disease. Of the 141 patients, 37 (26%) presented with disease progression at the first detection (median level 18%); the other 104 (74%) presented with MRD (median level 0.3%), 86 of them (83%) were detected within the first 90 days post-SCT (early post-SCT MRD). A total 42 of 136 (31%) patients with pre-SCT MRD had subsequent progression as compared to 48 of 310 (15%) patients without pre-SCT MRD ($p<0.01$); 39 of 86 (45%) patients with early post-SCT MRD progressed at a median interval 41 days from the first MRD detection, whereas 20 of 309 (6.5%) patients progressed without early post-SCT MRD ($p<0.01$). Among the 309 patients, 4 of 59 (6.8%) with pre-SCT MRD had subsequently progressed versus 16 of 251 (6.4%) patients without pre-SCT MRD ($p=0.77$).

Conclusions: Peri-transplant MRD detected by MFC is a significant risk factor for post-SCT disease progression that is likely associated with clinical relapse. The significance of pre-SCT MRD diminishes when early post-SCT MRD is taken into consideration, suggesting that post-SCT MRD may be a stronger predictor of relapse than pre-SCT MRD.

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1561 EBER-Positive Lymphoplasmacytic Colitis in Patients Status Post Solid Organ Transplantation

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Background: Patients who have undergone solid organ transplantation are at increased risk for developing diarrhea, generally in the setting of immunosuppression, secondary to infection, medication effect, or even varying malignancies—particularly Epstein-Barr virus (EBV) mediated post-transplant lymphoproliferative disorders (PTLD). Secondary to the offending agent, lymphoplasmacytic or inflammatory bowel disease-like colitis is not uncommon in such patients. Since the gut is one of the largest lymphoid organs and thus prone to the EBV infection, it is crucial to identify whether EBV is involved in the gut inflammatory process and to distinguish EBV-mediated colitis from early/polymorphic PTLD in patients status post solid organ transplantation.

Design: All endoscopic colonic biopsies in patients status post solid organ transplantation with any diagnosis of colitis or suggestive of colitis were included for analysis. A total of 50 cases met criteria, and all cases were reviewed on hematoxylin and eosin. In-situ hybridization (ISH) with Epstein-Barr virus-encoded small RNAs (EBERs) was performed for all cases with proper positive and negative controls.

Results: In this large cohort, the types of transplants included kidney (23/50), heart (8/50), liver (13/50), and multiple organs (6/50). All patients were on immunosuppression at the time of biopsy, and the time from transplantation to biopsy ranged from less than one year to 27 years. All 50 cases displayed minimal active inflammation with a heavy lymphoplasmacytic infiltrate in the lamina propria or submucosa. A total of 20% (10/50) of cases showed either rare or diffusely positive staining on EBER ISH in the lymphoplasmacytic infiltrate; these cases included 2 kidney transplants, 1 heart transplant, 5 liver transplants, and 2 multiple organ transplants. Three cases were further confirmed as polymorphic PTLD. All cases were ruled out for cytomegalovirus (CMV) colitis by immunohistochemistry and PCR, as well as mycophenolate-induced graft-versus-host disease-like colitis.

Conclusions: Our study indicates that EBER-positive lymphoplasmacytic colitis is not uncommon in patients status post solid organ transplantation. Furthermore, an EBER stain should be suggested in patients presenting with repetitive diarrhea displaying prominent lymphoplasmacytic infiltrate in a colon biopsy. Whether scattered EBER-positivity in these cases of colitis represents an early onset PTLD or EBV-mediated colitis is unclear and further studies are needed to elucidate these patterns.

1562 Minimally Invasive Autopsy as a Tool for the Diagnosis of Cause of Death Related To Infections Diseases in Developing Countries

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Background: The practice of complete diagnostic autopsies (CDA) remains the gold standard in the determination of cause of death (CoD). Performing CDAs in developing countries is challenging due to limited facilities and human resources, and poor acceptability. Minimally invasive autopsy (MIA), a combination of high tech imaging studies with selected puncture biopsies, has been recently proposed as an alternative to CDA. We aimed to develop and test a simplified MIA procedure involving organ-directed sampling with microbiology and pathology analyses implementable by trained technicians in low-resource income settings.

Design: A standardized scheme for the MIA has been developed and tested in a series of 30 autopsies performed at Maputo Central Hospital. The procedure involves an ultrasound scan examination, the collection of 20 mL of peripheral blood and cerebrospinal fluid (CSF) and puncture of key organs (liver, lungs, heart, spleen, kidneys, bone marrow and brain in all cases plus uterus in childbearing age women) using 14G biopsy needles.

Results: The sampling success ranged from 67% for the kidney to 100% for blood, CSF, lung, liver and brain. The amount of tissue obtained in the procedure varied between less than 10 mm2 for the lung, spleen and kidney, and over 35 mm2 for the liver and brain. A severe disease considered as a possible CoD was identified in 60% (18/30) cases with 14 cases being infectious diseases, 4 cases being malignant tumors (three of them of viral origin and one large B cell lymphoma). The MIA tended to yield a CoD diagnosis more frequently in patients 35-year-old or younger than in patients older than 35-year-old, although the difference was not statistically significant ($p=0.288$). Microbiological tests confirmed the pathological results and provided a specific etiologic diagnosis in 77%(14/18) of the diagnosed cases.

Conclusions: A simplified MIA technique allows obtaining adequate material for histological and microbiological analyses from body fluids and major organs. This procedure could greatly improve the determination of causes of death related to infections diseases in developing countries.

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1563 Utility of Reflexive Mycobacterial and Fungal Culture on Residual Frozen Section Tissue With Granuloma

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Background: Culture-based identification of mycobacteria and fungi is the gold standard for species level identification. Although this method is slow, it is imperative to guide antimicrobial therapy. It is not uncommon that tissue demonstrating granuloma(s) microscopically in surgical specimens is entirely formalin fixed and not submitted for culture. Our objective was to evaluate a protocol whereby if granuloma was detected on frozen section, specimens were referred by pathology for mycobacterial and fungal culture.

Design: This was a retrospective study evaluating surgical specimens with granuloma(s) present on frozen section examination over 2 years at a large academic medical center. Pathology results of AFB and GMS special stains were compared to culture results when available.

Results: 233 surgical specimens with granuloma(s) present on frozen section examination were identified. 83 (36%) specimens were sent for culture: 62 by clinicians, 16 by pathologists, and 5 by both. Table 1 describes the organisms recovered by culture.

Organisms Recovered in Culture		
Physician Initiated	Granuloma Protocol	Both
M. avium-intracellulare complex^	M. avium-intracellulare complex	M. avium-intracellulare complex
M. tuberculosis complex	M. kansasii	
M. tuberculosiscomplex*	Cryptococcus neoformans	
Aspergillus fumigatus*	Exophilia spp.	
Blastomyces dermatitidis	Serratia marcescens^	
Histoplasma capsulatum		
Cornyebacterium spp.^		
*no special stains performed; ^special stains interpreted as negative; all other special stains interpreted as positive		

Of those specimens sent for culture, 63 (76%) had corresponding GMS or AFB staining where organisms were identified in 24 specimens (16 fungi consistent with *Histoplasma*, 1 fungus consistent with *Blastomyces*, 1 other fungus, and 6 acid-fast bacilli). By culture, a species level identification was possible in 4 of 6 specimens (2 *M. avium-intracellulare* complex, 1 *M. kansasii*, 1 *M. tuberculosis* complex) which stained positive for AFB. In 13 specimens with fungi detected via GMS staining, no organisms were recovered in culture.

Conclusions: In clinical specimens, culture of frozen tissue permits improved species level identification of AFB, but imparts minimal increased yield for fungal identification. The improved species level mycobacterial identification as a result of reflex culture of specimens with granuloma is an important quality assurance initiative for optimal patient therapy and infection control measures.

1564 HPV Attribution in Non Cancerous, Precancerous and Invasive Penile Lesions: A Laser Capture Microdissection (LCM)-PCR Study

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Background: In many patients with penile neoplasia there are multiple histologically different lesions. Precise HPV attribution is needed to understand the role of HPV types in benign, premalignant and malignant lesions. The purpose of this study was to investigate presence of HPV types in each lesion by whole tissue section (WTS) and LCM-PCR of HPV+ specimens.

Design: Eighty six paraffin blocks were sectioned by the sandwich technique for histological and PCR analysis. Multiple LCM-PCR samples were taken from individual lesions from 51 patients. Categories defined by consensus of 3 pathologists with final review after disclosure of HPV results (Table 1). HPV analysis on WTS and LCM selected regions were performed by SPF 10-DEIA-LIPA 25 (version 1). DNA quality of all HPV negative cases was confirmed by RNaseP/PhHV qPCR.

Results:

Table 1, HPV positivity in penile lesional areas by WTS-PCR and LCM-PCR.

Final lesion diagnosis	Lesions (n=315)	HPV +	
		n	%
Condylomas	59	41	69
Squamous hyperplasia	34	4	12
Lichen Sclerosus*	25	4	16
Differentiated PeIN	53	3	5
Undifferentiated PeIN	110	104	94
Invasive Warty and/or Basaloid SCC	25	23	92
Invasive poorly differentiated Solid grade 3 SCC	11	4	4
Other Invasive SCC**	24	2	8

* Includes cases withfeatures of Differentiated PeIN and Undifferentiated PeIN
** Including veruccous,pseudohyperplastic and papillarycarcinomas.

Conclusions: HPV was frequent in Condylomas, Undifferentiated PeIN and especial invasive carcinomas (warty, basaloid and poorly differentiated). Variable HPVs were present and a lesion usually revealed one HPV type. Rarely associated with HPV were squamous hyperplasia, lichen sclerosus, differentiated PeIN, invasive usual SCC, low grade variants and sarcomatoid SCCs. LCM-PCR assigned individual HPVs to specific histological areas in heterogeneous lesions. LCM-PCR allows precise assignment of HPV to a lesional area and is necessary to understand the complexity of penile (pre-) cancer, and associated lesions, and define the true role of HPV.

1565 Correlations Among Histopathological Characteristics, Viral Distribution, and Cytokine/Chemokine Expression Level Within an Individual With A/H1N1pdm09 Infection Induced ARDS

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Background: Some cases of 2009 pandemic influenza virus (A/H1N1pdm09) infection were complicated with acute respiratory distress syndrome (ARDS). Previous studies have shown that A/H1N1pdm09 infected alveolar epithelial cell and induced inflammatory response, which caused collapse of the epithelial-endothelial barrier and edema in the alveolar lumen. In this study, we collectively evaluated and compared among histological characteristics of diffuse alveolar damage (DAD), A/H1N1pdm09 distribution and inflammatory response in various parts of the formalin fixed and paraffin embedded (FFPE) autopsied lung to elucidate whether there are localized immunological responses to A/H1N1pdm09 infection that lead to specific pathological changes.

Design: Two autopsy cases with A/H1N1pdm09-ARDS were examined. The FFPE samples were taken from several parts of lungs, including at least one section from each lobe. All samples were analyzed by three methods: H&E stain for histological findings of DAD severity, immunohistochemistry and *in situ* hybridization for the distribution of A/H1N1pdm09, and real time RT-PCR of cytokine/chemokine mRNA for the immunological responses.

Results: Both cases died on day eight post onset of the disease. There were some similar findings between them. Histological analysis showed exudative phase of DAD and there were variation in cytokine/chemokine expressions among different parts of lungs. The first case showed a significant variation in the DAD severity in histological analysis although viral antigen was detected broadly within the lung tissue. The second case showed homogenous DAD appearance but there was only a localized distribution of viral antigen in the lower lobe of lungs. Overall, there seems to be correlations among the DAD severity, viral distribution and local cytokine/chemokine expression levels in the lung.

Conclusions: Unlike seasonal influenza, A/H1N1pdm09 infection can cause ARDS. We found correlations among the DAD severity, viral distribution and local cytokine/chemokine expression levels in the autopsied lung with A/H1N1pdm09-ARDS. Our data supports the hypothesis that local and heterogeneous inflammatory response is the key to understand the underlying pathogenesis of ARDS induced by viral infection.

1566 The Presence and Expression of AmpC-Type Beta-Lactamases May Lead To False Identification of Ertapenem Resistant Enterobacteriaceae as a Result of the Lowered CLSI Breakpoints

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Background: The growing threat of multi-drug resistant microorganisms, in particular, carbapenem resistant Enterobaceriaceae (CRE) has emerged as a top concern plaguing modern medicine. With the relative paucity of new antimicrobial medications to combat this onslaught, judicial use of appropriate antimicrobial therapy is crucial. In 2012, the Clinical Laboratory Standards Institute (CLSI) lowered the definition of carbepenem resistance from ≥8.0 ug/ml to ³2.0 ug/ml. Bacteria which were once considered of intermediate resistance are now being classified as CRE, potentially forcing clinicians to escalate antimicrobial therapies. We sought to determine whether these bacteria in fact harbored a carbepenem resistance gene (KPC), typical of true CRE, or if they were rather AmpC carriers, with an additional non-specific resistance mechanism, such as porin mutation or efflux pump, atypical for true CRE.

Design: We prospectively collected organisms with minimum inhibitory concentrations (MICs) to ertapenem between £0.25 ug/ml to ≥64.0 ug/ml as identified by automated antimicrobial susceptibility testing (AST). Carbapenem resistant and susceptible isolates were also included as positive and negative controls. Our cohort was composed of 31 *Enterobacter* cloacae, 13 *Klebsiella pneumoniae*, 7 *Escherichia coli*, 2 *Klebsiella oxytoca*, 3 *Proteus mirabilis*, 4 *Serratia marcescens*, 1 *Morganella morganii*, and 1 *Pseudomonas aeruginosa*. DNA was extracted and amplified using realtime PCR with various primers and probes specific for AmpC genes and KPC primers confirmed with melt-curve analysis.

Results: A total of 62 isolates were tested: 33 were AmpC+/KPC-, 6 were AmpC+/KPC+, 4 were AmpC+/KPC equivocal, 7 were AmpC-/KPC+, 3 were AmpC-/KPC equivocal and 9 were negative for both. Of the 35 isolates which would have previously been classified as ertapenem intermediate but now resistant due to the breakpoint change, 18 are positive for an AmpC gene and negative for KPC. 7 of these isolates had an MIC of 2; 11 had an MIC of 4.

Conclusions: Our results suggest that the new breakpoints falsely categorize some AmpC possessing bacteria as CRE. Further investigation will better characterize the type of AmpC gene carried, and evaluate for presence of a porin mutation or efflux pump. We will also better characterize those isolates that were equivocal for the KPC gene. Lastly, we will ascertain the clinical impact of these results, and determine if escalated antimicrobial therapy led to patient harm.

1567 Evaluation of the Diagnostic Accuracy of Fungal Identification in Histopathology and Cytopathology Specimens

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Background: Invasive fungal infections are becoming increasingly common worldwide. The primary tools for detection and identification of fungi are microscopic examination and culture of tissues and body fluids, each of which has strengths and limitations. Microscopic examination allows for rapid, presumptive identification of fungal infection, but can have limited sensitivity and specificity, and a species level identification can rarely be made on histopathology alone. Fungal culture is the "gold standard" for species level identification, but can take weeks. Though these two approaches are often complementary, there are cases where the results of microscopic examination and culture are discrepant. Such discrepancies may be clinically significant, causing inappropriate or delayed initiation of antifungal therapy. Our primary objective was to evaluate the diagnostic accuracy of histologic identification of fungal infection. Our secondary objective was to evaluate the frequency with which tissue specimens with evidence of fungal infection are submitted for culture and identify methods to improve this ratio.

Design: An 18-year (1996 to 2014) retrospective review was performed on all surgical pathology and cytology specimens from which a pathologist diagnosed a fungal infection at a large tertiary care medical center. Cases of previously known fungal infection were excluded due to possible bias in pathology diagnoses. Demographic characteristics were recorded for all specimens for which a concomitant fungal culture was performed. All discrepancies between morphologic and culture-based diagnoses were recorded, and available slides from these cases were reviewed to perform a root cause analysis for misidentification.

Results: Of 837 cases where a morphologic diagnosis was rendered, only 359 had a concomitant culture. In 124 (35%) cases, the concomitant culture had no growth. Of the 235 of cases for which cultures had growth, 220 (94%) had concordant results. The remaining 15 (6%) discrepant cases most commonly involved interpretative errors between *Aspergillus* species and *Zygomycetes* in lung tissue. No statistically significant difference in use of special stains between concordant and discrepant cases was identified.

Conclusions: The identification of fungi in tissues is usually accurate and relatively rapid compared to culture, however, reporting should be standardized to take into account morphologic mimics and the limitations of histology. Education regarding the morphology of *Zygomycetes* in tissue is needed.

1568 Mucosal Inflammation in Candida Esophagitis (CE) Has Distinct Features

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Background: Scant literature exists characterizing the histology of CE. Apart from fungal forms admixed with neutrophilic exudate, epithelial changes have been deemed nonspecific. However, the histologic diagnosis of CE can be challenging due to sloughing of fungal exudate. The goal of this study was to better characterize inflammatory changes in CE to aid in differentiating it from other types of active esophagitis.

Design: We reviewed 167 consecutive esophageal biopsies diagnosed with CE at our institution from 2003-2014. 96 showed features of mucosal inflammation/active esophagitis (MI) and 71 had no MI. The 39 most recent cases with MI and 40 cases without MI were selected for further characterization. Distribution of intraepithelial neutrophils was recorded. Intraepithelial eosinophils (IEE) were counted per total biopsy when ≤6 or per one 400x most affected field of view (HPF) when present in higher numbers. Intraepithelial lymphocytes (IEL) were counted similarly per HPF. Normal cutoff was 42 IEL/HPF (Putra et al. Mod Pathol 2014, 27 Suppl2, 198A). CD4 and CD8 T-cells were analyzed by immunohistochemistry. The CD4:CD8 ratio > 1 and ≤ 1 indicated the predominance of CD4 and CD8 T cells respectively. Data is presented as mean ± SD.

Results: Patients with CE and MI (age 56 ± 18; M:F=1.2:1), and patients with CE, but without MI (age 57 ± 17; M:F=1:1.2) did not differ in the prevalence of GERD (52% and 55% respectively), antibiotic use (10% and 5% respectively) or factors associated with immunosuppression, such as steroid inhalers, systemic steroid use, cancer or severe diabetes (56% and 60% respectively). Microscopically, 97% (38/39) of patients with MI

had intraepithelial superficial neutrophils which showed a band-like infiltrate restricted to the upper half of the epithelium in 74% (29/39). No IEE were found in 44% (17/39) and few (≤6/biopsy) in 26% (10/39). Eosinophilia was therefore absent or insignificant in 70% of cases. IEL (95 ± 47, range 38 – 211) were increased in 97% (38/39) of cases. CD4-predominant IEL were present in 74% (29/39) of biopsies (CD4:CD8=3.0 ± 2.4), while CD8-predominant IEL were present in 26% (10/39, CD4:CD8=0.4 ± 0.3). Some degree of spongiosis was present in all 39 cases (100%).
Conclusions: A superficial, band-like neutrophilic infiltrate, absent or rare IEE and increased CD4-predominant IEL are common histologic characteristics of MI in CE. These distinctive features could aid in the differential diagnosis of difficult cases of active esophagitis.

1569 Lyme Carditis: Autopsy Findings of 5 Patients With Sudden Cardiac Death

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Background: Fatal Lyme carditis caused by the spirochete *Borrelia burgdorferi* is rarely identified. In 2013, CDC reported three cases of fatal carditis associated with Lyme disease; here we describe the pathologic, immunohistochemical and molecular findings of these, and two additional cases.
Design: Autopsy tissue samples from the 5 patients were evaluated by light microscopy, Warthin-Starry stain, immunohistochemistry (IHC) and PCR for *Borrelia burgdorferi*, and IHC for complement components C4d and C9, CD3 and CD79. Testing for other tick-borne pathogens was also performed.
Results: These sudden cardiac deaths associated with Lyme carditis occurred from late summer to fall, ages ranged from young adult to late 40's, and 4 patients were men. Cardiomegaly was seen in all cases, as were varying degrees of hepatosplenomegaly. Interstitial lymphocytic pancarditis in a characteristic "road map" distribution was present in all cases; cardiomyocyte necrosis was minimal, mild fibrosis was present and plasma cells were prominent. Underlying heart disease was evident in 4 cases. By Warthin-Starry stain and IHC, spirochetes were found in heart tissue from all cases. Minimal to mild lymphocytic meningitis was present in 4 cases, with rare spirochetes seen in the leptomeninges of 2 cases by IHC. Mild portal hepatitis was present, and one case had cirrhosis. Spirochetes were not found in other organs; no joint tissue was available for examination. By IHC, cardiomyocyte complement deposition was negative, and the myocardial infiltrate contained more T-cells than B-cells.
Conclusions: Although rare, sudden cardiac death due to Lyme disease may be an under-recognized entity. Immunohistochemistry using a commercially available *B.burgdorferi* antibody is a sensitive screening assay. Fatal Lyme carditis is characterized by pancarditis and marked tropism of spirochetes for cardiac tissues.

1570 Modified In Situ Hybridization AT-Tailing To Visualize the Gene Expression in Formalin-Fixed and Paraffin-Embedded Tissues

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Background: We previously developed *in situ* hybridization AT-tailing (ISH-AT) as a novel ISH method. ISH-AT uses a oligonucleotide probe that has $(AT)_{10}$ at the 3' end. Briefly, this $(AT)_{10}$ of the probe is elongated by self-priming and GenTaq polymerase in the presence of dATP, dTTP, and biotin-16-dUTP in the tissue after hybridization. Through this process, the target is labeled with many biotin molecules. The streptavidin (SA)-alkaline phosphatase (AP) or SA fluorescent conjugates were applied on the section, and signals were visualized by chromogenic or fluorescence detection. Recently, we simplified and improved the protocol of ISH-AT. In this study, we present a new protocol of ISH-AT and show its superiority in comparison to a commercial ISH method.
Design: The expression of 2009 pandemic influenza virus (A/H1N1pdm09)-RNA and severe fever with thrombocytopenia syndrome virus (SFTSV)-RNA in formalin-fixed and paraffin-embedded (FFPE) autopsied tissue sections were examined by ISH-AT or QuantiGene ViewRNA ISH Tissue Assay (ViewRNA) (Affimetrix, Santa Clara, CA, USA). ViewRNA is also a novel ISH method that uses simultaneous branched DNA signal amplification. The copy number of the target RNA in the FFPE tissue sections was also quantified by real-time RT-PCR to evaluate the detection sensitivity. Double staining with immunohistochemistry was also performed. The advantage and disadvantage of ISH-AT in comparison with ViewRNA was thus elucidated.
Results: We can detect A/H1N1pdm09-RNA and SFTSV-RNA in FFPE tissue sections using either ISH-AT or ViewRNA. ISH-AT with fluorescent-based visualization can show the colocalization of RNA and an antigen as a merged image by combining with immunofluorescence assay. It is difficult to show the colocalization in FFPE tissue sections using ViewRNA. The sensitivity of the ISH-AT method with 2 oligonucleotide probes was comparable to ViewRNA with 20 oligonucleotides pairs.
Conclusions: Visualization of the gene expression in FFPE autopsied tissues by ISH is much more difficult than in cell samples. The sensitivity of ISH-AT was comparable to that of ViewRNA. The ISH-AT method costs less than ViewRNA and is a strong tool to detect virus RNA in FFPE autopsied tissues.

1571 Grocott Methenamine Silver Stain: An Adjunct Diagnostic Tool in the Identification of Bacteria in Tissues

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Background: The tissue Gram stain has been an important diagnostic tool for over 100 years. However, exposure to antibiotic therapies, rapid growth or tissue environment can affect bacteria morphology and staining characteristics. Grocott methenamine silver stain (GMS) can react with polysaccharide components of microorganisms and is traditionally used in the histologic diagnosis of fungi. Many bacterial pathogens contain polysaccharide in their cell wall or capsule and can also be highlighted with GMS. This study aims to demonstrate the utility of GMS as an adjunct diagnostic tool in the histologic identification of bacteria.
Design: A total of 68 cases, consisting of 22 different bacterial species with various Gram-staining and morphologic characteristics, were selected for review. The bacterial diagnoses were previously confirmed with either immunohistochemistry or PCR. All cases of less commonly detected bacteria (<10 cases) were included, while cases of more commonly detected bacteria (>10 cases) were included if atypical Gram stain findings were previously reported, such as unexpected gram-negativity or weak/absent staining. Histopathologic evaluation, Gram stain, GMS and Warthin-Starry stain (W-S) were performed.
Results: GMS detected *Bacillus anthracis, Bacillus cereus, Clostridium sordellii, Enterococcus faecalis, Fusobacterium nucleatum, Neisseria meningitidis, Nocardia species, Propionibacterium acnes, Rothia species, Staphylococcus species, Streptococcus species, and Tropheryma whipplei*. GMS was positive and Gram stain was non-contributory in 8 cases involving 5 distinct organisms. GMS was positive and W-S was negative in 8 cases involving 4 different bacteria. GMS demonstrated more bacteria than Gram stain in 2 cases involving 2 distinct organisms, and more than W-S in 1 case. Occasionally GMS yielded unexpected results. For example, large yeast-like cocci were seen in 2 cases of endocarditis, one caused by *Staphylococcus cohnii* and the other by viridans group *Streptococcus.* One case of *Propionibacterium acnes* showed filamentous bacteria with terminal bulbs, which were not seen on Gram stain or W-S.
Conclusions: Identification of bacteria on tissue sections can often be challenging. While the Gram stain continues to be the primary special stain used to diagnose bacterial infections, GMS can be a useful ancillary tool and sometimes demonstrate bacteria when Gram stain is weak or negative. Therefore, we recommend performing GMS when the Gram stain result is not supported by the histopathologic findings or clinical data.

1572 Characteristics of Respiratory Illness Associated With an Outbreak of Enterovirus D68 Infection in the Lower Hudson Valley, New York, 2014

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Background: An outbreak ofsevere respiratory illness associated with *Enterovirus* D68 (EV-D68) was first reported in Missouri and Illinois in mid-August, 2014. As of September 26, 2014, the Centers for Disease Control and Prevention (CDC) and state public health laboratories have confirmed a total of 277 persons with EV-D68 infections from 40 US states. At the beginning of September 2014, elevated numbers of children with respiratory illness were noticed in healthcare facilities in the lower Hudson Valley, New York. The study summarizes our findings on the clinical characteristics of laboratory-confirmed cases and approach for routine laboratory detection of EV-D68.
Design: Nasopharyngeal (NP) swab specimens from patients with respiratory illness were collected and examined for the presence of *Rhinovirus/Enterovirus (RhV/EV)* by using the FilmArray™ Respiratory Panel (RVP) assay (Biofire™, Salt Lake City, Utah). Positive specimens were further analyzed using *Rhinovirus*- and *Enterovirus*-specific real-time RT-PCR. Samples testing positive by the enterovirus-specific real-time assay were further analyzed by molecular typing with DNA sequencing of the VP-1 region. Clinical data were obtained by reviewing medical records of laboratory-confirmed cases.
Results: FilmArray™ RhV/EV-positive NP swab specimens from 64 patients were examined. EV-D68 infection was confirmed in 22 of the 64 (34%) patients. Of 22 EV-D68 laboratory-confirmed patients, 14 (64%) were male, and age ranged from 8 months to 13 years (median = 5 years). Common clinical syndromes were asthma exacerbation (n=10), acute respiratory distress (n=7) and dyspnea (n=2). Eighteen patients were hospitalized and 3 of these were admitted to the pediatric intensive care unit. Interestingly, a specific RVP profile (negative for both *Enterovirus* primer sets and positive for *Rhinovirus* primer sets 1, 2 & 4 or 1 & 4) was observed in 17 of 22 (77%) patients with confirmed EV-D68 infection but only in 4 of 42 (9.5%) patients with non-EV-D68 infection ($p < 0.0001$), corresponding to a PPV of 81% and a NPV of 88% for our patient population.
Conclusions: An outbreak of severe respiratory illness associated with EV-D68 in children of the lower Hudson Valley, New York was investigated. Further study would elucidate the potential of the observed RVP PCR profiles based on current epidemic EV-D68 strains, as a screening tool for EV-D68 infection in clinical practice.

1573 Whole Genome Sequencing-Based Molecular Epidemiology of Vancomycin-Resistant Enterococcus faecium Pre Versus Post Daptomycin Use in a Suburban New York City Medical Center

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Background: Vancomycin-resistant *Enterococcus faecium* (VREf) belonging to clonal cluster 17 (CC17) has been emerging globally since the 1990's and is now among the predominant group of enterococci causing nosocomial infections of the bloodstream, urinary tract, skin and soft-tissues. The aim of this study was to assess the clonality of temporally spaced VREf clinical isolates using whole-genome sequencing analysis.

Design: Thirty-four and 113 VREf clinical isolates recovered from patients in a tertiary medical center in suburban New York City in 1995 and 2013, respectively, were selected and analyzed. Whole-genome sequencing was performed on the Illumina MiSeq™ or HiSeq™ System by using paired-end methods. Multilocus sequence typing (MLST) and single nucleotide variations (SNVs) data were derived from the genome sequences of each isolate. The genetic relatedness of different enterococcal isolates and sequence types were explored using the goeBURST program.

Results: The predominant strain type for VREf isolates from 1995 was ST17 (79.4%), followed by ST18 (8.8%) and ST323 (8.8%). By contrast, a newly described clone, ST736, accounted for 48.7% of VREf isolates in 2013, followed by ST18 (24.7%) and ST412 (21.2%). Population genetic analysis established that ST736 is a novel clone within CC17 with differences in two alleles from its prototype ST17.

Conclusions: Whole-genome sequencing analysis of VREf clinical isolates demonstrated a dramatic change in dominant strain types and the emergence of a novel clone ST736 in our patient population over the past 18 years.

Informatics

1574 Clinical Genome Analytics (CGA): An Automated Analytical Pipeline for Detection and Annotation of Clinically Relevant Genomic Variants

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Background: The development of next generation sequencing (NGS) and associated target sequence enrichment technologies has enabled time and cost effective detection of clinically relevant molecular alterations in hundreds of genes using a single clinical assay, thus paving the path for personalized medicine. Although data generation is no longer the major hurdle, accurate and time-effective bioinformatic and statistical analysis, and biologically meaningful interpretation of the data remain challenging.

Design: We created CGA (Clinical Genome Analytics), an automated data analysis pipeline that is implemented using Civet, an in-house engineered XML-based framework for building analytical pipelines for efficient multi-step data processing.

Results: CGA includes capabilities for automatic scheduling of jobs, monitoring analysis runs, and automatically saving information about each run (including command line options, versions and standard output/error for each tool). It puts together 17 different tools, from quality control and alignment through local realignment around indels and base quality recalibration to calling SNPs, indels and CNVs, and finally assigning genomic annotations to these variants. CGA takes an average of 6 hr to process samples containing approximately 25M paired-end reads, and ensures high (> 99.4%) sensitivity and specificity for the detection of >= 5% frequency SNPs and indels (<= 50bp) and of copy number changes >= 6.

Conclusions: CGA is an automated analysis pipeline that ensures accurate and sensitive detection and clinical annotation of mutations. It is currently being used as part of an NGS-based molecular diagnostic assay that detects actionable mutations in solid tumors in a CLIA-certified laboratory. It provides not only an accurate analysis of samples in a clinically acceptable short turn-around time, but also the ability to document quality metrics and information on all of the tools, their versions and options, and all reference genomes and databases used, which is key to ensure reproducibility and traceability in a clinical set-up.

1575 Utility of Automated Bioinformatic Pipeline in the Analysis and Interpretation of SNP Microarrays in Neoplasia

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Background: SNP microarrays are being widely used as a tool in a routine oncology diagnostic workflow. The main advantage is its ability to accurately identify the composition of derivative chromosomes & marker chromosomes commonly observed in clonal populations. One of the biggest challenges of interpreting oncology arrays is the lack of bioinformatics tools facilitating the analysis and interpretation of microarray data. Another major challenge is to rule out the common polymorphic genomic variants stored in databases such as DGV, as well as having the ability to tease out potentially relevant copy number variable (CNV) and Loss-Of-Heterozygosity (LOH) regions possibly contributing to the pathogenesis of neoplastic disorder. The aim of this study was to access and configure the utility of an automated bioinformatic pipeline in the analysis and interpretation of SNP Microarrays in neoplasia.

Design: For constitution SNP array's our lab has been using various automated bioinformatics pipelines for data analysis and reporting. For oncology SNP array data analysis and interpretation we collaborated with Cartagenia Inc. Aim is to create a different variant assessment and filtration strategies that can be established and automated for the analysis of SNP microarrays in various leukemias and the utility of the Bench platform in the identification of pathogenic changes. We selected array SNP array data from 42 cases of cytogenetically normal cases of CMML, Myelofibrosis and ALL and subjected to the customized bench.

Results: Our studies have allowed us to establish specific filters for the determination of potentially significant copy number changes (like mosaic deletions and mosaic duplications). SNP microarrays also have the added advantage of identifying regions of LOH which play a very important role in the 2 hit hypothesis in somatic cancers. Our filtration allowed removing germline mutations, stored in databases such as DGV, as well as alignment of genomic variants with control populations or healthy tissue samples. We have also been able to establish an automated filtration and reporting pipeline that allows the identification and reporting of regions of LOH harboring important cancer genes such as CDKN2A and JAK2.

Conclusions: We propose that an automated bioinformatic pipeline for the analysis and interpretation of neoplastic disorders will benefit the labs and oncologists ordering a high-resolution chromosomal microarray in a diagnostic setting.

1576 Diagnostic and Educational Uses of Google Glass in Anatomic Pathology

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Background: Google Glass is a wearable intelligent device permitting the capture and communication of photography and video via its hands-free, voice recognition capabilities. Multiple institutions are investigating the use of Glass technology in health care, most notably within surgical and internal medicine specialties. Glass utilization within pathology has been limited to very few reports. The first study investigated live-streaming of routine specimen dissection; another study described the acquisition and quality of still images in forensic autopsy findings. To date, no studies have applied the use of Glass-acquired data, specifically videos, for subsequent diagnostic and educational purposes in anatomic pathology.

Design: Google Glass (Explorer edition) photography and videography was implemented as an adjunct to routine gross specimen documentation by hand-held digital photography during intraoperative consultations and/or routine permanent processing. Specimens included pulmonary, gynecologic, and gastrointestinal resections. The edited dissection videos were less than 40 seconds in length. Data was obtained, stored, and accessed in the same secure and HIPAA-compliant methodology currently in place for digital gross photography. Specimen photography and videography were evaluated in several clinical and educational capacities, including but not limited to consultations among residents and attendings, gross specimen review at the time of microscopic examination, pathology resident education, and inter- and intra-departmental conferences.

Results: Glass technology obtained images and videos with quality comparable to those by digital photography but without disruption or increase in processing time to pre-existing grossing procedures. The use of Glass was described as more convenient and efficient than conventional photography. Dissection videos of complex resection specimens, beginning from their intact and fresh states to their final sectioning, were reported to be of greater diagnostic and educational utility than both photography and gross specimen review of the fixed and sectioned tissues.

Conclusions: Glass has demonstrated clinical and educational value within anatomic pathology. The analysis of surgical specimens in pathology almost always requires specimens to become disrupted and altered in fixative following dissection. Maintaining quality photographic and video records of the intact and fresh specimens at the time of initial dissection is important for diagnoses and reporting, medical education, and potential error reduction.

1577 FISH Digital Image Capture and Automated Segmentation Improves Laboratory Workflow Efficiency

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Background: Fluorescence in situ hybridization (FISH) is a laboratory testing modality where fluorescent probes highlight specific chromosomal sequences which are then localized by fluorescence microscopy. Limitations to FISH interpretation include identification of individual cells showing fluorescent signal without cellular overlap. These limitations are exacerbated by manual counting and interpretation, leaving manual FISH analysis subjective and time consuming. As FISH testing is increasingly incorporated in diagnostic pathology, turnaround time with accurate reproducibility of results is of utmost importance to workflow and patient care. We examined if incorporation of automated segmentation protocols on digitally captured images improves cell detection and laboratory workflow efficiency.

Design: FISH cases (hematologic and non-hematologic) were analyzed (5 Her2/neu, 3 FKHR, and 8 MYC) using automated FISH analysis systems (GenASIs, Applied Spectral Imaging, Carlsbad, CA). H&E slides were reviewed and marked by a pathologist. Following image capture by GenASIs, manual and automated segmentation protocols were performed on a minimum of 60 cells (hematopoietic cases) and 120 cells (non-hematopoietic cases) from four to five digital image frames. Manual segmentation involved a technologist circling appropriate cells for analysis with the GenASIs mouse. Automated segmentation by the GenASIs Omni software, was performed on identical frames with manual deselection of inappropriate cells. Both methods were compared for efficiency and accuracy.