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EpiCARE – a network for rare and complex epilepsies

HP-ERN-2016 European Reference Networks / Framework Partnership Agreement

D6.2 SOP for transfer of samples

Work Package: 6

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1. Version log

Version	Date	Released by	Nature of Change
1	25/01/18	I.Blumcke UKER	1st version

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2. Definition and acronyms

Acronyms	Definitions
DICOM	Digital Imaging and Communications in Medicine
EDTA	Ethylendiamintetraacetat
EEBB	European Epilepsy Brain Bank
FFPE tissue	formalin-fixed paraffin-embedded brain tissue
ILAE	International League against Epilepsy
ISO	International Organization for Standardization
PACS	Picture Archiving and Communications System
sCMOS camera	scientific complementary metal-oxide-semiconductor camera
SOP	Standard Operating Procedure
WSI	Whole slide imaging

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3. Introduction

Workpackage 6 (e-Neuropathology) promotes a European Tissue Bank and Reference Pathology Service for histological and molecular-genetic analysis of human epilepsy surgery specimens, which will be accessible to all EpiCARE partners.

Two deliverables were instrumental for this goal: to establish international guidelines for histopathologic diagnosis of epilepsy surgery specimens (D6.1) and to create high quality tissue biospecimens for clinically-applicable histopathology and molecular analysis, that can be also transfered amongst partner institutions (**D6.2**).

E-neuropathology will offer the large experience in standardized neuropathological examination protocols (i.e. ILAE guidelines and classification systems), tissue preservation and banking to all partner of EpiCARE. A strong asset for the EpiCare consortium will be to also generate a pipeline for reference histopathology review. Whereas the gold standard for transfer of samples and histopathology referral is postal shipping of human brain tissue, embedded tissue blocs or stained glass slides, our WP will also develop and validate the benefit of a web-based virtual microscopy reading and teaching platform.

4. Activities carried out and results

Activities carried out

Our previous work helped to establish international recommendations for the neuropathologic work-up of epilepsy surgery human brain samples (Blumcke et al. 2016). The second step will be to develop SOPs for tissue exchange in order to allow all European partners a cost-effective second look histopathology review at the e-Neuropathology Reference Center for Epilepsy Surgery (currently based in Erlangen, Germany, and equipped with a histopathology wet-lab and automated embedding and staining apparatus). As a gold standard, the contemporary approach is postal shipping as summarized below (see technical annex I-III). In order to reach out further and combine existing expertise across European countries as a collaborative e-Neuropathology Reference center approach, a web-based virtual microscopy reading and teaching platform should be developed. We investigated existing technical solutions (commercial and open source) for whole slide imaging and cross-platform virtual microscopy viewer (see Annex IV). Technical solutions for big data storage, data safety and linkage to the EpiCare database need further clarification and negotiation with EU representatives, as implementation and financial support is

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mandatory to develop such innovative European neuro-/pathology reference center network.

Results

Technical Annex I: SOP for sending native surgical tissue samples in formalin

• Native surgical brain samples must be shipped in shock-protected jars pre-filled with 10% formalin. All material (available for various tissue volumes) can be requested from the Department of Neuropathology in Erlangen, Germany (for more information, please see **www.neuropathologie.uk-erlangen.de**). The jar should be labeled with an encoded Pat-ID. Clinical history and other disease-relevant patient information should be send by separat postmail or electronically (web-based health information exchange platform to be provided by EU).

• Upon arrival, the reference center will allot a new Pat-ID for safety double encoding. The formalin-fixed surgical specimen will be processed according to international recommendations for the neuropathologicxal work-up of epilepsy surgery brain samples (see D6.1), including paraffin-embedding, 4µm thin sectioning followed by a predefined protocol of routine and immunohistochemical stainings to scientifically validate diagnosis (Blümcke et al. 2017).

• A comprehensive histopathology report will be prepared in English language and send by post mail or electronically (health information exchange platform to be established) within 3-5 working days from tissue arrival. Local IRB approval for long-term tissue storage is available and regularly adopted to meet most current safety regulations, including separat storage of tissue and clinical records as well as safe-locked rooms for tissue archival.

Technical Annex II: SOP for sending fresh-frozen surgical tissue samples on dry ice

• Native tissue together with a 5 ml EDTA-blood sample can be send to the Reference Center in Erlangen for further diagnostic work-up or research purpose. Naive samples should be stored in specialized jars (can be requested from the Department of Neuropathology in Erlangen, Germany) and shipped in cooling boxes provided from commercial carrier or requested from the Department of Neuropathology in Erlangen, Germany. The box must be filled with dry ice to maintain deep cooling. Patient consent is required for long-term storage (see below), genetic research or data sharing with international consortia. Patient consent forms in English language can be requested from the Reference Center in Erlangen. Clinical history and other disease-relevant patient information should be sent by separat postmail or electronically (web-based health information exchange platform to be provided by EU).

• Upon arrival, the reference center will allot a new Pat-ID and store matched blood-brain samples at -80°C until further use. Local IRB approval for long-term tissue storage is available and regularly adopted to meet most current safety regulations, including separat storage of tissue and clinical records as well as safe-locked rooms for refrigerator.

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Technical Annex III: SOP for sending FFPE tissue samples

• Surgical brain samples can also be processed at each Hospital's own pathology lab and embedded into paraffin for long-term storage and shippment. FFPE blocs, unstained or prestained glass slides can be shipped with European carriers to the Department of Neuropathology in Erlangen, Germany (for more information, please see www.neuropathologie.uk-erlangen.de). Clinical history and other disease-relevant patient information should be sent by separate postmail or electronically (web-based health information exchange platform to be provided by EU).

• FFPE samples will be further processed according to international recommendations for the neuropathological work-up of epilepsy surgery brain samples (see D6.1), using a set of routine and immunohistochemical stainings to scientifically validate diagnosis (Blümcke et al. 2017).

• A comprehensive histopathology report will be prepared in English language and send by post mail or electronic data exchange platforms (to be established) within 3-5 working days from tissue arrival. Local IRB approval for long-term tissue storage is available and regularly adopted to meet most current safety regulations, including separat storage of tissue and clinical records as well as safe-locked rooms for the tissue archive.

Technical Annex IV: A fully digitized pipeline for histopathology review (European e-Neuropathology reference center)

• Digital technology is already well established across medical disciplines and often essential to mundane routines and specialist activities. Whole slide imaging (WSI), affordable storage media and computation power are driving image analysis routines into the methodologic core of pathology. Undoubtedly the resultant progress in automation and algorhythmization while delivering huge benefits will bring novel threats to the welfare of patients and practitioners. The torrents of data created by high-throughput processes can easily submerge even the most conscientious diagnosticians, if sufficiently detailed methodological knowledge is not provided by postgraduate training and continuous medical education. This calls for updated curricula, detailed guidelines and accreditation procedures encompassing the relevant aspects of digital technology to avoid jeopardizing pathology's essential function as the foundation of medical practice.

• In the arena of epilepsy surgery, neuropathology is ideally positioned to play a major role in shaping a digitized future. The currently available combinations of large sensor multimegapixel sCMOS cameras, graphics processing units supporting frame rates above 60Hz and large ultra-high resolution (up to 8K) monitors are providing detailed, jitter-free and wide-screen visual experiences now surpassing those delivered via the ocular lenses of traditional microscopes. WSI-scanners considerably evolved from the first "virtual microscopy" patents (Bacus & Bacus, 2001) now digitize standard and fluorescence microscopic sections approaching high through-put level in various formats (Table 1). It currently takes less than one

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minute to scan conventional and special stains of a 15x15 mm2 section area under bright field illumination with a 20x objective. Individual sections are identified by their 1D- or 2D-bar coded tabs, while each partially overlapping „slide shot“ is exposure corrected and tiled by instrument-specific proprietory or open-source software in realtime or post-acquisition. Consequently, the rapid and general introduction of glass-less pathology archives even at academic centers is faced with considerable costs and the complex infrastructural demands necessary to maintain sufficiently performant retrieval and back-up logistics (Stathonikos et al. 2013).

Table: Technical features of current commercial WSI-scanners

Company	Model	Mode	Capacity	SS	File formats
3DHistech	Pannoramic 250 Flash III, 1000	BF, FL	250, 1000	st	mrxs
Hamamatsu	S60	BF	60	St, J	ndpi
Leica	Versa	BF, FL	200	st	scn
Aperio	ScanScope AT2	BF	400	st	svs
Olympus	VS120-L100-W	BF, FL	100	st, J	vsi
Zeiss	Axio Scan.Z1	BF, FL	100	st, J	czi
Huron	TissueScope LE120	BF	120	st, J, wm	hdf

Abbreviations: BF – bright field, FL – fluorescence, SS – slide size, st – standard (25mm x 75mm), J – jumbo (50mm x 75mm), wm - whole mount (150mm x 200mm)

• WSI files are principally formatted in a pyramidal fashion encompassing several levels of resolution to allow continuous and rapid zooming through fields of view/regions of interests at minimized data transfer rates and RAM requirements. Unfortunately, many microscope manufacturers and biomedical software providers have developed proprietary versions of the generic pyramidal format in an attempt to entrap the user in their ecosystems („walled gardens“) of locally installed or server-based applications. These irritant business policies in blatant disregard of the true scientific spirit have spawned a number of initiatives which develop platform-spanning alternative applications for different operating systems (Windows, Mac OS, Linux) under the GNU General Public License (GPL) model. Exemplarily, OpenSlide developed by Carnegie Mellon University School of Computer Science (http://openslide.org/) offers C libraries with Java and Python bindings to convert the WSI formats of most scanner manufacturers for viewing in standard browsers supporting HTML5 (Goode et al. 2013). Ideally, however, WSI files should comply with the „Digital Imaging and Communications in Medicine“standard, synonymous with ISO standard 12052 whose supplement #145 defines objects containing very large image data sets common to pathology (Singh et al. 2011). The creation of interoperability with the „Picture Archiving and Communications System (PACS)“ long established in most hospitals for radiology images represents a major

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integrative effort which notwithstanding the abortive attempts of a few manufacturers has not been addressed in a coordinated fashion.

• As X-ray archiving has moved from film to PACS, pathology institutes are now *en route* to divest their slide cabinets for data troves of truly gigantic capacities. The resultant demands on storage media, networking infrastructure and data security are immense. In view of the growing importance of global access to medical image for reproducible subtyping of large collectives, reference pathology of rare entities, training medical professionals and international research communication in general investing in adequate hardware and resilient infrastructure for medical purposes should have considerable priority in global policy decisions. If medical data can be entrusted in the long term to commercial cloud services or only to institutional agencies remains to be seen given the ever increasing exfiltration risks and criminal threats to large data storage installations of all kind. Fortunately browser-based "open source" solutions like OMERO (http://www.openmicroscopy.org/site/products/omero) have sufficiently matured now offering all pathology-relevant functions such as granular access rights management, platform-independent WSI-rendering, slide mark-up and annotation on a par with their commercial alternatives (Allan et al. 2012).

5. Conclusions

We established SOP for tissue exchange within the EpiCare consortium using courier services and postal shipping of fresh frozen, formalin-fixed and/or FFPE tissue specimens. Future navigation of the digital route towards web-based slide review and pathology teaching platforms will face a number of technological challenges which urgently seek continuous technical and financial support from the EU. The roadmap towards an internationally integrated European e-Neuropathology reference center will encompass

Freedom of data and software:
Without doubt every procedure in medicine should allow for its stringent and effective control. In the context of computerized applications and algorhythmic diagnostics this requires complete transparency regarding source codes, data formats, processes and results. Of foremost importance is the effective implementation of the DICOM Standard which has been developed with an emphasis on diagnostic medical imaging including pathology. It is already actively endorsed by the EU (https://ec.europa.eu/eip/ageing/standards/ict-and-communication/other-ict/dicom_en), however, the urgent need for its adoption needs to be impressed more forcefully on manufacturer of WSI equipment worldwide.

Securitization of data and networks:

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Local and global exchange of medical data is critically dependent on a performant network and storage infrastructure, which must be provided by the ERN hosting organization of the EU.
The European Parliament has adopted the *General Data Protection Regulation (GDPR EU 2016/679)*, that will be directly applicable in all Member States of the EU and effective from May 25th, 2018 replacing *Directive 95/46/EC.* Equally relevant in this context is the EU's *Medical Device Regulation (EU) 2017/745 (MDR)* which will be in effect on 26 May 2020. To meet the current and future e-Health requirements of European cybersecurity frameworks (http://www.consilium.europa.eu/media/31666/st14435en17.pdf) all pathology-related metadata and image files will have to be end-to-end encrypted using current state-of-the-art cryptography.

Increased performance analytics:
Developments in the microscopic visualization of molecular details will reveal additional layers of complexity directly impacting on the practice of personalized medicine and theranostic capabilities of every pathologist. The recognition of morphologic and mutational diversity will require a dramatic increase in diagnostic power which will only be attained in a concerted European effort to fully incorporate computerized image analysis, machine learning and diagnostic algorhythms into practice. The need to derive prognostic information from conventionally or immunohistologically stained sections will necessitate the use of artificial intelligence (AI) techniques, such as deep neural networks (DNNs) generated complex multi-parametric decision algorithms which can match and even outperform expert-level human diagnosticians (Djuric et al. 2017).
Establishing a European e-Neuropathology reference center will undoubtedly help in the promotion and validation of these emerging technologies across ERN pathology groups and accelerate their adoption in the pathology community at large.

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