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SFORL GUIDELINES

Pre-therapeutic histological and cytological assessment in head and neck squamous cell carcinomas. French Society of Otorhinolaryngology Guidelines — 2012

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KEYWORDS
Practice guideline;
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Pathology

Summary
Objectives: The authors present the French Society of Otorhinolaryngology (SFORL) guidelines for histopathologic assessment of head and neck cancer.
Materiel and methods: A multidisciplinary workgroup set up by the SFORL performed an exhaustive review of the literature according to levels of evidence, following the 2000 guidelines of the French national health approvals and assessment agency (ANAES).
Results: Comparison between histologic and clinical data is essential. In case of discrepancy between clinical, radiological and histological findings, reinterpretation or new biopsy may be required (professional consensus). Mere suspicion of carcinoma on fine-needle aspiration lymph-node biopsy only exceptionally warrants aggressive treatment (professional consensus). Exploration for HPV is not recommended as routine practice, being without therapeutic impact (professional consensus). Anti-p16 immunohistochemistry is optional, for epidemiological purposes (professional consensus). Tumor-bank tissue storage must conform strictly to prevailing legislation and good practice rules for sampling and preservation (professional consensus).

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Conclusion: Pathology assessment is mandatory in suspected H&N squamous cell carcinoma. The present guidelines are intended to optimize management.
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The present article successively deals with the French Society of Otorhinolaryngology (SFORL) guidelines for:

- the contents of histology biopsy reports, and their role in the management of head and neck squamous cell carcinoma;
- the role of cytopathology examination in initial head and neck cancer assessment;
- the interest of exploring for human papilloma virus (HPV) in diagnostic biopsy of head and neck squamous cell carcinoma;
- the use of a tumor bank in initial head and neck cancer assessment.

Histology biopsy reports in the management of head and neck squamous cell carcinoma

Anatomopathology reports include an opening section comprising administrative and clinical data, a diagnostic section comprising macro- and microscopic description, and a conclusion. The pathologist is responsible for their presentation. They contain information that is essential for patient management. This information is detailed in the specialty reference documents. Diagnosis is based on collating the available clinical and biological data.

Report contents (according to version 2, 2009) of the anatomy and cytopathology good practice guidelines (RBPACP: *Référentiel des bonnes pratiques en anatomie et cyto-pathologiques* v2) of the French Association for Quality Assurance in Anatomy and Cytopathology (AFAQAP: *Association française d'assurance qualité en anatomie et cytologie pathologiques*)

The report first presents administrative and clinical data. These are partly supplied by the clinician and should be as precise and informative as possible, mandatorily including:

- the address of the anatomy and cytopathology (ACP) structure;
- patient identification (surname, given name(s), date of birth, PIN or other registration number);
- type of sample (fine-needle aspiration biopsy?), number of vials sent and number of vials received;
- name and address of prescribing physician;
- if possible, names and addresses of corresponding physicians;
- name and signature of the reporting pathologist;
- dates of sampling, recording and reporting;
- ACP structure record number;
- clinical data provided by the biopsy physician, including patient history.

Macro- and microscopic description

The report is intended to diagnose any malignancy, guiding the clinician with regard to lesion aggression. Macroscopic management is a fundamental stage. It should be stated whether samples were delivered fresh or fixed and if any apposition or freezing was performed; whether the biopsy delivered is oriented; and whether extemporaneous examination had been performed.

In diagnostic biopsy, macroscopic examination usually consists in specifying the number and size of the samples. If the biopsy is oriented, resection margins should be tattooed in different colored inks, respecting specimen orientation.

The description should follow a logical order: either that of the vials received or that of the size of the samples. It should include:

- confirmation of infiltration; description of dysplastic lesions, if any;
- histologic type, if possible determining whether or not it is a variant: verrucous, spindle-cell, adenoid or acantholytic, basaloid, papillary, adenosquamous or, in the oral cavity, cuniculatum squamous cell carcinoma (Fig. 1);
- if possible, the degree of differentiation corresponding to the histologic grade: well, moderately or poorly differentiated;
- presence of nerve sheathing or vascular emboli: although often difficult to determine on biopsy, should be reported if visible [1—4].

As well as the carcinoma, any associated lesions (dysplasia, etc.) may also be described, and especially *in situ* carcinoma lesions (Fig. 2). Two precancerous lesion classifications (Table 1) are available, to be selected in liaison with the clinicians [3,5].

Optionally (and, in practice, rarely), infiltration thickness and inflammatory infiltrate data may be provided.

Data should correspond to the prevailing guidelines and recommended classifications [3]. Complementary techniques (special staining, immunohistochemistry and/or *in situ* hybridization) should be described and interpreted. Immunostaining is rarely needed to diagnose squamous cell carcinoma; in relatively undifferentiated forms, however, total and/or CK5/6 cytokeratin expression can usefully be detected.

HPV exploration is dealt with in the third section.

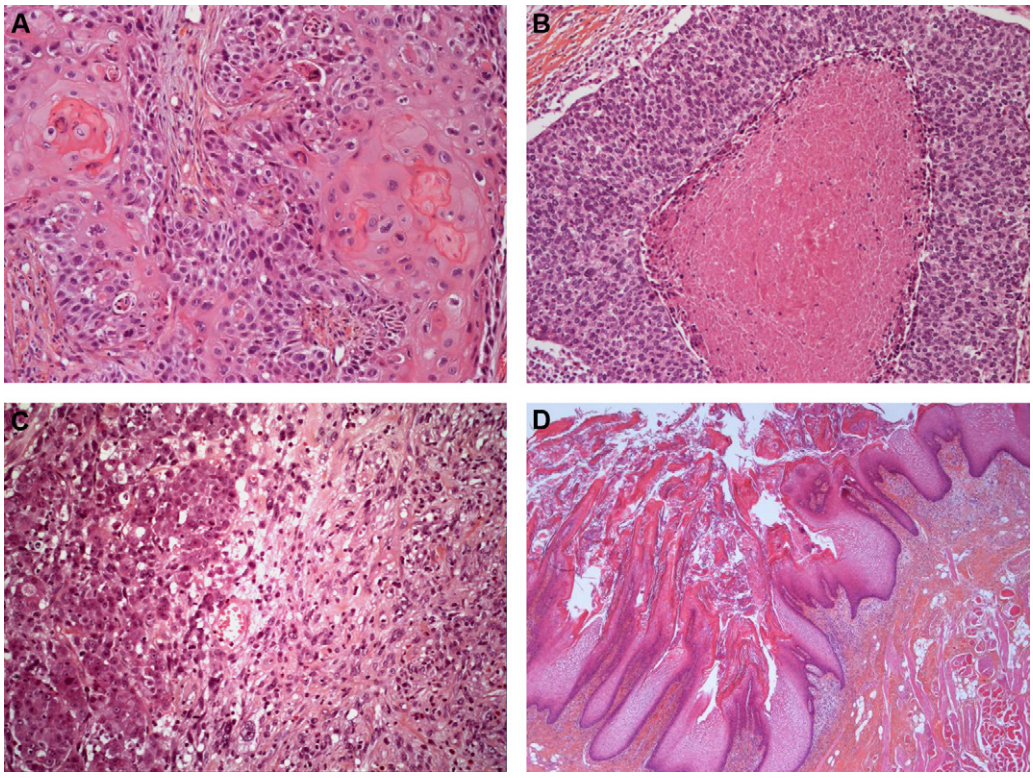

Figure 1 Invasive squamous cell carcinoma: some histologic variants. A. Conventional squamous cell carcinoma. B. Basaloid squamous cell carcinoma. C. Spindle-cell carcinoma. D. Verrucous squamous cell carcinoma.

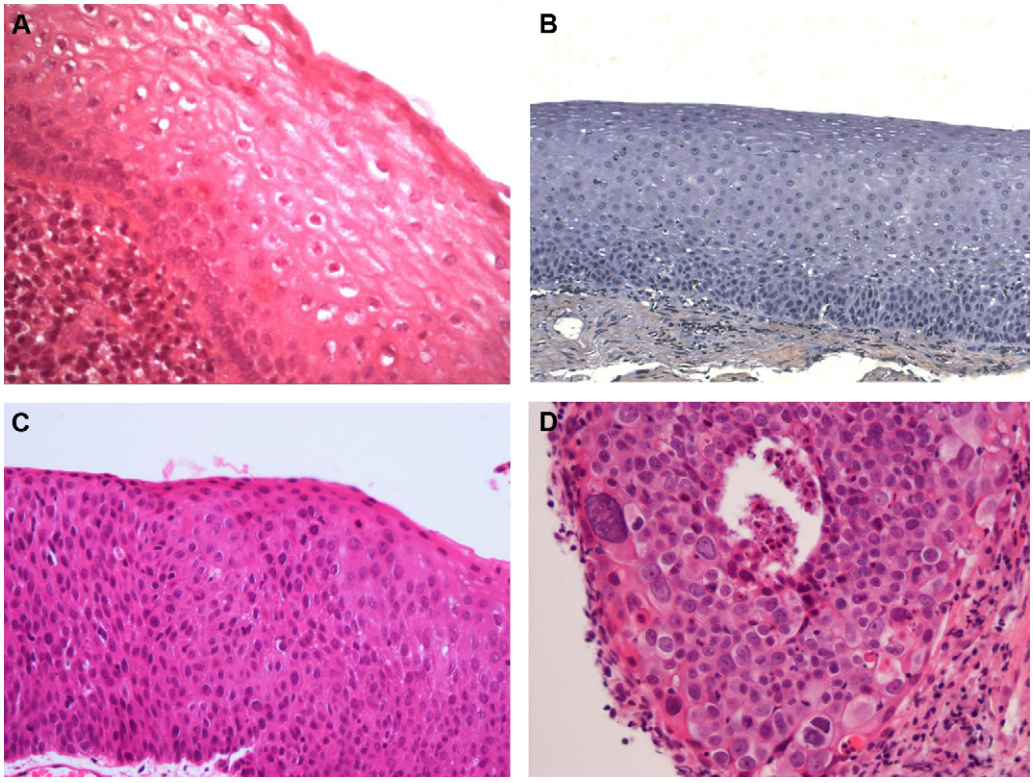

Figure 2 Dysplasia and invasive squamous cell carcinoma (HES × 40). A. Normal malpighian epithelium. B. Mild to moderate dysplasia. C. Severe dysplasia/*in situ* carcinoma. D. Invasive squamous cell carcinoma.

Table 1 Precancerous lesion classification.

	World Health Organization (WHO)	Hellquist (Ljubljana)
Benign	Simple hyperplasia	Simple hyperplasia Abnormal hyperplasia
Precancerous with low risk of invasive carcinoma	Dysplasia grade I Dysplasia grade II	Atypical hyperplasia
Precancerous with high risk of invasive carcinoma	Dysplasia grade III Carcinoma *in situ*	Carcinoma *in situ*

In conclusion

Great care should be given in filling out the pathology request form. So far as possible, a diagnosis should be suggested for each sample, with the degree of proliferation (invasive, *in situ*). The conclusion should provide a synthesis, using national and/or international classifications. In case of discrepancy between clinical, radiological and histological findings, pathology and clinical data should be compared, with possible re-analysis of the sample or complementary sampling.

Histology biopsy reports in the management of head and neck squamous cell carcinoma: SFORL guidelines.

- Great care should be given in filling out the pathology request form (professional consensus).
- The practitioner should check, in the report, the various carcinoma characteristics, and especially the degree of invasion (professional consensus).
- Pathology and clinical results should be compared, and sampling be renewed or re-analyzed if necessary, after discussion with the pathologist (professional consensus).

Cytopathology in initial assessment for head and neck cancer

Histologic examination to confirm malignancy is mandatory before initiating treatment of head and neck cancer. When no mucosal tumor is accessible for biopsy, diagnosis may be founded on cervical adenopathy. Two types of sampling may be performed: fine-needle lymph-node aspiration, which may be guided by imaging, or resection biopsy (adenoidectomy). Indications for biopsy by trocar or lymph-node surgical biopsy are highly controversial, rarely performed, and are no longer recommended in current practice (expert consensus).

Fine-needle aspiration cytology is a simple, inexpensive and relatively non-invasive examination enabling differential cytologic diagnosis between lymphoma, metastasis and chronic adenitis [6]. It can easily be performed at the bedside or on an outpatient basis. False-positives and false-negatives are frequent, although it is fairly effective in diagnosing lymph-node metastasis from squamous cell carcinoma [7,8]. Slide interpretation is highly investigator-dependent. In poorly differentiated tumor, cell typing may be difficult; some teams, when possible, perform complementary immunohistochemical staining.

In practice, when primitive tumor biopsy is not feasible, two situations may arise:

- for adenopathy without obvious primitive lesion but with a strong clinical suspicion of squamous cell carcinoma metastasis, in which clinical assessment (ENT examination, CT and PET) failed to find the primitive tumor, fine-needle aspiration can diagnose malignancy in almost 90% of cases [9]. Lymph-node surgical biopsy is contra-indicated as worsening the rate of lymph-node recurrence and metastasis [10], with a risk of seeding the cutaneous trajectory [8]. The logical attitude [11,12], when no primitive tumor is found, is tonsillectomy or tonsil biopsy if aspiration indicates squamous cell carcinoma metastasis, with possible exploratory cervicotomy and extemporaneous histologic examination. The patient should be advised of the possibility of associating lymph-node curage to the operation;
- if the patient's general health status precludes general anesthesia and the tumor is not accessible orally or by flexible endoscopy, diagnosis requires fine-needle aspiration cytology. Biopsy of non-resectable lymph-nodes, by trocar or surgery, is only rarely indicated to confirm diagnosis before initiating curative or palliative treatment.

Fine-needle aspiration cytology is not sufficient to initiate therapy, but contributes to guiding assessment. Before any invasive surgery or radiation and/or chemotherapy, diagnosis and exact histological type should be confirmed on classic histology in a tumoral or lymph-node biopsy sample.

It should be stressed that interpreting fine-needle aspiration samples of cervical cysts, especially in region II, is difficult: it is often impossible to distinguish between a second-groove (tonsillar) cyst and cystic metastasis of a (usually oropharyngeal) well-differentiated squamous cell carcinoma. It should be borne in mind that, in patients over 40 years of age, metastasis is more likely than congenital cyst and that histologic examination of the resection specimen is required.

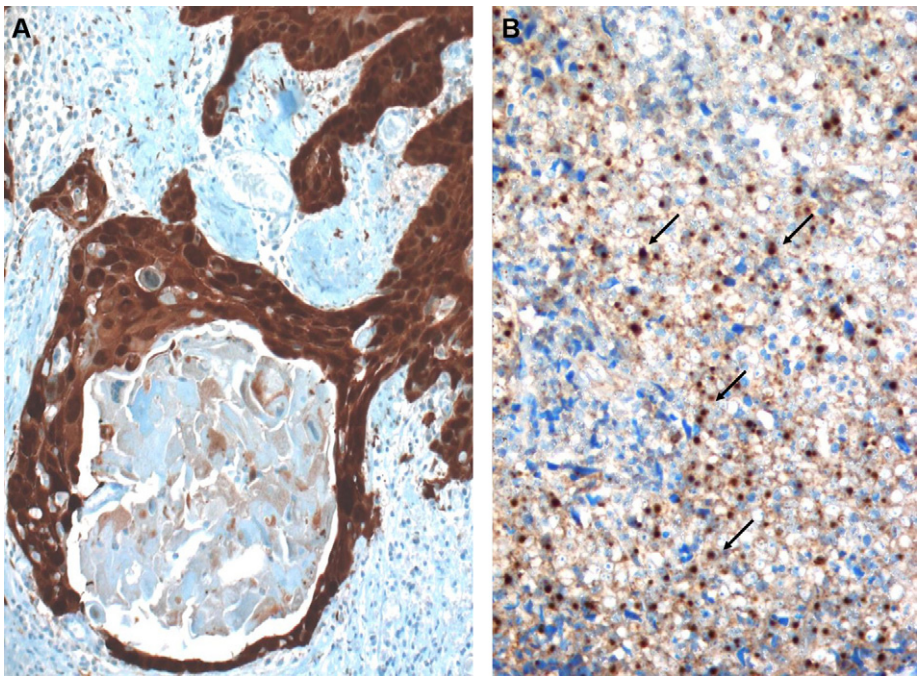

Figure 3 *In situ* identification of HPV infection. A. Immunohistochemical study with anti-p16; the tumor cells are uniformly stained (cytoplasmic and nuclear staining). B. HPV *in situ* hybridization (oncogenic HPV cocktail). The tumor cells are stained in the nucleus, showing the integration of the virus into the genome (arrows).

Cytopathology examination in initial assessment of head and neck cancer: SFORL guideline.

Mere suspicion of squamous cell carcinoma based on fine-needle lymph-node aspiration is insufficient to initiate aggressive therapy (surgery, radiation and/or chemotherapy) (professional consensus).

Role of human papilloma virus (HPV) exploration in diagnostic biopsy of head and neck squamous cell carcinoma

In certain (notably oropharyngeal) locations, oncogenic HPV subtypes (mainly HPV16 and HPV18) may be the only environmental factors found. HPV rates reach 50 to 70% in tonsillar cancer [13—19]. These small, icosahedrically symmetrical non-enveloped DNA viruses have an oncogenic effect mediated by viral proteins E6 and E7, respectively disturbing the functioning of tumor-suppressor genes p53 and pRb.

Epidemiological data show that the HPV rates depend on tumor location, predominating in oropharyngeal sites (tonsil and tongue base), and on the patient's geographic origin, with the highest prevalence in Europe being in Scandinavian countries. In France, the single retrospective study to be published, involving 12 hospital centers and 523 oropharyngeal or oral cavity biopsies, found HPV in 46.5% of oropharyngeal and 10.5% of oral cavity squamous cell carcinomas [20].

Several cohort studies confirmed presence of HPV in at least 30% of tonsil cancers. HPV prevalence in 5046 head and neck squamous cell carcinomas (all locations taken together) was 25.9% [16]; oropharyngeal locations (35.6% of HPV-associated cancers) were more frequent than the oral cavity (23.5%) or larynx (24%).

In head and neck squamous cell carcinomas, HPV seems to be associated with better radio- and chemosensitivity, and is a factor of good prognosis in terms of survival [13,21—23].

Oncogenic HPV infection may thus serve as a major biological marker, both therapeutically and prognostically. It is therefore increasingly important to screen for oncogenic viruses, using reliable tools, so as better to adapt treatment by better understanding of the virus-induced micro-environment.

Virologic exploration uses PCR on a tumor fragment, fresh, frozen or included in paraffin, or *in situ* hybridization by DNA probe on material included in paraffin (usually with a cocktail corresponding to the most frequent HPV oncogenes) [24,25].

In immunohistochemistry, p16 overexpression by the tumor is an indirect marker of HPV (Fig. 3). However, anti-p16 antibody staining needs to be correctly interpreted (diffuse staining of the majority of tumor cells) [26]; moreover, p16 overexpression may also be found in certain HPV-negative squamous cell carcinomas on *in situ* hybridization or PCR. With or without association with HPV, p16 overexpression is a factor of fairer prognosis [27—29].

Tumor banks: guidelines in initial head and neck cancer assessment

Tumor cryopreservation banks were initially set up for research purposes, and are now indispensable tools in cancer research. Patients with one or more cryopreserved sample

Role of human papilloma virus (HPV) exploration in diagnostic biopsy of head and neck squamous cell carcinoma: SFORL guidelines.

- HPV should not be researched for systematically, in absence of therapeutic impact (professional consensus).
- In the particular case of oropharyngeal carcinoma and/or patients free of smoking and alcohol risk factors, immunohistochemistry with anti-p16 antibody is optional for epidemiological purposes (professional consensus).

are listed in a computerized data file which, in accordance with French public health law, is submitted to the health research data processing consultative committee, as stipulated in article 40-2 of Act nº 78-17 of January 6, 1978 concerning computerized data files and liberty; as stipulated in the Civil Code (articles 16-1 and 16-6), the samples may not be sold and patients receive no payment.

Just as a bank holds money and a blood-bank conserves blood samples, a tumor bank conserves tumor fragments. Over time, as well as tumor fragments and serum samples, fragments of healthy tissue (exposed to toxins or not), skin, nodes, and exfoliated cells in saliva are also sampled for a given patient: the term ''biological collection'' or ''biobank'' is in fact more apt than ''tumor bank'', which is rather reductive; to avoid confusion, however, the term ''tumor bank'' will be used hereinafter. Conservation and use of biological material has, since the bioethics law of August 6, 2004, been strictly controlled by laws and regulations, themselves partly founded on EU provisions (notably, Directive 2004-23 of March 31, 2004, amended by Decree 2007-1220 of August 2007). Moreover, good practice guidelines have been progressively developed in this field.

There are tumor banks for health purposes, comprising samples stored for diagnostic and/or prognostic purposes or for therapeutic decision-making, based on investigations requiring frozen material. In 2006, the French hospital admission and care organization authority *(Direction de l'hospitalisation et de l'organisation des soins*: DHOS) and national cancer institute (*Institut national du cancer*: INCA) set up an expert group to determine which tumors should be sampled in health-service tumor banks [30]. The tumors selected were: malignant hemopathies, sarcomas, brain tumors and childhood tumors. Some colorectal and pulmonary tumors initially selected were later excluded under the new 2011 INCA guidelines (Tumor-bank indications and guidelines update, available on the INCA website). Head and neck tumors are thus not included. The experts' choice, however, was a snapshot of the state of knowledge in molecular and tumoral pathology, obviously requiring constant updating. Moreover, the same expert group strongly recommended that, whenever possible, a sample of any malignant tumor should be cryopreserved for research purposes, including retrospective studies. Abnormalities of recognized health interest concern DNA and are represented by gene rearrangement, translocation, amplification, deletion and mutation, shown on direct DNA or RNA study. More exceptionally, abnormal quantitative expression may be of interest. Interest may be diagnostic or therapeutic. If such abnormalities are detected in one or more head and neck tumor subtypes, a health tumor bank should be set up. At present, cryopreservation is not mandatory for head and neck tumors, regardless of location.

Which physicians are concerned?

Any physician or surgeon, in the public or private sector, may decide to cryopreserve a head and neck tumor fragment. If the practitioner is remote from any tumor bank, an agreement on inter-institution sample management needs to be drawn up.

Which tumors should be sampled?

For head and neck tumors, no law or professional guidelines specify which are to be sampled. Targeting is usually up to hospital teams doing research in head and neck tumor biology, depending on their line of research.

Place of sample storage

In practice, tumor banks tend to be located in health structures and particularly in biological resource centers. The organization requesting cryopreservation should first submit the project to a Committee of Protection of the Individual (*Comité de protection des personnes*: CPP) for approval, then make a declaration to the Ministry of Research and to the director of the relevant regional health authority to obtain authorization according to article L-1243-C of the Public Health Code. Hospital tumor banks come under the 2006 good practice rules for clinicians and researchers drawn up by INCA in cooperation with Inserm, the Paris AP—HP hospitals board and the national cancer center federation (CLCC), one of the ''professional guidelines'' published by INCA and available at http://www.e-cancer.fr/expertises-publications-de-l-inca/rapports-et-expertises/recherche under ''*Conservation et utilisation des échantillons tumoraux en cancérologie*'' (conservation and use of tumor samples in oncology). Part of the collection may be given over to another organization (mainly, research teams) for scientific use, either free of charge or in exchange for payment after approval by the tumor bank management and the scientific committee of the Biological Resources Center or Platform.

Good practice rules

The French Health Authority (HAS) guidelines (available on the HAS website, under ''*Cryopréservation*'') are:

- it is necessary to inform the patient and be sure there is no opposition to the biological samples being used for non-diagnostic purposes; the information should be delivered by a qualified physician and confirmed by duplicate signed written consent, one copy given to the patient and the other kept in the medical file; the consent form should detail the managers(s) of the collection, with titles and

addresses; it should be specified that the patient may at any time demand that his or her samples be destroyed; a separate specific consent form is to be provided to and signed by the patient in case of genomic identification;
- it is important that tumor samples be frozen as quickly as possible (within 15 minutes, if possible) to ensure good nucleic acid conservation; sampling should be from a non-necrotic region of the tumor, using sterile material; the sample should be taken quickly to the pathology department for freezing, or be immediately placed in RNA-later medium to allow transport at room temperature as soon as possible (especially suitable in the private sector); after conditioning in the pathology department, the sample is to be taken for storage in the biological resources center;
- a ''mirror'' inclusion block (corresponding to the other face of the frozen sample) should be systematically created for the sample; stained slides are to be stored in the pathology department;
- an information sheet concerning the sample and a copy of the pathology report should be sent to the tumor bank;
- non-tumor samples (blood, saliva, healthy tissue, etc.) should be taken in the same step and undergo specific conditioning, transport and storage;
- cryopreservation is a medical act, and should be performed by a pathologist, or possibly delegated to a laboratory technician, but only under the supervision of a pathologist;
- the stored sample may be used for studies based on nucleic acids or proteins; the biological resources center's technical facilities should be adapted to the study to be undertaken.

Tumor banks: SFORL guidelines.

Any tumor-bank storage must respect prevailing legislation, especially as regards informed consent, and scrupulously follow good practice rules for sampling and storage (professional consensus).

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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