2051 Synchronous Lung Adenocarcinoma and Primary Pulmonary MALT Lymphoma: An Underdiagnosed Entity Associated with KRAS Mutations

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Background: Pulmonary extranodal marginal zone lymphoma (MZL / MALT) is a rare entity accounting for less than 0.5% of primary pulmonary malignancies. The occurrence of lung adenocarcinoma (AD) and primary pulmonary MALT lymphoma as collision tumors have only been rarely reported. We investigated the concurrent incidence of these two entities in a large cohort of lung AD cases submitted for routine molecular diagnostic testing.

Design: Consecutive lung AD cases were reviewed and categorized based on the presence or absence of a chronic inflammatory lymphoid infiltrate and scored as 0 - absent/minimal infiltration, 1+ moderate or 2+ intense. Results of molecular testing for recurrent mutations in EGFR, KRAS, BRAF, HER2, PIK3CA, AKT, MEK1 and fusions involving ALK were recorded. IgH rearrangement studies and immunohistochemical stains for CD3, CD20, Kappa and lambda were performed in cases with intense (2+) lymphoid infiltrates.

Results: A total of 600 lung adenocarcinoma cases were reviewed. None of the patients had prior history of a lymphoproliferative disorder. Lung adenocarcinoma driver mutations were identified in 389 cases (65%). In 319 (53%) tumors the lymphoid infiltrate was scored as 0, 264 (44%) as 1+ and 17 (3%) as 2+. A score of 2+ was most commonly associated with a KRAS mutation (10/17, 59%, p=0.03). Among the cases with intense lymphoid infiltrates, 4 (24%) had clonal IgH rearrangement as well as morphologic and immunophenotypic features consistent with MALT lymphoma (including a nodular distribution, predominance of CD20+ B cells and light chain restriction). In all cases, the AD was intimately associated with the lymphoma component and there was no clinical evidence of a systemic lymphoproliferative disorder. A KRAS mutation was identified in 3 cases, 1 case was wild type. The incidence of MALT lymphoma associated with lung AD is 0.7% in this series.

Conclusions: The synchronous occurrence of lung AD with an intimately associated MALT lymphoma is higher than previously reported. The distinction between an exuberant reactive lymphocytic infiltration and a MALT lymphoma, in the setting of lung AD, can be challenging and may be often underdiagnosed if not fully investigated. While the etiology is uncertain, the presence of a KRAS mutation in 3 out of the 4 synchronous lesions is an interesting finding which warrants further investigation in terms of its implications for tumor immunology and newer immunotherapy approaches.

Quality Assurance

2052 Factors That Determine Patient Consent for Research BioBanking and BioSpecimen Research: Lessons for Optimizing Specimen-Driven Pathology Research

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Background: With rapidly increasing interest in understanding, measuring, and optimizing the patient consent process for research biobanking, the BioSpecimen Sciences Program (BSP) set out to study its current research consent process. We attempted to establish comprehensive baseline consent rates for all disease site groups in a multi-campus academic health system. We set out to discover any variability among disease site groups as well as to determine whether consents obtained through direct patient encounters of BSP staff were superior over traditional consent mechanisms.

Design: During a one-year period, all surgical patients were reviewed for individual research consent status as one of the four categories: 'Signed' (acceptance or refusal), 'Not signed' (no declaration), 'No consent' (no form in clinical chart), or 'No chart' (chart unavailable). Patients were further divided according to clinical subspecialty group. In addition, daily pre-admission schedules were screened for patients who were eligible for specific institutional research studies, and BSP coordinators approached these patients in person.

Results: 4007 patients had research consent forms that could be evaluated. Of these, 2740 (68.4%) consents were signed. 2730 (68.1%) patients agreed to biobanking for research while only 10 (0.2%) refused explicitly. The highest response rates of consent came from patients seen by the genitourinary (91.6%) and gynecological site groups (91.4%), whereas dermatology (45.5%) and plastic surgery (9.2%) were lowest. 246 patients were approached in person by BSP coordinators. This produced a response rate of 98.8% with 234 patients (95.1%) agreeing to biobanking, 8 (3.3%) refusing, 1 (0.4%) withdrawing, and 3 (1.2%) undecided. BSP staff increased the number of patients who agreed to biobanking rom 116 (previously consented by non-BSP staff) to 234.

Conclusions: The surgical preadmission package is an effective mechanism for research consenting. However, there was significant variability between different clinical site groups (9.2-91.6%). Reasons include staff buy-in, consent logistics, competing research consents, and heterogeneity of research volume and clinical trial activities. Pathology/BSP research coordinators are highly effective in optimizing research consent rates (98.8% response, 95.1% acceptance). Personal involvement of BSP staff more than doubled enrollment of our "highest-value" patients. Personal involvement of both pathologists and biobank staff is key for achieving optimal consent rates.

2053 Temperature-Based Antigenicity Preservation Methods for Tissue Microarrays: Longitudinal Prospective Study

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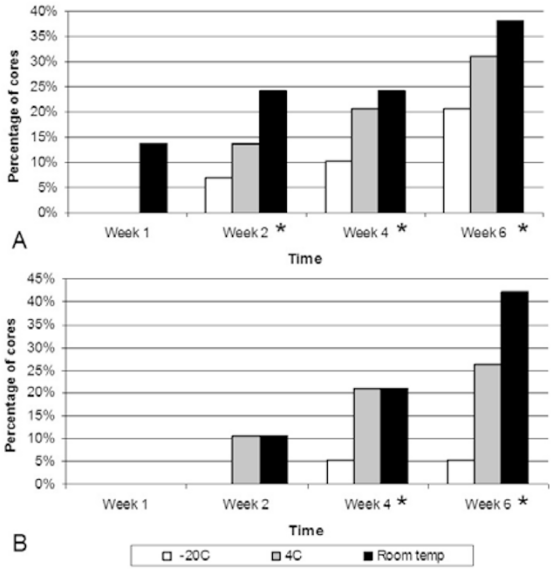
Background: Studies have shown time-dependent loss of antigenicity for a variety of diagnostic, prognostic and predictive antibodies used on precut paraffin slides. However, there are no established guidelines for storage methods of precut controls, patient tissue, or microarrays (TMAs). We sought to determine loss of antigenicity and potential for preservation by refrigeration in a longitudinal prospective study.

Design: Selected diagnostic or prognostic antibodies included p53, IDH-1, Ki67, synaptophysin, and androgen receptor (AR). TMA with 22 cores from small cell carcinomas, prostatic adenocarcinomas, and gliomas was constructed; 125 slides were cut at 4 microns at time 0. Slides were stored exposed to air at room temperature (RT), 4C, or -20C; IHC was performed on the Leica Bond III at time 0, weeks 1, 2, 4, and 6. Each tissue core was scored for overall intensity (0 to 3+) and % of cells staining (100 cells within hotspot). Loss of antigenicity was defined as a decrease of staining intensity by one order and/or loss of≥10% of positive cells compared to time 0.

Results: By 6 weeks, Ki67, p53 and AR antibodies had progressive loss of detectable immunoreactivity, which was most prominent for those stored at RT ($p<0.05$). For Ki67, significant loss of antigenicity was first detected at 1 week on slides stored at RT; storage at -20C preserved % positive cells until 4 weeks; at 6 weeks, storage at all 3 temperatures had loss of expression. p53 had sustained loss of antigenicity at 4 weeks ($p<0.05$) in slides stored at RT and 4C, which manifested as decreased intensity rather than % of reactive cells. AR had loss of % staining at 6 weeks at RT only. Synaptophysin and IDH-1 had no loss of reactivity regardless of storage temperatures.

Loss of ≥ 10% cells staining positively in hotspots, and/or loss of intensity of staining for Ki67 (A) and p53 (B), * ($p<0.05$)



Conclusions: Loss of antigenicity is antibody dependent and most notable for slides stored at RT. Refrigeration of slides significantly delayed loss of antigenicity for all affected antibodies, especially at -20C. Progressive decrease in Ki67 and p53 % positivity and staining intensity could affect reporting of prognostic results; thus refrigeration should be considered for long-term storage. Monitoring of antigenicity loss at longer time-periods as an ongoing part of the study will define optimal storage recommendations.

2054 Flocculant Artifact Caused by Sterile Lubricant in Fine Needle Aspirates (FNAs)

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Background: The use of sterile lubricant in place of conducting gel while performing ultrasound guided FNA of the thyroid is common practice. As a result of a new purchasing policy at our institution, our usual sterile lubricant (Surgilube®) was replaced with an alternative gel (Allegiance®). Soon thereafter, reports of non-diagnostic thyroid FNA cytology results due to obscuring artifacts became evident. The purpose of this study is to test three brands of lubricating gel to determine their propensities for causing artifacts in FNA specimens.

Design: A pannus, removed for obesity, was identified. Four 3x3cm areas, labeled A, B, C, and D, were designated on the skin. Equal amounts of Surgilube®, Allegiance®, and E-Z Lubricating Jelly® were applied to areas A, B, and C, respectively. No gel was applied to area D. Three containers of CytoLyt® were designated for each area. A 25 gauge needle was used to aspirate the pannus through the gel, one needle per pass. Three passes were rinsed into each container. In total, 12 ThinPrep® slides were prepared. The randomized and de-identified slides were evaluated by a cytopathologist for amount of flocculant artifact (0=none, 1=mild, 2=severe) and number of tissue fragments.

Results:

Container # (Gel Type)	Artifact Score (0-2)	# of Tissue Fragments	Mean Number of Tissue Fragments±Standard Deviation
A1 (Surgilube®)	0	4	5.0±1.73
A2	0	4	
A3	0	7	
B1 (Allegiance®)	2	1	2.3±1.53
B2	2	4	
B3	2	2	
C1 (E-Z Jelly®)	1	0	0.7±0.58
C2	1	1	
C3	1	1	
D1 (None)	0	5	5.0±1.00
D2	0	6	
D3	0	4	

Flocculant artifact was severe in area B, mild in area C, and was not present in areas A and D. The mean number of tissue fragments was highest for areas A and D, intermediate for area B, and lowest for area C.

Conclusions: The substituted gel (Allegiance®) resulted in significantly more contamination and aspiration of fewer tissue fragments. Upon investigation, it was found that carbomer-containing gels can interfere with the ThinPrep® system. The substituted gel is a carbomer-containing product, while our original gel (Surgilube®) is not. Heightened vigilance should be paid to the type of gel in use and its potential for interference. We recommend the use of non-carbomer containing gels for thyroid FNAs in ThinPrep® systems as its use resulted in the least contamination and aspiration of the most tissue fragments.

2055 The Processing of Surgical Specimens with Forensic Evidence: Lessons Learned from the Boston Marathon Bombings

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Background: Following the Boston Marathon bombings, pathology departments at hospitals across Boston were inundated with limbs, and surgical specimens from trauma surgeries. Each department faced uncertainties in grossing these specimens. Many departments have protocols for processing items such as recovered bullets; however, there is currently no defined protocol to guide a department on processing specimens containing forensic evidence.

Design: Our pathology Department Chair contacted Department Chairs of four major hospitals in Boston to ask for collaborators in developing a protocol for processing of specimens with forensic evidence. Although there was limited interest in collaborating to develop a protocol, our department had been in contact with pathology assistants and residents from each of these hospitals, and we were able to make general outlines of the practices at three of the hospitals. Based on these outlines we consulted Boston's Cheif Medical Examiner to get the opinion of an expert in forensics and a prosecuter to learn what information would be helpful in court. Collaboration with the Federal Bureau of Investigations is in progress currently.

Results: Our experience in combination with input from the medical examiner and other Boston area hospitals led to the development of a robust protocol for the processing of surgical specimens containing forensic evidence.

Figure 1. Protocol for handling of surgical specimens containing forensic evidence

Specimen arrival and identification

- Identify one person or a team of people who will handle all of the specimens and reporting of results
  - *Recommendation: One pathology assistant and/or resident and one attending physician*
- Specimen arrives via courier from operating room/emergency department
  - Accession case, and confirm that each case has three patient identifiers
  - Sealed containers received from operating room with foreign bodies should remain sealed

Grossing of surgical specimen

- Obtain plain film radiographs of specimen in several views (i.e., antero-posterior, side views)
- Photograph and diagram specimen as it is received
  - *Recommendation: Photographs should have limited, password protected access*
- Remove any superficial loose objects (e.g., make-shift tourniquets, clothing etc.) and place items in a container for FBI/police with three patient identifiers
- Describe lacerations, fractures, burns, remaining foreign bodies (locations, types), and results of radiograph
  - Note: Examine and comment on viability of tissue at the resection margin(s)
- Wash specimen, and take additional pictures
  - Document any additional information obtained after cleaning the surgical specimen
- If there is an indication (e.g., osteomyelitis, diabetes) submit sections of resection margin(s)
- Remove foreign bodies from the surgical specimen, photograph them, and place them in a sealed container labeled with three patient identifiers
- All foreign bodies should be weighed, described, and measured
  - *Note: If foreign bodies are received in a sealed container, weigh them in their container, then subtract the weight of an empty container of the same type and record the true weight in the gross description*

Disposition of surgical specimens and foreign-body containers after grossing

- Place surgical specimens and sealed containers with foreign bodies in a locked, secure area with limited access
- Contact FBI or local police to inform them that foreign-body evidence is ready
- Hold surgical specimens until patient is discharged (overflow specimens may need to be held in the morgue or alternative location)
- In the case of a patient death, surgical specimens and foreign bodies should be transferred to the medical examiner's office

Important points in this protocol include assigning the task of processing the specimens to one individual or one team of individuals, photographing specimens before and after washing, obtaining a radiograph of each specimen, and having a locked, secure area to store forensic evidence. Furthermore, we recommend that such surgical specimens should be processed as "gross only", i.e., standard histological sections are not required unless there is a specific indication or an individual request.

Conclusions: When acts of terror occur, especially when there are mass injuries, a comprehensive protocol can help pathology departments process surgical specimens efficiently, securely, and thoughtfully to allow for optimal patient care and appropriate gathering of forensic evidence.

2056 Representative Sections Are Sufficient for Adequate Intraoperative Assessment of Gastric Margins in Most Gastrectomies

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Background: Positive resection margins in gastric or esophageal cancer are associated with worse survival. Evaluation of the gastric resection margin is frequently requested by intraoperative consultation (IOC). The decision to freeze the entire versus representative gastric margin is often based on institutional tradition rather than evidence based data. We evaluated gastrectomy specimens to determine the variables associated with a positive gastric margin and identify a data-driven approach for IOC margin assessment.

Design: 110 consecutive gastrectomies for neoplasia with IOC margin assessment in a 3 year period were reviewed. The following variables were studied: patient age/gender, tumor size and distance to margin, histologic diagnosis, complete or representative margin examination (including the number of blocks), and presence of lymphovascular invasion (LVI).

Results: Our study group consisted of 61 esophago-, 36 partial-, and 13 total gastrectomies. The most frequent diagnoses were intestinal-type adenocarcinoma (55%), diffuse/signet ring cell carcinoma (25%), squamous cell carcinoma (5.5%), and GIST (3.6%). 206 IOCs were performed using 561 blocks. In 72% of cases, the entire gastric margin was examined by frozen section using a mean of 6 blocks (range 1-16). Of these, 12.7% were positive. In 20% of cases, representative margins were examined by frozen section using an average of 2 blocks (range 1-4). Of these, 13.3% were positive. In 8% of cases, the extent of examined margin was not documented. Variables showing significant association with positive margin were:1)diffuse/signet ring cell adenocarcinoma ($p=0.001$), 2)LVI ($p=0.000$), 3)tumor size (>2.3 cm, $p=0.04$) and 4)distance to margin (4.9 ± 3.7 cm from negative margin versus 1.9 ± 2.4 cm from positive, $p=0.034$). Importantly, no margin partially examined at IOC was positive when subsequently entirely submitted (0/18). Gender and age did not influence margin status.

Conclusions: Our analysis suggests that representative examination is sufficient to adequately assess the margin status in gastrectomy cases with a histologic diagnosis of non-diffuse or mixed type gastric adenocarcinoma, small tumor size ($\leq$2.3 cm) and a distance to the margin of $\geq$5 cm. By limiting the number of blocks, the labor-intensive frozen section effort may be reduced, leading to shorter turn around time. In addition, limiting the number of blocks may have the benefit of reducing labor costs associated with paraffin block embedding, permanent slide generation and block and slide storage.

2057 Molecular Validation Study of Nucleic Acids Extraction from Vacuum Sealed Surgical Pathology Specimens

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Background: Formalin is used as the universal fixative in surgical pathology laboratories world over. However, it is highly toxic, irritant, potentially carcinogenic and requires diligent care in its usage. To eliminate formalin use for specimen transport to the laboratory, we placed the surgical specimens in plastic bags under-vacuum sealing. Our aim was to assess if this method of transport could be used routinely without compromising integrity of the nucleic acids in this molecular era.

Design: 15 surgically resected specimens from 3 anatomic sites (5 colon, 5 lung, 5 uterus) were selected for this pilot study. Specimens were placed in plastic bags under-vacuum sealing and transported at 4 °C. 4 normal tissue samples using a 5 mm skin biopsy punch were collected at 0, 1, 2, 3 hour in duplicate; one snap frozen at -80C & the other processed as formalin fixed paraffin embedded tissues (FFPE) [60 fresh, 60 FFPE]. Manual DNA column extraction protocol for genomic DNA isolation & total RNA isolation protocol to isolate total RNA were run. DNA/RNA quantities were evaluated by spectrophotometry. DNA integrity was assessed by amplification of commercial Control Size Ladder mix generating a series of amplicons of 100, 200, 300, 400 base pairs (bp). RNA integrity was assessed by real-time quantitative reverse transcription PCR by measurement of expression of β2 microglobulin (B2M) transcripts. Cycle threshold (Ct) values of 30 fresh & 30 for FFPE tissue samples were used with a cut-off of > 37.0 Ct for acceptable RNA quality.

Results: H & E stained sections from all the FFPE samples showed optimal histologic preservation. The yield of extracted DNA & RNA was adequate with good purity for both fresh & FFPE tissues [A260/A280 OD ratios >1.10 (range DNA:1.10-2.11, RNA:1.32-2.73)]. Fresh tissues: Total recovery between 6.46ug to 238.51 ug, the amplified DNA yielded product sizes of 300 bp for 54/60 samples, 400 bp for 53/60 samples. RNA from all the samples was of good quality, with acceptable B2M amplification in 28/30 samples. FFPE tissues: Total recovery between 3.12 ug to 81.09ug, the amplified DNA yielded product sizes of 300 bp for 60/60 samples, 400 bp for 60/60 samples. RNA from all the samples was of good quality, with acceptable B2M amplification in 29/30 samples.

Conclusions: Our data suggests that the DNA as well as RNA integrity is very well preserved even after 3 hours of vacuum sealing of the surgical specimens without formalin fixation. Vacuum-based preservation of surgical specimens is a viable, molecular friendly and environmentally-safe option for tissue transport to laboratory.

2058 Should Clinician-Initiated Preordering of CMV IHC Be Allowed on GI Specimens?

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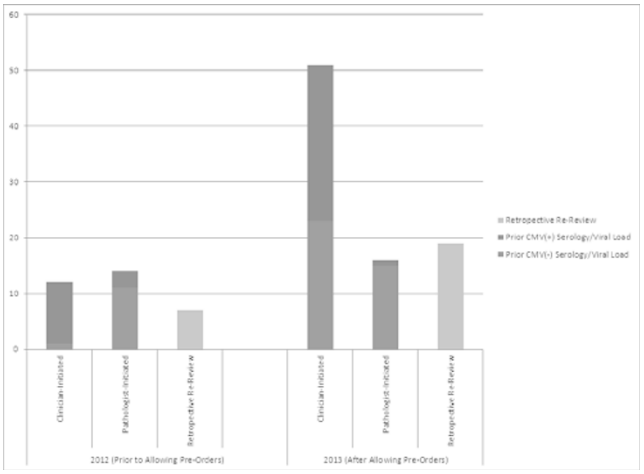
Background: Cytomegalovirus (CMV) infections of the gastrointestinal (GI) tract may become life-threatening if not identified and treated promptly. CMV immunohistochemistry (IHC) is helpful when classic viral inclusions are not identified in suspicious cases. Clinician preordering of CMV IHC may increase the number of tests performed without added benefit. Due to pressure from clinicians, we recently agreed to temporarily allow preordering on clinically suspicious cases. In this study, we sought to compare the number of clinician-initiated orders prior to and after allowing preordering to objectively determine the value of this practice.

Design: We reviewed all non-liver GI specimens with a CMV IHC order over a comparable 6-month period when preordering was not allowed (April to September 2012) and when preordering was temporarily allowed (April to September 2013). We retrospectively reviewed the reasons for clinician-initiated orders. We then re-reviewed all specimens in the study to determine whether CMV IHC would have been ordered based on the histopathologic features.

Results: The total number of clinician-initiated orders increased by 425%, which consists of 76.1% of total CMV IHC orders in 2013. Pathologist-initiated orders increased only by 14% (Table 1 and Figure).

Table 1

	2012 [n = 26]		2013 [n = 67]	
	Clinician-Initiated [n = 12 (46.2%)]	Pathologist-Initiated [n = 14 (53.8%)]	Clinician-Initiated [n = 51 (76.1%)]	Pathologist-Initiated [n = 16 (23.9)]
Prior CMV(-) Serology/Viral Load	1	11	23	15
Prior CMV(+) Serology/Viral Load	11	3	28	1



A pathologist, on re-review, was able to identify all IHC-proven positive specimens (Table 2).

Table 2

	2012 [n = 26]		2013 [n = 67]	
	Negative [n = 24]	Positive [n = 2]	Negative [n =61]	Positive [n = 6]
Clinician-Initiated CMV IHC Orders	12	0	46	5
Pathologist-Initiated CMV IHC Orders	12	2	15	1
CMV IHC That Would Have Been Ordered on Retrospective Re-review of Histologic Specimens	5	2	13	6*

*All specimens were randomly and blindly re-reviewed, with selected ones blindly reviewed twice. One IHC-proven CMV(+) specimen was identified only at the second challenge.

Conclusions: The results demonstrate that preordering of CMV IHC is often unnecessary and should be discouraged. Pathologists can reasonably determine the need for CMV IHC on an individual basis.

2059 Comparison of Methods Used to Determine Workload for Surgical Pathology Specialists

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Background: With the shift towards subspecialized sign-out in SP, it has become challenging to determine equivalent workloads due to significant variation in the type and complexity of cases. Physician work has traditionally been measured by relative value units (RVU) which have known inherent flaws that over or underestimate work. This study compares the RVU system with slide counts and the Royal College of Pathologists (RCP) point system which weights workload by subspecialty.

Design: Surgical pathology [breast, gynecologic (GYN), gastroenterology (GI), genitourinary (GU), general, medical renal/liver] and cytopathology workload for one representative month (November 2012) at Stanford Hospitals & Clinics was calculated using three different methods: 1) RVU; 2) RCP 3rd ed. Guidelines on Staffing and Workload for Histopathology and Cytopathology Departments; 3) University of Washington slide count equivalents. The 2011 mean RVU (6007) for academic SP practices as reported by the Medical Group Management Association (MGMA) was used to calculate hours of work per day based on a 6 hour work-day (127 work days per year). Simple regression analysis was performed to compare the three methods.

Results: Regression analysis shows RVU positively correlates with RCP (0.93, $p<0.01$) and slide counts (0.86, $p<0.01$). RCP also correlates with slide counts (0.70, $p=0.05$).

Table 1: Hours of work per day

	RVU	RCP	Slide Counts
Breast	3.7	3.0	6.1
GYN	4.4	3.7	4.0
GI	9.7	7.8	8.5
GU	2.7	2.1	3.3
General	5.9	7.6	7.0
Medical Renal/Liver	3.5	5.4	2.3
Cytology	6.8	6.0	6.0

Conclusions: RVU and RCP (prospective measures of workload) correlate well with each other but less well with slide counts (retrospective measure of actual work). RVU gives more weight to GI; slide counts, breast; and RCP, medical renal/liver. RVU is known to overestimate work in specialties such as GI that are rich in small biopsies and to underestimate work in specialties with complex cases such as medical renal biopsies. Slide counts similarly underestimate work for medical renal cases and can potentially overestimate work if large specimens are oversampled. RCP appears to provide a better estimate of GI workload but may underestimate breast workload which has high slide counts. To be credible for determining workload distribution, the method used must be viewed as fair across services. None of the systems evaluated is entirely satisfactory but a modification that takes the best characteristics of each has promise.

2060 Discrepancy Rates in Liver Biopsy Reporting

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Background: The reporting of medical liver biopsies takes place in both general (local) and specialist (referral) hospital settings. With relatively small case numbers however, maintenance of competency in a highly specialised field is potentially challenging in

a general setting. This study evaluates the reporting discrepancies identified between cases referred to a specialist centre and the referring general pathologists.

Design: Fifty consecutive recently referred cases were selected and original and final reports were compared. Discrepancies were classified as per the Royal College of Pathologists (UK) guidelines: B1 (a surprising discrepancy), B2 (a discrepancy occasionally seen), B3 (a common discrepancy where inter-observer variation is well recognised) or C (discrepancy due to failure in clinicopathological correlation). Discrepancies were also scored for potential clinical impact (1: none, 2: minor impact, 3: major impact).

Results: The overall rate of any discrepancy was 40% (20 cases, see Table 1). The most common discrepancy (45%) type was B3 and these were mostly due to difficulties in recognising bile duct abnormalities and fibrosis staging. Most of the cases however had discrepancies which were not inter-observer dependant; six (12%) had B2 discrepancies, mainly due to misinterpretation of inflammatory infiltrates, one B1 discrepancy was found where steatohepatitis was reported by the referring pathologist as normal and there were four cases (8%) where poor clinicopathological correlation resulted in diagnostic discrepancies. Most discrepancies (70%) were of a major potential clinical impact (14% of all referrals) and none were considered to have no potential clinical impact.

Table 1. Breakdown and characteristics of discrepancies

Discrepancy Type	Number of Cases	Characteristics
B1	1 (5%)	ASH reported as normal
B2	6 (30%)	Misinterpretation of inflammation, special stains
B3	9 (45%)	Fibrosis upstaging, bile duct abnormalities not recognised
C	4 (20%)	Failure to correlate with high alcohol intake, choledocolithiasis

ASH = alcoholic steatohepatitis

Conclusions: Liver biopsy reporting discrepancy rates are high and although many of the difficulties are recognised as challenging areas of hepatopathology, the majority are avoidable and the potential clinical impact is usually great. With the increasing trend to sub-specialty reporting there is a strong argument for a review of liver biopsy reporting practices to include mandatory referral of all or selected cases and regular audit in the general setting.

2061 Positive Predictive Value of "Atypia" Diagnosis on Breast Biopsy; a Valuable Quality Assurance Tool

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Background: The pathologic designation "atypia" is used when appearances are suspicious but not diagnostic of malignancy. Multiple histopathologic appearances are included in the umbrella term "atypia", including atypical ductal hyperplasia (ADH), columnar cell change with atypia (CCCWA) or flat epithelial atypia (FEA), and "atypia NOS". A biopsy diagnosis of atypia leads to a clinical recommendation for excision. As only a proportion of these patients will have a malignant diagnosis at excision, precision and consistency in the diagnosis of atypia are extremely important. Currently, there is no QA system in breast pathology analogous to the BI-RADs radiology system, whereby trends in an individual radiologist's positive predictive value (PPV) are monitored over time. The PPV of breast atypia diagnosis within individual institutions, and between individual pathologists over time could be an important QA tool to measure diagnostic consistency and detect diagnostic trends.

Design: All MSKCC patients in 2008-12 with a breast biopsy atypia diagnosis and subsequent excision were identified (n=652), pathology reports were reviewed and the departmental PPV was identified. An anonymized analysis was performed to calculate the PPV of an atypia diagnosis for each of the breast pathologists at MSKCC over time. The subgroup "ADH bordering on DCIS" (ABD) was submitted to more in-depth analysis in terms of clinical and pathologic features in order to identify parameters which could be used to further refine the PPV rate.

Results: The departmental PPV for atypia diagnoses was 23.6%, and was similar for both in-house biopsies and referred cases. Overall, 2.6% of in-house biopsies had an atypia diagnosis. Inter-pathologist analysis demonstrated remarkable concordance between pathologists for both rate of atypia diagnosis (2.13-3.27%) and PPV of an atypia diagnosis (PPV range 22.7 -25% between 5 of 6 pathologists). The diagnostic rate of atypia was stable over the study period. The PPV for the individual atypia diagnoses were 9% (CCCWA/FEA), 10.5% (atypia NOS), 30% (ADH), 48.5% (ABD). Analysis of multiple clinical and pathologic parameters for ABD failed to demonstrate any statistically significant differences between those with and without malignancy on excision.

Conclusions: Assessment of the PPV of atypia diagnoses on breast biopsy is a potentially useful quality assurance tool in breast pathology. This patient cohort (n=652) is part of an ongoing clinicopathologic-radiologic correlation study to develop a nomogram to guide clinical management of breast atypia.

2062 Variation of CD34+ Stem Cell Enumeration in Post-Stimulated Leukapheresis Specimens Using FC500 and FACSCanto II Systems

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Background: Accurate enumeration of CD34+ cells in leukocyte-rich cell suspensions is critical for clinical decision-making in stem cell transplant. Recently, we experienced that quantitation of the CD34+ stem cell count using the FC500 flow cytometer (Beckman Coulter, Inc., Brea, CA) was higher than that obtained from National Marrow Donor Program® (NMDP) network of banks. Therefore, we analyzed the CD34+ stem cell count using both the FC500 and FACSCanto II (BD Biosciences, San Jose, CA) systems.

Design: Unstimulated peripheral blood specimens and granulocyte colony stimulating factor stimulated leukapheresis specimens from 10 patients were assessed for CD34+ stem cells using a FC500/Coulter Stem-Kit and a FACSCanto II/BD Stem Cell Enumeration kit. Stem cell quantitation was also performed in a local reference lab using a FACSCanto II/home brew kit.

Results: The average CD34+ stem cell count in unstimulated peripheral blood specimens was 0.086%, 0.068%, and 0.068% obtained from FC500, FACSCanto II, and reference lab, respectively. The difference between the FC500 and FACSCanto II was significant ($p=0.003$). The correlation coefficient (r^2) was 0.98 between FC500 and FACSCanto II, while it was 0.92 and 0.87 between reference lab and FC500 or FACSCanto II. On the other hand, the average CD34+ stem cell count in stimulated leukapheresis specimens was 1.08%, 0.385%, and 0.465% obtained from FC 500, FACSCanto II, and reference lab, respectively. The difference between the FC500 and both FACSCanto II systems was significant ($p=0.004$ - 0.006). The correlation coefficient (r^2) between FACSCanto II and reference lab was 0.99 whereas it was only 0.76 and 0.74 between FC500 and FACSCanto II or reference lab. Lastly, the difference of the correlation coefficients (r^2) between FC500 and both FACSCanto II systems was again significant ($p=0.015$ - 0.002).

Conclusions: Enumeration of CD34+ stem cells in stimulated leukapheresis specimens using the FC500 flow cytometer was 2.3 to 2.8-fold higher than the FACSCanto II, despite comparable levels in unstimulated peripheral blood samples. In addition, the linear correlation between FC500 and FACSCanto II was reduced in stimulated leukapheresis samples. These findings underscore the importance of variation in CD34+ stem cell counts using different flow cytometers. This variation in CD34+ stem cell enumeration may be due to differences in anti-CD34 antibody, gating strategy, or interference in post-stimulated samples.

2063 Look before You "LEEP": A Retrospective Quality Assurance Study

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Background: Loop electrosurgical excisional procedure (LEEP) is an established diagnostic and treatment modality for high- and some persistent low-grade squamous intraepithelial lesions (HSIL, LSIL). At our institution, pathologists noted many LEEPs without HSIL which did not appear to conform to guidelines.

Design: Quality assurance review was performed for all 326 LEEP specimens from 1/2011 - 8/2013. Available clinical and pathologic materials including surgical pathology, cytology, and colposcopy results were reviewed. Diagnoses were coded to the highest grade of pathology (e.g., CIN 1-2 as CIN 2) to give the benefit of the doubt to the clinicians. Appropriate indications for a LEEP included a biopsy (or endocervical curettings (ECC)) with HSIL or duration >20 months (guideline: ≥ 24 months). Possible appropriate indications included HSIL on Pap only, "atypical" or LSIL pathology on ECC, and colposcopy indications (unsatisfactory or extension into the endocervical canal). Not appropriate indications included a remote history of SIL, ASC-H Paps, and no indications that could be determined on chart review.

Results: Our patients ranged in age from 19-65 with a median of 31 years and 75% of the patients were age 38 or younger. Results are presented by specific indications and guideline appropriateness (both p<.0001, Chi-Square). Of patients with not appropriate/possibly appropriate indications, there were 3 cases with pregnancy complications attributed at least in part to the LEEP.

Presence of HSIL by LEEP indication

Indication	HSIL on LEEP	No HSIL on LEEP	Total
Biopsy	147 (67%)	74 (35%)	221
Duration	4 (22%)	14 (78%)	18
HSIL Pap	2 (14%)	12 (86%)	14
ECC	0	9	9
Colposcopy	0	9	9
ASC-H Pap	0	14	14
History	0	6	6
None	1* (2%)	34 (98%)	35
Total	154	172	326

*CIN 1-2

Presence of HSIL by appropriateness

Appropriateness	HSIL on LEEP	No HSIL on LEEP	Total
Yes	151 (63%)	89 (37%)	240
Possibly	2 (6%)	29 (94%)	31
No	1* (2%)	54 (98%)	55
Total	154	172	326

*CIN 1-2

Conclusions: 17% and 26% of our cases had not appropriate or not appropriate/possibly appropriate indications, and of these, 97% had no HSIL on LEEP. The strongest indication was HSIL biopsy/ECC, followed by duration (all cases were ≥ 24 months). These results support a more conservative approach with stricter adherence to guidelines. Clearly pathology results can be used as a basis for outcomes research to improve patient safety and minimize risks.

2064 Melanoma Sentinel Lymph Node Immunohistochemistry in an Era of Cost Containment – Analysis of 2559 Consecutive Blocks Using a Consistent Protocol

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Background: Lymph node status in melanoma has crucial therapeutic and prognostic implications; even rare immunohistochemistry (IHC)-detected cells carry adverse prognosis. Our standard protocol for sentinel lymph nodes analysis (SLN) is to do IHC (S100, MelanA (MA)) on histologically-negative nodes (3 levels) or confirm obvious melanoma metastases (MM). This study aims primarily to assess value and financial impact of using S100 in addition to MA and secondarily at finding other cost-effective testing strategies.

Design: We searched our LIS for SLN blocks with S100 and MA reported between 2004 and 2013. Reports and select slides were reviewed to distinguish true MM from false positives (nodal nevi and dendritic cells) and determine SLNs in which IHC was the only evidence of MM from those where IHC was to confirm MM. Metastasis size was recorded. The primaries of MM were reviewed for factors that predict metastasis. Cost estimates used 2013 Medicare global payment (CPT 88342).

Results: Of 2559 SLN blocks (1098 patients), 97 blocks (72 patients) were positive for MM (3.8%/6.6%) with S100 and/or MA. MA was positive on 99 MM blocks (97 S100+/MA+, 2 S100-/MA+) and S100 positive in 98 (97 S100+/MA+, 1 S100+/MA-). The one S100+/MA-(0.04%) MM was a desmoplastic MM with other obvious metastasis. All other metastases were MA+. Review of available slides from 85 SLN nodes with metastases showed that 35 were detected by IHC alone and 19 were <0.1mm; IHC was for confirmation in the remaining cases. Screening IHC thus detected 35 occult metastases among 2497 histol- blocks at a cost per occult metastasis of $6,876. In contrast, the cost per incremental node found for performing S100 on histol- blocks to identify one MA- metastasis was at least $240,685. The primary melanomas for the 35 occult metastases included tumors of all Breslow thicknesses, Clark's levels and mitotic activity. Nevi were detected in at least 90/2559 (3.5%) IHC stained blocks.

Conclusions: It is safe and cost-effective to rely on MA to screen for occult MM and reserve S100 for special types (e.g. spindled/desmoplastic). Screening with only MA is far more cost-effective than MA + S100. We have not found histologic features of primary melanomas that would obviate screening of certain cases to further lower costs. In histologically-negative SLNs, incidence of nevi is comparable to frequency of MM, with implications for methods like RT-PCR that detect melanocytic mRNA but may not distinguish nevi from MM.

2065 Focused and Ongoing Professional Practice Evaluations in Anatomic Pathology

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Background: In 2008, the Joint Commission (TJC) introduced focused and ongoing professional practice evaluation (FPPE/OPPE) requirements. These use an evidence based approach to evaluating physician competence and performance and trends which may impact patient safety or quality of care. Five years since their introduction, little consensus exists on how FPPE/OPPE data should be collected within anatomic pathology. We aim to: 1) assess current FPPE/OPPE policies in 4 institutions, and 2) develop an efficient and unified FPPE/OPPE policy by reconciling the TJC requirements with existing anatomic pathology accreditation standards.

Design: FPPE/OPPE policies of anatomic pathology departments from 4 institutions affiliated with a single academic center in the western United States were compared. Also, TJC FPPE/OPPE elements of performance were aligned with medical test site standards of the WA state department of health (DOH) and select '12 College of American Pathologists (CAP) accreditation checklists: Anatomic Pathology, Laboratory General, All Common. The result is an efficient FPPE/OPPE data collection system to evaluate performance of anatomic pathologists.

Results: Significant variability in FPPE/OPPE policies existed across 4 institutions. Two of 4 anatomic pathology departments did not have dedicated FPPE/OPPE policies, but collected data under various departmental policies. The remaining 2 institutions' FPPE/OPPE policies focused heavily on diagnostic accuracy. Moreover, triggers for initiating FPPE, measures to resolve performance issues, and how this data is used to revoke/limit privileges were not clearly defined. Only 1 institution incorporated the required 6 core competencies, provided data sources for each metric, addressed continuing medical education documentation, and developed an OPPE data collection scheme. Therefore, to establish a unified FPPE/OPPE policy, TJC requirements were compared to the standards of existing accrediting organizations. Based on this comparison, a proposed system to assess professional competencies specific to anatomic pathology is broadly categorized into: diagnostic accuracy, report completeness, communication, turn-around-time, and practice volume.

Conclusions: Documentation of physician competency has been a requirement of hospital accreditation since '08. However, these findings indicate that performance evaluation measures are variable in anatomic pathology departments, even those affiliated with the same academic medical center. Future directions include adaptation of the data collection system developed here for efficient compliance with TJC requirements.

2066 Development of a Semiautomated Method for Subspecialty Case Distribution and Prediction of Intraoperative Consultations in Surgical Pathology

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Background: In many surgical pathology labs, operating room (OR) schedules are prospectively reviewed to determine specimen distribution to different subspecialty services as well as to predict the number/nature of potential intraoperative consultations (IOCs) for which prior medical records and slides require review. At our institution, such OR schedules are converted into easily-interpretable, "surgical pathology-friendly" reports to better facilitate the above activities. This conversion, however, was time-consuming and arguably a non-value-added activity. Our goal was to develop a more lean solution.

Design: A dynamic Microsoft Excel spreadsheet was developed to automatically convert published OR schedules into different tabular formats. Based on the listed surgical procedure description in the schedule, a keyword search was used to sort cases by subspecialty, while a weighted list of linked phrases was utilized to predict potential IOCs. After two trial and optimization cycles, the spreadsheet method was incorporated into our standard operating procedures. The impact of implementing this method on our practice is described below including a comparison of six months' worth of manual IOC prediction with two months' worth of automated prediction.

Results: The spreadsheet was able to select cases for distribution to the appropriate subspecialty and to accurately predict IOCs. On direct survey, users indicated that they spent 1-2 hrs less/day on this activity than before. Comparison of the manually and automatically compiled IOC predictions showed the mean daily difference in predicted versus actual IOCs performed underwent no statistically significant changes before and after implementation for all subspecialties except breast/endocrine, which underwent significant structural changes during the time period (Table).

	Manual	Automated	P-value
Bone/soft tissue/skin	1.22	1.34	0.59
Breast/endocrine	0.85	1.90	<0.0001
Gastrointestinal	0.74	0.73	0.97
Genitourinary	0.62	0.90	0.09
Gynecologic	0.38	0.39	0.94
Head/neck/lung	1.61	1.37	0.27

Conclusions: A well-designed, Lean and simple "information technology" solution (spreadsheet) to determine subspecialty case distribution and prediction of IOCs in surgical pathology is at least as accurate as the "gold standard" manual method, and requires less time to generate.

2067 Tumor Cellularity as a Quality Assurance Measure for More Accurate Clinical Detection of *BRAF* Mutations in Melanoma

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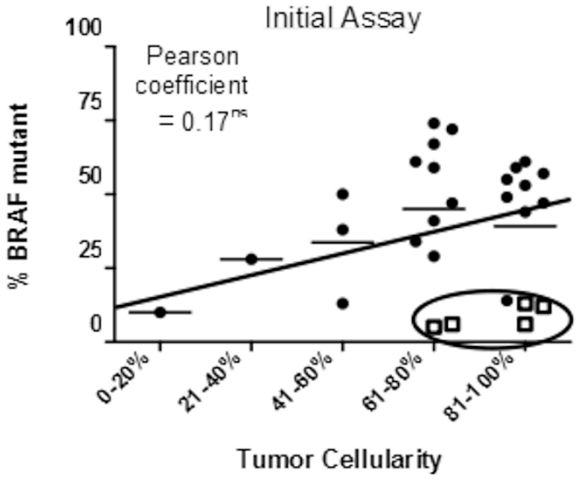
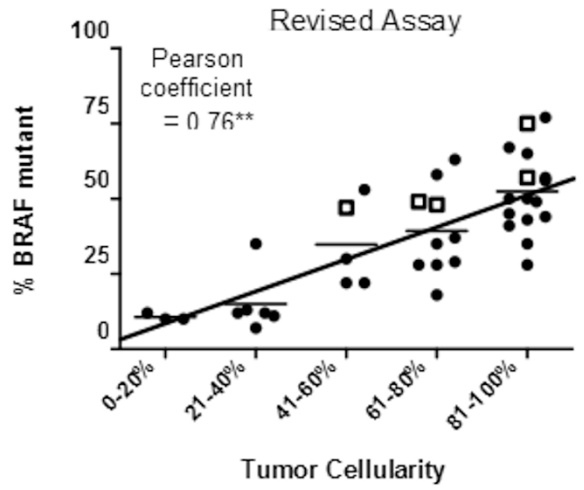
Background: Detection of *BRAF* mutations is a standard of care to predict small-molecule inhibitor (Vemurafenib) response in metastatic melanoma. We applied pathologist-generated estimates of tumor cellularity as a quality control measure for detection of *BRAF* mutations -particularly the analytic sensitivity of pyrosequencing for BRAF V600E vs non-V600E.

Design: We reviewed 62 formalin-fixed melanomas, assessed BRAF mutational status via pyrosequencing and correlated mutant % vs tumor as % of total cellularity. Additional 131 specimens were studied in a revised pyrosequencing assay.

Results: BRAF mutations were seen in 27/62 (44%) of melanomas, with V600E comprising 25/27 (93%) and non-V600E 2/27 (7%) of *BRAF*–mutated samples. Overall, the assay showed poor correlation of % BRAF mutant vs tumor cellularity (ρ=0.17, $p \leq 0.3$), primarily because a minority (n=5, 19%) of samples showed low V600E signal despite high tumor cellularity. This prompted a quality assurance investigation, which revealed our initial pyrosequencing assay had 'false positive', weak p.V600E signal in specimens with a V600K mutation. A revised pyrosequencing assay was designed. It was performed in 131 specimens, with comparable rate of BRAF mutations (50/131 or 38%), but higher rate of non-V600E BRAF mutations (15/50 or 30%). The revised assay showed strong correlation between % BRAF mutant and tumor cellularity (ρ =0.76, *p<0.01). Repeat testing of previously discordant samples showed them to have high p.V600K signal rather than low p.V600E signal.

Conclusions: Pathologists play important roles in molecular diagnostics, beyond identification of correct cells for testing. Accurate evaluation of tumor cellularity not only ensures sufficient material to maintain appropriate analytic sensitivity, but also provides an independent measure that can be used for quality assurance of the assay.





2068 Standardization of Quality Control in IHC Testing for Breast Cancer Biomarkers

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Background: Current quality control (QC) practices for clinical immunohistochemistry (IHC) use both human tissues obtained from clinical specimens and cell lines in microarrays with various levels of antibody expression as controls, which are run in parallel with the test specimens for ER/PR and Her2 testing of breast carcinoma. Archived tumors are less than ideal for controls due to inherent heterogeneity between and within specimens, unquantified expression levels of the antibody of interest, as well as unknown variations in pre-analytical processing. Control material obtained from cell lines may overcome some of the limitations inherently present in archived human tissue; nonetheless, the major drawback of using cell lines is that they do not replicate the spatial relationship between the tumor cells and the relationship of the tumor cells with the stroma i.e. the histologic pattern of tissues as the cells are propagated *in vitro.* In an effort to overcome some of the disadvantages of the current control methodologies and to generate standardized controls for breast cancer testing by IHC, we have developed breast cancer cell line derived xenografts (CDXs) as biomarker expression level reference standards; not only to control for tumor heterogeneity and pre-analytical variability, but also to eventually use this type of standardized control material to assess a wide-range of analytical variations in IHC techniques.

Design: Well-characterized breast cancer cell lines were analyzed by immunoblot to identify and select cell lines with varying steady-state levels of ER/PR expression, corroborated by IHC. The selected cell lines were used to generate CDXs in immunodeficient mice. Assessment of biomarker staining was performed on CDX tumors and compared to that of a range of previously analyzed breast tumors displaying varying levels of biomarker expression.

Results: Breast cancer cell lines MCF-7, T-47D, BT-474 and MDA-MB-468 with known range of ER/PR expression levels were selected based on immunoblot and IHC data. The CDX tumor tested for ER/PR revealed concordant results when compared with the respective cell lines from which the xenograft tumors were generated. The consistency of histologic homogeneity was observed in CDX tumors as well.

Conclusions: The consistency of differential ER/PR expression in different CDX tumors suggests their utility as suitable external quality control aid in IHC testing of invasive breast cancer and also as a tool for antibody validation and IHC methodology.

2069 GATA3 Immunohistochemical (IHC) Expression: A Tissue Microarray Analysis (TMA) of Expression Profiles in Malignancies

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Background: GATA binding protein 3 is a transcription factor with two zinc fingers at the carboxyl terminus and belongs to a distinct family of tumor genes that has tumor suppressor function. GATA3 binds to consensus DNA sequences in the promoters of genes unlike other tumor suppressor genes. The GATA3 family has a variety of roles including regulation of cell development in different tissues in both hematopoietic (T cells) and non-hematopoietic tissue (kidney, nervous system, skin, breast). Loss of GATA3 is a known factor in pathogenesis of breast cancer. We validated its IHC expression in a wide array of malignancies.

Design: TMAs of two 1mm cores of each of 504 different tumors were immunostained for GATA3. The anatomic sites and number of cases of the represented tumors included the following: breast carcinoma (77); urothelial carcinoma (79); hepatocellular carcinoma (100); colon carcinomas (81); pancreatic ductal adenocarcinoma (28); gastric adenocarcinoma (31); endometrial carcinoma (27); ovarian serous carcinoma (27); lung adenocarcinoma (27). Malignant melanoma (27) were used as negative controls.

Results: The pattern of GATA3 staining when positive was intensely nuclear, within the clusters of malignant cells. No cytoplasmic staining was noted.

IHC profile

	POSITIVE (%)	NEGATIVE (%)	TOTAL
BREAST DUCTAL CARCINOMA	35 (45.4)	42 (54.5)	77
UROTHELIAL CARCINOMA	56 (70.8)	23 (29.1)	79
HEPATOCELLULAR CARCINOMA	0 (0)	100 (100)	100
COLONIC ADENOCARCINOMA	0 (0)	81 (100)	81
PANCREATIC ADENOCARCINOMA	0 (0)	28 (100)	28
GASTRIC ADENOCARCINOMA	0 (0)	31 (100)	31
ENDOMETRIAL ADENOCARCINOMA	0 (0)	27 (100)	27
OVARIAN SEROUS CARCINOMA	0 (0)	27 (100)	27
LUNG ADENOCARCINOMA	0 (0)	27 (100)	27
MALIGNANT MELANOMA	0 (0)	27 (100)	27

Statistical Analysis for GATA-3 in Breast and Urothelial Carcinoma

	Breast Carcinoma	Urothelial Carcinoma
Sensitivity (%)	45.4	70.8
Specificity (%)	100	100
PPV (%)	100	100
NPV (%)	92	95.4

Conclusions: GATA3 is a sensitive and highly specific marker for diagnosis of urothelial and breast ductal carcinoma. Hence, it is useful for diagnosis/confirmation of primary and metastatic urothelial carcinoma and plays a role in diagnosing breast ductal carcinoma, especially cases with an estrogen-receptor (ER) positive profile.

2070 Changing Physician Practice Standards: Implementation of Pathologist Report Cards

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Background: Healthcare reform and the implementation of Accountable Care Organizations (ACO) emphasize quality patient care and cost containment. Many hospital based pathology practices will likely face institution wide mandates to decrease costs while increasing turnaround times to result in highly efficient patient care. Financial analysts site physician practice standards as the number one hurdle in providing more streamlined, low cost care. Academic centers may be particularly affected in that laboratory utilization may be affected by education needs of resident pathologists and the complexity of cases encountered. In this study we aim to determine if confidential report card distribution to attending academic pathologists results in a change of practice in utilization of the anatomic pathology (AP) laboratory.

Design: AP laboratory utilization was defined by the ordering of additional H&E sections (levels) and Immunohistochemical (IHC) stains. Data was collected for the group as a whole and for each individual. Rates of ordering were calculated by taking the total number of levels or IHC ordered divided by the number of blocks signed out. Each pathologist was given a confidential report card with their lab utilization rates and the average rate for the group for the previous quarter. The exercise was repeated the following quarter and change in practice behavior was assessed.

Results: The rate of additional H&E ordered prior to distribution of report cards was 2.0%. The rate for the quarter following distribution was 1.5%. 6/8 pathologist showed a decrease after the intervention, 1 showed no change, and 1 showed a small 0.4% increase. The rate of IHC ordering was 8.1% prior to the intervention and decreased to 7.3%. 7/8 pathologists had a rate decrease after the intervention. Overall lab utilization decreased by 1.3%.

Conclusions: AP lab utilization will likely become increasingly important with the overall focus on value in healthcare. Few studies have looked specifically at AP utilization rates. We began to collect and report data and found that the act of making pathologist self-aware of their practice standards and how they compare to their colleagues resulted in a change in behavior. We find these results incredibly interesting and see them as a springboard for increased practice standardization that may have broad based implications in cost containment and implementation of changes in practice standards. We plan to continue lab utilization surveillance and reporting as well as continue to assess the utility of these implementations.

2071 Proactive Auditing for Inappropriately Ordered 1,25-Dihydroxyvitamin D

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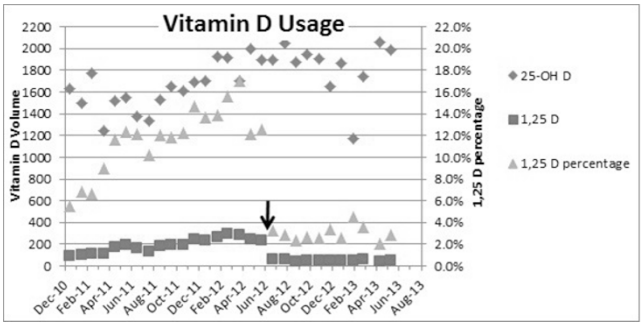
Background: Vitamin D deficiency has been linked to a number of diseases, increasing demand for laboratory testing. For nutritional assessment of vitamin D status the appropriate test is 25-hydroxyvitamin D (25-OH D), however, 1,25-dihydroxyvitamin D (1,25 D) is often erroneously ordered. 1,25 D is a poor marker for nutritional status due to short half-life and large within-patient variability. A review of vitamin D ordering practices at our institution revealed an unexpected increase in 1,25 D orders, prompting further study.

Design: A retrospective analysis of 1,25 D and 25-OH D orders as well as the percent 1,25 D (1,25 D/[1,25 D + 25-OH D]) from December 2010 to June 2012 was performed. Starting in June 2012, all 1,25 D orders were proactively audited by pathology staff and deemed appropriate or inappropriate based on accepted indications such as renal failure/transplant and calcium disorders. When deemed inappropriate, the provider was contacted and questioned about specific testing reasons, followed by education on which vitamin D test was proper for nutritional assessment.

Results: Throughout the study period the 1,25 D volumes increased to a high of 290 monthly orders with a 1,25 D percentage of 14.6%. Immediately following initiation of the proactive audit the mean monthly 1,25 D volume plummeted to 53, with a 1,25 D percentage of 2.9% (Figure 1, arrow).



Conclusions: 1,25 D has narrow clinical utility and is only relevant in a few clinical scenarios. Inappropriate assessment of 1,25 D for nutritional vitamin D status may lead to misdiagnosis and inappropriate treatment. Our results show a dramatic drop in completed orders of 1,25 D following initiation of a proactive audit. This activity resulted in better diagnostic practice as well as significant financial savings. To elaborate, 25-OH D is an in-house test at our institution while 1,25 D is performed at a reference laboratory at additional cost. As we have shown, the majority of 1,25 D requests were erroneously ordered thus adding cost with no diagnostic value. Through proactive audit, we reduced our mean monthly 1,25 D expense from $6,401 to $1,561 with an extrapolated annual savings of $56,000. By educating providers, inappropriate 1,25 D volumes decreased and remain low, indicating continued savings and better overall diagnostic practices.

2072 The Impact of Pre-Signout Random Review of Surgical and Cytologic Cases on Error Rate in Anatomic Pathology

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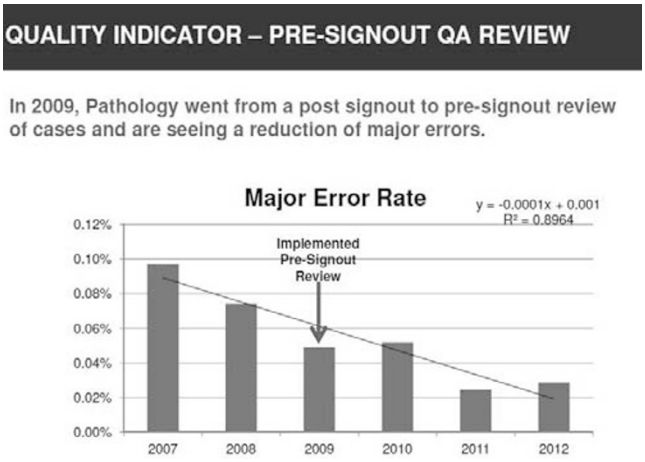
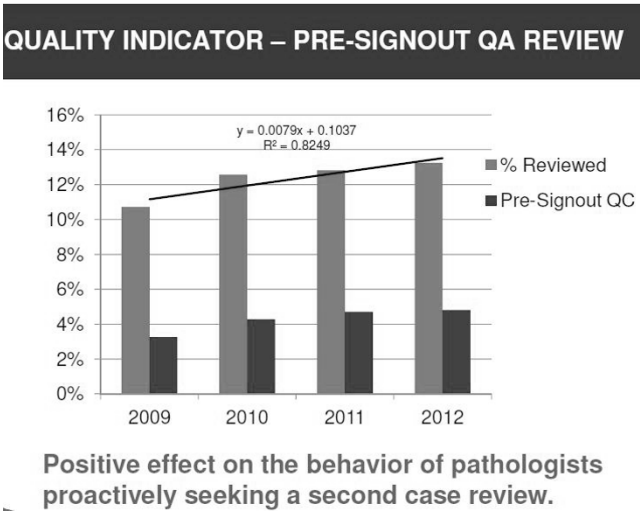
Background: In 2009, UPMC Presbyterian Shadyside implemented an automated random pre-signout review of surgical and cytologic cases within a Center of Excellence (COE) model. The previous practice was to perform a review post-signout, one to two months after the diagnosis was reported. The pre-signout review was piloted in select surgical COEs and then rolled out to the rest of the surgical pathology group, followed by the cytology COEs. Pathologists have always had the opportunity to consult with a colleague prior to signout of any case. With the institution of the pre-signout review, if a colleague had seen the slides of a case randomly selected, that pathologist could enter the formal review on the chosen case in the computer.

Design: Since 2007, an annual pathologist performance review has been conducted which includes the error rate of individual pathologists as well as the department as a whole. By comparing the departmental error rate pre and post implementation of the random pre-signout review, it is possible to determine the effect of this unique quality assurance tool. In addition, examination of the number of colleague consultations pre and post implementation provides insight into the behavior patterns of the pathologists.

Results:



In 2009, Anatomic Pathology went from a random, manual, labor intensive post-signout review of cases to an automated, random, pre-signout review. Our Department has seen a continuing reduction in major errors ever since.



The percentage of cases receiving a second review by a colleague prior to the signout of a pathology case has increased since the implementation of pre-signout QA.

Conclusions: The establishment of an automated, random pre-signout selection of cases for a quality review has resulted in a decrease in the number of major errors as well as had a positive effect on the behavior of the pathologists who proactively seek a second review of their cases.

2073 The Impact of Knowledge Transfer Strategies on the Detection of Venous Invasion in Colorectal Cancer

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Background: Venous invasion (VI) in colorectal cancer (CRC) is an independent prognostic indicator that is widely under-reported. This study aimed to determine the impact of knowledge transfer on VI detection among pathologists who had previously participated in an interobserver variability study of VI detection in CRC (*Kirsch R et al., Am J Surg Pathol. 2013;37:200-210*).

Design: Knowledge transfer strategies following the interobserver variability study included: (1) feedback on individual and overall VI detection rates (2) illustration of morphologic clues for VI detection and (3) advice on benchmark VI detection rates. Eighteen months later, participants were invited to submit reports from all CRC resections signed out 18 months prior to, and 18 months following completion of the study. Reports were reviewed for presence or absence of VI and other mandatory elements of the College of American Pathologists protocol. The number of tumor containing blocks and elastin stains/case were recorded. A minority of cases required slide review to determine the type of vascular space invasion present.

Results: Eight of the 12 original pathologists (5 GI, 3 non-GI) participated in the study. The number of reports examined was 220 pre-study and 206 post-study. There was a two-fold increase in VI detection post-study (overall: 19.1% to 39.3% [P<0.001]; GI: 18.2% to 38.4% [P<0.001]; non-GI: 21.8% to 44.7% [P<0.02]). The mean number of elastin stains/case increased from 0.10 (std. error, 0.41) to 2.51 (2.06) (P<0.001); the mean number of tumor containing blocks was similar (8.28 [6.69] vs. 8.12 [4.07]; P=0.761). Rates of perineural invasion (PNI) (13.9% to 22.3%, P=0.032) and discontinuous tumor deposits (12.3% to 22.8%, p=0.005) were increased. There were no significant differences in small vessel invasion, TNM stage, tumor location or neoadjuvant therapy pre- and post-study.

Conclusions: Participation in an interobserver study of VI and subsequent knowledge transfer were associated with a two-fold increase in VI detection. Increased use of elastin stains and awareness of morphologic clues for VI were likely responsible. The reasons for the increase in PNI and discontinuous tumor deposits are less clear, but may have been influenced by AJCC 7th edition reporting changes during the period of this study.

2074 Collaboration between a Pathology Department and Cancer Tumor Registry Enhances Compliance of Pathology Cancer Reporting

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Background: The American College of Surgeons' Commission on Cancer (CoC) requires that, greater than 90% of pathology cancer resection reports contain all necessary scientifically validated data elements as defined by the College of American Pathologists (CAP) surgical case summary checklist. This is a major requirement for cancer treatment programs wanting to maintain or seek CoC accreditation. Adherence to this requirement by anatomic pathology laboratories is a critical link that validates continued accreditation by the CoC and hence increased referral for cancer related work.

It also ensures that labs conform to the data reporting and formatting requirements of the CAP Laboratory Accreditation Program. We report on our success of achieving the above stated aims, at a large tertiary hospital based anatomic pathology laboratory within our health system.

Design: We have developed a model which involves collaboration between the Cancer Tumor Registry (CTR) registrars at the hospital site and the clinical and administrative leadership of our pathology department. The program was started in 2010 when we were not consistently meeting the 90% threshold. The CTC registrars started reviewing all pathology cancer reports daily. All reports were reviewed for completeness of data elements as mandated by CAP synoptic checklists. Every week a summary of all outliers was sent to the chief of anatomic pathology service. Based on this weekly report, individual pathologists who signed out incomplete reports were contacted and report addendums were issued to correct for any missing data elements.

Results: This iterative process has continued for the last 3 years and we have seen dramatic improvements in our compliance rates for cancer reporting. From 2010, when our our average monthly compliance rate was 63.85%, we have seen sustained and steep improvements in compliance rates for 2011 and 2012. In 2013, our monthly compliance rate for all tumor types is consistently greater than 95% (and even 100% for some tumors).

Percent compliance rate of completeness of pathology cancer reports as defined by CAP case summary check-list.

Year	% Compliance Rate
2010	63.85
2011	89.8
2012	97.5
2013	96.6

Data from North Shore University Hospital, Manhasset, NY.

Conclusions: Novel ways of collaboration and communication between pathology departments and CTRs can help maintain and improve compliance with cancer reporting requirements.

2075 Cancer Tracking: Clinician Compliance and Program Outcome

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Background: The results of certain laboratory tests are critical for patient care and must be communicated to the patient care team in a timely and clear fashion. The College of American Pathologists (CAP) has developed guidelines for establishment of "critical values" in laboratory medicine. More recently, the CAP along with the Association of Directors of Anatomic and Surgical Pathology (ADASP) have developed a set of recommendations for timely reporting of significant or unexpected surgical pathology findings. Among the urgent diagnoses requiring rapid reporting to the clinical care team are new diagnoses of malignancy and the unexpected finding of malignancy. The University of Missouri has established a "cancer tracking" protocol in which all diagnoses of malignancy require acknowledgement of receipt of the report by the responsible clinician.

Design: To determine compliance with the "cancer tracking" policy, we reviewed five months of clinician compliance with the signoff requirement on surgical pathology reports identifying a malignancy. The protocol requires the Department of Pathology to send a letter to the responsible clinician requesting signoff acknowledgment of receipt of the report. 1155 reports of malignancy with requests for confirmation of receipt were sent to the physicians named on the request form as the treating physician and/or the physician performing the biopsy.

Results: 692 (60%) signed acknowledgements were received following the first letter. In 463 cases, a second letter was sent requesting acknowledgement of review of the report. Three hundred and fifty-six (77%) acknowledgements were received following the second letter. In 107 (9%) cases, no response was received within a three-week follow up period.

Conclusions: Confirmation that the physician caring for a patient receives a pathology diagnosis is important for patient management and quality assurance in the reporting process. While the surgical pathology report was placed in the chart, it was impossible to confirm that the responsible physician had received and reviewed the report in a timely fashion in 9% of cases reviewed in our study. Failure to communicate pathology results to the treating clinician appears to be a significant problem and improved techniques for communication and confirmation of transmission of important anatomic pathology diagnoses need to be developed.

2076 ThinPrep CytoLyt Fixation Has No Effect on ER and Cytokeratin Staining on Normal Breast Tissue

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Background: ThinPrep CytoLyt is a methanol-based fixative commonly used in FNA procedures, including FNA of the breast. Aspirated material may be the only tissue available for receptor testing. The common practice of cytology laboratories is to prepare cell blocks from CytoLyt fixed material. The American Society of Clinical Oncology/ College of American Pathologists guideline recommends formalin fixation time for optimal immunohistochemical testing of estrogen receptor to be between 6 and 72 hours. However, currently, there are no standard recommendations regarding the accepted time for tissue to be maintained in methanol based fixatives for receptor testing. The aim of this study is to determine if delayed formalin fixation due to CytoLyt preservation has any effect on immunohistochemical staining on breast tissue.

Design: Breast tissues were collected from 10 mastectomy specimens. Tissues were placed in ThinPrep CytoLyt solution for 4 hours and 15 hours before transferring to neutral buffered formalin (NBF) for an additional 6 hours. Immunohistochemical stains of estrogen receptor (ER) and pan-cytokeratin (CK, clone AE1/AE3) were performed on each specimen using the Ventana Autostainer. The slides were compared to controls (breast tissue from the same patient directly fixed in 10% NBF).

Results: One of ten specimens has no glandular tissue and is excluded from the study. For ER staining, one specimen showed mildly decreased intensity after 15 hours in CytoLyt. ER staining for tissue fixed in CytoLyt for 4 hour and 15 hours in eight of the remaining nine specimens show staining intensity and percentage of positive cells comparable to controls directly fixed in 10% NBF. For cytokeratin staining, all 9 cases of tissue fixed in CytoLyt for 4 hours and 15 hours show strong staining intensity and staining patterns comparable to tissue fixed in NBF directly.

Conclusions: Breast tissues exposed to CytoLyt fixative for 4 hours and 15 hours has no effect on staining quality for ER and pancytokeratin. Our data indicates fine needle aspiration material collected by FNA can be preserved in CytoLyt for at least 15 hours without compromising ER and cytokeratin immunostain on breast tissue.

2077 Rapid Improvement in Workflow Steadiness after Implementation of Lean Concepts

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Background: The HUGTP Pathology Department serves 3 hospitals and 15 primary care centers in the Barcelona metropolitan area. A total of 25,000 surgical pathology cases are diagnosed every year, with a 20% increase in the last 10 years. The cases accessioned every day vary greatly in number (65-150) and complexity. As a result of this variability, bottleneck situations were common in the histology lab, leading to slide delivery delays and turnaround time prolongations. We resorted to Lean concepts in an attempt to solve these problems.

Design: Our work focused on the accessioning area, cutting room and histology lab. Situational analysis of the starting point was carried out through direct observation by quality staff and subsequent building of a value stream map identifying the valueless parts of the process. Analysis was completed with a precedence diagram method to establish the most efficient process sequence. A 5S approach was used to optimize working conditions in the cutting room. A flow diagram prescribing both the daily processing of a limited number of paraffin blocks and preferential treatment of urgent biopsies was proposed. Block number daily variability, satisfaction levels of pathologists, residents and technicians, and diagnosis turnaround times were used as result indicators.

Results: Poor availability of surgical instruments, untidy shelving of specimens, cutting room overcrowding at peak hours, cassette printing bottlenecks, and unevenness in the daily number of blocks were identified as main problems at the starting point. Establishment of personalized kits of instruments, ordered shelving of specimens, shifts of cutting residents and technicians, beforehand cassette printing, and a limited number of daily blocks (a maximum of 325) along a 3-month period significantly improved working conditions in the cutting room and histology lab, increased personnel satisfaction levels, reduced the block daily number standard deviation from 116 to 60, and diminished the diagnosis turnaround time from 7.4 to 5.6 days.

Conclusions: Lean concepts provide low-cost, highly efficient tools for optimization and standardization of work. Application of these concepts to common problems of pathology departments result in greater steadiness of workflow, shorter diagnosis turnaround time, and unanimous elevation of personnel satisfaction levels.

2078 Improvement of Renal Biopsy Quality after Implementation of On-Site Evaluation by Cytotechnologists

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Background: Renal biopsy is an essential tool in the diagnosis and surveillance of kidney disease. Pathologists need to evaluate renal biopsies by light microscopy, immunofluorescent staining, and electron microscopy (EM). It is crucial that the clinician obtain an adequate biopsy including several glomeruli, and that the biopsy be triaged and placed into fixative immediately. At our institution, we trained our cytotechnologists to perform on-site evaluation and triaging of renal biopsies, which was implemented in the beginning of 2013. Previously, renal biopsies were delivered to the frozen section room via a courier and evaluated for adequacy by a pathologist or a pathology assistant. The aim of this study was to compare the quality of renal biopsies submitted before and after implementation of on-site evaluation by cytotechnologists.

Design: In this retrospective study, we reviewed renal biopsies performed at our institution between June 2012 and July 2013. Biopsies evaluated first in the frozen section room were designated as group I, while those in which cytotechnologists performed on-site evaluation were designated as group II. In both groups, tissue was transferred carefully onto a glass slide and evaluated for adequacy under the light microscope to identify glomeruli. For adequate specimens, the tissue was divided into 3 pieces and submitted in the appropriate fixative or transport solution for light microscopy or EM, and snap frozen for immunofluorescence microscopy. In group II, if the initial biopsy yielded insufficient glomeruli, the cytotechnologist requested that the nephrologist or radiologist either obtain another core or specify which of the 3 tests should be given priority. For all biopsies, we evaluated the presence or absence of glomeruli as well as swelling of podocytes and endothelial cells (an indicator of preservation injury). These parameters were compared between the 2 groups.

Results: 313 cases were retrieved from surgical pathology archives, including 186 cases in group I and 127 cases in group II. In group I, no glomeruli were identified by EM in 30 (16%) cases, while in group II, no glomeruli were identified by EM in 5 (4%) cases. The difference was statistically significant (P = 0.0004). Podocyte and endothelial cell swelling was reported in 47 (25%) and 21 (16%) cases in group I and group II, respectively (P = 0.0434).

Conclusions: On-site evaluation by cytotechnologists at the time of renal biopsy reduces preservation artifact and improves the yield of diagnostic tissue, as measured by the presence of glomeruli.

2079 Getting on the Same "Level:" A Single Institution Survey Shows Lack of Standard Definitions for Deeper Tissue Sections May Lead to Missed Pathology

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Background: The terms "recut," "level," and "deeper" are commonly employed by pathologists (PTs) and histotechnologists (HTs) to indicate how far to trim into a paraffin block to obtain tissue for glass slides. However, lack of standard definitions and the resulting expectation-interpretation mismatch may lead to repeat requests for additional sections, increased workload and turn-around time, and the potential to miss lesions within the remaining tissue. To assess our PTs' and HTs' definitions for these terms, a survey was conducted.

Design: The authors distributed a survey to 8 PTs and 6 HTs with 11 open-ended questions about standard section thickness, definitions for "recut," "level," and "deeper," and number of sections discarded between stained slides for each of those terms. Surveys for PTs and HTs asked similar questions, with slight wording changes to reflect each group's role.

Results: 1. Survey responses for standard section thickness:
3 µm: 3 PTs, 5 HTs
4 µm: 3 PTs, 1 HT
5 µm: 2 PTs
2. Number of sections discarded between each of the following:
(*e.g., # sections* (*# respondents*))
Levels: 1(2PTs, 1HT), 2(2PTs, 1HT), 3(2PTs, 3HTs), 5(1PT), 10(1PT, 1HT)
Deepers: 2(1PT, 1HT), 4(2PTs, 1HT), 5(2PTs), 6(3HTs), 10(2PTs), 12(1PT), 20(1HT).

Conclusions: There was slightly less agreement for section thickness between PTs (range: 3-5 µm, mean: 3.88 µm) than between HTs (range: 3-4 µm, mean: 3.17 µm). Both groups exhibited wide variation in the number of sections discarded between levels (PTs: 1-10, HTs: 1-10) and deepers (PTs: 2-12, HTs: 2-20). Defining "level" and "deeper" by the number of sections discarded may lead to dramatic differences in tissue sampling via compound effects of user variation in section thickness. Expectation-interpretation mismatch between PTs and HTs may result in shallow tissue sections, leading to missed diagnoses (Fig.1). Quantitative standards should be defined for the number of microns of tissue discarded between levels and deepers, thus allowing slight variation in section thickness for different tissue types or personal preferences.

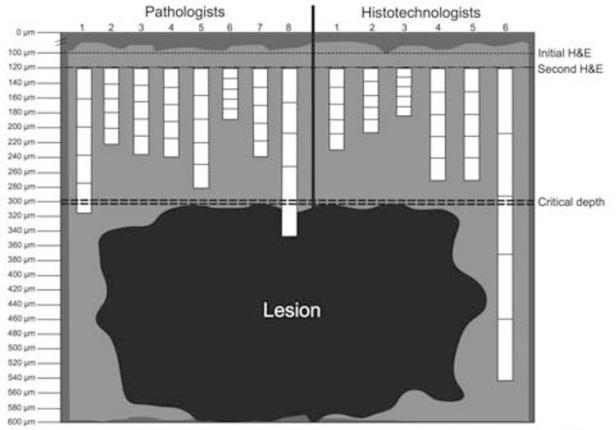

Figure 1. Theoretical scenario: Initial slides from this paraffin block suggested an underlying malignant process; five deeper sections were ordered. Horizontal black lines within numbered white bars represent the depth at which deeper sections would be cut, per survey respondent (# sections discarded x section thickness). Only 2/8 PTs and 1/6 HTs would obtain a section with lesional tissue.

2080 The Clinical Utility of Abdominal Fat Pad Fine Needle Aspiration in the Diagnosis of Amyloidosis: An Institutional Experience

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Background: Fine need aspiration (FNA) of the anterior abdominal fat pad is a low-cost, low-morbidity procedure, but its diagnostic utility in the diagnosis of amyloidosis is unclear. While its specificity is high, previous reports in the literature cite conflicting rates of sensitivity, ranging from 19% to 78%. In this study, we evaluated the utility of fat pad FNA in the diagnosis of amyloidosis at our institution.

Design: A retrospective review was performed of all abdominal fat pad FNAs between January 1, 2012 and June 30, 2013 at our institution. For each case identified, electronic medical records were reviewed to record clinical history, additional procedures performed for amyloid detection, and final clinical diagnosis. Unstained, fixed, direct smears from these procedures were stained with congo red, with adequate controls.

Results: Eighty-nine abdominal fat pad FNAs were performed for evaluation of amyloidosis over this 18-month time period. Of the 89 cases, 9% (8) were positive for amyloid by congo red staining, 85% (76) were negative, and 6% (5) were inadequate for analysis. In 6 of the 8 positive cases, abdominal fat pad FNA provided the initial diagnosis of amyloidosis (primary AL amyloidosis). Of the 76 negative cases, 8 had a subsequent diagnosis of amyloidosis by another method and 6 had previous diagnoses of amyloidosis documented by biopsy of other sites. In 28 of the negative cases, the FNA results were assumed to definitively rule out amyloidosis, obviating the need for further studies despite clinical findings. In 19 patients, additional work-up and biopsies confirmed the absence of amyloid.

Conclusions: Our institutional review resulted in a calculated specificity of 100% and a sensitivity of 36% for this procedure. Abdominal fat pad FNA demonstrates high specificity and, when positive, represents a useful diagnostic test to confirm amyloidosis as an alternative to more invasive procedures. However, it suffers from a relatively low sensitivity and does not rule out the diagnosis in patients in which there is a high clinical suspicion, signaling the need for further diagnostic testing in this population.

2081 Patient Safety and Timeliness of Pilot-Co-Pilot (PCP) Pathologist Team Sign-Out

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Background: Based on the cognitive theory of slow and fast thinking (Kahneman and Tversky) pathologist diagnostic errors in precision and accuracy are secondary to failures in pattern recognition consensus and bias, respectively. We used an aviation industry approach of the PCP for real-practice pathologist team sign-out and evaluated the frequency of error and timeliness of diagnosis.

Design: Using a two-headed microscope, a team consisted of a pilot who moved slides on a stage and a co-pilot who could call a timeout at any time to look at any field or at any objective power. The PCP team reached diagnostic consensus or obtained a consult if consensus could not be reached. Two PCP pathologist teams signed out a total of 480 cases. We used an observational method to measure the time to case sign-out and compared these data to historic single pathologist case sign-out times. Two senior pathologists secondarily reviewed all cases. We measured interobserver variability of the teams and two senior pathologists with the 480 cases and 50 randomly selected cases of the individual PCP team members. We measured diagnostic accuracy by using expert consultation of all discrepant cases signed out by the PCP teams and the individual pathologists.

Results: For the PCP teams and individuals, the mean case sign-out time all cases (bigs and biopsies) was 8 and 14 minutes per case, respectively. The interobserver kappa value was good to excellent for the PCP teams and senior pathologists and moderate to good for the individual team members and senior pathologists. The diagnostic accuracy of the teams was higher than for individuals, although a larger sample size is necessary to test for statistical significance.

Conclusions: We found that PCP pathologist teams make diagnoses faster and that are more accurate and precise compared to individuals. This method has the possibility to change the paradigm of pathology sign-out and further could be augmented with digital imaging. We believe that the team approach encourages the reaching of consensus on patterns recognition and markedly reduces bias.

2082 Value in Dermatopathology: Decreases in Slide Quantity Does Not Affect Patient Care and Reduces Costs

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Background: In our current dermatopathology practice for biopsies, we routinely process two initial slides each with two tissue sections. It was our impression that the second slide rarely changed or aided in diagnosis, and we sought to investigate their utility. Also, we wanted to investigate how often additional deeper levels aided in diagnosis, and to examine the costs involved in processing second slides and deeper levels. As healthcare moves forward, we must find ways of cutting costs and saving time while concurrently ensuring that the highest level of patient care is being met.

Design: The time frame spanned 12 weeks from June to August 2013. For the 1019 biopsies examined during this period, we noted how often the second initial slide aided in diagnosis. In addition, as a second arm of the study, we looked at the rate of deeper levels performed on all biopsies, the reasons for ordering them, and how often they helped. Lastly, we investigated the costs involved in both of these standard processes.

Results: Of the 1019 biopsy cases, the second slide did not aid in diagnosis in any of the cases. The approximate total cost of processing 2nd slides was $1,141. Deeper levels were ordered on 12.2% of all cases (124 cases). When deepers were ordered, they aided in diagnosis 19.4% of the time. The reasons for ordering deepers included to rule out malignancy (32 cases), for clarification (84 cases), non-diagnostic initial levels (6 cases), and technical/embedding issues (2 cases). The total cost of cutting three additional slides with deeper levels for each of these cases was $915.

Conclusions: Automatic preparation of a second slide did not aid in the diagnosis of any of the cases. Reduction of slide preparation for routine dermatopathology cases would save approximately $4560 per year. This is a direct cost that does not account for pathologist time and mental fatigue from looking at additional slides. Also, one slide preparation would save on storage space and filing efforts. With this data, we plan to change our dermatopathology practice in the near future to cutting only one initial slide with two tissue sections per biopsy case. By doing so, we will be saving money and time without sacrificing patient care. Also, by establishing a baseline rate of deeper level ordering and its utility, we will be able to compare our new upcoming practice to our old one to aid in further refinement our practice to maximize both efficiency and quality.

2083 Is There a Need for Rapid *On Site* Cytological Assessment in the Endoscopy Suite for Gastrointestinal EUS-FNA Specimens?

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Background: Endoscopic ultrasound guided fine needle aspiration biopsy (EUS-FNA) is the preferred method of obtaining tissue from lesions within and adjacent to the gastrointestinal (GI) tract, since morbidity and mortality are low in the setting of high diagnostic accuracy. In many institutions rapid *on-site* cytological evaluation within the

endoscopy suite is considered to be the standard of care, given the immediate feedback regarding quality and quantity of the sample, but is associated with a considerable resource commitment. Our institution does not perform *on-site* evaluation and routinely prepares a cell block for possible immunohistochemical analysis (IHC). This study analyzes our overall diagnostic yield and incidence of non-diagnostic cases to determine the validity of this strategy.

Design: Data, encompassing clinical information (including presence or absence of mass lesion), procedural records (gauge of needle and needle passes) and cytological assessment were analyzed for all consecutive GI EUS-FNA procedures (n=85) performed at Vancouver General Hospital from August 2011 to January 2013. Clinical follow-up information was collected for all patients to determine diagnostic accuracy.

Results: 85 biopsies were performed in 78 patients with an average of 2.6 needle passes, from sites including pancreas (n=62), stomach (n=8), duodenum (n=1), lymph nodes (n=6), retroperitoneal masses (n=4), para renal mass (n=1), bile duct (n=2) and porta hepatis (n=1). Malignancy was diagnosed in 45 (53%) biopsies, while 24 (29%) encompassed benign entities. 'Suspicious for malignancy' and 'atypical' was recorded in 8 (9%) and 6 (7%) biopsies, respectively. Within the suspicious and atypical category 9 (60%) patients showed malignant pathology on resection, while no follow-up was available for 5 patients. Only 2 (2%) biopsies received an initial cytological diagnosis of 'non-diagnostic'; subsequent rebiopsy confirmed a malignant neoplasm. Of the malignant diagnoses IHC was performed in 23 (51%) instances and was essential for definitive final diagnosis in 20 (87%) of these.

Conclusions: Given these findings our study highlights that without rapid *on-site* cytological evaluation diagnostic accuracy can be achieved with a low average number of needle passes and within an acceptable diagnostic range. Additionally, the ability to perform IHC increased our diagnostic accuracy considerably.

2084 Analysis of the Utility of Routine Histologic Examination of Specimens Generated by a Hospital-Based Podiatry Service

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Background: The current economic situation is leading to renewed efforts at cost containment in medicine. Exisiting medical literature suggests that some types of pathology specimens, such as elective joint replacement specimens for osteoarthritis, have such low rates of discrepancy between the pre-operative clinical diagnosis and the histologic diagnosis that the costs of histologic analysis may not be warranted. Podiatry services are another source of pathology specimens that are considered by some to be of low clinical impact, but the utility of routine histologic review of podiatry specimens has not yet been fully explored.

Design: We identified all cases submitted for histologic review by the podiatry service at our institution for a three year period from July 2010 through June 2013 (n=247). We omitted cases consisting solely of skin biopsies (n=47, mostly plantar warts) and one case that was insufficient for diagnosis. For the remaining 199 cases, the pathology reports and clinical notes were reviewed to evaluate the compatibility of the pre-operative clinical diagnoses and the histologic diagnoses. Cases were also classified as being predominantly either an infectious/inflammatory process or a mass-forming process.

Results: Of the 199 reviewed cases, 26 (13%) had a histologic diagnosis that was not compatible with the pre-operative clinical diagnosis. The discrepancy rate was significantly higher for mass-forming processes (23 of 101 cases; 23%) than for infectious/inflammatory processes (3 of 98 cases; 3%). While some of the discrepancies would likely have no impact on clinical management, at least a few would have, including one case with a pre-operative diagnosis of synovial cyst that was diagnosed as an adnexal tumor with features suspicious for malignancy by histology. The discrepancy rates for selected specific pre-operative clinical diagnoses are displayed in Table 1.

Discrepancy Rate for Specific Pre-Operative Clinical Diagnoses

PRE-OP DIAGNOSIS	TOTAL #	# DISCREPANT (%)
Cellulitis	5	0 (0)
Ganglion Cyst / Synovial Cyst	15	5 (33)
Giant Cell Tumor	2	1 (50)
Gout	9	0 (0)
Neuroma	31	5 (16)
Osteomyelitis	52	0 (0)
Plantar Fibromatosis	11	2 (18)
Rheumatoid Nodule	4	0 (0)

Conclusions: The routine review of pathology specimens generated by a podiatry service results in a significant percentage of histologic diagnoses that are discrepant from the pre-operative clinical diagnosis, and thus can be argued to be of clinical value. The clinical value of histologic review appears to be much higher for mass-forming lesions than for infectious/inflammatory lesions.

2085 Practicing Rimm's Lab Algorithm: Determination of the Specificity of Novel Immunohistochemistry Antibodies

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Background: Immunohistochemistry (IHC) is a favored technique to identify and semi-quantitate protein expression in anatomic pathology laboratories around the world. The conventional utility of IHC in clinical pathology laboratories extend from diagnostic to prognostic services in the line of patient care. Since laboratories do not have a standardized process of antibody validation, we would like to present our experiences regarding novel IHC antibody validations by following Rimm's Lab Algorithm.

Design: We have implemented Rimm's lab algorithm on a total of 43 IHC antibody validations in our core facility in the last 2 years. The antibodies ranged from novel transcription factors, signal transduction moieties and structural proteins with no validation information existing in the literature to well standardized stains. We followed rigorous antibody selection criteria, regulation of pre-analytical variables, design and development of clonal controls with wide dynamic range, comprehensive optimization work up including multiple antigen retrieval methods and antibody titrations.

Results: From the 43 novel antibodies (32 unique markers), 19 (46%) antibodies failed to demonstrate the specificity. The satisfactory validation rate was 53% with clear demonstration of specificity.

Novel IHC Antibody Validation Success Rate

Abs	D.S.B with first Ab purchase	D.S.B with second Ab purchase	D.S.B with nth Ab purchase	Cell lines used to test Abs	D.S by Abs
43	11	9	3	23	19

D.S.B (Demonstration of Specificity of a Biomarker); Ab (Antibody)

Conclusions: Comprehensive utility of clonal specific cell lines are critical to determine specificity of antibodies. Significant number of novel antibodies sold by commercial companies could not establish specificity. Antibody specificity disclosure (pictorial) is paramount for the scientific community to have confidence in the data presented in the literature. We believe Rimm's lab algorithm is a worthy design to replicate in validation of novel antibodies across pathology laboratories.

2086 Concordance between Whole-Slide Imaging and Light Microscopy for Surgical Neuropathology

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Background: Whole slide imaging (WSI) technology is a promising tool, especially attractive in the setting of distant consultations. The purpose of this study is to determine diagnostic concordance between interpretations using WSI and standard light microscopy in neuropathology cases.

Design: Neuropathology cases diagnosed between April 15th and June 15th, 2013 were retrospectively reviewed and 97 cases were included in the study. Diagnoses related to degenerative disc disease, pituitary adenomas, metastatic carcinomas and melanomas, vascular malformations, and other benign or descriptive diagnoses (e.g. meningocele, dermoid cyst, focal cortical dysplasia, gliosis) were excluded. Consultation cases without available slides were also excluded. All slides were reviewed by one of the authors (MP), and a diagnostic slide was selected for WSI. Slides were scanned at 40x with ScanScope® XT (Aperio Technologies Inc, Vista, CA). A neuropathologist (TT) reviewed the images and independently provided a diagnosis and WHO grade for all cases. The same neuropathologist later performed a standard microscopic evaluation of the same slides without access to the original pathological or WSI-based diagnoses. Concordances between three diagnoses (original pathology report, slide-based and WSI-based) were evaluated.

Results: There was complete agreement between all three modalities in 88 of 97 cases (90.7%). Slide- and WSI-based diagnoses were in agreement in 92 of 97 cases (94.9%). Three cases were assigned a lower grade on WSI, and the main limitation was the difficulty in identifying mitotic figures on the screen. Oligodendroglial components were missed in two of the glioma cases, representing the difficulty of appreciating the chromatin pattern on the screen. Slide review and pathology report were discrepant in four cases due to differences in the interpretation of tumor grade.

Conclusions: The concordance between WSI and slide based diagnoses in this study is slightly lower than previous reports. This may be secondary to exclusion of relatively simple diagnostic entities from the study. Main limitations of WSI include identification of mitotic figures, and chromatin structure in selected cases, which may undergrade some of the neoplasms commonly seen in neuropathology practice.

2087 Sampling Error Is a Major Contributor to the Low Sensitivity of Anal Cytology as a Screening Tool for High Grade Dysplasia

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Background: Anal squamous cell carcinoma is a relatively rare carcinoma with squamous dysplasia as a well-defined premalignant phase. Due to the infrequency of anal carcinoma, screening is only recommended in high-risk groups. Prior reports have suggested that the performance characteristics of anal cytology are not as good as those typically seen in cervical cytology. Unpublished data from our institution has shown that the sensitivity of anal cytology for all squamous dysplasia was 88%, but when evaluating only for high grade dysplasia the sensitivity was significantly lower at 25%. In this study, we focused on anal cytology samples that were NILM or LSIL on pap smear, but had a high grade or higher diagnosis at biopsy. The goal of this study is to determine whether the low sensitivity of anal cytology as a screening tool for high-grade dysplasia is due to errors in sampling versus errors in the interpretation/diagnosis of the cytology specimens.

Design: We reviewed 55 anal cytology samples that were diagnosed as benign or low grade; however, were diagnosed as high grade upon follow-up biopsies.

Results: Out of the 55 cases, 2 were eliminated as unsatisfactory. 8 cases were diagnosed as NILM, 4 cases were diagnosed as ASCUS, 25 cases were diagnosed as LSIL, 15 cases were diagnosed as LSIL-ASCH, and 1 case was diagnosed as HSIL. Thus, 37 out of 53 cases (70%) belonged to negative, ASCUS or low-grade category; indicating that the low sensitivity of anal cytology for high-grade dysplasia is largely due to errors in sampling.

Conclusions: Our previous data suggests that the overall sensitivity of anal pap smears for squamous dysplasia is comparable to the reported sensitivity of cervical pap smears. However, anal cytology tends to perform poorly in the identification of high-grade lesions. The distinction is important, since current guidelines for management of anal dysplasia recommend only spot treatment of high-grade lesions. With this study, we have established that this poor sensitivity is in large part a function of sampling error. Perhaps evaluation and review of sample collection techniques could improve the sensitivity and in turn, the diagnostic ability of cytology for high grade anal dysplasia.

(Abbreviations: NILM=negative for intraepithelial lesion or malignancy; LSIL=low-grade squamous intraepithelial lesion; HSIL=high-grade squamous intraepithelial lesion; ASCUS=atypical squamous cells of undetermined significance; ASC-H=atypical squamous cells, cannot exclude HSIL).

2088 Tissue Identity Testing by STR Genotyping: An Eight-Year Experience at a Single Tertiary Medical Center

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Background: Specimen identification and accessioning errors occur in approximately 6% of cases in surgical pathology leading to mislabeling of a significant number of cases (mismatch). Specimen handling and processing errors lead to extraneous tissue (floaters) in up to 3% of all slides that can cause significant diagnostic uncertainty. Tissue identity testing by short tandem repeat polymorphism (STR) analysis is a powerful molecular technique used in resolving these issues. Here we report our experience of STR genotyping analysis at a tertiary medical center over an eight-year period.

Design: 102 consecutive cases from July 2005 to July 2013 were included in the retrospective evaluation: 59 mismatch and 43 floater cases. Formalin fixed paraffin embedded tissue, cytology and frozen section slides were used as starting material. The original slides were reviewed and target tissues of concern were confirmed by H&E stain, followed by microscopic dissection and DNA extraction. Genotyping was performed by AmpFlSTR Identifiler PCR Amplification system followed by capillary electrophoresis and data analysis using GeneMapper 3.7 software (Applied Biosystems, Inc.). For mismatch analysis, genotype was matched to the patient's normal tissue.

Results: Pathologists or physicians requested all cases except one. Tissue genotyping confirmed or ruled out sample mismatch and floaters in 95% (97/102) of the cases. Among 59 mismatch cases (44 biopsies, 15 resection specimens), genotyping was conclusive in 57 cases: 38 match and 19 mismatch. Of 43 floater cases (33 biopsies, 10 resections), 40 had a conclusive genotyping results with floater confirmation in 27 cases. Failure in 5 cases was due to insufficient starting material or poor DNA preservation. In one case mismatch between tumor and patient's tissues was attributable to tumor microsatellite instability.

Category	Specimen Type	Conclusive	Inconclusive
Mismatch(n=59)	Biopsy*(n=44)	Match= 28	1
Physician request= 58		Mismatch= 15	
Patient request=1	Resection(n=15)	Match=10	1
		Mismatch=4	
Floater(n=43)	Biopsy(n=33**)	Floater=21	3
		Not floater=8	
	Resection(n=10)	Floater=6	0
		Not floater=4	

TABLE 1. Genotyping results by category (*includes biopsies, curettage and cytology specimens, **one mismatch due to MSI)

Conclusions: Applicable to a wide variety of tissue types, STR polymorphism analysis offers a powerful quality control measure in surgical pathology that can resolve 95% of diagnostic problems due to mislabeling errors and extraneous tissue. Microsatellite instability in cancerous tissue samples should be ruled out when a tissue mismatch is observed by STR genotyping.

2089 Impact of the Introduction of an Automated Embedding System on Performance in a University Hospital Histopathology Department

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Background: Automation in the laboratory can improve efficiency and quality of service. In light of increased pressure on resources an automated Embedding system (Autotec ®) was acquired, with the aim of reducing the time required for embedding and improving Turn Around Times (TAT). The Autotec allows tissue pieces to be held in place within a specially designed cassette during processing, such that embedding is not reqiured and the case can be moved directly from processing onto section cutting.

Design: A verification process was undertaken, in which benign resection specimens (e.g. prolapsed uteri) were initially processed on the automated embedder with subsequent review by 2 pathologists. This was gradually expanded until all resections, LLETZ specimens, curettings and skin excisions >2cm were processed using the automated embedder such that it was used for at least 50% of blocks daily. TATs and block numbers for a 3 month period were compared before introduction of the Autotec® and after achieving 50% throughput. Pathologists were surveyed regarding the perceived impact on workflow in the cut-up room and on the quality of sections.

Results: TAT improved by 4 days for cancer resections and endoscopic biopsies and by 5 days for all other surgical specimens. Time rostered for embedding by medical scientific staff was reduced from 5.5 hours to 3 hours/day. Block numbers did not increase significantly (<1%). Of nine pathologists who were surveyed, 4 believed that the autotec had increased the time spent in cut up, ranging from a slight increase up to 25%. Two reported that the time spent had not increased and the remaining 3 did not specify. The increased time spent was in all cases, a direct result of the use of the specific cassettes required for the Autotec, which require the use of a plastic "insert" prior to placing the tissue in the cassette and are difficult to close. This impacted less when assisted by a medical scientist expreienced in the use of these cassettes. Five of 9 pathologists reported a minor negative impact on the quality of sections. The most common issue was edge artefact, which occurs as a result of overfilling of cassettes.

Conclusions: Introduction of the Autotec lead to dramatic improvements in TAT, without increased staff. Its use increased the amount of time spent by pathologists in the cut-up room, possibly negatively impacting the training of resident staff. Minor problems with the quality of sections were common but in no case did this prevent accurate histological evaluation.

2090 Impact of the Introduction of a Voice Activated Dictation System (Dragon®) on Quality in a University Hospital Histopathology Department

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Background: Increased workload with a concomitant decrease in clerical staff negatively impacted Turn-Around Times (TAT) in 2011. A Voice Activated Dictation System (Dragon®), which generates a typed report directly from the pathologist's voice, using a microphone head-set, was introduced. This eliminates the need for a typist. All pathologists were offered approxiamtely 1 hour of individual training in its use.

Design: One year later pathologists were surveyed to assess frequency of use and satisfaction with the system. Of 11 pathologists, 8 maintained the use of Dragon®. Six reported using it for 70% or more of surgical pathology reporting, 4 for 90% and 1 for 50%. One did not specify. Seven users were entirely satisfied with the system. One reported an increased clerical burden on them but wished to continue to use the system. The 3 who did not undertake or continue the use of Dragon® gave reasons including external noise interference, discomfort with the technology and insufficient time to train. TAT from the point at which slides were issued to the pathologist until the point of first authorisation, for endoscopic biopsies and cytology specimens, were compared from Jan-Jun 2011 with Jan-Jun 2013, using the day on which >80% of cases were authorised as a cut off point. All amended and corrected reports for these periods were also reviewed.

Results: TAT had improved by 2 days for endoscopic biopsies and by 1 day for cytology by the second quarter of 2013, despite an increase in specimen numbers. The number of amended reports increased from 2 to 6 from Jan-Jun 2011 to Jan-Jun 2013. In no case did the amendment relate to use of voice activated dictation. The total number of corrected reports issued from Jan-Jun 2011 was 13. The total number issued from Jan-Jun 2013 was 54. 23/54 corrected reports were issued by the 3 non-Dragon® users and 31/54 by the 8 Dragon®. users. The average number of corrected reports was higher in the non-Dragon® users (n=7.6) when compared to Dragon® users (n=3.8). 2/31 corrected reports were directly related to the use of the voice activated system.

Conclusions: The introduction of the voice activated dictation system improved TAT. Two corrected reports were identified which related directly to its use. The overall rate of corrected reports in the department was <0.5% and was lower for those using Dragon® than for the non- Dragon® users. The overall increase in corrected and amended reports most likely represents a change in pathologists' behaviour, resulting in improved documentation and reporting of error.

2091 Effect of Cold Ischemic Time on Ki67 Expression in MCF-7 Cell Line Derived Xenografts

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Background: Ki67 is a nuclear marker expressed in all phases of the cell cycle except G0. Immunohistochemical staining of Ki67 is widely used to assess the degree of proliferation in breast cancer. Recently, the 2011 St. Gallen Consensus Conference recommended Ki67 staining for all early breast cancers. Variation in scoring, intra-tumor heterogeneity and an overall lack of standardization have hindered the uptake of this recommendation into routine practice. Some retrospective studies have suggested that Ki67 is tolerant of pre-analytical variables. Further characterization of Ki67 expression will contribute to standardizing methodology, facilitating its valid clinical use. The effect of various cold ischemic times (CITs) on Ki67 staining within and outside previously studied ranges on a homogenous breast cancer cell line in a controlled setting was assessed.

Design: Well-characterized breast cancer cell lines were analyzed by immunohistochemistry to select a cell line with variable estrogen and progesterone receptor expression. The selected MCF-7 cell line was used to generate cell derived xenografts (CDXs) in an immunodeficient mouse. Immediately after surgical resection, six CDXs were divided into smaller portions. Three portions were subjected to a different CIT. Tissue was processed using 10% neutral buffered formalin. All Ki67 staining was scored by five observers.

Results: The MCF-7 breast cancer cell line best illustrated variable staining and therefore, was used to generate CDXs in an immunodeficient mouse. As CIT increased, Ki67 staining decreased as shown in Table 1.

Table 1. Ki67 Staining for Various Cold Ischemic Times

CIT (hours)	Ki67 Staining - Specimen 1 (%)	Ki 67 Staining - Specimen 2 (%)	Ki67 Staining - Specimen 3 (%)
0	>95	>95	>95
0.5	>95	>95	
2	75-95	>95	>95
4	>95	>95	>95
8	50-75	50-75	75-95
12	50-75	50-75	
48	50-75	30-50	30-50
72	20-30	20-30	

Conclusions: Prolonged cold ischemic time negatively affects Ki67 expression in a cell line derived xenograft model, with some of the decay in staining beginning after two hours and the decay fully established at eight hours. Both of these time periods are beyond the one hour cold ischemic time established by the CAP/ASCO guidelines for breast biomarkers.

2092 Inadequate Reporting of *Helicobacter pylori* Infection Status in Gastric Adenocarcinoma Resections

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Background: *Helicobacter Pylori* (HP) is a carcinogen that causes gastric adenocarcinoma. Several studies have shown that eradication of HP decreases the risk of future malignancy. Patients with partial gastrectomy for early gastric cancer have

significantly reduced cancer recurrence with HP eradication. Reporting HP gastritis is an optional part of the most recent College of American Pathologists gastric cancer checklist. This study investigates the frequency of reporting HP infection status in the setting of gastric adenocarcinoma resection.
Design: All pathology reports and available clinical notes from primary gastric adenocarcinoma partial resections from two tertiary care institutions were reviewed (1984 to 2013). Total gastrectomy cases were excluded. Carcinomas arising in Barrett's type mucosa and/or the gastro-esophageal junction were excluded. Reporting of HP either by routine or special stains was noted and was correlated with clinical follow-up.
Results: Reports from 318 partial gastric resections were identified. The median age was 65.5 y (range 29 to 92 y); female = 121 patients. HP was reported in 10.4% (n=33) of partial resections. When HP status was reported, 51.5% (n=17) of cases were positive for HP gastritis. Clinical follow-up was available for 16 of the 17 HP positive cases; only 5 of the 16 cases (31.2%) were treated for HP gastritis. In a subset of cases that were stage 1B or lower (n=83), only 9.6% (n=8) had a reported HP status. Of the 8 patients with HP status, 6 were positive for HP gastritis; the majority of patients with HP gastritis in early gastric cancer were treated for infection.
Conclusions: In this two institution study, there was a low rate of reporting HP status in the setting of gastric adenocarcinoma resection. Overall, only 10.4% had a report of HP status; a subset of patients with early disease (Stage 1B or lower) had similar low reporting (9.6%). A secondary clinical concern identified by this study is the low level of treatment for HP-positive cases. Overall, only 31.2% were treated for HP infection. Based on this data, we believe current practice may be inadequate and that HP status in partial gastrectomy should be more frequently examined in partial gastric adenocarcinoma resections.

2093 Image Cytometric HER2 Quantitation: Cut-Off Values for the Equivocal Range

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Background: *HER 2* status for selection of breast cancer patients for targeted therapy, is assessed by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), for protein overexpression and gene amplification respectively. Less-than-optimal concordance rates between IHC and FISH are attributed to lack of standardized protocols, and variations of interpretation. ASCO/CAP guidelines, to standadize HER2 testing, require 95% overall concordance between IHC usually assessed visually, and FISH. Our goal was to improve concordance between results of in-house image cytometric IHC quantitation and FISH, as low IHC 3+ positive (> 2.2 – 2.6) were frequently discordant with FISH non-amplified results.
Design: 160 breast carcinomas were stained by Herceptest (DAKO), analyzed using the Automated Cellular Imaging System III (DAKO), and scored as the mean score from 6 areas in a 40 x objective. Scores of below 1.8, within 1.8 – 2.2, and above 2.2 were interpretated as 0 to 1+ (not expressed), 2+ (equivocal), and 3+ (overexpressed) respectively. FISH for HER2 amplification was performed using the PathVysion HER2 DNA probe kit (Abbott Molecular). The HER2/centromere 17 ratios were calculated and interpreted according to ASCO/CAP guidelines.
Results: 66 of the 160 cases (41%) showed IHC scores above 2.2 (3+), 56 had FISH amplification (84.4% concordance). Of 12 with low IHC positive 3+ scores (> 2.2 – 2.6), only 5 (41.7%) were FISH amplified. 84 (53%) showed IHC scores between 1.8 – 2.2 (2+); 13 (15%) were FISH amplified. 10 (6%) had IHC scores below 1.8 (0, 1+); one showed FISH amplification. Based on a modified IHC equivocal score of 1.8 – 2.6, 54 of 160 (34%) showed IHC scores more than 2.6; 51 were FISH amplified (94.4% concordance). 96 (60%) show IHC scores between 1.8 – 2.6; 20 (13%) with FISH amplification.
Conclusions: The ACIS digital interpretation scoring algorithm of negative, equivocal, and positive HER2 is variable among different studies. We show improved concordance (84.4 to 94.4%) between image cytometric scores and FISH amplification when the 2+ equivocal range is changed from 1.8 – 2.2 to 1.8 – 2.6. Low positive (3+) specimens (> 2.2 – 2.6) are now included in the equivocal group. This change in the upper equivocal range cutoff improves concordance and eliminates need to FISH test IHC 3+ cases.

HER 2 concordance between IHC scores of ACIS and FISH

IHC score by ACIS	> 2.2 (positive) (n=66)	> 2.6 (positive) (n=54)	1.8 - 2.2 (equivocal) (n=84)	1.8 - 2.6 (equivocal) (n=96)
FISH amplified	56	51	13	20
FISH not amplified	10	3	71	76
Concordance	84.8%	94.4%	78.6%	84.5%

2094 Automated vs. Manual Paraffin Embedding: A Cost-Benefit Analysis

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Background: Increasing financial pressures have forced laboratories to implement methods that lower costs and improve efficiency. Employing automation as a means to achieve these goals does not yet have wide use in surgical pathology. The robotic autoembeder (RA) (Tissue-Tek® AutoTEC® Automated Embedding System with Tissue-Tek Paraform Sectionable Cassette System) eliminates manual embedding, one of the most labor intensive steps in tissue processing. However, the specialized cassettes (TEC) required for these instruments are more costly and hold smaller tissue volumes than conventional cassettes (CC) and also present some technical challenges at microtomy. In this study we report our experience with RA in a community hospital from a cost-benefit perspective.
Design: A 1 year analysis of surgical specimens included both manually embedded (ME) and autoembeded (AE) cases. The cost of ME was calculated using the price of CC plus the histotechnologists salary/hr of embedding time. The assumption was made that a technologist could manually embed 100 large specimen cassettes/hr and 50 biopsy cassettes/hr. The cost of AE was the cost of TEC. A subset of ME radical prostatectomies (RP) was compared to a similar group of AE cases to see if the smaller cassette size of the TEC resulted in a greater number of cassettes/specimen weight and if the microtomy challenges of the TEC resulted in the generation of more recuts.
Results: 69,664 accessions generated 249,034 blocks. AE accounted for 50.42% (60,964/120,902) of biopsies and 94.69% (121,332/128,132) of large specimen blocks. Volume and cost statistics are presented in Table 1.

Table 1: Cost evaluation of automated vs. manual embedding process

Parameters	AE		ME	
	Bxs	Large	Bxs	Large
Total cases	29,672	10,184	29,238	570
Total cassettes	60,964	121,332	59,938	6,800
Cost of cassettes: TEC @ $0.41/cassette; CC @ $0.14/cassette	74,596		9,400	
Time to ME hrs			1,199	68
Cost to ME @ $25/hr			29,975	1,700
Embedding cost/cassette ($)	0.41		0.62	

AE=automated embedding; ME=manual embedding; Bxs=biopsies; CC=conventional cassette; TEC=specialized cassette

There were no significant differences in number of blocks or recuts for AE and ME RP specimens (Table 2).

Table 2: Automated vs. manual embedding for radical prostatectomies (n=65 each)

Parameters	AE	ME
Weight (range)	52.8 g (24-233)	53.1 g (21-200)
Average % volume submitted	98.9	95.1
Recuts	53	51
Total cassettes	2982	2732

Conclusions: RA reduced costs by 34% and reduced histotechnologist work time by 6.54 hrs/day. AE technology is cost effective provided there is sufficient volume to offset capital investment.

2095 Determining Specimen Adequacy for Breast Carcinoma Predictive Markers on Cytology Specimens

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Background: Studies have shown a variable degree of correlation of predictive markers between cytology and surgical specimens however, few have addressed the underlying factors leading to this variability.
Design: A 2 year retrospective study identified fine needle aspiration with cell blocks (CB) and high gauge needle core biopsies (NB) of metastatic mammary carcinoma (N=46) with IHC analysis for ER, PR and/or Her2. The patient's receptor status on primary tumor or most recent recurrence was confirmed by prior surgical pathology review or documentation in the electronic medical record. Quality metrics (QM) measured on each specimen were defined as: estimated cellularity, preservation (1-4 point scale), obscuring elements (1-4 point scale) and percentage of blood present.
Results: 25 CB and 21 NB from body sites of liver (6), pleural fluid (10), peritoneal fluid (3), bone (2), lung (5) soft tissue (3), Lymph node (6) and breast (2) were reviewed. Discrepancies identified were 4 (16%) for CB and 6 (28.6%) for NB. 40% of discrepancies occurred in bone specimens.

Quality Metrics on Cell Block Material

	Cellularity	Preservation	Obscuring	Blood
Discrepant	81 cells	2.75	1.75	46.3%
Nondiscrepant	170 cells	2.5	0.8	39.2%

Mean values

Quality Metrics on High Gauge Core Needle Biopsies

	Cellularity	Preservation	Obscuring	Blood
Discrepant	433 cells	2.67	0.6	18.3%
Nondiscrepant	364 cells	2.63	0.73	20%

Mean values

Conclusions: Cellularity and obscuring elements were the quality metric identified with the highest effect on reliability for CB and NB, respectively. Preservation and the amount of blood in the specimen did not have an obvious effect on the discrepancy rate. Evaluation of QM in cytology specimens can be an effective tool to determine which specimens are adequate for predictive marker testing.

2096 Sentinel Lymph Nodes in Cervical Cancer: A First Cut Analysis

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Background: Ultrastaging (US) of cervical carcinoma (CxCa) sentinel lymph nodes (SLNs) increases the detection of nodal metastases (mts), yet improved sensitivity requires more technical and professional resources. The current healthcare climate of declining reimbursements and technical workforce shortages requires examination of the effect caused by protocols such as US. This is a cost benefit analysis of the US protocol for CxCa SLNs that we used at our institution for a period of 10 years.
Design: The institutional database was searched for all cases of CxCa, squamous (Sq) and nonsquamous (NSqCa) with SLNs over a ten-year period (1998-2007). All SLNs were serially sectioned at 2mm intervals perpendicular to the long axis of the SLN and entirely submitted to obtain one H&E stained slide per block (standard processing). SLNs that were negative by standard processing were subjected to the following US protocol: 5 H&E levels with unstained slides accompanying each level were cut at 40 um intervals. If the US levels were negative, a keratin cocktail immunostain (consisting of AE1/AE3, CAM5.2, Cytokeratin MNF116, and Keratin 8&18) was performed on the first unstained slide. We recorded the number of SLNs per case, blocks per SLN part, the presence of mts and at which stage of the US protocol they were identified. The technical cost (materials and labor) and professional cost (pathologist labor), as well

as the reimbursement rates for SLN (CPT code 88307) from CMS, were examined.
Results: The range of blocks per SLN part was 1-5. The technical and professional costs per block were $90.82 and $17.36, respectively. Reimbursement per SLN part with keratin was $428.31 and without keratin was $238.56. For results, see Table 1.

Pathology Findings and Cost of Cases of CxCa SLNs

	SqCa	NSqCa	Total
Cases Identified	49	12	61
Avg SLNs/ Case	4.18	5.08	4.3
Pos SLNs by SP	22 (10.7%)	2 (3.2%)	24 (9.0%)
Median Blocks/ SLN Part	1	1	1
Avg Blocks/ SLN part	1.14	1.25	1.17
Sensitivity of SP	88%	40%	80%
Pos SLNs by US	3 (1.6%) *	3 (5.1%)	6 (2.5%)
Avg US Cost/ Case	$474.66	$682.03	$515.45
Avg Cost/ SLNs diagnosed by US	$7,752.70	$2,728.11	$5,240.40

A financial deficit was created once the number of blocks per SLN part exceeded 3.
Conclusions: US of CxCa SLNs has improved sensitivity but at a greatly increased technical, labor, and professional workflow cost. Optimizing our protocols to balance sensitivity and revenue expectations of US protocols is critical. This first cut analysis demonstrates that US, with the benefit of increased sensitivity, does not generate a financial deficit until the number of blocks per SLN part exceeds 3.

2097 Assessment of Inter-Observer Agreement in Identification of Histologic Features of Antibody Mediated Rejection in Cardiac Allograft Biopsies

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Background: The 2010 International Society for Heart and Lung Transplantation (ISHLT) consensus meeting proposed a grading and reporting scheme for antibody mediated rejection (AMR) in cardiac allograft biopsies that includes assessment of both histologic and immunopathologic findings. These recommendations emphasize that AMR can be a diagnosis rendered by the pathologist (pAMR1(h+) or pAMR2) even in the absence of clinical features of cardiac dysfunction or donor specific antibodies (subclinical AMR). Histologic interpretation is subject to inter-observer variability. The aim of this study was to assess inter-observer agreement (IOA) of histologic features associated with AMR in cases with immunofluorescent (IF) studies.
Design: H&E slides of all cardiac allograft biopsies with positive IF studies performed at our institution from January 2010- July 2013 as well as an equal number of randomly selected cases with negative IF were independently reviewed by three pathologists who routinely assess these biopsies. All cases were reviewed blindly for histologic features associated with AMR (capillary endothelial changes (CEC), macrophages or neutrophils in capillaries, interstitial edema, hemorrhage and fibrin thrombi). The results were tabulated with calculation of the kappa coefficient for IOA and correlation with IF results.
Results: There is substantial IOA with an average calculated kappa coefficient of 0.64 (Figure 1). Two pathologists identified two of the same histologic features (CEC and interstitial edema) in 42% of the cases with positive IF; and identified at least one feature in 74%. The histologic feature with the highest correlation to IF status was CEC.

Figure 1: Interobserver Agreement of Histologic Features of AMR

Histologic features	Interobserver Agreement (kappa)	IF result	Number of cases with histologic findings (n=38)		
			Pathologist 1	Pathologist 2	Pathologist 3
Capillary endothelial changes	0.75	+	13	14	15
		–	4	4	0
Macrophages/ neutrophils in capillaries	0.77	+	7	5	9
		–	1	1	0
Interstitial edema	0.77	+	11	7	13
		-	2	2	3
Hemorrhage and fibrin in vessels	0.98	+	1	0	1
		-	0	0	0
	0.82 overall average				

Conclusions: There is substantial IOA in the assessment of histologic features of AMR. At least two of three pathologists identified at least two of the same histologic features in 68% of cases with IF positive results, suggesting that histologic assessment of AMR has a high rate of reproducibility. The highest level of correlation of histologic changes with IF results was the identification of capillary endothelial change. While the reliability of the histologic features in predicting AMR needs to be further validated, our findings support the utility of the histologic features in identifying AMR.

2098 Root Cause Analysis of the Frequency and Etiology of Discrepancies between Intraoperative Frozen Section Results as Reported in Pathology Reports Versus Surgical Operative Notes

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Background: Anecdotes of surgical operative notes (SON) reporting intraoperative frozen section (IFS) results that differ from those recorded in the corresponding pathology report (PR) are common, frustrating, and create a potential for suboptimal patient care. Our insitution reports IFS results to the OR via phone with a verbal readback. We examined the relationship between IFS diagnoses recorded in the PR and those recorded in the SON, and sought to explain the basis of identified discrepancies.
Design: A retrospective 5 month review of SON and PR of surgeries with at least one IFS identified a) documentation of sending IFS in the SON, b) documentation of IFS results in the SON, and c) discrepancies between the PR and SON. Discrepancies were jointly reviewed by all authors and categorized according to etiology of discrepancy (see Table 2), the use of words of uncertainty (e.g. favor, possibly), and the presence of a statement deferring to permanent sections.
Results: A total of 351 surgeries generating 592 IFS specimens were identified. The SON mentioned sending at least one specimen for IFS for 304 surgeries (87%), and the SON reported IFS results for at least one specimen for 275 surgeries (78%). When IFS results were present in the SON (275 surgeries, 441 specimens), discrepancies were identified for 47 sugeries (17%) and 53 specimens (12%).

Etiologies of Discrepancy with # and % of Discrepant SON

Suppression of Uncertainty	20 (43%)
Additional Information of Unknown Origin	15 (32%)
Information Transfer Deficit	10 (21%)
Lumping	2 (4%)
Suggestive of Purposeful Misrepresentation	2 (4%)
Addition of Uncertainty	1 (2%)
Loss of Information	1 (2%)

Words of uncertainty were present in the PR IFS diagnosis in 40% of discrepant cases. A statement deferring to permanent sections was present in the PR IFS diagnosis in 19% of discrepant cases.
Conclusions: This study shows that IFS results as recorded in the PR and SON are frequently discrepant, and that both performance-related and psychological barriers inhibit accurate transmission of IFS diagnoses between pathologist and surgeon. The etiology behind such communication errors is broad, and includes cognitive biases such as heuristics bias and belief bias, process impediments to communication such the requirement for non-face to face communication, and the lack of adequate documentation of communicated ideas. This study identifies multiple potential targets for better communication resulting in an enhanced intraoperative decision making process.

2099 HER2 Testing in Gastric Cancer by Immunohistochemistry: Assessment of Interlaboratory Variation

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Background: Immunohistochemical (IHC) testing for HER2 overexpression is becoming the standard of care for guiding adjuvant treatment of gastric carcinoma with trastuzumab. The goal of this study was to assess inter-laboratory variation in IHC staining and interpretation across multiple medical laboratories.
Design: A tissue microarray consisting of 45 cores from 23 gastric cancer resections was distributed to 37 laboratories for HER2 staining and interpretation. IHC results were compared against expert scores at an academic institution, and correlated with *in situ* hybridization results from the originating resection specimen. Interlaboratory agreement was calculated using Cohen's kappa statistic.
Results: The survey demonstrated that there are several variations in IHC methods utilized by participating laboratories, including the primary antibodies in use. There was excellent agreement amongst laboratories in HER2 positive (IHC 3+) cases (Kappa 0.80 ± 0.01), and very good agreement amongst laboratories in HER2 negative (IHC 0 or 1+) cases (Kappa 0.58 ± 0.01). Less agreement was observed amongst laboratories when scoring equivocal (IHC 2+) cases (Kappa 0.22 ± 0.01). Sensitivity and specificity of HER2 IHC were 99% and 100% respectively when measured against expert review and consensus score as a reference standard.
Conclusions: There is substantial inter-laboratory agreement in the interpretation of HER2 IHC despite variability in staining protocols. Although HER2 IHC is a highly sensitive and specific test, the primary antibody selection may significantly impact IHC results. Furthermore, gastric tumors require a unique scoring system and expertise in interpretation. Intratumoral heterogeneity has a significant impact on HER2 scoring by IHC. Ongoing quality assurance exercises between laboratories will be important to ensure optimization of HER2 testing.

2100 Analysis of Image Cytometric Quantitated Immunohistochemical Controls with Levey-Jennings Quality Control Charts

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Background: Our immunohistochemically (IHC) stained slides for estrogen receptor (ER), progesterone receptor (PR), HER2/neu (HER2), and Ki-67 controls (Dako Envision + Dual Link System-HRP) are image cytometric (ICM) quantitated by a software program (Dako ACIS III). Results are reported as a unitless stain intensity. There is no formal process for reviewing the quantitative control data over time. We investigate the application of Levey-Jennings quality control (QC) charts and the Westgard rules to IHC control quantitation results.
Design: Daily ER, PR, HER2, and Ki-67 IHC control results were analyzed over two months. Slides were inspected to determine when the control specimen was changed. Stain intensity by control specimen was analyzed using the student's t test (SAS JMP Pro). Levey-Jennings QC charts were constructed using the means and standard deviations for each control over the entire period (MS Excel 2013).

Results: IHC controls for 7/2-9/6/2013 (n=42) only demonstrated significant differences in stain intensity by control specimen in a minority of cases (Figure 1). Levey-Jennings QC charts for each control demonstrated no violations of the most commonly applied Westgard rules (Figure 2), although some results were non-consecutively greater than 2 standard deviations (SD) from the mean.

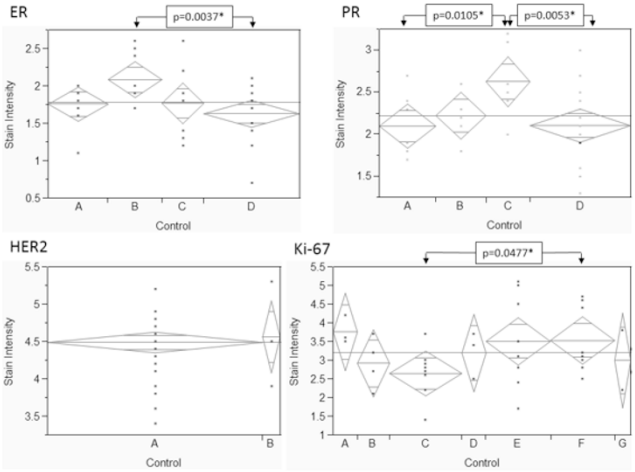

Figure 1. Stain intensity by IHC control specimen. Only p-values for statistically significant differences are shown.

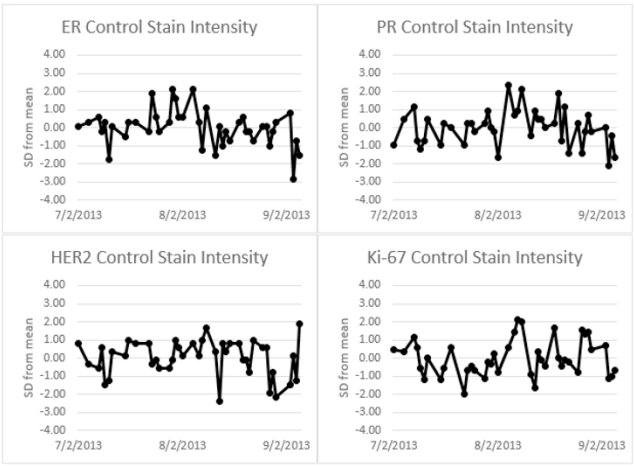

Figure 2. Levey-Jennings QC charts for IHC controls. Stain intensity is normalized (SD from mean).

Conclusions: Changes in the control specimen only significantly impact the quantitation of an IHC control in a minority of cases. Levey-Jennings QC charts with subsequent application of the Westgard rules may be a useful approach to introduce objective QC principles to the analysis of ICM quantitated IHC controls. This type of QC analysis is routinely performed in clinical pathology laboratories.

2101 Clinical Significance of CD4/CD8 T-Cell Ratios by Flow Cytometry in Bronchoalveolar Lavage Specimens in Sarcoidosis

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Background: Bronchoalveolar lavage (BAL) specimens are frequently submitted for flow cytometric analysis to determine the CD4/CD8 ratio. The clinical utility of this test in the diagnosis of sarcoidosis and other pulmonary diseases is unclear.

Design: The CD4/CD8 ratios of BAL specimens collected from 2006–2012 were correlated with biopsy-proven sarcoidosis versus non-sarcoidosis diagnoses. Results were compared using a t-test, where $p<0.05$ was considered significant.

Results: Of the 145 BAL specimens analyzed, the median age was 55 years old with a male to female ratio of 0.8:1. 17 patients had biopsy proven sarcoidosis. The median CD4/CD8 ratio for sarcoidosis was 3.15 (range 0.3 to 20) compared to 1.7 (range 0.2 to 19) in non-sarcoidosis patients (p=0.11). In the non-sarcoidosis patients the median ratios were variable depending on the disease state (ARDS 1.15 (n=8), autoimmune disease 2.9 (n=4), drug 2.6 (n=6), infection/pneumonia 1.95 (n=25), interstitial lung disease 1.5 (n=18).

Conclusions: Although the median CD4/CD8 ratio for sarcoidosis is higher than other pulmonary disease states, it is not statistically significant. Therefore, there appears to be little utility for BAL CD4/CD8 ratios as an aide in the diagnosis of sarcoidosis.

2102 Inter-Institutional Pathology Consultation: The Importance of Breast Pathology Subspecialization in a Setting of Tertiary Cancer Center

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Background: Inter-institutional pathology consultation (IPC) has shown to be significant in patient care. We intended to evaluate the value of reviewing breast core biopsies by breast pathologists in a setting of tertiary cancer center.

Design: The pathology reports of a total of 502 consecutive consult cases of breast core biopsies were reviewed. In real time, the actual cases were read by two subspecialized breast pathologists. In case of discordance with the outside report, the case was reviewed by both pathologists and consensus was reached. Discordant cases were divided into minor and major based on the impact on patient care. Major discordance is defined when the patient undergoes different therapy modality based on the changed diagnosis. The outside pathologists were divided into two groups, senior and junior. Discordance is subdivided into malignant, premalignant, biomarkers, fibroepithelial lesions, and others. Pathologic and clinical follow-up for all major discordant diagnoses was performed.

Results: There were 104 (20.7%) discordant cases, of which 40 (8%) with major discordance and 64 (13%) with minor discordance. Clinical follow-up showed a change in management plan for 15 of 40 (37.5%) patients with major discordance. Major discordance rate for the subcategorized diagnostic groups was: malignant 5 (12.5%), premalignant 16 (40%), biomarkers 10 (25%), fibroepithelial lesions 6 (15%), and others 3 (7.5%). There was no statistically significant difference between junior or senior pathologists groups.

Conclusions: Our findings support the value of IPC review in decreasing the likelihood of diagnostic errors that may lead to significant impact on patient care. It is necessary that outside pathology material in the referral settings been reviewed by a specialized breast pathologist.

2103 A Population-Level Approach to Improving Turnaround Times for Colorectal Cancer Resection Reports

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Background: The turnaround time (TAT) of cancer resection reports is an important dimension of quality. In colorectal cancer (CRC), the time interval from surgery to treatment with adjuvant therapy is an independent prognostic factor for survival. The pathology TAT is an integral component of this interval. The goal of this project is to improve TAT for CRC resection reports in Ontario (population 13.3 million) utilizing data derived from the Ontario Synoptic Cancer Pathology Reporting System coupled with strong physician engagement.

Design: For the purpose of this study TAT is defined as an interval between the date of surgery and the date on which the electronic pathology report is available centrally at Cancer Care Ontario (CCO). Over 95% of completed reports are received electronically by CCO's Ontario Cancer Registry within 24 hours of verification by the pathologist. TATs were measured at provincial, regional (LHIN) and institutional levels. The provincial clinical program and regional leads set a target of 90% of cases to be reported within 14 calendar days. Data is reported quarterly to sites and annually to the public. New indicators for all disease sites are in development.

Results: Over 10,000 CRC reports were received between July 2012 and June 2013. First quarter results for 2013/14 show 8 of 14 regions and 32 of 51 institutions are within target. Two regions and 8 institutions are within 10% of the target and 4 regions and 11 institutions are more than 10% below target.

Post-Surgical TAT: Percent of regions and institutions approaching or meeting performance target, Ontario, Apr-Jun 2013.

	Region (N=14)		Institution (N=51)	
	Number	Percent	Number	Percent
At or above target of 90%	8	57.1%	32	62.7%
Within 10% of target	2	14.3%	8	15.7%
More than 10% below target	4	28.6%	11	21.6%

Conclusions: The establishment of a population-level TAT metric for CRC reports along with a clinically relevant target focuses attention on quality improvement in this important area of cancer pathology. Monitoring of pathology related metrics by a central agency focusing on quality improvement will draw attention to resource gaps and potential process improvement areas and ultimately lead to improved quality of care and outcomes for the cancer patient.

2104 Frozen Sections for the Evaluation of Sentinel Lymph Nodes after Neoadjuvant Therapy for Breast Cancer Are Specific but Not Sensitive

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Background: Most data concerning the relative high accuracy rate of frozen section (FS) for the detection of metastatic carcinoma in sentinel lymph nodes (SLNs) for breast cancer (BC) patients comes from the adjuvant setting. Though the frequency of FS SLN node analysis in the adjuvant setting is decreasing in light of results from the ACOSOGZ11 trial, the use may be increasing in the neoadjuvant setting where any positive result, even when the metastatic tumor burden is small, would likely trigger an immediate axillary lymph node dissection at the time of surgery. However, after neoadjuvant therapy, SLNs often have histopathologic changes that may affect the sensitivity and specificity of the FS procedure.

Design: Surgical pathology records from 2011-2013 were searched for all BC patients treated with neoadjuvant therapy who underwent FS analysis of a SLN. The results from the FS were compared with the final pathologic interpretation of the SLN to determine the sensitivity and specificity of the procedure. Sensitivity and specificity were calculated for FS compared to the results on final pathology.
Results: Overall, FSs were performed on 132 separate SLNs from 47 different patients who had received neoadjuvant treatment for BC. Of the 132 SLNs, 17 were positive at the time of FS, and 115 SLNs were negative. On permanent sections, all of the 17 positive SLNs at the time of FS were confirmed as positive. However, an additional 21 SLNs were actually positive on permanent section that were called negative on FS. This results in an estimated sensitivity of 44.7% (95% CI: 28.6-61.7%) and specificity of 100% (95% CI: 96.1-100%). When examining these findings on a per patient basis, 11/47 patients (23%) were found to have at least one positive SLN at the time of FS, but an additional 12 patients (26%) in this cohort actually did have metastatic disease in one or more SLNs on permanent section, suggesting a per-patient sensitivity of 47.8% (95% CI: 26.9-69.4%) and specificity of 100% (95% CI: 89.9-100%).
Conclusions: Our findings reveal that FS analysis of SLNs following neoadjuvant therapy is specific but not sensitive on individual SLNs and on a per-patient basis. While all of the positive SLNs at the time of FS were confirmed on permanent section, approximately half of the patients with true SLN involvement had false negative results at the time of surgery. Pathologists should communicate with surgeons that the use of the FS procedure to diagnose SLNs after therapy is limited.

2105 Frozen Section Turn-Around-Time in a Large Tertiary Care Teaching Hospital: Recommendations for Current Practice

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Background: With the increasing sub-specialization of surgical pathology and case complexity, a re-evaluation of frozen section turnaround time (FS-TAT) recommendations is warranted. The most recent large-scale analysis of FS-TAT (1997 CAP Q-Probe) found that 90% of FS cases worldwide were reported in ≤20 minutes, forming the basis of the CAP AP checklist recommendation (ANP.11820) that all AP practices complete 90% of single part FS in ≤20 min. However, this checklist item was removed in 2012, leaving institutions to determine their own FS-TAT standards. The goal of this study is to evaluate FS-TAT 15 years after the CAP Q-Probe in a large (>500 bed) urban academic teaching hospital and to provide up-to-date recommendations for FS-TAT in this type of institution.
Design: FS worksheets from Apr-Sept 2012 and Apr-Sept 2013 were collected, data input into MS Excel, and analyzed according to the following parameters: 1)month-year performed, 2)time specimen received, 3)time results reported, 4)TAT, and 5)level of complexity, i.e. noncomplex (NC) or complex (C). Complex specimens were defined as having one or more of the following characteristics: multiple cases, same time; multiple specimens, same case, same time; multiple blocks, same specimen; complex gross evaluation (inking, sectioning, photography); complex histologic evaluation (deeper levels needed); or intradepartmental consultation required.
Results: Overall, 1870 FS worksheets (cases) were collected. 55% were complex (n=1029); the complexity rate did not vary per month or year. 44% of all FS and 68% of NC-FS were reported in ≤20 minutes. 88% of NC-FS cases were reported in ≤30 minutes (in keeping with the 1997 CAP Q-Probe results, in which the slowest large hospitals reported 90% within 30 minutes). 89% of C-FS cases were reported in ≤40 minutes.
Conclusions: The CAP 1997 Q-Probe reported TATs of ≤20 min in 90% of FS cases performed worldwide, but also demonstrated increased TAT in large hospitals and those with teaching programs (the 20 min goal was achieved in only 50% of large hospitals). The current study stratified cases by complexity, and showed that □90% NC-FS cases were reported within 30 min and □90% of C-FS cases were reported in 40 min. The 20 min FS-TAT goal is likely unattainable in large teaching hospitals with a high percentage of complex cases, and in light of the removal of the CAP FS-TAT checklist recommendation (ANP.11820), we recommend that each institution set individualized, data-driven FS-TAT goals based on their size, teaching status, and case complexity.

2106 The Utility of a Tissue Microarray in the Validation of a Non-FDA Approved Nonpredictive Antibody (Napsin-A)

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Background: The College of American Pathology (CAP) requires that the performance characteristics of every assay used in the immunohistochemistry laboratory be appropriately validated prior to clinical use using a written protocol. However, a recent CAP survey identified that for non-FDA approved, nonpredictive markers, 32% of labs do not have a written procedure and 14% did not validate their most recently utilized antibody. This highlights the fact that an easily used and cost effective process is needed in order to validate newly introduced antibodies and for repeat validation for ongoing changing analytic variables. We propose the incorporation of a tissue microarray (TMA) with an adequate number and appropriate spectrum of specimens as an effective, efficient and cost-effective tool for antibody validation.
Design: We identified 100 cases of primary and secondary lung malignancies between 2007 and 2013 for the construction of a TMA for the validation of the Cell Marque Napsin-A immunohistochemical rabbit polyclonal antibody. The primary cases included 45 adenocarcinomas, 27 squamous cell carcinomas, 1 small cell carcinoma, 1 large cell neuroendocrine carcinoma, 1 large cell carcinoma, 1 carcinoid, and 2 adenosquamous carcinomas. All grades and stages were represented. The secondary tumors included 2 thyroid carcinomas, 4 colorectal carcinomas, 1 endometrioid carcinoma, 1 esophageal adenocarcinoma, 1 renal cell carcinoma, 1 melanoma, 1 synovial sarcoma, 1 osteosarcoma, 1 paraganglioma, 1 carcinoma of unknown primary, 1 germ cell tumor, and 7 leiomyosarcomas.
Results: The cost of creating and staining a single TMA with 100 1.5 mm samples for Napsin is $11.56 for in-house creation and $911.56 for an outside vendor. The cost to individually analyze 100 cases at $11.56/test is $1156. This results in a cost savings of either $1144.44 or 244.44/validation respectively.
Conclusions: The recent CAP survey has identified a critical need for easily performed and cost effective processes for the validation of newly introduced nonpredictive immunohistochemical markers. The use of TMAs is an effective, efficient, and cost effective tool for the initial validation of newly introduced immunohistochemical markers and would result in even greater cost savings for ongoing revalidation due to changes in analytic variables. Savings are greatly enhanced if onsite staff is capable of creating TMAs. This should also foster greater compliance with CAP standards.

2107 Low Cost Workflow Improvement Reduces GI Block Use 25% by Altering Classical Histotechnology Teaching

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Background: To reduce the histology workload and improve efficiency, we evaluated the workflow as applied to gastrointestinal (GI) biopsies which represent a significant percent of total histology. Previously, GI biopsies were limited to 4/cassette in order to facilitate the tedious orientation of multiple tissue fragments in a block on a diagonal. This practice follows published histotechnology teaching that biopsies be oriented along a diagonal so that the microtome blade would strike each tissue fragment independently.
Design: We implemented three specific changes: 1. Up to 10 GI biopsies could be placed in each cassette. 2. Histotechnologists would no longer orient GI biopsies. 3. Embedding would be in a straight line rather than diagonal.

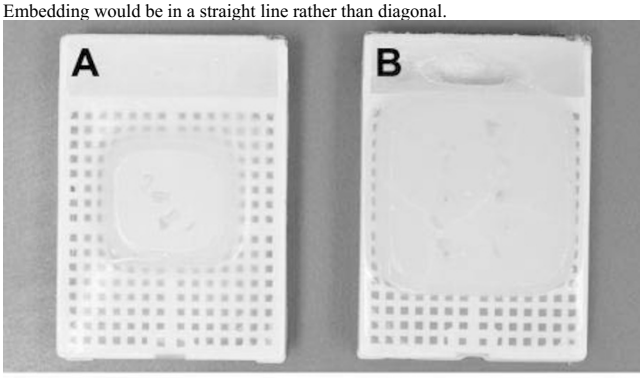

Figure 1: A, prior embedding method. B, new embedding method.

assess the quality both before and after we subjected 100 cases (50 before/50 after) to a quality control assessment which were evaluated by an independent, trained national histotechnology evaluator. We surveyed both histotechnologists and pathologists for their overall satisfaction in making these changes.
Results: Slide quality assessment revealed no significant differences of the fixation, microtomy, or staining between the two groups. GI biopsy cases before and after implementation (475 total) were assessed. The average number of cassettes used prior to implementation was 1.43 cassettes per part, whereas after implementation the average number of cassettes per part was 1.16 (p-value = <0.001) – a 25% reduction. Amounting to a savings of one cassette per 1.19 cases accessioned. Pathologists reported improved work satisfaction and increased speed both grossing and performing microscopy. Histotechnologists reported increased job satisfaction citing increased ease and speed of embedding and microtomy.
Conclusions: The change in the biopsy workflow increased the speed of embedding and microtomy independent of the number of blocks and objectively decrease the number of blocks processed, nearly 5 blocks saved for every 6 cases accessioned. This simple, low-cost, low-effort process change yielded immediate and significant time savings for both grossing and histology staff and increased job satisfaction amongst histotechnologists and pathologists. This change in biopsy handling challenges conventional histotechnology teaching.

2108 Heat Induced Epitope Retrieval for Immunohistochemistry at High Altitude: A Quality Assurance Study

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Background: Heat induced epitope retrieval (HIER) of formalin-fixed paraffin embedded tissues is now standard practice in immunohistochemistry (IHC). We aimed to determine the optimal HIER temperature at high altitude, the hypothesis being that, because of lower barometric pressure, staining patterns will be improved with lower HIER temperatures at high altitude.
Design: This study was performed at IHCtech in Aurora, Colorado, 5,471 ft above sea level (designated "high altitude") and Emory University Hospital in Atlanta, Georgia, 1,050 ft above sea level (designated "sea-level"). Appropriate tissues were stained with 4 commonly used antibodies: melan A, CD3, MIB-1 and CK20, using the Bond-Max™ automated IHC stainer (Leica Microsystems). 3 HIER temperatures (90°C, 95°C, and

100°C) were tested on 10 serial sections for each antibody in each lab, generating a total of 240 slides. Staining quality was scored by the authors in a blinded fashion, noting the area of the slide stained (0-negative; 1-<25%; 2-25-75%; 3->75%) and the staining intensity (0-negative, 1-weak, 2-moderate, 3-strong). Statistical analysis was done using the Mann-Whitney U test.

Results: Result scores are summarized in table 1.

Table 1

Antibody	HIER Temp	Average Area Score (Sea Level)	Average Intensity Score (Sea Level)	Average Area Score (High Altitude)	Average Intensity Score (High Altitude)
Melan A	90°C	3.0	2.0* (p-value = <0.0001)	3.0	3.0
Melan A	95°C	3.0	3.0	3.0	3.0
Melan A	100°C	3.0	3.0	3.0	3.0
CD3	90°C	3.0	2.5* (p-value = 0.0137)	3.0* (p-value = 0.0006)	2.8* (p-value = 0.0353)
CD3	95°C	3.0	3.0	3.0* (p-value = 0.0006)	3.0* (p-value = 0.0057)
CD3	100°C	3.0	3.0	1.4	1.8
Ki-67	90°C	2.8	2.2* (p-value = 0.0004)	2.5* (p-value = 0.0464)	2.8
Ki-67	95°C	3.0	3.0	3.0* (p-value = 0.007)	3.0* (p-value = 0.0143)
Ki-67	100°C	3.0	3.0	1.7	2.3
CK20	90°C	1.0* (p-value = <0.0001)	1.2* (p-value = <0.0001)	1.2	1.7
CK20	95°C	2.0* (p-value = <0.0001)	2.0* (p-value = <0.0001)	2.2	2.1
CK20	100°C	3.0	3.0	1.3	1.3

* denotes statistically significant difference ($p < 0.05$) compared to 100°C epitope retrieval at the same altitude

HIER temperature significantly impacted staining quality. At high altitude, lower HIER temperatures (90°C or 95°C) resulted in more complete and even staining of tissue sections and stronger staining intensity. This effect was seen with all antibodies tested except melan A.

Conclusions: We show that optimal HIER temperatures for labs at high altitude vary from those for labs at sea level. Alternate epitope retrieval recommendations for laboratories at high altitude are warranted.

2109 Prolonged Ethanol Exposure Adversely Affects Fluorescence In Situ Hybridization

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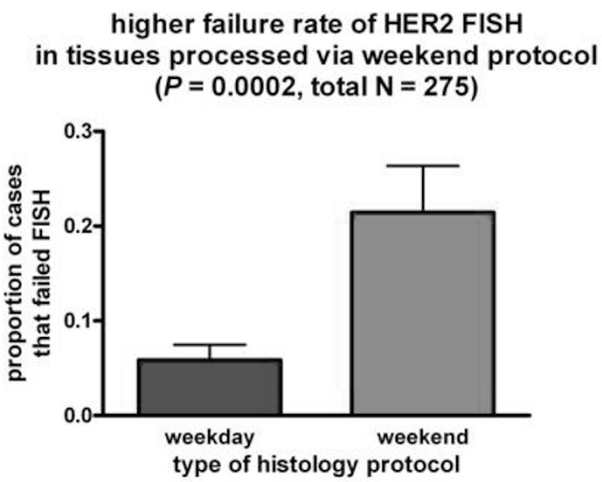
Background: Amplification of the *HER2* gene and/or overexpression of the protein is found in up to 25% of invasive breast cancers. *HER2* amplification status is important in the prognosis and treatment of patients with breast cancer; determination of *HER2* amplification identifies a subset of patients that may benefit from therapy with the monoclonal antibody trastuzumab. Guidelines published jointly by the College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) recommend that tissue be fixed in formalin for at least 6, but not longer than 48 hours for optimal *HER2* testing. For laboratories without weekend histology services these guidelines are difficult to satisfy. Our histology lab attempted to do this by increasing fixation time in 75% ethanol on cases processed on weekends. We subsequently identified an increase in the number of cases that failed FISH testing; this lead us to retrospectively review failure rates in tissues processed using the weekend versus weekday protocols.

Design: All surgical pathology specimens tested for *HER2* amplification using FISH at our institution since the adoption of the weekend processing protocol were reviewed (n=275). Breast specimens received Monday through Thursday were processed according to standard protocol while Friday specimens were processed using the weekend protocol. For weekday cases, the total amount of time spent in varying concentrations of ethanol was 5 hours. Cases subject to the weekend protocol spent 28 hours in varying concentrations of ethanol. *HER2* FISH analysis was performed using standard FDA-approved procedures.

Results: Of the 275 cases reviewed, 70 underwent weekend processing. Twenty-one percent of those weekend cases had unreadable FISH results due to high autofluorescence, compared to only 6% failed cases using the regular weekday protocol ($P = 0.0002$).



Conclusions: Prolonged ethanol fixation is detrimental to scoring *HER2* copy number using FISH and should therefore be avoided.

2110 Incorporation of Tissue Microarrays in HER2 Immunohistochemical Assay Validation

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Background: The College of American Pathology (CAP) requires that the performance characteristics of every assay used in the immunohistochemistry laboratory must be appropriately validated using a written protocol prior to clinical use. 2010 and 2013 CAP surveys identified that for HER2 validation most laboratories felt the minimum number of 25 cases was appropriate; however, only 56% of laboratories used this minimum number and only 66% of predictive markers were revalidated after introduction. This highlights the fact that an easily used and cost effective process is needed in order to validate and revalidate HER2. We question whether the incorporation of tissue microarrays (TMAs) with an adequate number and appropriate types of specimen could serve as the gold standard for antibody validation, in the validation and revalidation of HER2.

Design: Seventy five specimens with mammary carcinoma were identified and added to two TMAs by using TMA Master (Caliper 3D HISTECH) with 34 and 41 samples (1.5mm each) respectively, and stained using HER2 antibody (Ventana, 4B5 clone). All grades and stages of mammary carcinoma and ranges of ER/PR status were used. All cases were compared using fluorescence in-situ hybridization performed by two other academic centers.

Results: The cost per individual immunohistochemical test for HER2 in our laboratory is $52.09. The cost to validate HER2 using 75 individual cases is $3906.75. The cost to create and stain the two TMAs ranges from $104.18 using onsite house staff to $904.18 using outside vendor for TMA creation, resulting in savings ranging of $3802.57 and $3002.57 respectively.

Conclusions: The recent CAP surveys have identified a critical need for easily performed and cost effective processes for the validation and revalidation of HER2. The incorporation of tissue microarrays into routine daily practice is an effective and cost effective tool for the initial validation of HER2 and will result in even greater cost savings for ongoing validation due to changing analytic variables. Savings are greatly enhanced if onsite staff is capable of creating TMAs.

2111 Impact of Multidisciplinary Team Meetings on Intradepartmental Consultation Rate; an initial Finding of the Irish National Quality Assurance Programme in Histopathology

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Background: Peer review (PR) is an essential component of quality assurance (QA) in surgical pathology. One of the commonest forms of PR is intradepartmental consultation (IDC) where an opinion is sought from a second pathologist prior to release of the report. Departmental policies are increasingly mandating secondary review prior to authorization of cases with several national QA guidelines proposing target review rates of 10%. Review of cases listed for discussion at multidisciplinary team meetings (MDTM) is another form of PR but differs from IDC in that the cases reviewed are not selected by pathologists. MDTM activity has increased substantially over the past decade and is a significant additional workload for pathologists. In Ireland a national QA programme in histopathology was launched in 2009 in which all 33 laboratories submit anonymised specimen-linked QA coded data to a central database that includes all cases subjected to IDC and MDTM review.

Design: The national central database is managed by a novel information technology system, the National Quality Assurance Intelligence System-Histopathology (NQAIS-H), that was designed to process and display the QA data that is regularly extracted from the individual laboratory information systems. A review of cases submitted to NQAIS-H over a 12 month period (June 2012 to May 2013) was performed and those cases that were coded as being subjected to IDC and MDTM review were

identified. Histology cases were divided into 4 categories - small biopsy, GI endoscopic biopsy, cancer resections, and non-biopsy non-cancer resection specimens. Turnaround times (TAT) of cases were also captured.
Results: A total of 415,838 histology and cytology cases were identified over the 12 month period with IDC recorded in 18,078 cases (mean = 4.3%) and MDTM review in 40,498 cases (mean = 9.8%). Both PR activities were coded in 5,077 cases (1.2%) coded which gives a combined mean PR rate of 12.9%. TAT of cancer resection cases was longer when subjected to IDC (mean TAT difference of 3 days) compared to no IDC but MDTM review of cancer resection cases did not effect TAT.
Conclusions: The IDC rate in Irish histopathology departments is significantly lower than published recommendations but when combined with MDTM review a PR rate of 12.9% is achieved. Despite the effect on TAT IDC is an essential form of proactive PR that should be integrated with MDTM review in a QA programme.

2112 Bilateral Bone Marrow Biopsies for Diagnosis and Follow-Up of Plasma Cell Myeloma

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Background: The utility of bilateral bone marrow biopsy (BBMB) to stage patients (pts) with lymphoma is well known, but it has not been systematically studied in plasma cell myeloma (PCM). The practice of BBMB for PCM has been in use for 4 decades at our institution. In this study, we retrospectively reviewed unilateral (UBMB) and BMBBs from patients with PCM to determine the value of the contralateral biopsy.
Design: We reviewed BMB data corresponding to PCM pts at the University of Minnesota between 01/2009 and 06/2011 – including UBMB and BBMB. Each case had immunohistochemical (IHC) stains (CD138, kappa and lambda) and flow cytometry (FC). Biopsies less than 1 cm were excluded from this study. Any BMB showing CD138 positive plasma cells with monoclonal light chain restriction by IHC with or without positive FC result was considered positive. The percent of discordant cases on BMBB was assessed using these criteria: positive BMB with negative concurrent contralateral BMB study by morphology, IHC and FC and/or significant % discordance of BM involvement bilaterally (>2 fold difference).
Results: A total of 174 BM biopsies (125 BBMB and 49 UBMB) were performed on 68 pts (51% males). 93% (163/174) of the total biopsies were follow-up for PCM of which 72.4% (118/163) were BMBB. 63.6% of initial diagnostic biopsies were also bilateral (n=7/11). Of the total follow-up biopsies, 35.5% (58/163) were involved by PCM (average BM involvement: 14.6% (1-95%). 26.3% (43/163) showed bilateral involvement among the BBMB, and 9.2% (15/163) showed unilateral involvement among the UBMB. None of the bilateral BM cases showed positive biopsy by morphology/IHC contralateral to negative aspirate, morphology, and IHC/FC results. Only 11.6% (5/43) of BBMB showed > 2 fold difference in involvement on either side by PCM on follow up. No significant difference in lateral % involvement was seen on BMBB among the diagnostic biopsies.
Conclusions: PCM is considered a patchy disease, but our results show that obtaining a contralateral biopsy is unlikely to affect the overall rate of positivity for newly diagnosed or residual PCM. In a minority of residual PCM cases, the % of PCM may have lateral variability; however, we propose that protein studies may be more representative of disease response/progression than biopsies in these cases. Based on the data reviewed, we suggest that the contralateral biopsy, at diagnosis and follow-up, is of limited value. In addition to sparing patients an extra procedure, we estimate that only performing UBMB on our PCM pts could save payors over $100,000 annually.

2113 On-Site Cytofixation Improves Cell Block Cellularity and Overall Quality in Endobronchial Ultrasound Guided Fine Needle Aspiration of Mediastinal Lymph Nodes

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Background: Endobronchial ultrasound guided fine needle aspiration (EBUS-FNA) is rapidly becoming the modality of choice for assessment of mediastinal lymphadenopathy. When combined with rapid on site evaluation for adequacy, it has sensitivity exceeding 90% in some reports and specificity of 100%. Cell blocks have become standard and are virtually indispensable for conducting ancillary studies. Therefore, optimizing their cellular yield and quality is paramount. However, there is limited literature on the impact of on-site specimen fixation on the quality and yield of cell blocks for EBUS-FNA specimens. We compared the quality and yield of cell blocks prepared from EBUS-FNA specimens submitted in saline and those submitted in CytoRich® Red (CRR) Preservative Fluid, an alcohol based fixative solution.
Design: We evaluated 44 hematoxylin and eosin (H&E) stained slides from cell blocks prepared from mediastinal lymph node EBUS-FNA specimens. 22 specimens were submitted in saline while the other 22 were immediately fixed on-site in CRR; specimen collection and cell block preparation were otherwise identical and followed standard laboratory protocols. We scanned the cell block slides with the Aperio ScanScope® CS slide scanner at 20x magnification and then performed quantitative assessment of the number of cells per unit area via image analysis using a modified version of the nuclear analysis algorithm in Image Scope®. Additionally, two pathologists (K.E.T and P.N.S.) independently reviewed each slide and assigned scores on a scale of 0 to 3 based on adequate cellularity for diagnosis and extent of preservation of cytomorphologic detail, with discrepancies resolved by consensus. Statistical significance was assessed with the two-tailed t-test using MedCalc® statistical software.
Results: There was a marked difference in both cellularity and overall quality between non-fixed and fixed cell blocks. The mean cellularity per unit area was 344 and 1051 cells in the non-fixed and fixed samples respectively, a mean increase of 707 cells per unit area (p = 0.030). Similarly, the non-fixed samples had a mean quality score of 1.8 while the fixed samples had a mean score of 2.4 (p = 0.039).
Conclusions: On-site cytofixation significantly improves the cellularity and morphologic preservation of cell blocks and should become standard practice for EBUS-FNA specimens submitted for cell block preparation.

2114 Use of Interobserver Variability as a Means of Assessing Intraobserver Variability during Validation of Whole Slide Imaging

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Background: Whole slide imaging (WSI) converts glass slides into digital images that can be viewed remotely. We have an ongoing validation project for WSI scanners in our institution using the draft guidelines released by the College of American Pathologists in 2011.
Design: Key slides from 200 consecutive cases were selected from the following categories that we intend to WSI scan: consults, frozen sections, malignancies, and special or immunostains. The cases, in 2 sets of 100, were used to validate 4 machines each, over 2 phases. The slides were scanned at 20x magnification using standard image quality on Ventana iScan Coreo Au scanners. Pathologists viewed half of the cases as glass slides first and half as WSI first, then reversed after a delay of at least 3 weeks. Brief history was provided. 35 pathologists participated. Each case was viewed by 2 pathologists in each phase.
Results: Intra- and interobserver agreement were compared over two validation phases (4 scanners per phase).

Comparison of intra- and interobserver agreement

	Phase A	Phase B
Both intra- and interobserver agreement	121	98
Intraobserver agreement without interobserver agreement	15	24
Neither intra- nor interobserver agreement	6	4
Uninterpretable on WSI	0	6
Other (see below)	58	68

The remaining 58 cases in Phase A and 68 cases in Phase B had one pathologist with intraobserver disagreement while the other pathologist had intraobserver agreement. In Phase A, the discrepant diagnosis relative to the other pathologist was made on the glass slides in 25 cases and WSI in 33 cases. In Phase B, the discrepant diagnosis was made on the glass slides in 29 cases and WSI in 39 cases.
Conclusions: By cross-analyzing intra- and interobserver agreement, the effect of intraobserver variability due to case difficulty and WSI limitations can be somewhat disentangled. Relative to the other pathologist, many of the discrepant diagnoses were on glass slides (43%), indicating that most but perhaps not all of the intraobserver variability is due to case difficulty rather than WSI limitations, making identification of those limitations problematic. Use of very straightforward cases for validation would solve this problem, but WSI would not be well-tested. Establishing a benchmark of intraobserver variability on the glass slides prior to scanning would double the effort needed for validation and would lack precision with such small numbers.

2115 Reproducibility of Basal-Like Breast Carcinoma Biomarker Staining: An International Immunohistochemical Survey of Diagnostic Pathology Laboratories

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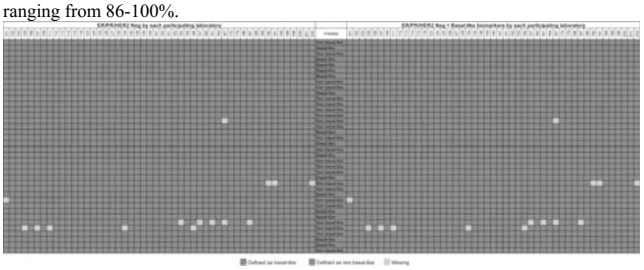
Background: Gene expression-based molecular approaches are not yet widely available in hospital laboratories, therefore surrogate immunohistochemical definitions for basal-like breast cancer have been proposed, typically relying on lack of expression of ER, PR and HER2. Specificity of this triple negative definition can be improved with inclusion of positively expressed basal-like biomarkers, such as CK5 and EGFR. Assessment of the performance of the triple negative definition and basal-like immunopanels (e.g. triple negative and CK5 positive) for identification of basal-like breast cancer cases in clinical laboratories is presented.
Design: A breast cancer tissue microarray (n=40, of which 38 have PAM50 subtype assignment) enriched for the basal-like subtype was stained in 50 clinical laboratories for ER, PR, HER2, CK5 (or CK5/6) and an optional extra basal-like biomarker of choice (e.g. EGFR, p63, CK14) according to established protocols at each facility. Staining was evaluated by designated personnel at each facility using established methods.
Results: Against a PAM50 gene expression profile gold standard, clinical laboratories using a triple negative definition were able to successfully identify cases of basal-like breast cancer with a sensitivity ranging from 27-100% and a specificity ranging from 76-100%. With inclusion of a specific basal-like biomarker(s) of choice in addition to ER/PR/HER2, those same laboratories were able to successfully identify cases of basal-like breast cancer with a sensitivity ranging from 13-93.3% and a specificity ranging from 86-100%.



Conclusions: This study by the Canadian Immunohistochemistry Quality Control provides the first evaluation of basal-like breast cancer diagnostics in clinical laboratories. Surrogate immunopanels are specific but can lack sensitivity, and show considerable interlaboratory variability. Efforts to standardize staining and

interpretation of basal-like immunopanels are necessary before clinical application. Regular participation of laboratories in external quality assurance and proficiency testing programs will facilitate implementation.

Techniques

2116 In-Situ Redox Profiling of Diffuse Large B-Cell Lymphoma

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Background: Methods for the histochemical detection of tissue thiols have been described in the literature. These methods are incompatible with routine histology given their complex chemistries and need for specialized microscopy. Further, there is scant data evaluating the in-situ distribution and concentration of free protein thiols (i.e. -SH or sulfhydrals) and reducible protein thiols (i.e. disulfides, mixed disulfides, nitrosothiols, and sulfenic acids) in human tissues.

Design: Contemporary literature has recently likened post-translational protein thiol oxidation to *O*-phosphorylation, with regard to its role in cell signal transduction, enzyme function, protein structure, and disease pathogenesis. We therefore sought to explore the distribution and concentration of free and reducible protein thiols, by developing a staining technique using modern histochemical reagents that allow for the visualization of tissue protein thiols in-situ, under bright field microscopy.

Results: The thiol staining technique was developed and validated using routinely available control tissue. The thiol staining technique was then used to assess benign tonsillectomy specimens, benign surgical lymph node specimens, and excisional lymph node biopsy specimens diagnosed as involved by diffuse large B-cell lymphoma (DLBCL). In both the tonsils and lymph nodes, we observed a robust presence of free protein thiols. Germinal center cells contained appreciably more free protein thiols than the surrounding small lymphocytes. When we examined the localization and concentration of reducible protein thiols, staining was exclusive to germinal center macrophages and sinus histiocytes. When we applied this technique to excisional lymph node biopsies diagnosed as involved by DLBCL, we observed a robust concentration of free protein thiols within the malignant cells that was comparable to the staining observed in benign germinal center cells. When we examined the localization and concentration of reducible protein thiols, we observed that in contrast to the benign tissue, the malignant cells demonstrated pronounced and diffuse staining.

Conclusions: We describe a novel and reproducible histochemical method for detecting protein thiols in-situ. In addition, we present the first in-situ data examining the distribution and concentration of free and reversibly oxidized protein thiols in high grade lymphoma specimens. Taken together, our findings demonstrate tissue thiols as a novel biomarker for further defining tumor histopathology.

2117 From HER2 FISH to DISH

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Background: In our institution HER2 status is assessed on all newly diagnosed breast cancers. HER2 FISH is performed on cases equivocal by immunohistochemistry (4B5 and SP3 clones). Dual in situ hybridization (DISH), an alternative to FISH, has advantages that include the use of light microscopy and slides can be archived and retrieved indefinitely.

Design: We performed HER2 DISH on a Benchmark Ultra (Ventana Medical Systems Inc., Roche) on 91 samples with equivocal IHC results that had previously been assessed by HER2 FISH (Pathvysion, Abbot). DISH was not interpretable in 1 case (technical failure), 7 cases were excluded; 6 due to clustered heterogeneity, and one had insufficient tissue. DISH was assessed by a breast pathology fellow and breast pathologists for inter-observer reproducibility. DISH was compared to FISH results obtained by two cytogenetics technologists.

Results: The fellow and pathologists had complete agreement between the FISH and DISH (positive, negative or equivocal) in 77% cases with no major discrepancies (positive vs negative). Minor discrepancies (negative vs. equivocal; or equivocal vs. positive) occurred in 22.9% of cases. HER2/CEP 17 ratio was≥1.6 in 0% of cases with major discrepancy and in 77% with minor discrepancy. The inter-observer agreement between DISH and FISH was not significantly different. DISH cases with ratios <1.6 or >2.2 showed 98% concordance of results with FISH. We have incorporated our results into practice as follows: DISH ratios <1.5 or >2.5 can be reported after counting a minimum of 20 nuclei. Ratios between 1.5 and 2.5 require counting of an additional 20 nuclei. If the ratio remains 1.6 to 2.2, an additional score by another observer is obtained. FISH is currently being performed within this range as well to gather more data within this range. We plan to re-evaluate this testing algorithm over time to optimize efficiency of HER2 testing.

Conclusions: HER2 DISH appears comparable to FISH. Major discrepancies between DISH and FISH results can be minimized by ensuring a second scorer within the ratio of 1.6 to 2.2.

2118 Comparison of C-Met Immunoreactivity in Surgically Treated Gastroesophageal Adenocarcinoma Using Two Commercially Available Antibodies

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Background: C-met, hepatocyte growth factor (HGF) receptor, overexpression is known to be associated with tumor progression in lung, colon, and stomach carcinomas. Many ongoing trials are investigating c-met targeted therapy in these patients. In gastroesophageal adenocarcinoma (GEA), c-met is thought to confer resistance to anti-Her2 therapy. Currently, no guidelines exist as to interpretation of c-met immunohistochemistry (IHC) in GEA. The aim of our study was to compare c-met immunoreactivity in GEA using 2 commercially available antibodies and examine its relation to clinical outcomes.

Design: 48 cases of treatment-naïve GEAs were assessed for c-met expression using Ventana SP44 monoclonal antibody (prediluted/antigen retrieval with CC1 solution) and Leica NCL-CMET monoclonal antibody (1:80 dilution/antigen retrieval with Citra solution). Appropriate controls were evaluated. C-met membranous and/or cytoplasmic reactivity was graded as follows: 0=absent, 1=weak, 2=moderate, 3=strong and percent positive tumor cells was also recorded. Cases with <2+ or 30% staining were considered negative, and cases with≥2+ and 30% staining were considered positive. In addition, clinicopathologic features of GEAs were recorded.

Results: Median age was 66 yrs (range 37-83), with a 4:1 M:F ratio. Barrett's esophagus was seen in 92% of cases. Most GEAs (37/48) were low stage (T1-T2), and 10 had nodal involvement (N1/N2). The overall death rate was 33%. Positive staining was observed in 27 patients using either antibody; however, the concordance of positive cases using both antibodies was 16/27. The only variable that correlated significantly with survival was tumor stage (p = 0.007).

Conclusions: Our study demonstrates that caution must be exercised when assessing c-met status in GEA using different commercial antibodies. Pathologists and oncologists alike should be aware of the potential differences in staining that may impact treatment plans.

Analysis of c-met positive GEA using 2 different antibodies

	Ventana + cases (n=27)	Leica + cases (n=27)
Stage T1/T2	21	20
Stage T3/T4	6	7
Lymph node metastasis (N1/N2)	6	6
Recurrence	3	4
Deceased	10	11

22 Discrepant cases of c-met expression in GEA

	Ventana + but Leica – (n=11)	Leica + but Ventana – (n=11)
Stage T1/T2	9	8
Stage T3/T4	2	3
Lymph node metastasis (N1/N2)	2	2
Recurrence	2	3
Deceased	3	4

2119 Automated 3D Reconstruction of Digital Pathology for Registration to 3D Imaging

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Background: Whole slide scanning opened new avenues for large-scale image analyses of pathological sections in clinical practice and research. Coupled with special stains, immunohistochemistry and manual or electronic annotations, digital images host a great deal of data. While images and data can be quickly acquired in 2D, an increasingly common goal is to acquire a 3D rendering of the pathology and a high resolution gold standard for imaging studies of the same.

Design: A goal of the Canadian Atherosclerosis Imaging Network is to develop improvements in imaging of carotid atheromas through comparative studies with excised plaque specimens. Sectioned atheromas are to be manually annotated for regions of interest (lipid core, calcification, etc.) and analyzed by electronic algorithms (eg. colorimetry of antigen expression). These semi-serial images are then to be reassembled in 3D for registration to in-vivo and ex-vivo ultrasound, CT, PET-CT and MRI.

Results: Recruited patients underwent carotid endarterectomy. Fixed plaque specimens were semi-serially sectioned, stained, scanned then annotated manually and with electronic algorithms. Resulting 2D images were then rendered in 3D in an automated fashion using ex-vivo micro-CT as a spatial reference for the individual slices.

Conclusions: Reconstruction of the pathology in 3D is greatly facilitated by this methodology in comparison to manual slice-by-slice methods. Since slice transforms are guided by a pre-existing model (micro-CT) the reconstruction is not only faster, but has greater objectivity and fidelity. With embedded annotations, the resulting 3D map contains abundant qualitative and quantitative data for such studies.