the patient.

As a result of the above description, **it is** therefore **imperative to apply tracking systems**. Such systems are usually used in the laboratories of clinical biochemistry, microbiology etc., to guarantee the monitoring of the processes of analysis and to avoid errors such as the loss of the sample, the exchange of samples, etc.

3.2 TRACKING OF THE SAMPLE

3.2.1 DEFINITION

The terms traceability and backward-traceability are often used as synonyms. In fact, to identify two mirrored processes, not by chance the English language uses the term *tracking* for traceability and *tracing* for backward-traceability.

The ***traceability/tracking*** is the process that follows the product from upstream to downstream of the production chain and causes, at every stage through which passes, appropriate traces (information).

The ***retrace-ability/tracing*** is the inverse process that needs to be able to gather the information previously released.

In the first case, the main task is to determine which procedures and what information should "leave trace"; in the second, this is mainly to highlight the technical instrument more suitable for tracing these traces.

It goes without saying, that the two processes are strongly interlinked and based on a system that, in the absence of specific references, will be unified in the term "tracking".

3.2.2 REGULATIONS AND LITERATURE

According to the *International Organization for Standardization* UNI EN ISO 9000:2005: "Quality Management Systems: Fundamentals and vocabulary", for tracking is meant "*the ability to trace the history and use or the location of an entity by means of recorded identification".*

These standards, up to now, have not been made compulsory in services of pathological anatomy. It should be pointed out that, in pathological anatomy the application of procedures of tracking, with respect to a laboratory of clinical biochemistry, is more complex, since the work process is not fully automated. A multi-institutional analysis has demonstrated that the errors of identification of the samples in the laboratories of pathological anatomy have more or less the same frequency in all stages from the acceptance, processing and cutting of the paraffin block containing the tissue (incorrect identification of the blocks in paraffin 0.17 % and slides the 0.11 % of the errors of identification) (14). In the USA, the guidelines of the CAP (4, 14-16) have defined rules for the tracking of the histological samples during all phases pre-analytical (sampling, collection and transport, acceptance, procedures for the production of preparation through fixation and embedding in paraffin, cut of the sections, staining and/or other analyses of

immunohistochemistry and/or molecular), analytical and of archiving of the blocks of tissue in paraffin. Some of the recommendations, recognizing the possibility of loss of the surgical specimen, indicate the strategies needed to avoid this event (16-19).

3.2.3 **GUIDELINES: TRACKING OF THE SAMPLE**

Because of the large number of samples and of the many passages which may affect the material itself, from its excision/sampling up to storage, considering also, any use subsequent to archiving (consulting, review, insertion into cohorts for research purposes), **it is made mandatory for the part of the reference structure to guarantee the tracking of the material, which must follow systematically and strictly all the steps of the pipeline** (sampling-clinics, surgical areas, etc., transport and processing path and archiving).

The **procedures of tracking** of the material taken for exams of pathological anatomy **must be computerised within the same organization providing for the use of barcode systems** (20, 21**), devices of radio frequency** (22) **or something alike**.

With regards to the **material coming from external institutions**, where there is no sharing of the informatics system, **it is necessary to arrange the definition of specific procedures for the tracking of the samples**, as shown above, also for the purposes of protection of the patient.

Regarding the determination of **criteria for the identification of the material and the various methods of transport**, it is necessary, therefore, to instruct Institutions to stick to the rules and the requirements dictated by the CAP, Laboratory Accreditation Manual 2013 Edition (in particular, p. 41 onwards) (23). This is periodically updated, and expresses indication of different or additional procedures to be part of internal legal system.

The loss of a sample in pathological anatomy has to be regarded as serious occurrence often being the sample irreproducible. Pathologists must therefore put in place all the procedures that avoid this risk by engaging in a chain of custody adequate with the identification of individuals responsible for the procedure. Whenever the patient requires the **material for consultation or further investigations outside of the reference structures**, each institution must provide dedicated forms to arrange and record the storage mode (slides and/or blocks) and return by the patient.

4. COLLECTION, TRANSPORT AND PRESERVATION OF SAMPLES OF CELLS AND TISSUES FOR DIAGNOSTIC INVESTIGATIONS OF PATHOLOGICAL ANATOMY

The method of collection and transport of samples at the service of pathological anatomy are fundamental in order to ensure the stability of structural and biological components of the tissue removed. As indicated by numerous studies, the time of ischemia (8-10), the method of preservation during the collection and of transport can cause irreparable damage the molecular features of the tissue (24).

4.1 COLLECTION, PRESERVATION AND TRANSPORT OF THE SAMPLE

4.1.1 REGULATIONS AND LITERATURE ON FORMALDEHYDE AND CHEMICAL RISK

Formaldehyde, gas of pungent smell and irritant, is produced and marketed normally in the form of an aqueous solution, with the name of formalin. In health care facilities, the formalin can be used for:

- ✓ the collection and as a means of transport of tissues derived from surgical excision at the surgical wards and of biopsy sampling at clinics (endoscopic, radiologic clinics etc.)
- ✓ the fixation of samples in pathological anatomy. The formalin is the fixative for excellence of the tissues collected for histopathological diagnosis, because it preserves the cell morphology and the architecture of the specimen. In addition, most of the commercially available antibodies for immunocytochemical investigations are produced to recognize antigenic sites whose structure is preserved by fixation in formalin. National guidelines and international (11, 13, 25), recommend the use of buffered formalin for histologic and immunohistochemical and molecular (gene mutations) examination**. Until now, a feasible alternative to formaldehyde as a fixative of tissues in the services of pathological anatomy is not yet available, but this essential use of formalin should not prejudice the applicability of mandatory preventive procedures to protect the health of the individuals exposed**.
- ✓ the conservation of the tissue residual to the sampling phase (grossing of tissues for histological preparations), leftover of biological material to be discarded when the diagnosis is concluded.

The formaldehyde used in the laboratories of pathological anatomy is Neutral Buffered Formalin (NBF) to prevent acidification due to its tendency to be oxidized to formic acid. The buffered solution increases the formation of monomeric formalin (glycol methylene) as a reagent for fixing. Formaldehyde, by being a small molecule is lightweight and can evaporate easily. The kinetic of evaporation depends on the temperature, humidity and airflow. The half-life in the environment of the formaldehyde is a very short one, since in the air it is quickly removed by photochemical processes, precipitation and is quickly biodegradable.

Formaldehyde has a tissue penetration of about 1mm/hour (due to the methylene glycol) and produces a slow cross-linking (due to covalent bonds of the carboxylic groups of the formalin with proteins, glycoproteins, nucleic acids and other molecules). This chemical property, which represents a limit for fixation of the surgical tissue samples of large dimensions, limits *in vivo* damages, because it facilitates the elimination through breathing before the damage to the airway occurs. On the other hand, the high water solubility of formaldehyde determines a high absorption by the mucus of the respiratory tract and of the first airway, particularly the nose and the sinuses. Sufficient scientific evidence has defined an alleged action as carcinogen in the nose-pharyngeal tract and paranasal sinuses, while it remains controversial that for myeloid leukaemia (26).

With EU Regulation No. 895/2014 of the commission of the August 14, 2014, amending Annex XIV of the EC Regulation N. 1907/2006 of the European Parliament and of the Council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) was stated (first recital) that formaldehyde meets the criteria for classification as a carcinogen (category 1 B) (according to the regulation (EC) n. 1272/2008 of the European Parliament and of the Council) and therefore it meets the criteria for inclusion in Annex 14 of the EC Regulation N. 1907/2006 Referred to in Article 57 letter A to this Regulation. The new classification, active since April 1, 2015, involves the need to consider the carcinogenic risk for the purposes of the management of the health and safety also with reference to exposure to formaldehyde and involves the applicability for the processes that involve the use of the formaldehyde of the Italian D. Lgs. April 9, 2008 N. 81 (Protection from carcinogens and mutagens). Also in the USA, the Department of Health and Human Service, Public Health Service in the National Toxicology Program Report lists the formaldehyde as carcinogen (27). The International Agency for Research on Cancer (I.A.R.C.) has sanctioned the carcinogenic properties of formaldehyde in 2006 (28, 29). The World Health Organization has set a limit of air quality for the formaldehyde of 0.1 mg/m^3

over 30 minutes of sampling. In Italy, the document of the Ministry of Health no. 57 of 22 June 1983 addressed the problems linked to the suspected carcinogenic effect of this substance. The Plan of Prevention and Promotion of the health and safety in the workplace 2005-2007 of the Veneto region (30) lists the environmental exposures in the operating theatres during the step of container filling for biological samples with 4% formalin and in the pathology laboratories during the different phases of manipulation of the specimen. Given the small amounts used, the risk of exposure in all of these steps is usually moderate and therefore these activities, as well as any manipulation of formalin, must be carried out under a fume hood or adequate vacuum system. Studies on the levels of formalin in grossing rooms (site of manipulation and cutting of biopsy specimens) of the laboratories of pathological anatomy has demonstrated a higher exposure compared with other tasks and then the need for adequate technology (hoods with ventilation, adoption of fume hoods and aspirated benches) and equipment of workers with appropriate means of individual protection, and secondary prevention with increase of the periodicity of the health surveillance (31, 32). Other authors have demonstrated that the exposure in the grossing room is due to the manipulation of surgical samples immersed in formalin and is higher for large complex samples (33) and that the levels of formalin evaluated with detection systems on a single operator on equal terms are variable from one operator to another (34).

4.1.2 REGULATION AND LITERATURE ON COLLECTION AND TRANSPORT OF SAMPLES

Institutional rules at national level specifying how to collect and transport of specimens from the surgical theatre to pathological anatomy have not been established yet. In the “Manual for the Safety in the operating room: Recommendations and Checklist” produced in 2009 by the Italian Ministry of Health is cited “Mode of preservation of the sample (“fresh” without fixative or in fixative) (Objective 3. Correct identification of the surgical samples, point 4) (5). The same guidelines recommend that the “individual directions of the hospital develop a written procedure for the proper mode of transport intra- and extra-hospital of the biological material from the operating room at the service of pathological anatomy or other diagnostic service, indicating the responsibility and the tracking of the process”.

In the US guidelines of the College of American Pathologists (CAP) (23) give clear indications on how to collect and transport the sample of tissue. **The Italian Society of Pathological Anatomy and Cytology (SIAPEC) is responsible for drafting procedures to ensure implementation of the recommendations provided in this document.**

4.1.3 PROCEDURES IN USE OF COLLECTION AND PRESERVATION OF TISSUE SAMPLES

Here below are the main modalities currently in use for the collection, preservation and transport of biopsy samples and surgery:

FRESH

This approach of preservation is compulsory for the intraoperative consultations, but it can be used in other cases, provided that the logistic and organization conditions allow an immediate transfer of the samples in pathological anatomy and an immediate beginning of processes for the preparation of samples for diagnostic investigation (grossing, fixing etc.). Dried materials or material exposed to any fixative may not be subjected to extemporary intra-operative examination.

VACUUM PACKAGE, AT 4 °C

The use of the vacuum package in the operating room for the storage and transportation of surgical samples allows the elimination of formalin from the environment (35, 36). The vacuum sealing and cooling method can be used for immediate dispatch of the sample for extemporaneous intra-operative examination or for any other histological examination of size equal to or greater than about 1 cm. The conservation of fresh surgical samples under vacuum is based on the principle of removing oxygen that limits the growth of the aerobic flora and allows the conservation for a time 6 times greater than that of the conservation not under vacuum. The procedure includes that the removed surgical sample is immediately subjected to the under-vacuum procedure in dedicated apparatus, stored at 4 °C and preserved at 4 °C even during transport. Scientific studies show that with this procedure the specimen can be preserved in an optimal way up to 24 hours (36, 37). A time vacuum of 48 hours at 4 °C still guarantees a good vitality of the cells (38) and conservation of histological and biological features of the tissue (36, 37). Longer times are not recommended. European guidelines for the laboratories that perform molecular investigations on tissues regard the use of the method of conservation under vacuum in cold conditions as a solution for the monitoring of the time of ischemia (13), until the beginning of fixing and consequently the time of fixation (39).

- **IMMERSED IN FORMALIN**

Liquid formalin as a means of storage and transport **is recommended for small biopsies**.

The immersion in formalin is one of the methods of collection and transport of **surgical samples**. However, there are some limitations for the storage and transportation in formalin of surgical samples:

- during the step of container filling there is the possibility of dispersion of formalin fumes into the environment, therefore such activities has to be carried out under a fume hood and with all the necessary precautions to avoid dispersion of the fumes in the environment;
- the formalin has a power of penetration of about 1mm/hour, then follows the step of fixation that requires even more time; as a result, the immersion in formalin of surgical samples *in toto* does not guarantee an optimal conservation of the specimen;
- if the specimen in formalin is not completely fixed, it can be potentially infective.

4.1.4 GUIDELINES: TISSUE PRESERVATION AND COLLECTION

❖ **SMALL BIOPSIES**

- ✓ use containers preloaded with buffered formalin according to the present regulations;
- ✓ if the transfer of the biopsy specimen in formalin to laboratories of pathological anatomy is delayed, storage on the premises where doing sampling must ensure both the preservation of tissue quality (keeping the sample away from sources of heat) and the tracking of the material.

At present, the use of fixatives other than buffered formalin has severe limitations in ensuring all special assays to be run on specimens.

❖ **SURGICAL SAMPLES**

- ✓ for examination during surgery: immediate transfer the unfixed biological material in bio-box at low temperature or under-vacuum at 4 °C.
- ✓ for definitive histological examination: immediate dispatch in cool containers or if the sending is not immediate, maintain the sample under-vacuum at 4 °C.

Exposure to formalin in the operating room should be limited, and the exclusive use of alternative procedures validated scientifically should be reached within a period not exceeding 3 years.

4.1.5 **GUIDELINES: TRANSPORT OF SAMPLES**

Methods of transport must ensure the tracking of the sample (e.g., transport times) **and its proper storage**. The transport of the tissue sample **under-vacuum** must ensure the **maintenance of the temperature at 4 °C**.

The Hospital, also on the basis of the Circular of the Ministry of Health "Recommendations for the safe transport of infectious materials and diagnostic samples" n. 3 of 8 May 2003 (40), **must develop and implement a written procedure for the proper methods of transport of the biological material from the operating room and/or clinics in the service of pathological anatomy or other diagnostic service** (both intra and extra-hospitals), indicating the responsibilities of the staff that is in charge and specifying the tracking of the process.

For the transport of biological material, it is also necessary to apply the existing rules and regulations on safety (41).

5. PRESERVATION AND ARCHIVING OF SAMPLE IN PATHOLOGICAL ANATOMY

5.1 ARCHIVE MATERIALS

The term **"archive material"** must be understood as inclusive of all the tissues and cells that are taken in accordance with the various functions described in chapter 2, once they have accomplished, either immediately or in the future, the function for which they were taken.

When referring to "storage of the material", *rectius* namely "archival material", there is a distinction of time and teleological, which is independent of the type of assay to be performed. Therefore, the linguistic term "material" concerns, in general terms, all of the objects of the sampling described by the above chapter 2, as well as the descriptions made in article 3 (a), (b) of the legislative decree 191, 2007[1]. The linguistic term "archive" refers to the deposit of the material in a dedicated place of storage, for a not pre-determined term and regardless of its function.

From this preliminary definition a first distinction should be derived between the **material processed for sampling** and the **unprocessed material**, the so-called **leftover material not sampled**. While for the first category of material, there is a problem of long-term archival, for the second category, the needs underlying the relative preservation are limited and substantiated temporally and functionally.

Even without taking a position on the delicate issue of the right of ownership of the material (25, 42-44), the article 22, indexed as "use an excised part of the human body", from the Oviedo Convention of April 4, 1997, Convention for the protection of human rights and the dignity of the human being with regard to the application of biology and medicine: Convention on Human Rights and Biomedicine, foresees that "*whenever a part of the human body has been taken in the course of an intervention, this cannot be stored and used for a purpose distinct from*

[1] The Legislative Decree 6 November 2007, n. 191, Implementation of Directive 2004/23/EC on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells, published in Italian Gazzetta Ufficiale 9 November 2007, n. 261 - Suppl. Ordinario n. 228, at the article 3, entitled "definition", intends for " a) «cells»: single cells or set of human cells not connected by any form of connective tissue; b) «tissue»: all parts of human body formed by cells.

that for which it was taken in origin, according to the procedures of information and consent appropriate". By this arrangement, therefore, functional constraint emerges on the ways and purposes for which the samples may be used; a functional constraint must exist between the purpose of the sampling and the function of the same, with the possibility of a patient's consent also extended to different areas and sectors (for example, for the purposes of study or research).

5.2 LEGISLATION ON THE TERM OF CONSERVATION

Regarding the retention period of the material there is the need to reconcile a plurality of public and private interests, which do not always coincide. We must move from a premise, **the setting of a minimum period of conservation concern the diagnostic and medico-legal purpose of the material. We have therefore to exclude any purposes of study and research beyond the control of the object of the present work and subject to a different consent to data processing on the part of the patient, in consistency with the causal link,** requested by the Convention of Oviedo[2], between the removal of the material and its preservation or its use (45).

In the present legal system, a discipline of positive terms of preservation of archive material is not properly defined (46). The acts of the SIAPEC and of the Ministry of Cultural Assets and Environmental restrict themselves to define the period of retention of the histological diagnostic material, seeing it as an integral part of the remaining diagnostic documentation, and maintain as mandatory a term of twenty years, while there are still five years for the cytological preparations. In particular, in the Ministerial Circular n. 61 The December 19, 1986 N. 900.2 / AG. 464/260, having as a topic the retention period of the health records at the public health institutions and private nursing homes, it is stated that the clinical records (the health file that contains all the documents, i.e. diagnostic reports, progress notes, annotations counselling, verbal operators, etc., which can be used to describe the diagnostic and therapeutic process of a patient being treated in hospital, both in urgency wards and ordinary as well as in D. H/D. Surgery), together with the related reports, should be stored indefinitely as they represent an official act indispensable to ensure legal certainty, as well as constitute valuable information source for historical researches health.

[2]As highlighted it follows that for the purposes of the study it seems necessary to consider a different consent from the patient and a different juridical treatment of the events that may interest the material itself.

With reference to the x-rays, by not holding the features of official acts, it is believed that under the medical, medical-legal, administrative and scientific profile a period of twenty years of storage is regarded as correct. Such indication refers to the minimum period of preservation, being allowed for institutions that consider it necessary a longer storage time. According to the above-mentioned ministerial circular "In analogy to what established for X-rays it has been taken into account that the same retention period of twenty years can be applied to the remaining documentation diagnostics until any changes may intervene to amend the predicted limit".

On October 14th, 1987 the third section of the Italian Superior Council of Health intervened on the relationship between the "remaining documentation diagnostics" and diagnostic material made from the cytological and histological slides and the inclusions in paraffin, pointing out that only the diagnostic histological material (consisting of the slide and of its related inclusion), regardless of the positivity or negativity of the report, is considered an integral part of the remaining diagnostic documentation referred to in the circular December 19, 1986 n. 61, Concerning the retention period of health records at public and private institutions for the care and treatment and, therefore, must be retained for twenty years. It has also still stated that the guidelines expressed in Circular n. 61 are to extend to the provisions contained in the Decree of the President of the Council of Ministers the February 10, 1984 only for the histological preparations and for the relating inclusions, it being understood that in every case that the cytological preparations must be retained for a period of five years.

From examination of the provisions and the opinions listed above the material of archive object of the present study cannot be likened to that described in the circular n. 61 The December 19, 1986, also in consideration of the ontological difference between the material in question, not shown or described in a document in the technical sense, and the "remaining diagnostic documentation". **The archival material**, moreover, **does not constitute a public act and the discipline of its conservation does not respond**, therefore, **to the same purpose that is behind the conservation of medical records**.

This implies therefore **the need to regulate the retention of the above-defined material**. It should be premised that **the time limit that we intend to fix is a minimum value**, hence the structure hosting the material may choose to keep the material for a term that is longer than the one fixed.

5.3 MINIMUM PERIODS OF STORAGE AND ARCHIVAL

5.3.1 *TISSUE NOT SAMPLED*

It is easier to determine the term in respect of the leftover tissue, which is not sampled, i.e., the material that is residual, left by the operators after having selected the necessary samples for diagnostic purposes. The need for conservation, in this hypothesis, it is limited in time and has to match with the function performed by the same material, i.e. by the need to integrate with additional samples that should be taken, in the case of a lack or unfitness for the diagnosis.

The leftover material not sampled, moreover, is bulky and perishable. The costs for a conservation, according to rules may be, therefore, very large and the maintenance of suitable premises is somewhat complex.

In this case, therefore, **the preservation of the material must be guaranteed up to the formulation of the diagnosis**, date from which the same has no more a particular diagnostic utility or legal value.

It follows that for the conservation of the material forming the so-called reserve not sampled it seems appropriate and consistent with the diagnostic and medico-legal purpose, the provision of a **requirement for the conservation of 15 days**, **following (i.e. starting from) the date of validation of the diagnostic report**.

With regard to the conservation of the leftover tissue, this should take place in appropriate environments and with systems to guarantee the security, tracking and the conservation suitable to ensure a possible use for further investigation. It is suggested, in particular, the preservation of material with vacuum systems. Please refer for these profiles to what is indicated in the subsequent paragraphs 5.4 and 5.5, to be considered as applicable also to the “not sampled tissue” the paragraphs on the place of conservation in the Manual of the College of American Pathologist (23).

5.3.2 ***SAMPLED MATERIAL***

With regards to sampled material (blocks in paraffin, and slides), the provision of a minimum term of preservation is more complicated and requires examining a plurality of competing factors.

In view of this, and as already stated, **fixing a time limit of minimum duration implies that at the end of its term there is no obligation for destruction or disposal of the material, but only the extinction of the obligation of the conservation of the material**. The health care facility may, therefore, continue to hold the material for a longer period than indicated.

The need to regulate derives by the lack of any applicable law and by the ontological and teleological differences between the material from the archive with respect to medical records and other medical documentation. It seems appropriate to point out that, unlike the material under examination, the patient clinical record is a public act, expression of the power certification, and participates in the public nature activity in the field of health care, which is referred[3]. It follows that the requirements related to the conservation of the same cannot be assimilated to those related to preservation of the material from the archive, of which they do not share the nature of a public act.

The obligation for the conservation of the material from the archive, imposed on health structure, becomes increasingly more expensive and difficult to implement over time and with the progressive increase of the number of samples that the same structure is required to retain. The cumbersome, including economic, of obligation stands out further if correlated with the peculiar method of preservation and archiving of samples (47). It seems appropriate, on this specific topic, to explore also the possibility of an externalisation of the obligation of preservation in favour of structures that satisfy the requirements for the conservation of the above-mentioned material.

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[3] In this sense Cass., Sez. 3, 30 novembre 2011, n. 20547, in Ced Cassazione.
These conclusions are derived from the examinations of the same Circolare 19 dicember 1986 n. 61 of Ministry of Health, General Hospitals Directorate, div. II, where it is specified that the patient clinical records, together with the diagnostic reports, have to be conserved indefinitely to guarantee the legal certainty . They constitute a valuable source of documents for research of health history.

Having stated the need to put **a minimum period of duration of the obligation of storage**, indicating the need for regulatory action on the point, it is believed that this period **can be, congruently, determined in ten years**.

In this sense it supports, first of all, the need to introduce a time limit to a bond of conservation that, in the absence, would have unlimited duration. Needs of legal certainty and factual situations require, in fact, to set a final date to the period of the bonds.

The term of 10 years is suitable to adequately protect the patient as regards the diagnostic needs underlying the conservation of the material and to safeguard his good health.

With regards to the medico-legal needs and possible defence of the health care facility or of the doctor in the course of a possible judgment, civil or criminal (48), it must be pointed out that the term under consideration involves only the end of an obligation to conservation of the material, while subsists, as already stated, the right of the structure to hold the material for a longer period (for example, in all cases where there is a dispute with a patient or with one of its successor). In case of indefinite duration of obligation, the structure is required to retain and deliver the sample at any time to the civil judicial authority and the non-delivery of the same can fall under art. 116 Italian Civil Procedure Code. By fixing a time limit of duration, the possible non-delivery of the material (in the case of the destruction or loss) after the expiry of the term itself, cannot be persecuted under art. 116 Italian Civil Procedure Code.

A further modification to the existing scheme with reference to the allotment of burden of allegation and proof between the parties to a civil judgment do not seem to be emerging. In the case where the documentation is required in the area of the administration of justice after the term of 10 years (a circumstance not to exclude both for the judicial interpretation of the date of commencement of the term of effect of the limitation period, both for the existence of the damage so-called long latent, still in consideration of possible need of the material only in the course of proceedings or in the course of any action of recourse proposal by the health care facility in respect of the doctor), remains the problem of the distribution of burden of proof, as recent jurisprudential applications continue to weigh on the obligor (health facility or doctor) the risk arising from uncertainty about the existence of a causal link between behaviour of the doctor

and the damage[4]. The limited number, however, of the disputes that, after the lapse of such time limit, may affect the health care facilities and the possible care to prevent the destruction of the material sampled in cases in which both pending a judgment civil or criminal charges being filed against the facility or doctor are appropriate topics for believing preferable the fixing of a time limit for the conservation of the material in question, in a manner consistent with the one provided for other reports confidentiality (for example, a requirement for the conservation of accounting records, as provided for art. 2220 Italian Civil Code). The needs of protection of the patient, in this case, are not different from those emerging for other confidentiality reports.
An interest in the preservation of the material appears to remain in cases in which both introduced a judgment after the expiry of the period in question and the material has been destroyed, but it is a hypothesis that can hardly happen and it does not appear that any unsuccessful in a civil judgment is prevalent with respect to the interest to the containment of the expenditure incurred for the conservation of the same material in an appropriate manner.

Finally, considering that, with the increasing amount of samples held by the health care facility, both in material and in digital format, can increase equally the risks associated with a possible loss (46), in which case, in the presence of the obligation of conservation, it would be easier to draw arguments of test to the disadvantage of the healthcare facility and the physician in civil judgment pursuant to art. 116 Italian Civil Procedure Code.

It should be noted that, according to article. 3, First paragraph, h), the Law 30 march 2001 n. 130 (Provisions in the field of cremation and scattering of ashes, published in the Official Journal no. 91, 19 April 2001, indexed changes to regulation of mortuary police, approved by decree of the President of the Republic September 10, 1990, n. 285),

[4] From the last Cass., Sez. 3, 30 september 2014, n. 20547, in Ced Cassazione, under which, in a trial of compensation of the damage derived from medical-surgical activities, the damaged actor has the burden of proving the existence of the contract (or the social contact) or the onset (or worsening) of the pathology and to describe the non-compliance of the behaviour by the obligor, abstractly adequate to cause the damage. Instead, the physician and/or the health structure have to demonstrate that the non-compliance has not occurred or that it was not the cause of the damage. As a result, if, at the end of the trial, the causal link is still uncertain, the burden imposed with the debtor..(Specifically, the Supreme Court set aside the judgment of merit that, excluding the casual link, had rejected the proceedings brought by the family of the deceased patient, because the expert appointed by the court has assigned an identical grade of possibility to two causes of death technically conceivable, a single one attributable to the conduct of the doctor). The problem is equally analysed for the determination of the subjective element.

not activated[5], "the obligation of the pathologist physician to collect from the corpse and retain liquid

biological samples and cutaneous adnexa, irrespective of funerary practices chosen, for any enquiries for the sake of justice, is determined in minimum duration of ten years".

The provision of a term of ten years appears, therefore, reasonable and adequate in relation to the purpose of archiving and preservation of the subject material.

For what concerns the commencement, it seems appropriate to fix it with effect from the moment the same begins to carry out the functions for which it was collected and stored. Therefore, **the period of ten years must be determined with reference to the date of the validation of diagnostic report** (45). **This term is also corroborated by guidelines of the CAP** (23).

The term of ten years is a minimum time limit after which the structure no longer has the obligation to keep the material. In any case, if legal proceedings, civil or criminal, are in course, the healthcare facility, heard the interested doctor, is required to evaluate the opportunity for the conservation of material, even beyond the period of ten years.

5.4 METHOD OF ARCHIVING

Samples fixed and embedded in paraffin (blocks in paraffin) are perishable material if not kept in suitable spaces as shown in the paragraph below (para. 5.4.). They must also be compliant with all regulations on tracking and traceability as reported in paragraph 3.2 and subsequent 3.2.1 and 3.2.2 of the present document. With regard to the method of preservation of the material in the archive it must be considered that, as regards the slides, the same can also be stored in digital form, using techniques that allow to maintain the characteristics of the material and related medical and legal diagnostic needs which is stretched in archiving. It specifically refers to the mode of dematerialization of the clinical documentation for agreement

[5]. The first part of the first paragraph of the rule states that: within six months of the entry into force of the present law, with regulation adopted under article 17, paragraph 1, of the law 23 August 1988, n. 400, and following amendments, under proposal of the Minister of Health, after consultation of the Interior Minister and of the Minister of Justice, after consulting of the members of the commission of Parliament, it is provided the change of the regulation of the mortuary police, approved with decree of the President of the Republic 10 September 1990, n. 285, based on the following principles.

within the meaning of Article 8 paragraph 6 of the law of 5 June 2003 NO 131, between the government and the regions and autonomous provinces of Trento and Bolzano on document "Guidelines for the dematerialization of the clinical documentation in diagnostic imaging-rules and practices" Rep. Acts N. 81 The April 4, 2012 (49).

On the specific point, it should be noted that, with reference to medical records, it is explicitly provided that the conservation of the same can be done, without new or more burden on public finances, even if only in digital form, in compliance with the legislative decree 7 march 2005, n. 82, And of the legislative decree 30 June 2003, n. 196. In the same way, as regards the slides and as long as it remains unaltered the function, must be considered as having been fulfilled adequate to allow the storage and the storage of the sample even in a different form, as long as the shape of conservation will not affect the function of the material. The problem for many healthcare institutions of storage environments large enough to contain thousands of catalogued specimens requires to reconcile both the essential requirements of the protection of the probative value and balance sheet of the data contained within a histological preparation and the undeniable need for budget (economic and structural) of health care organizations calls to ensure the preservation of histological data. Recent advances in computer technology allow today to performing the so called digitisation of histological examination, of any type: cytology, biopsy, postoperative histological or from a cadaver. Thanks to digitisation, in fact, it is possible to proceed with a substantial scanning of what appears on the slide, the different magnifications and the different colours: on the one hand this allows to store in a manner substantially unlimited the given testimony to the vision of the exhibit frozen in electronic format in such a way as to be able to be reviewed by other eyes and on the other, the free the structures from burden to store for long times thousands of slides holding space (44). The digitisation in fact could allow the structures that have something to store (the slides of histological and cytological preparations) to store the material according to this method.

The obligation of conservation can be adequately fulfilled, in cases in which can and will not affect the functionality of the material and then of the slides by the use of the digital form. **For the slides**, therefore, **preservation in digital form or material may be understood as an alternative mode, left to the discretion of the structure**.

PRESERVATION OF ARCHIVAL SAMPLES

OBJECT	TIME	DECURRENCE	MODALITY
1) "leftoover"	15 days	validation of the report	material
2) sampled tissue:			
a) blocks	10 years	validation of the report	material
b) slides	10 years	"	material or digitalized

5.5 ARCHIVE LOCATION

Specific problems emerge regarding places of storage of the sample fixed and embedded in paraffin, in particular among others the safety of the structures and of the personnel and the quality of preservation. It follows that the structures in which tissue material can be stored must comply with the requirements dictated by the CAP, Laboratory Accreditation Manual 2013 Edition (in particular p. 41 onward) (23), which is periodically updated, unless expressed indication of different or additional procedures on the part of internal legal order is declared.

	TEMPERATURE	UMIDITY	PARASSITES	FLOODING
Min. Beni Cult. e Amb. UNI 10829	19-24°C	40-60%		
Muller J Comp Path 2011	22°C	30-50%		
NCI Biorepository 2011	<27°C	controlled	controlled	
CLIA	Fresh location	Dry location		
Farmacopea USA	<25°C			
BIG - IBCSG	<26°C	>30 e <70%	controlled	controlled

5.6 GUIDELINES: PRESERVATION AND ARCHIVING OF MATERIAL

NOT SAMPLED LEFTOVER MATERIAL

Time: 15 days, starting from the date of validation of diagnostic report

Mode: conservation of the material with vacuum systems or the like in order to avoid environmental dispersion of fumes of formalin (EU Regulation 895/2014 amending Annex XIV of the EC Regulation 1907/2006).

Place: environments and/or systems suitable to ensure the security, tracking and the preservation suitable for possible use for further investigation.

SAMPLED LEFTOVER MATERIAL

BLOCKS

Time: 10 years, starting from the date of validation of diagnostic report

Mode: the preservation of the material must be implemented with assurance of tracking.

Place: ambient temperature (< 27 °C) and humidity controlled (>30% and < 70 %), with control systems for the parasite infestation.

SLIDES

Time: 10 years, starting from the date of the validation of the diagnostic report

Mode: the preservation of the material must be implemented with assurance of tracking, it is to be hoped that systems of digital preservation will be considered.

Place: environments and/or systems suitable to ensure the security, tracking and conservation suitable for possible revision.

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