**Abstract Title:** The effects of formic acid versus Cal-EX tissue decalcification on immunohistochemical staining

**Introduction:** Exposure to acid is the most commonly used decalcification technique for routine surgical specimens. Our centre uses Cal-EX, a proprietary decalcifying agent containing hydrochloric acid, to decalcify routine surgical and autopsy specimens. However, hydrochloric acid can cause chemical alterations in tissues that interfere with ancillary testing, particularly immunohistochemistry (IHC). In this study, we assess formic acid (FA) as an alternative to Cal-EX with respect to its effect on IHC staining.

**Methods:** Formalin-fixed tonsillar tissue from 7 patients was exposed to 10% formic acid or Cal-Ex for 2 hours, 24 hours, 48 hours or 7 days, respectively, prior to routine processing and embedding. Each block was stained for 21 IHC markers relevant to the evaluation of lymphoproliferative disorders. The intensity of immunostaining of each slide was compared to a section of tissue from the same specimen without decalcification and scored as 2 (intact), 1 (attenuated) or 0 (absent).

**Results:** Scoring of the 7 specimens demonstrated at least some attenuation of IHC staining with Cal-EX in every specimen and with every IHC marker tested. Decreased staining intensity with FA was seen in every specimen but with only 17/21 markers. Decreased intensity of immunostaining was time-dependent; for 16/21 markers, Cal-EX impaired staining in at least one specimen within 2 hours while FA required at least 24 hours (6/21 markers). Complete absence of staining occurred in at least one specimen for 7/21 markers with Cal-Ex.

**Discussion:** These results suggest that compared to Cal-EX, formic acid is a gentler decalcifying agent with respect to its effect on IHC staining. Future validation studies would use different tissue types in order to assess FA’s effects on staining with other markers and the length of time required for decalcification.

**Abstract Title:** Somatotroph adenoma with dual transcription factor expression

**Introduction:** A 20 year old male presented with evidence of gigantism/acromegaly. Endocrinological investigations identified elevated growth hormone levels and a failed glucose tolerance test. Imaging revealed a macroadenoma expanding the sella with encroachment on the optic chiasm and cavernous sinuses. Trans-sphenoidal resection was undertaken and a gross total removal was achieved.

**Results:** Histopathological features were typical of a densely granulated somatotroph adenoma with abundant growth hormone expression, scattered prolactin expression and sparse examples of fibrous bodies. Unexpectedly, the adenoma not only expressed PIT-1 but also SF-1 transcription factors. This finding suggests that the adenoma may have been pluripotent. The prognostic significance of this finding is uncertain although the patient is stable from an endocrinological and imaging perspective approximately one year post-op.

**Discussion:** Use of transcription factors in characterization of pituitary adenomas is a relatively new addition to the WHO classification of these tumors and a pituitary adenoma of this nature has not been previously reported.
**Abstract Title:** Lymphomatosis Cerebri as a Presentation of Secondary Central Nervous System Lymphoma (SCNSL)

**Introduction:** A lymphoma involving the brain diffusely without formation of a discrete mass lesion is best characterized as lymphomatosis cerebri. While such a presentation is well-described in primary central nervous system lymphoma (PCNSL), our autopsy revealed a clinically undiagnosed lymphoma outside the CNS which disseminated secondarily to the brain.

**Case:** A 72-year-old female presented with abdominal pain. A CT showed bilateral adrenal hemorrhages. Treatment with steroids resulted in symptomatic improvement. However, she soon developed confusion, stroke-like symptoms, and decline in consciousness. Serial MRIs demonstrated progressive extensive asymmetrical leukoencephalopathy with abnormal enhancement but no mass effect. Multiple investigations were nonrevealing. Given a working diagnosis of demyelinating encephalomyelitis, she was managed with more steroids and intravenous immunoglobulin without a biopsy diagnosis. She eventually developed refractory status epilepticus, and died shortly afterwards. Post-mortem examination revealed extensive infiltration of the brain, left kidney, and adrenal glands by a diffuse large B cell lymphoma, which also involved the lung and peripancreatic tissue. CNS involvement included cerebral grey and white matter, brainstem, cerebellum, and minimally the leptomeninges. Lymphomatous infiltrate was mostly angiocentric. The neuropathology was typical of a lymphomatosis cerebri.

**Discussion:** Lymphomatosis cerebri is a lymphoma exhibiting diffuse infiltration of the CNS without forming any discrete mass(es). It is often mistaken for non-neoplastic entities such as inflammatory or demyelinating conditions. While it is known as a very uncommon presentation of PCNSL (review of 42 cases, Izquierdo et al., Neuro Oncol. 2016 May; 18(5): 707–715), its occurrence in SCNSL is exceptionally rare. Our case reinforces the concept that a radiological presentation of leukoencephalopathy may actually be a lymphoproliferative neoplasm. Furthermore, the neoplasm may be secondary, necessitating extensive clinical investigations to rule out a lymphoma outside of the CNS.
**Abstract Title:** Characterization of gingival fibromas amid other lesions of the gingiva

**Introduction:** Gingival fibromas (GFs) are fibrous lesions of the gingiva that are not well defined in the literature. However, they are histologically similar to reactive, periosseous lesions of the gingiva called peripheral ossifying fibromas (POFs). Although thought to be distinct, both are characterized as cellular proliferations of dense fibrous tissue, with POFs differing as they demonstrate foci of ossification. Previous unpublished investigations have shown that GFs and POFs have similar immunohistochemical staining profiles suggesting that GFs are periosteal, osteoblastic lesions that may be related to POFs. We aim to expand upon the immunohistochemical characterization of GFs, and to confirm their osteoblastic phenotype. In addition, we will also compare them to classical fibromas that originate from the gingiva, as they are another type of reactive fibrous gingival lesion. This investigation will hopefully shed more light on pathological reactive processes that occur in the gingiva and the lesions that arise from them.

**Methods:** Formalin fixed, paraffin embedded GFs (n = 10), POFs (n = 10) and fibromas of the gingiva (n = 10) were acquired from the Western University Pathology Department tissue archives. Slide sections were cut and routine hematoxylin and eosin staining was performed. Immunohistochemical staining was performed for special AT-rich sequence binding protein 2 (SATB2), runt-related transcription factor 2 (RUNX2), osteocalcin and smooth muscle actin (αSMA). Slides were evaluated under microscopy and the immunohistochemical staining patterns were assigned immunoreactive scores (IRS) based on the assessed percentage of positive staining cells and intensity of staining.

**Results:** GFs, POFs, and fibromas of the gingiva all stained positively for osteoblastic markers SATB2, RUNX2 and osteocalcin. GFs and POFs stained positively for myofibroblast marker αSMA while fibromas of the gingiva did not. When comparing GFs and POFs, the staining patterns of SATB2, RUNX2 and αSMA were similar.

**Discussion:** These findings further demonstrate that GFs and POFs exhibit a similar immunohistochemical profile, and supports that GFs are osteoblastic lesions possibly related to POFs. They additionally suggest that classical fibromas originating from the gingiva express an osteoblastic phenotype.
Fixing formalin safety and specimen quality perceptions in the workplace

Introduction: Neutral buffered formalin is the gold standard fixative agent to preserve cellular structures for microscopic examination. However, formalin has also been associated with both short-term and long-term occupational health risks, including ocular and respiratory irritation, carcinogenicity, and reproductive complications. These risks are often balanced with the impact on specimen quality since insufficient formalin usage can result in unsatisfactory tissue fixation. Recently, several strategies have been implemented to decrease the occupational risk to formalin. Due to ongoing concerns about safe levels of occupational exposure, we sought to probe the depth of understanding amongst the laboratory staff with a targeted survey.

Methods: An incentivized online survey was issued to a total of 104 technical and medical staff within the Division of Surgical Pathology within Pathology and Laboratory Medicine (PaLM, London, Ontario). The survey consisted of 17 multiple choice and free-text questions addressing various aspects of formalin handling. These questions were structured to identify safety concerns and desired improvements to the work environment. Additional questions were targeted toward specimen types that are frequently received with inadequate formalin.

Results: A total of 58 participants (25 medical staff and 33 technical staff) responded, with a 56% (58/104) response rate. 95% (55/58) handle formalin in their department. For short-term risks, 95% (55/58) report a perceived risk of ocular and respiratory irritation, and 67% (39/58) perceive a risk of breathing difficulties and aggravation of other medical conditions. 19% (11/58) report other perceived risks, such as skin irritation and headaches. For long-term risks, respondents report a perceived risk of developing cancer (76%, 44/58) and worsening existing medical conditions (71%, 41/58). 47% (27/58) report concerns about reproductive complications. 65% (38/58) feel that occupational risks can be mitigated with proper PPE (Personal Protective Equipment) and improved ventilation. Additionally, 86% (50/58) desire a formalin handling training module. For specimen quality and handling, 86% (51/58) are aware of the recommended ratio for optimal tissue fixation. However, respondents feel that specimens are received in containers with either unsatisfactory amounts of formalin (41%, 24/58), or inadequate container size (66%, 38/58).

Discussion: These data show a significant prevalence of concerns regarding poor ventilation, short-term and long-term health risks, and inadequate specimen fixation. Significant efforts have been made to maximize the rate of air exchange within the current ventilation infrastructure and decrease the occupational risk to formalin exposure. However, directed initiatives to communicate these changes may provide awareness and mitigate perceived occupational risk. Additionally, respondents indicated an openness to formal safety training and monitoring formalin exposure. These data highlight the main areas of occupational concern and provide direction for future endeavours. The lab may therefore benefit from the implementation of a formalin handling training module, improved ventilation systems and further education about the perceived and actual risks associated with formalin exposure.

Abstract Title: To Add or Not to Add – Identifying Trends and Reasons for Submitting Additional Sections in Surgical Pathology and Implications for Gross Room Protocols and Turnaround Time

Introduction: Accurate grossing of surgical specimens is crucial for appropriate diagnosis. After initial gross examination, additional sections (adds) can be requested by the pathologist for various reasons. The objective of this study is to identify trends in types of specimens requiring adds and the reasons for requesting them. Secondary objectives include determining trends in the personnel grossing the specimens, diagnosis and impact on turnaround time.

Methods: A retrospective review of surgical specimen adds requests from January 2018 to November 2020 was conducted. Data including specimen type, reason for request, number of adds, gross examiner, diagnosis, and time between initial gross examination and completion of adds request were collected. Data were analyzed using quantitative statistics.

Results: 1. Overall, 1.9% (1168/60389) of all cases from Jan 2018 to Nov 2020 required adds. Of these, the most common categories requiring adds were gastrointestinal (GI) (0.76%), gynecological (GYNE) (0.54%), breast (0.15%) and genitourinary (GU) (0.13%). An average of 6.4 additional sections were submitted for each case. The most common reasons for adds were lymph nodes, additional sections of mass, or submission of remaining tissue.

2. 4.4% of GI specimens required adds, with colon specimens accounting for 2.1%. Of the colon specimens requiring adds, the most common reason was for additional lymph nodes (59%) and 86% of these cases were for malignant diagnoses. 1.4% of all GI adds requests were for appendices which was usually to submit in toto (88%). 86% of appendix diagnoses were benign, of which acute appendicitis was the most common (27%). Of liver specimens, 13% required adds. 54% of the liver add requests were for additional sections of the mass/cyst/nodule.

3. 1.5% of GYNE specimens required adds, with uterine specimens accounting for 1.2%. Of total uterine specimens, 3.12% required adds. The most common add request was for submission of additional endometrium and endomyometrium (45%), where 77% of specimens had benign diagnoses.

4. 2.5% of all breast specimens required adds. Most commonly, adds of fibrous tissue were requested for mastectomy (66%) and lumpectomy specimens (43%). These were most often for malignant diagnoses (69%).

5. 2.2% of GU specimens required adds with kidney specimens accounting for 1.2%. 54% of the cases required adds of mass/nodules, which all resulted in a malignant diagnosis.

6. 71% of the specimens requiring adds were grossed by pathologists' assistants (PAs). There was no significant difference in adds requests for specimens grossed by senior (more than 5 years experience) or junior PAs. PA students, residents and MLTs accounted for 17%, 8% and 4%, respectively.

7. The average delay in turnaround time was 7.9 days, with a range of 1-46 days.

Discussion: Overall, add requests occurred more often for benign diagnoses, except for GU and breast specimens. The most common add request was for lymph nodes, likely due to insufficient sampling which increased turnaround time. It may be beneficial to review grossing protocols for appendices and complex specimens, such as liver and kidney due to the high proportion of add requests. It would be a useful practice for pathologists to document the specific reason(s) for add requests to determine the impact of the add on the diagnosis and highlight trends in specific grossing protocols that could be amended.
Abstract Title: More than just a blob? Using immunohistochemistry to decipher germ cell lineage

Introduction: The ability of pluripotent stem cells, such as embryonic stem cells (ESC), to self-renew and differentiate into a homogeneous population of specialized cells makes them an ideal candidate for use in cell replacement therapy to treat a broad range of diseases. Directed differentiation of ESCs, however, often result in cells in immature stages of development that are suboptimal for many applications. P66Shc, a member of the ShcA (Src homologous- collagen homologue) adaptor protein family, regulates autophagy, metabolism and cellular redox state. Modifications in p66Shc levels have been shown to alter stem cell identity and fate. Previously, Wild-type (WT) and p66Shc knock-out (KO) murine embryonic stem cells were transplanted into immunodeficient NOD/SCID IL-2Ry null mice and resultant teratomas harvested (Betts). Initial transcript profiler array data reported elevated pluripotent and progenitor markers and altered/reduced levels of mature lineage markers in p66Shc KO teratomas. In addition to standard microscopy, we propose to utilize immunohistochemistry (IHC) to characterize cells by stage of maturation (pluripotent, immature, mature) and germ cell lineage (ectoderm, mesoderm, endoderm). In this targeted pilot study, we hypothesize that p66Shc is critical for morphogenesis and cellular maturation of ectoderm lineage.

Methods: Three teratomas derived from WT and p66Shc KO murine embryonic stem cells were histologically assessed by standard hematoxylin and eosin (H&E) staining. Two (2) markers for IHC were chosen from the initial profiler array data: 1) Transcription factor Oct3/4, a marker of pluripotency was measured at much higher transcript levels in p66Shc KO teratomas relative to WT (+28.3-fold), and 2) Sox2, a marker of neuronal or epithelial cell lineage measured at modestly higher levels (+5.4-fold).

Results: Histologically, p66Shc KO teratomas are primarily comprised of undifferentiated cells while WT teratomas contain predominantly well-differentiated cells, primarily of ectoderm lineage. The majority of undifferentiated cells in p66Shc KO teratomas were negative for Oct3/4, but positive for Sox2. In contrast, most undifferentiated cells in the WT teratomas were positive for Oct3/4. The proportion of Oct3/4 cells, relative to whole sections, were similar amongst all groups.

Discussion: WT teratomas are characterized by predominantly well-differentiated, mature cells in this study. In contrast, p66Shc KO teratomas are largely composed of undifferentiated cells, which are negative for Oct3/4, but positive for Sox2, suggesting that these morphologically indistinct cells are not pluripotent, but have committed to ectoderm germ cell lineage (immature). Together, these data suggest a key role for p66Shc in maturation of ectoderm germ cell lineage, however, limited sample size and subjective measures restrict interpretation. A more robust study is required to confirm these findings, which include increasing sample size, additional markers to further distinguish maturation state and delineate ectoderm subpopulations. Future plans to comprehensively assess teratomas include distinguishing a panel of markers to histologically characterize cells of all three germ cell lineages and their maturation states.