SPECIAL REPORT

International recommendation for a comprehensive neuropathologic workup of epilepsy surgery brain tissue: A consensus Task Force report from the ILAE Commission on Diagnostic Methods

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Summary

Epilepsy surgery is an effective treatment in many patients with drug-resistant focal epilepsies. An early decision for surgical therapy is facilitated by a magnetic resonance imaging (MRI)—visible brain lesion congruent with the electrophysiologically abnormal brain region. Recent advances in the pathologic diagnosis and classification of epileptogenic brain lesions are helpful for clinical correlation, outcome stratification, and patient management. However, application of international consensus classification systems to common epileptic pathologies (e.g., focal cortical dysplasia [FCD] and hippocampal sclerosis [HS]) necessitates standardized protocols for neuropathologic workup of epilepsy surgery specimens. To this end, the Task Force of Neuropathology from the International League Against Epilepsy (ILAE) Commission on Diagnostic Methods developed a consensus standard operational procedure for tissue inspection, distribution, and processing. The aims are to provide a systematic framework for histopathologic workup, meeting minimal standards and maximizing current and future opportunities for morphofunctional correlations and molecular studies for both clinical care and research. Whenever feasible, anatomically intact surgical specimens are desirable to enable systematic analysis in selective hippocampectomies, temporal lobe resections, and lesional or nonlesional neocortical samples. Correct orientation of sample and the sample's relation to neurophysiologically aberrant sites requires good communication between pathology and neurosurgical teams. Systematic tissue sampling of 5-mm slabs along a defined anatomic axis and application of a limited immunohistochemical panel will ensure a reliable differential diagnosis of main pathologies encountered in epilepsy surgery.

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KEY POINTS

- *Neuropathology in epilepsy surgery*: Standardized neuropathologic examination of brain tissue obtained from epilepsy surgery allows classification of the clinicopathologic substrate of the patient's seizure disorder. It will also help predict a patient's risk for favorable or unfavorable postsurgical seizure control. Prominent examples of such efforts are ILAE's First Consensus Classification Systems for Focal Cortical Dysplasia and Hippocampal Sclerosis. It will also improve our understanding of the underlying etiology by using well-characterized human brain tissue for advanced brain research strategies
- *Recommendations for consensus protocols in the neuropathology workup*: According to the recent ILAE clinicopathologic classification systems, neuropathologic assessment of epilepsy surgery specimens and neuropathology reports should apply standardized consensus terminology following protocols. This is considered paramount to the implementation of evidence-based medicine, for example, by randomized controlled clinical trials, which remain incomplete in the field of epilepsy surgery. A purpose of our presented Task Force report is to attempt to standardize the neuropathologic workup of tissue procurement, handling, and processing, with the goal of establishing an essential infrastructure for systematic neuropathologic examinations in this specialized brain surgery
- *Neuropathology Task Force of the ILAE Commission on Diagnostic Methods*: The ILAE Commission on Diagnostic Methods covers all major diagnostic modalities to clinically characterize and diagnose a patient's epilepsy, namely electro-/neurophysiologic, neuropsychology, imaging, and neuropathology measures. Our objective is to provide standardized protocols, terminology use, and guidelines for a cost-effective diagnosis of epilepsy and their related comorbidities as well as use of consensus classification systems for underlying etiologies. The Neuropathology Task Force has been instrumental in recent clinicopathologic classification systems tested and disseminated by a collaborative virtual microscopy platform as well as a collaborative Summer School initiative, annually convening at different venues around the world, for example, 2013–2014 in Erlangen, Germany, and 2015 in Campinas, Brazil

Standardized operational procedures (SOPs) for inspection, distribution, and processing of epileptogenic brain tissue will reduce sampling errors in any pathology laboratory, ensure the best possible histologic assessment, and support research activities and brain-banking initiatives. Good communication and interaction between neuropathologists, epileptologists, and neurosurgeons are considered best medical practice and could start at multidisciplinary patient management conferences, preceding epilepsy surgery. Such communication would ensure the neuropathologist's knowledge of the clinical and radiologic diagnosis, and type and aims of resection prior to receipt of the surgical specimen, thereby optimizing the yield of histopathologic assessment. To support interdisciplinary communication and strategies for reliable tissue analysis in routine clinical applications and research, the International League Against Epilepsy's (ILAE's) Neuropathology Task Force developed a simple protocol for tissue handling that can be applied worldwide.

The SOP is based on systematic sampling of 5-mm interval slabs along an anatomically defined plane of section. For optimal neuropathologic precision, anatomically intact resections (also referred to as "en bloc") are preferable. However, surgical procedures in the dominant hemisphere or close to eloquent areas will often be directed by safety considerations, and large "en bloc" samples may not be feasible. In such cases, biopsies from electrophysiologically well-characterized epileptogenic areas or lesions detected by magnetic resonance imaging (MRI) are helpful as an alternative strategy to allow histopathologic validation of the epileptogenic substrate. In complex cases, the presence of a neuropathologist in the operating room is recommended to document anatomic landmarks of the surgical specimen. The neuropathology report should specify a final diagnosis and subtype of the epileptogenic lesion and any additional pathologies noted (Table 1), their localization, and extent in the samples submitted as well as in relation to clinical information provided, for example, epileptic lesion versus irritative versus ictal-onset zones.[1–3]

Although routine light microscopic assessment stains remain the benchmark, a set of well-characterized antibody immunoreactivities has been developed to identify aberrant patterns of disease-specific protein epitopes. Recent epilepsy classification schemes for subtype-specific clinicopathologic diagnosis are built on a selected and moderate number of such antibody immunoreactivities,[4,5] and supported by evidence from peer-reviewed research studies.[6–10] The Task Force recommends systematic application of these antibodies (or alternative probes when published with ample evi-

Table 1. Principal histopathologic categories of brain lesions associated with drug-resistant focal epilepsies submitted to epilepsy surgery

	n (%)	Mean age at Onset	Mean age at Surgery
Hippocampal sclerosis	2,071 (36.8)	11.4	33.6
Tumors	1,160 (20.7)	16.9	27.2
Cortical malformations	1,067 (19.0)	6.0	17.7
No lesion	363 (6.5)	13.1	28.0
Scars	321 (5.7)	10.9	25.4
Vascular malformations	305 (5.4)	23.4	34.5
Dual pathology	209 (3.7)	9.5	26.7
Encephalitis	95 (1.7)	11.3	18.4
Double pathology	12 (0.2)	6.8	11.9
Total	5,603	12.2	27.9

Data retrieved from the German Neuropathology Reference Center for Epilepsy Surgery. Age at onset/surgery = mean age of patients at onset of spontaneous seizure activity (in years) and surgery (in years), respectively. Dual pathology includes hippocampal sclerosis with another principal pathology.[5] Double pathology refers to two etiologically independent pathologies (hippocampal sclerosis not included).[6]

Table 2. Recommended antibodies for the diagnosis of epilepsy-associated brain lesions[a]

Staining	HS	MCD	Tumors	Vasc.	Infl.	Scars	No lesion[16]
HE	x	x	x	x	x	x	x
CV-LFB	x	x	x	x	x	x	x
GFAP	x[1]	x	x[9]	x	x	x	x
MAP2		x[4]	x[10]				x
NeuN	x[2,11]	x[5]	x[11]	x[11]	x[11]	x[11]	x
NFL		x[6]					x
Vim		x[7]					x
CD34		x[8]	x[12]				x
Ki67			x[13]				x
IDH1			x[14]				x
CD68			x		x	x	x
CD3	x[3]				x[15]	x	x

HE, hematoxylin-eosin; CV-LFB, cresyl violet-Luxol fast blue (LFB can be also combined with HE); GFAP, glial fibrillary acidic protein; MAP2, microtubule-associated protein 2 (clone HM2); NeuN, neuronal nuclei (clone A60); NFL, nonphosphorylated neurofilament protein (clone SMI32); VIM, vimentin; CD34, oncofetal class II epitope CD34 (clone QBenD10); Ki67, proliferation marker (clone Mib1); IDH1, R132H point-mutation specific antibody; CD68, antibody specific for macrophages and microglia, other epitopes shown to specifically recognize microglia can also be applied; CD3, antibody specific for T lymphocytes; HS, hippocampal sclerosis; MCD, malformation of cortical development; Vasc., vascular malformations including cavernomas and arteriovenous malformations; Infl., inflammation; Scars, resulting from brain trauma or vascular infarcts, information may be available from clinical history; no lesion, refers to histopathologic specimens in which none of the aforementioned principal histopathologic categories can be identified. This will usually require a more generous application of immunohistochemical investigations (see diagnostic values specified below).

x = recommended stains and immunoreactivities, as specified below (1–16). 1 = describe reactive gliosis in areas of neuronal loss and at surface boundaries (Chaslin's gliosis); 2 = subfield analysis of neuronal cell loss (ILAE classification of HS); 3 = to exclude cytotoxic (CD8+) T-cell infiltration in limbic encephalitis; 4 = heterotopic neurons in white matter; 5 = architectural abnormalities of cortical layering in FCD I, II, and III, and polymicrogyria; 6 = dysmorphic neurons in FCD ILAE type II, but present also in aged pyramidal cells of cortical layers III and V; 7 = balloon cells in FCD type II, but also expressed in reactive astrocytes; 8 = can be present in balloon cells in FCD type IIb; 9 = differentiates astrocytic from clear-cell oligodendrocytic-like differentiation; 10 = majority of glial cells in diffuse glioma; only neuronal expression in glioneuronal tumors; 11 = to exclude associated FCD type III a-d (ILAE classification 2011); 12 = majority of ganglioglioma and diffuse glioneuronal tumors, typically not expressed in low grade gliomas; 13 = low proliferation index (<5%) in glioneuronal tumors; 14 = reacts specifically with the R132H point mutation not present in glioneuronal tumors; 15 = to verify cytotoxic (CD8+) T-cell infiltration in limbic or Rasmussen encephalitis; 16 = use entire panel, as nonlesional epilepsy should be diagnosed only following exclusion of any other lesion pattern.

[a]Proposed stains and antibodies for immunohistochemical reactions have been selected from published ILAE consensus classification systems,[4,5] and ILAE agreement studies,[17] but can be adopted by specific laboratory expertise if required (see specifications below). This list does not cover the entire spectrum of antibodies available for the differential diagnosis of brain pathology or compete with recommendations of the WHO classification system for brain tumors.

dence) as minimum standard in neuropathology laboratories in clinical centers engaged in epilepsy surgery (Table 2), acknowledging that some national medical boards or public health services may not accept a given antibody or compensate for its diagnostic use.

Long-term tissue storage and archiving will become more important in modern diagnosis as new molecular tests are increasingly available, for example, *IDH1* mutation and 1p/19q codeletion analysis in the differential diagnosis of diffuse gliomas. Clinicians may increasingly start requesting retrospective investigations or additional review of stored tissue samples for patients who underwent epilepsy surgery in the past.[11] Advanced frozen storage facilities, adequate record keeping, and protocols for microscopic review of snap frozen samples will facilitate this process and improve diagnostic accuracy if lesional tissue is available for molecular testing or for evaluation in any advanced research study.

Methods

The recommendations build upon previous work of the ILAE Neuropathology Task Force of the Diagnostic Methods commission in 2009–2013, that also developed clinicopathologic consensus classification systems for hippocampal sclerosis[4] and focal cortical dysplasia.[5] A detailed survey of neuropathologic protocols and brain-banking initiatives was conducted among Task Force members and from published literature to identify common practices in the field.[12–15] Because most protocols are adapted to local conditions, practices, and legal regulations governing human brain tissue, this information was also helpful in defining minimum standard requirements that can be applied worldwide. With this background in mind, the Task Force for Neuropathology of the ILAE Commission on Diagnostic Methods (term 2013–2017) was charged with the task of developing a consensus recommendation for tissue inspection, distribution, and histopathologic examination. Input from our clinical and research colleagues helped define their expectations from an efficient and reliable neuropathology service working in close collaboration with the

different team members while minimizing disruptions to routine workflows. The results allowed us to develop a core protocol for handling human brain tissue obtained at epilepsy surgery. We also took into account experience gained from research efforts collecting and storing human brain tissue (e.g., European Epilepsy Brain Bank; German Neuropathology Reference Center for Epilepsy Surgery), panel discussions among international epilepsy neuropathologists during international epilepsy meetings (ILAE, European Congress of Epileptology, and American Epilepsy Society meeting), and participants of the International Summer School for Neuropathology of Epilepsy Surgery.[16] Because these recommendations are aimed primarily at clinical diagnosis in patient care, the important role of human tissue research using histopathologically well-characterized tissue samples will not be systematically reviewed and discussed, nor will protocols for research be provided here.

It is the consensus of this Task Force, that state-of-the-art neuropathologic workup of human brain tissue obtained during epilepsy surgery requires a minimum set of established and well-recognized stains and antibody immunoreactivities that can be utilized internationally by neuropathologists or general anatomic pathologists in most hospitals. Antibodies recommended in this work have been selected based on the following criteria.

1 Antibody immunoreactivities described in and recommended by the ILAE classification system for focal cortical dysplasia (FCD), for example, NeuN, nonphosphorylated neurofilament protein (SMI-32), vimentin, and Map2[5]
2 Histochemical stains and antibody immunoreactivities described in and recommended by the ILAE classification system for hippocampal sclerosis, for example, cresyl-violet-Luxol-fast-blue (CV-LFB), NeuN, and GFAP[4]
3 Antibody immunoreactivities recommended by an international FCD agreement study, for example, NeuN, SMI32, and vimentin[17]
4 For the differential diagnosis of epilepsy-associated brain tumors, the group strongly recommended on the basis of published reports the use of CD34,[7,15] as well as mutation-specific IDH1 and the proliferation marker Ki67[18] This selection of antibodies does not compete with the World Health Organization (WHO) classification system for brain tumors or any additional molecular test that may be required in the future to clarify each tumor's risk for progression or patient's treatment stratification.)
5 Microglial nodules and lymphocytic infiltration were hallmarks in Rasmussen and limbic encephalitis and should be validated with respective antibody immunoreactivities. The group proposes CD68 as a marker for microglia, and CD3 as a marker for lymphocytic T-cell differentiation. This does not rule out application of any other published marker (e.g., Iba1, CD8, and CD45) that is already established and used in a laboratory.

An Interdisciplinary Diagnostic Approach Is Required for Successful Epilepsy Surgery

Optimum histopathologic services can be achieved in a setting of collaborative interaction with epileptologists, neurosurgeons, and neuropathologists. Other disciplines involved in clinical workup including neuro-/radiologists, neurophysiologists, and neuropsychologists also contribute to increasing the expected yield from neuropathology services. This paper, therefore, will briefly review some concepts that distinguish epilepsy surgery from the neurosurgical treatment of other neurologic disorders, such as tumors and vascular malformations.

Patients undergoing epilepsy surgery generally have long-standing drug-resistant seizures.[19–23] A broad spectrum of histopathologic lesion categories can be encountered in this cohort, including tumors, degeneration (e.g., hippocampal sclerosis [HS]), brain malformations (e.g., FCD or cortical tubers in patients with tuberous sclerosis complex [TSC]), glial scars (traumatic brain injury, bleeding, perinatal infarcts, or any other ischemic insult), inflammation (e.g., Rasmussen's or limbic encephalitis), or vascular malformations (e.g., cavernomas, angiomatosis, arteriovenous malformations). However, in 6.5% of all patients, no specific microscopic abnormality is identified (Table 1).

Improved high-field structural and functional neuroimaging techniques allow presurgical detection of many potentially epileptogenic focal brain lesions.[24,25] The decision for surgical intervention, either as a curative-tailored complete resection, partial resection, or as palliative treatment (callosotomy, hemispherotomy, vagus nerve and deep brain stimulation), should then be discussed at interdisciplinary case management conferences. Counseling a patient about best available treatment options and optimum long-term risk–benefit tradeoffs requires careful considerations of all available information.[26] Ideally, the neuropathologists should be involved early, already at this point, to discuss expected results from histopathologic analyses of resected brain specimens and the likelihood of making a specific diagnosis if anatomically intact "en bloc" resections will not be made available. The extent of neurosurgical resection may largely depend on the location of a given lesion, as well as its relation to the epileptogenic, irritative, and ictal-onset zone, for example, whether it is localized in the dominant hemisphere or adjacent to eloquent cortical areas. Invasive electroencephalography (EEG) recordings

are frequently carried out as part of the presurgical workup, which will also influence the histopathologic examination (see below).

Concepts in epilepsy surgery differ from those in most other neurosurgical procedures in neurooncology, brain trauma, or palliative medicine, in which nonlesional brain tissue is only rarely resected. In contrast, the main goal in epilepsy surgery is to cure epilepsy and maintain long-term seizure control, rather than to only remove a suspected brain lesion. Consequences from this strategy are multilayered and also affect the histopathologic workup. The electrophysiologically defined epileptogenic area can be larger than or even separate from an MRI-evident brain lesion, and the surgical resection field could be, therefore, larger than anticipated for a lesionectomy. Consequently, histopathologic assessment may not reveal altered cortical brain structure in all surgical specimens, when samples were selected by a standardized operational procedure for inspection, distribution, and processing (see recommendations of this Task Force below). This does not imply that histologically "normal" tissue is functionally normal, as many molecular alterations that increase tissue susceptibility to seizures or decrease seizure threshold escape detection at the resolution level of light microscopy. With more advanced and refined analysis protocols, such alterations can often be identified, for example, acquired channelopathies and altered glial networks.[27,28] However, these abnormalities may not be revealed by a routine histopathologic workup and thus will not be discussed further here. Another challenge represents the large spectrum of secondary changes resulting from intracerebral diagnostic procedures. Implantation of intracranial electrodes, either with subdural grids, strips, or depth electrodes are used increasingly and always cause reactive cellular responses, for example, presence of subpial bleeding, traces of reactive cellular (inflammatory) infiltration, and microinfarcts along the trajectory of a depth electrode. It is beyond the scope of this Task Force report to discuss all possible scenarios. However, information about such procedures should be made available to the neuropathologist together with a short summary of the patient's epilepsy history in order to differentiate bona fide epileptogenic glial scarring or inflammatory infiltrates from iatrogenic secondary changes.

In summary, an interdisciplinary approach assists the epileptologist and the neurosurgeon in fashioning an ideal resection, helps the neuropathologist to understand the clinical question, and ultimately the patient by optimizing care. A standardized histopathology report is helpful for further management of each patient's epilepsy and will improve our understanding of the underlying etiology by using well-characterized human brain tissue for advanced brain research strategies.

A Comprehensive Histopathology Report in Epilepsy Surgery

All histopathology reports should refer to anatomic landmarks and orientation, and clearly state a histopathologic diagnosis according to current classification systems. Relevant auxiliary clinical and diagnostic information including MRI findings should be included in the "clinical history" section of the histopathology report. In many epilepsy specimens it may be possible to determine whether resection borders are lesion-free. This is particularly applicable to focal cortical dysplasias and glioneuronal tumors. Tissue from ultrasonic aspiration may be submitted to the pathology lab and provide additional information but limit the possibility of identification of anatomic landmarks (e.g., aspirates from the amygdala) as well as any assessment of completeness of resection. Postoperative imaging also provides further information on the extent of resection.

The neuropathology laboratory should be responsible for the standardized neuropathologic tissue procurement as proposed below, which can also allow for sufficient tissue harvest for related research projects. This will ensure that tissue for molecular or any other further analysis does contain expected cellular components (lesional, perilesional, or histopathologically unaffected normal region as control). Further histopathologic assessment should systematically apply recommended stains and immunoreactivities specified in Table 2 to achieve a specific diagnosis according to international consensus[4,5] or WHO classification systems.[29] The latter will require, however, more extensive immunohistochemical and molecular testing as different treatment strategies become increasingly available for molecular-genetic tumor subtypes.[11]

The standardized operational procedure for tissue inspection, distribution, and processing illustrated below anticipates most common clinical scenarios in epilepsy surgery: (1) selective amygdalohippocampectomy or combined with anterior temporal lobe resections; (2) lesionectomy or corticectomy, with or without invasive brain recordings; (3) large resections including hemispherectomy/hemispherotomy; (4) resections close to eloquent areas (which may yield, by clinical necessity, incomplete or fragmented anatomic samples); and (5) cortical resections of an MRI-negative electrophysiologically active seizure focus. The proposed protocol is, however, sufficiently flexible to adjust for local requirements, but it should encourage examination of routine practices and adoption of these recommendations where possible. This will allow for better comparison and reliable diagnostic workup of epilepsy surgery specimens.

Technical Annex

The Task Force has developed a standardized operational procedure (SOP) for tissue handling and procurement in the

setting of epilepsy surgery that can be applied in any laboratory worldwide, and can adapt to local institutional requirements to minimize disruptions to routine workflows. It refers to a standardized cutting scheme using anatomic landmarks, which can be supplied by the neurosurgeon, for example, by drawings, photographs, colored ink, stitches, or staples (Figs. 1–5). Key practice is systematic cutting of the specimen into 5-mm interval parallel slabs along a given anatomical axis, for example, anterior-posterior or dorsal-ventral, and sampling for routine histopathology of every second slice. This scheme can be used for most frequent clinicopathologic scenarios in epilepsy surgery, as exemplified in Figures 1–4, for example, selective hippocampectomy (Fig. 1), anterior temporal lobectomy (Fig. 2), lesionectomy with or without intracranial recordings (Figs. 3 and 4), or cortical resection of an MRI-negative epileptogenic brain region (Fig. 5). This present protocol will remain open to future modifications as additional data become available.

Standardized operational procedure for tissue inspection, distribution, and processing in epilepsy surgery

1 Whenever possible, anatomically intact tissue samples are recommended for histopathologic assessment (see Figs 1–5). The resected surgical specimen(s) should be procured in the operation room by the local neuropathology laboratory. Neurosurgeons could label the anterior-posterior or dorsal-ventral axis of each sample with staples or ink. Suspected lesions or other regions of interest should also be marked, such as the site of an epileptic focus determined by presurgical or intraoperative electrophysiologic recordings.

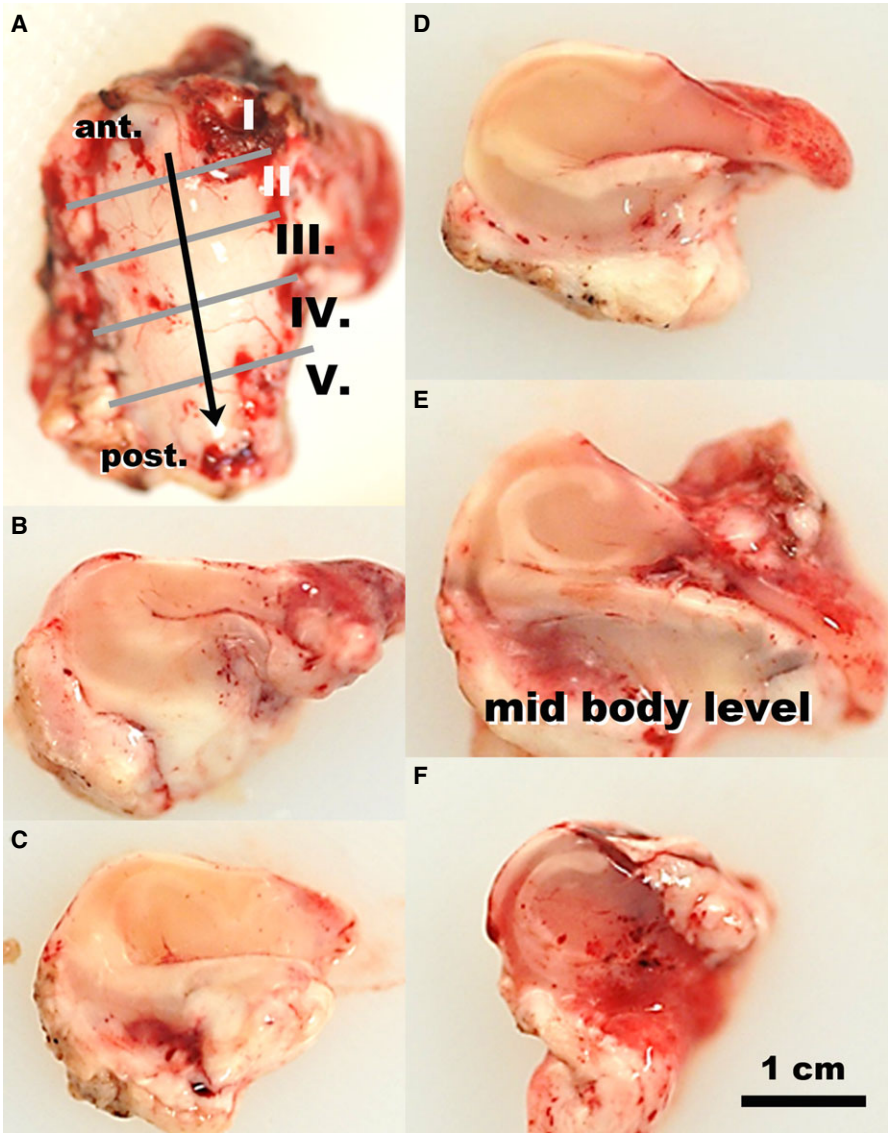

Figure 1.
Tissue procurement of anatomically intact human hippocampus (for snap frozen tissue banking). Identify the anterior-posterior axis of fresh sample (arrow in **A**). Dissect tissue specimen in 5-mm slabs perpendicular to anterior-posterior axis (I.–V. indicated by gray bars). Document order and chose slice from hippocampal mid-body level for histopathology (E, slice IV. in A). Fix this slice in 10% formalin overnight for paraffin embedding. Adjacent tissue can be used for tissue banking at −80°C. Always alternate between histopathology use (i.e., **C**, **E**), and snap frozen storage (**B**, **F**) or other research projects (**D**). Scale bar = 1 cm. Taken from Blümcke I., Sarnat H.B., Coras R. (2015), Surgical Neuropathology of Focal Epilepsies: Textbook and Atlas, with permission from John Libbey Eurotext.

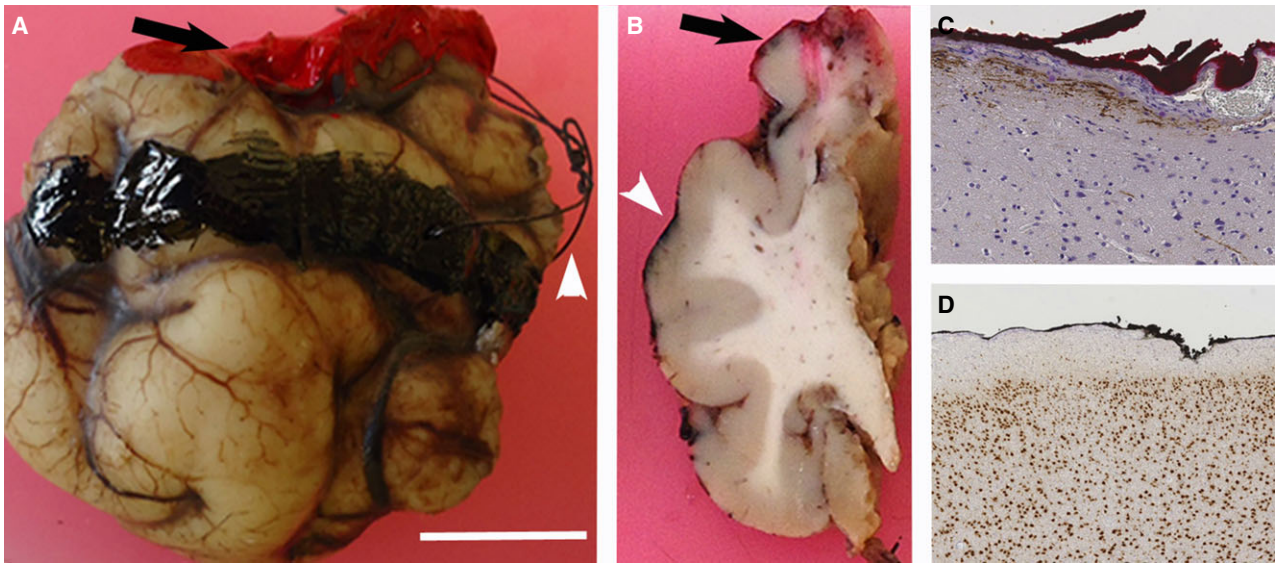

Figure 2.
Helpful orientation for tissue procurement of anatomically intact temporal lobectomy specimens. (**A**) A right anterior temporal lobe specimen received with a stitch marking the middle temporal lobe gyrus, which is inked black (arrowhead) from the pole to the posterior surgical margin. The superior resection margin is inked red (arrow). (**B**) The red- and black-inked gyri are visible on the sliced sections at 5 mm and also on the histology slices shown in **C** and **D**, respectively. Scale bar in **A** = 1 cm.

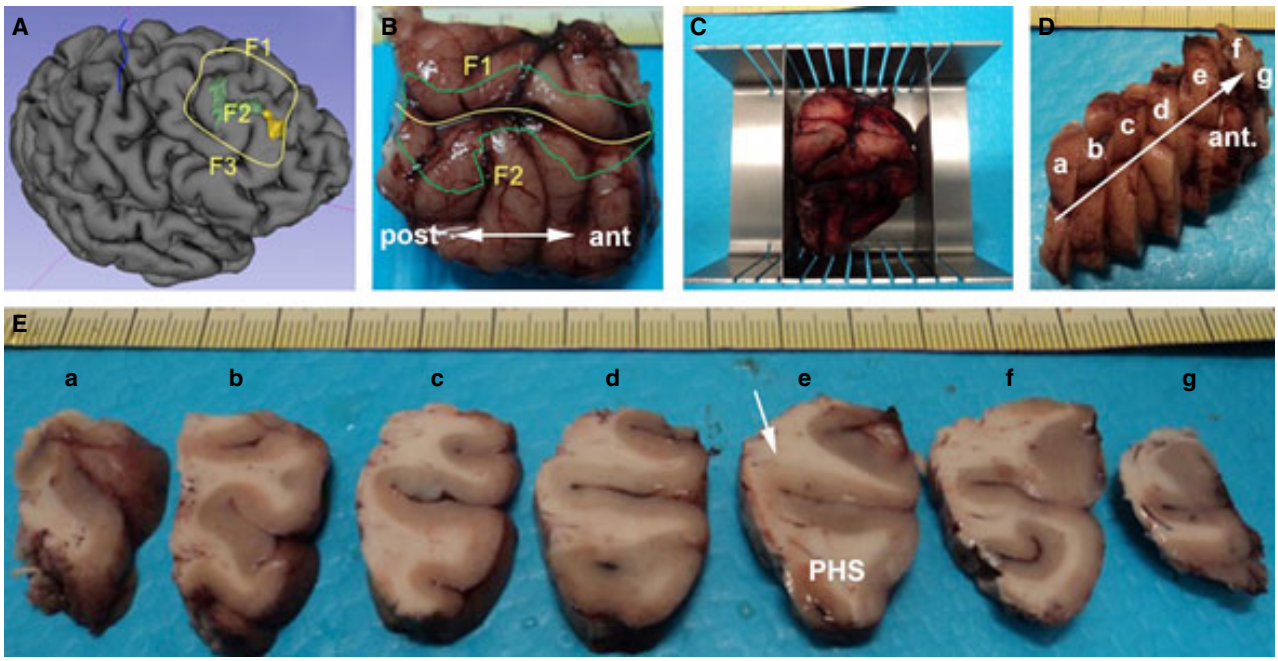

Figure 3.
Procurement of fixed cortical tissue with MRI-visible lesion following an anatomically intact surgical resection procedure. Identify the anterior-posterior axis of resected tissue specimen from brain map (**A**) and neuroanatomic landmarks (**B**). (**C**) Dissect formalin-fixed specimen in 5-mm slabs at coronal plane in anterior-posterior direction. Any device helpful for serial sectioning can be used. (**D**, **E**) Document order (slabs a–g) and choose every second slice for histopathologic analysis (Ea, Ec, Ee, and Eg). Label slice capturing the MRI-visible lesion (arrow in Ee indicating blurred white matter border at bottom of sulcus in principal histopathology slice, PHS) as such. Adjacent tissue can be used for approved and consented research projects (PFA fixed Vibratome sections for Ed and Ef, tissue banking at −80°C for Eb). Histopathologic abnormalities in tissue used for research can be always documented from alternating slabs selected for histopathology. Slices Ea and Eg represent the resection border and should be separately embedded into paraffin for histopathologic examination.

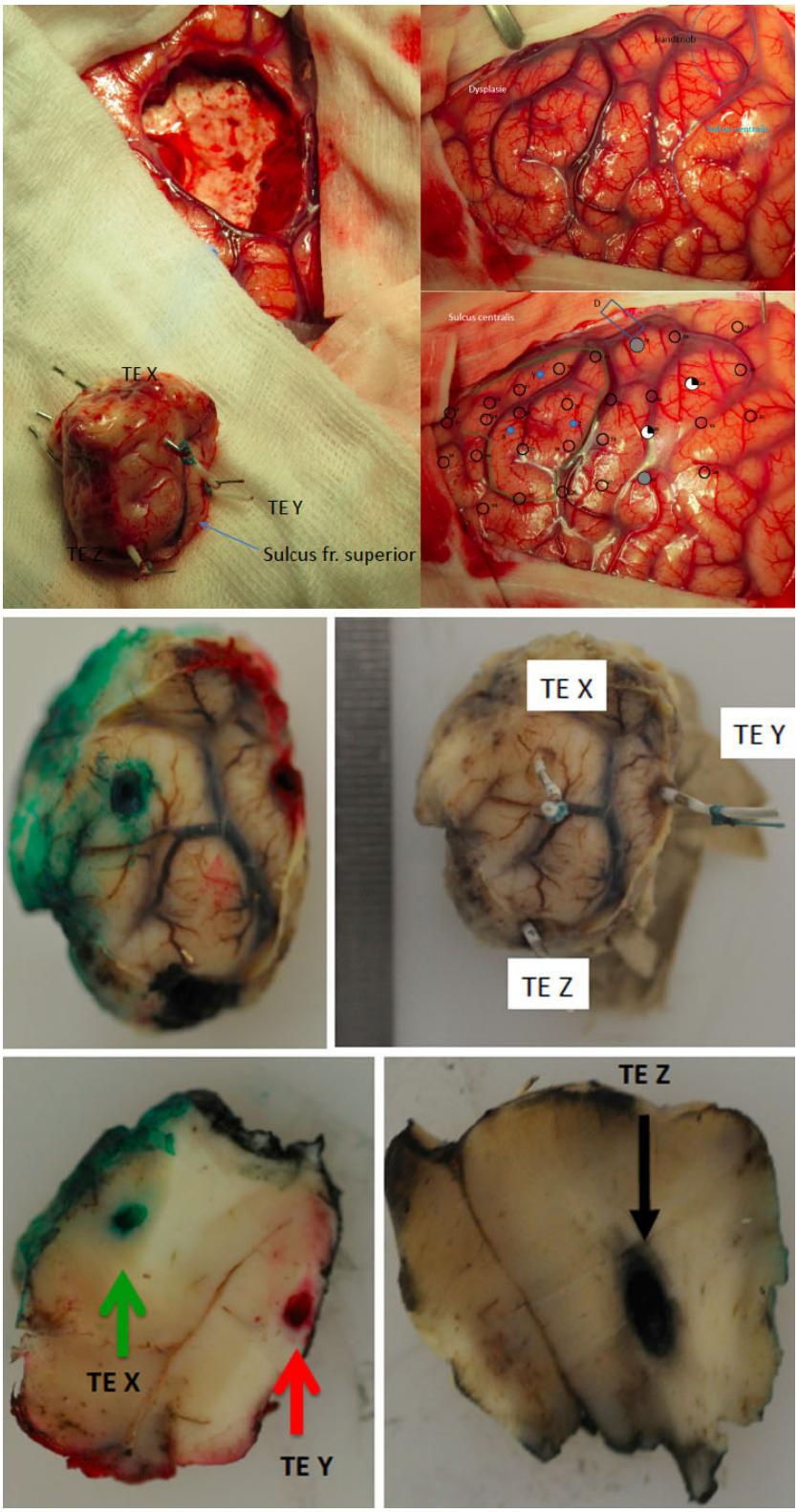

Figure 4.
Tissue procurement of anatomically intact brain tissue investigated by intracranial EEG procedures. Identify the anatomopathologic orientation of unfixed specimen and depth electrodes (preferable when left in situ). Label electrodes with differently colored ink. This specimen was used only for histopathologic examination and fixed, therefore, en bloc before further processing. Dissect tissue specimen in 5-mm slabs. Document order and fix slices in 10% formalin overnight before paraffin embedding. If required, use slides adjacent to histopathology examination for tissue banking at −80°C or other approved and consented research projects. Taken from Blümcke I., Sarnat H.B., Coras R. (2015), Surgical Neuropathology of Focal Epilepsies: Textbook and Atlas, with permission from John Libbey Eurotext.
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2 At the neuropathology laboratory, document the specimen weight in grams (specify prefixation or postfixation) and size of tissue specimen (preferably by a photograph that includes a metric ruler next to the tissue). Weights of ultrasonic surgical aspirates also provide estimates of total volume of brain tissue removed.

3 If snap frozen tissue will be required for molecular-biologic investigations, dissect fresh tissue specimen with

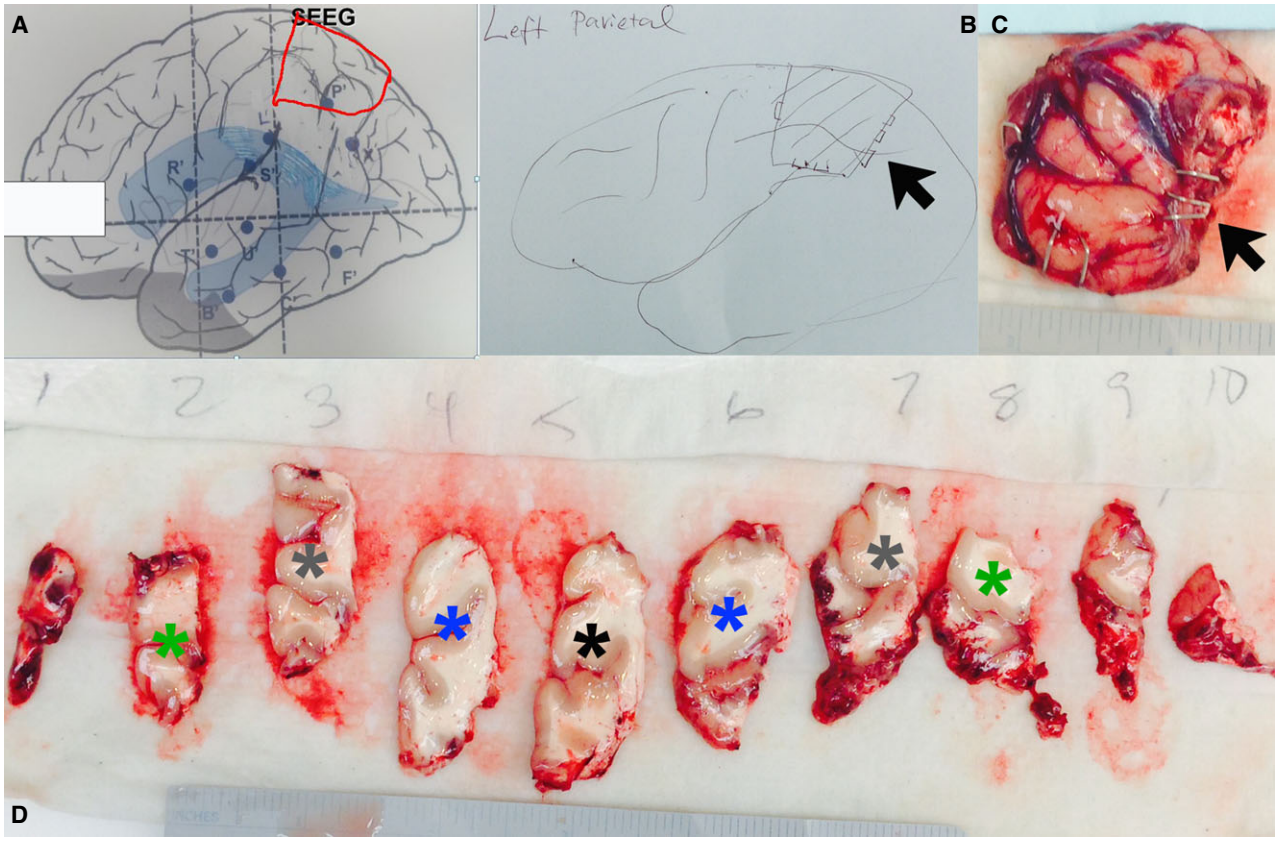

Figure 5.
Procurement of fresh cortical tissue (MRI-negative) following an anatomically intact surgical resection procedure. Identify the anterior-posterior axis of fresh sample from brain maps (**A**, **B**) and neurosurgical landmarks (**B**, **C**, staples indicating 3D orientation). (**D**) Dissect tissue specimen in 5-mm slabs at coronal plane in anterior-posterior direction. Document order (slab 1–10) and choose every second slice for histopathology (i.e., 1, 3, 5, 7, 9). Adjacent tissue can be used for tissue banking at −80°C (blue asterisks, slices 4 and 6) or other approved and consented research projects (green asterisks in slices 2 and 8). Slices 1, 9, and 10 represent the resection border and should be separately embedded into paraffin for systematic histopathologic examination. Taken from Blümcke I., Sarnat H.B., Coras R. (2015), Surgical Neuropathology of Focal Epilepsies: Textbook and Atlas, with permission from John Libbey Eurotext.

5-mm interval parallel slices according to the anatomic orientation (preferably at coronal planes along anterior-posterior axis, Figs. 1 and 5). Label all slices and document their order by photography, using an alphabetical or numerical system (e.g., I, II, III, . . . 1, 2, 3, . . . A, B, C, . . . a, b, c, Figs 1, 3, and 4). Labeling margins with different colored inks may be helpful in some cases. If possible, slices containing a macroscopic abnormality or the center of the epileptogenic zone as defined by imaging and/or electrophysiology should be labeled as such. Tissue from this region should be apportioned for histology or banking (for example, snap frozen, formalin or paraformaldehyde fixed, cell culture, electron microscopy) as determined appropriate in each case and dictated by size of lesion. The neuropathologist needs to make judgments with small samples in the allocation of tissue for research to ensure that there is no compromise to the histologic diagnosis. The remaining sections can be alternatively fixed (for histology) or frozen (for banking at −80°C). Use appropriate vials to store fresh frozen samples and label vials with deidentifying study number (preferably lab-number), slice number (see 5), and date of storage.

4 If snap frozen tissue is not required, the specimen should be immersed in fixative overnight (10% formalin or 4% paraformaldehyde for at least 12 h) and subsequently dissected into 5-mm interval parallel slices according to anatomic orientation (preferably at coronal planes along anterior-posterior axis; Figs. 2 and 3). Label all slices and document their order by photography, using an alphabetical or numerical system. Depending on the size of the specimen, alternate slices will be saved for histopathology and embedded into paraffin to allow thin cutting at 4–7 μm for routine histology, histochemical stains, as well as antibody immunoreactivities. If possible, the slice containing a macroscopic abnormality or capturing the center of the epileptogenic zone as defined by imaging and/or electrophysiology should be labeled as such. The

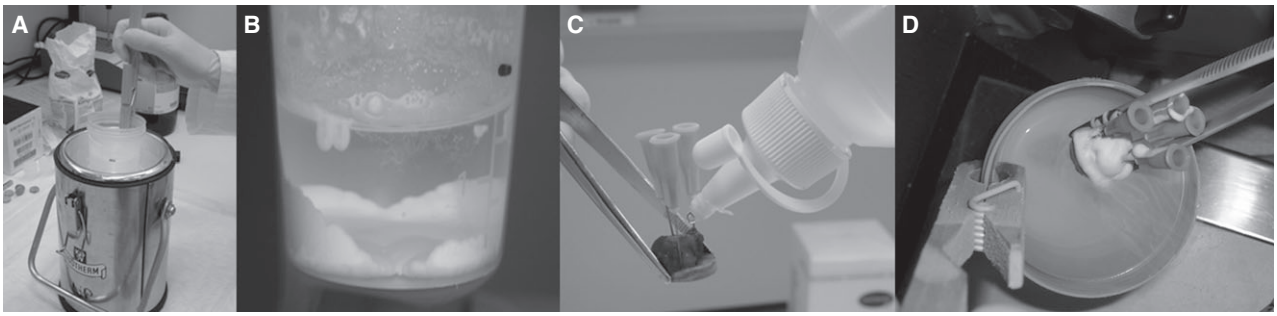

Figure 6.
Frozen storage of brain tissue. Technical equipment: Liquid nitrogen with container, isopentane (2-methylbutane) in plastic bin, test glass holder and petri dish, small and big tweezers, razor blades, cork pads, and consented patient information (also check for possible infectious agents), tissue embedding medium. Fill container with liquid nitrogen and plastic bin with isopentane. Chill plastic bin containing isopentane in liquid nitrogen (**A**, **B**). Patient's specimen should be mounted in tissue-embedding medium on cork plate (**C**) and freeze sample in chilled isopentane for 1 min (**D**). Next store sample in appropriately labeled cryo-vial in −80°C until further use. Always wear protective glasses and gloves when working with liquid nitrogen! Taken from Blümcke I., Sarnat H.B., Coras R. (2015), Surgical Neuropathology of Focal Epilepsies: Textbook and Atlas, with permission from John Libbey Eurotext.

remaining tissue blocks will become available for tissue banking and/or other research projects.

5 Slabs from the resection border should be labeled and embedded separately into paraffin for systematic histopathologic examination.

Tissue processing and storage protocols

A standardized paraffin embedding protocol is recommended using commercially available semi- or fully automated equipment.

Paraffin-embedded tissue specimens should be cut with a rotatory microtome at 4–7 µm thickness (preferably at 4 µm). Blocs should be cooled to −15°C before cutting. Sections should be stretched in heated water bath at 40°C before mounting on coated glass slides. Allow drying for 30 min at 60°C or overnight at 36°C.

For tissue biobanking, unfixed tissue should be snap-frozen in isopentane (2-methylbutane), cooled to the temperature of liquid nitrogen, and stored at −80°C in appropriate tissue container (Fig. 6). Tissue should be covered in compound cryostat embedding medium to prevent drying artifacts during long-term storage.

Standard immunohistochemistry protocol for paraffin-embedded sections (can be modified according to local requirements)

1 *De-paraffinize* (de-wax) sections in xylene 2 × 10 min; hydrate with 100% isopropanol 5 min; hydrate with 96% isopropanol 5 min; hydrate with 70% isopropanol 5 min; rinse in distilled water.

2 *Antigen retrieval* to unmask antigenic determinants: boil slides for 2 × 10 min in citrate buffer (e.g., microwave). Refill buffer after first round; cool for at least 10 min and rinse 2–3 times in Tris-buffered solution (TBS).

3 *Block endogenous peroxidase activity*: Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 15 min (45 ml methanol + 5 ml H_2O_2 [30%]) and rinse 2–3 times in TBS.

4 *Preventing nonspecific staining*: Blocking in 3% fetal calf serum/1% goat serum/0.1% triton X 100 in TBS for 1 h. Do not rinse! Carefully wipe away excess serum around the sections with tissue paper and apply primary antibody diluted in blocking solution. Incubate overnight at 4°C

5 *Visualize bound antibodies* (as recommended in Table 2): Rinse 2–3 times in Tris-buffered solution (TBS); apply biotinylated secondary antibodies and incubate for 10 min; rinse 2–3 times in TBS; apply streptavidin peroxidase and incubate for 10 min; rinse 2–3 times in TBS. Apply chromogen (i.e., diaminobenzidine tetrahydrochloride) mixture to tissue section for 5–10 min (visual control of immunoreactivity); rinse in distilled water; counterstain in hematoxylin (2–5 s); rinse in water; dehydrate samples (70% isopropanol, 96% isopropanol, 100% isopropanol, xylene, 10 dips per rinse); mount coverslip using appropriate medium.

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Disclosure

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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