and optimized staining protocol as previously described, each *ALK*-negative case resulted as either amplified at 2p23 or aneusomic for chromosome 3, and ultimately all 3 cases matched the established genotype. **Conclusions:** Fully automated bright field detection of NSCLC *ALK* gene status can be achieved under specific conditions in ThinPrep cytology specimens.

ST47. Evaluation of *MDM2* Amplification in Cases with Adipocytic Tumors Using Chromogenic *in Situ* Hybridization (CISH) Technique

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Introduction: Atypical lipomatous tumor/well-differentiated liposarcoma (ALT-WDLPS) and dedifferentiated liposarcoma (DDLPS) are often difficult to distinguish morphologically from benign adipocytic tumors and other high-grade sarcomas, respectively. ALT-WDLPS and DDLPS share the same basic cytogenetic abnormality characterized by a 12q13-15 amplification involving the *MDM2* oncogene. In contrast, none of the various benign tumors of adipocytes has an amplification of this locus. Therefore, fluorescence *in situ* hybridization (FISH) is used frequently to detect the *MDM2* amplification and aid with the diagnosis of these entities. Recently, bright-field *in situ* hybridization techniques such as chromogenic *in situ* hybridization (CISH) and silver-enhanced *in situ* hybridization (SISH), which combine features of immunohistochemical analysis and FISH, have been introduced for the determination of *MDM2* amplification status. These new techniques use a peroxidase enzyme-labeled probe with chromogenic detection, instead of a fluorescent-labeled probe, allowing results to be visualized by standard bright-field microscopy. **Methods:** The present study compared the results of the FISH and CISH methodologies for detecting *MDM2* amplification in cases with adipocytic tumors with an established histopathologic diagnosis. A total of 44 cases (Benign n=15, Borderline/malignant n=29) were tested with FISH and CISH. For each case, 50 cells were examined for the presence of red (chromosome 12 copies) and black (*MDM2* copies) signals using dual color *MDM2* and *CEN12* CISH probes (Ventana Medical Systems, Inc). The overall ratio of black to red signals was calculated, and a ratio of 2 or greater was considered to be amplified. **Results:** Eleven of the cases showed amplification with both FISH and CISH, and 24 cases showed no amplification. CISH technique was found to be equally effective as FISH for testing adipocytic tumors. **Conclusions:** CISH is advantageous for allowing pathologists to evaluate the histologic and molecular alteration occurring simultaneously in a specimen. Moreover, CISH is found to be more cost effective when used with automation, and the signals do not quench over time. This establishes clinical utility of CISH testing for MDM2 to establish diagnosis of adipocytic tumors.

ST48. Optimization of the Illumina TruSeq Amplicon Cancer Panel Protocol for Clinical Use

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Introduction: Implementation of a complex assay workflow, exemplified by next-generation sequencing, can be very challenging in a clinical laboratory. In this study, we evaluated the impact of a number of experimental parameters on the data quality produced by Illumina TruSeq Amplicon Cancer Panel (TSACP) to improve workflow for clinical implementation. **Methods:** Previously tested genomic DNA extracted from FFPE tumor tissue harboring hotspot mutations in *EGFR*, *KRAS*, *BRAF*, *IDH1*, *PIK3CA*, *NRAS*, and *KIT* were used for targeted resequencing library preparation using the Illumina TSACP assay and sequencing on an Illumina MiSeq. A batch size of 24 to 32 samples enabled the optimal use of reagents and achieved a mean depth of coverage >500x. Data analysis was performed using MiSeq Reporter. **Results:** We found that quantification of DNA by Qubit was a poor predictor of assay performance. Although using less than the recommended 250 ng of input DNA still resulted in a PCR product, the number of non-reproducible low frequency variants increased significantly. To reduce the noise in the data, we decreased the number of PCR cycles from 30 to 27, which decreased the average number of variants passing filter (AD>50, VF>10%) per case from 15 to 4, with 100% retention of reproducible calls. In an attempt to improve coverage for samples with a low PCR yield, we doubled the amount of PCR product pooled into the final library to be sequenced, which led to a significant increase in the average number of variants from 6 to 17 per sample, many of which had an allele frequency of 10% to 15%. An evaluation of inter-assay replicates showed the increase in the overall number of variant calls with low variant allele frequencies were not reproducible and therefore likely false positives. We found that the intensity of the PCR product on LapChipGX tracings was an accurate predictor of sequencing coverage with samples showing low intensity (less than 16) demonstrating low coverage and a high number of artifactual variants. Most notably, adjusting the sequencing read lengths from paired-end 2x150bp to 140+160bp improved the bidirectional coverage of known hotspot variants mutations that were located at the 5' end of several amplicons. **Conclusions:** We effectively optimized multiple components of the standard workflow to create an enhanced customized assay to better fit the demands and address challenges encountered in a clinical environment and improved confidence and reliability of the results.

ST49. Reproducibility and Accuracy of Cross Platform RT-qPCR Testing for the Detection of mRNA from Surface Squamous Cells Overlying Pigmented Skin Lesions

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Introduction: Clinical and pathologic identification of pigmented skin lesions is dependent on visual pattern recognition, leading to less than acceptable sensitivity and specificity for the accurate identification of melanocytic neoplasms. We previously demonstrated removal of epidermal cells overlying melanoma using tape stripping technology permits the isolation and measurement of mRNA that accurately discriminates benign pigmented skin lesions from malignant melanoma and *in situ* melanoma. The QuantStudio RT-qPCR platform previously was used to obtain clinical sensitivity in a multi-gene expression assay compared to histopathology of 100% with specificity of 85%. **Methods:** Fifty-seven specimens were obtained by lesional tape sampling, followed by RNA extraction and cDNA synthesis. Replicate samples were run on both the Biotrove OpenArray NT system and on the LifeTechnologies QuantStudio system. The resultant RT-qPCR data were scored using a previously determined classifier that accurately discriminates between melanoma and nevi. The classifier scores for each specimen on the two platforms were compared by clinical concordance and linear regression. Within-run precision was measured using 20 replicates of a pooled human reference RNA source. **Results:** Interinstrument concordance was 100% on melanoma/nevi classification calls, with r-squared of classifier scores = 0.97; the slope of the regression line was 0.98 with an offset of 0.01. The QuantStudio data were used to refine the classifier to 2 signal genes and 3 housekeeping genes, with an AUC-ROC = 0.94. The within-run %CV of genes in the classifier was ≤ 1.2%. **Conclusions:** We demonstrated perfect clinical concordance between two types of OpenArray instruments, maintaining the high sensitivity and specificity previously demonstrated for melanoma detection. This excellent between-instrument precision indicates that OpenArry RT-qPCR is a reproducible tool for the evaluation and optimization of gene signatures on cells isolated from pigmented skin lesions. In the future, molecular signatures of neoplasms may supplant the highly subjective nature of clinical examination and histopathologic examination of pigmented skin lesions. Melanoma is on the rise in the US and early detection and appropriate therapy will reduce overall morbidity and mortality from this neoplastic disorder. Molecular signatures using RT-qPCR technology will bring new opportunities to accurately screen, diagnose and monitor neoplastic diseases. The specific platforms adopted to clinically analyze mRNA will be critical to reproducibility and accuracy of the test results. The use of a reproducible molecular signature using a non invasive specimen collection technology should improve patient outcomes, reduce unnecessary expenditures and enhance patient satisfaction.

ST50. Utility of Thymidylate Synthase Genotyping for 5-Fluorouracil Chemotherapy in a Large,Colorectal Cancer Cohort Study

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Introduction: 5-Fluorouracil (5-FU) chemotherapy in combination with leucovorin is the routine regime for advanced colorectal adenocarcinoma. 5-FU targets the enzyme, thymidylate synthase (TYMS), which provides the only *de novo* source of thymidylate for DNA synthesis. Germline polymorphisms in the 5'-promoter enhancer region (5'-TSER) and the 3'-untranslated region (3'-UTR) of the TYMS gene have been variably correlated in the literature to TYMS expression levels, responsiveness to 5-FU therapy, and clinical outcome. Our previous colorectal cancer outcome study of 150 Korean patients identified TYMS genotyping as the best prognostic indicator of 5-FU efficacy, evaluating 5' and 3' polymorphisms independently. The current study assesses these and an additional 5'-TSER polymorphism (G>C SNP) for independent and combined effects. **Methods:** TYMS genotyping was assessed on 156 Korean surgically resected colorectal cancers with long-term follow up (mean overall survival 71 months, range 1-147, S.D. 50). Patients received identical chemotherapy and results were controlled for cancer stage, age, gender, and tumor location. The chemotherapy regimen consisted of 24 weeks of 5-FU at 425 mg/m^2 + leucovorin at 20 mg/m^2, given on days 1 to 5, every 4 weeks. The evaluated TYMS polymorphisms included a 6 base pair deletion of the 3'-UTR (DEL) versus the wild