Abstracts of the 2009 Technologist Award Recipients

TT25. Evaluation and Comparison of Automated & Manual Chromogenic In-Situ Hybridization (CISH) Assays Using Lean Process Improvement Analysis

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Chromogenic in-situ hybridization assays (CISH) provide clinical laboratories fast, sensitive and permanent records of a variety of hybridization assays with RNA and DNA targets. Lack of automation has limited their usage due to the intense manual labor involved. We validated kappa and lambda RNA-CISH assays using an automated immunohistochemistry (IHC) staining system and compared them to manual CISH assays and IHC stains for the purpose of conducting a Lean process improvement analysis. 30 cases (15 lymph nodes, 15 decalcified bone marrows) from patients with multiple myeloma, plasma cell dyscrasia, or lymphoma were evaluated. A Bond maX (Leica Microsystems Co) automated staining system was used with assay reagents from the same vendor. These results were compared to a manually performed (same methodology) CISH assay in 5 cases. For IHC, monoclonal anti-kappa light chains (clone L1C1, Lab Vision Corp) and rabbit polyclonal anti-lambda light chains (A0193) (Dako Inc) were applied to the same specimens. Assay time, total turn-around-time (TAT), technologist time (hands-on time), and quality of stains [0(poor, high background) – 4(excellent, clean background)] were evaluated for each stain. The mean automated CISH assay time was 4.6 hours (range 4.45 – 5.15 hours) compared to 7 hours (range 6.5 – 8 hours) for manual CISH. The mean total TAT for automated CISH assays was 14.5 hours (range 8–24 hours) compared to 29 hours (range 26-32 hours) for manual CISH. The mean technologist time for automated CISH assay time was 17 minutes (range 9-25 minutes) compared to 73 minutes (range 63-83 minutes) for manual CISH. The mean stain quality score for automated CISH stains was 3.7 (range 2-4) compared to manual CISH stains had a mean stain score of 2.5 (range 1-3) and to IHC stains with a mean stain score of 2.9 (range 0-4). Automated CISH substantially shortened the assay time, the total TAT and reduced the technologist hands-on time when compared to manual CISH assays and resulted in superior quality stains. Automated CISH assays using an automated platform is the preferred method of performing kappa & lambda stains in our laboratory. Other institutions may want to consider this approach to save money and improve workflow.

ID53. Title: Clinical Performance of Invader HPV v2 ASR Assay and Correlation with HPV Genotyping

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Introduction: Infection with high risk (HR) subtypes of human papillomavirus (HPV) is associated with cervical cancer and precancerous cervical lesions. To better understand the implications of HR HPV genotype on outcome, and potential for treatment decisions, we performed HPV genotyping on cervical pap samples that had been tested for HR HPV in a screening assay. Results of both assays were compared to evaluate and optimize the performance of the HR HPV assay and to detail the distribution of HPV types in our clinical population. **Materials and Methods:** 8,500 SurePath cervical specimens were tested for HR HPV using Hologis's Invader HPV v2 ASR (HPV ASR). Genomic DNA was extracted (Media Extraction kit, Qiagen MDx) and added to each of 3 master mix pools. Interpretation was based on the ratio of the mean fluorescent intensity of the 3 master mixes. A subset of samples with positive, negative, and equivocal results in the HPV ASR assay were tested on the Roche Linear Array HPV Genotyping Assay (HPV GENO) according to manufacturer recommendations to identify both high and low risk (LR) HPV genotypes and to correlate to the HPV ASR results. Results were also compared to pap smear findings. **Results:** HR HPV was detected in 3% of negative pap smears and 17% with a diagnosis of ASC-US. There was excellent concordance between the HPV ASR and HPV GENO assays. Discrepant samples were further evaluated. In particular, a small number (<5% of positive samples tested) were HR+ in the HPV ASR assay, but typed as LR types 67 and 70 by HPV GENO, indicating some cross-reacting LR types. The majority of equivocal samples were HPV GENO negative. Thus, the data collected from this comparison allowed refinement of the positive cut off values and better definition of the equivocal zones for the individual master mix pools in the HPV ASR assay. The HPV GENO results were also used to evaluate the distribution of genotypes in our patient population. HPV HR type 16 is most frequent, followed by types 39 and 51. HR type 66 was seen more commonly than expected. Mixed infections (multiple HR or HR plus LR) are more frequent than single genotype infections. **Conclusions:** While

the clinical role of HPV genotyping beyond types 16 and 18 is not entirely clear, it can be a useful tool in the clinical laboratory to resolve difficult interpretive situations as well as to monitor both HR and LR type frequency.

ID07. Influenza A Subtyping: Seasonal H1N1, H3N2, and the Appearance of Novel H1N1

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Background: In recent years, the molecular detection of influenza viruses has become routine, and is helpful in quickly identifying appropriate patients for antiviral therapy. However, antiviral resistance, especially to Tamiflu, has increased to encompass the majority of seasonal influenza A infections. Resistance was seen only in H1N1 isolates this year, making the differentiation of influenza A strains based on the H type clinically useful for determining appropriate antiviral therapy. **Methods:** Viral RNA was prepared from nasopharyngeal swab samples using an EasyMag nucleic acid extractor (Biomerieux, France). Detection of influenza A and B was performed on the Roche LightCycler using a commercially available ASR assay (Eragen, Madison, WI). FluA+ samples were reflexed to a second real-time PCR assay targeting the matrix protein gene (Stone et al., J. Virol. Methods 117:103–112, 2004) to determine subtype based on differential melting of FRET probes. The assay was validated in cooperation with the Illinois Department of Public Health (IDPH). Approximately 5°C difference in Tm was observed between seasonal H1N1 and H3N2. **Results:** From 2/17/09 to 4/21/09, 550 nasopharyngeal specimens were tested and 103 FluB+ and 107 FluA+ samples were identified, of which 86 were H1N1, 6 were H3N2, and 15 had very low Tm values that were distinctly different from seasonal H1N1 or H3N2; results paralleled the rates reported by the CDC and were useful in guiding antiviral use. On 4/22/09, a FluA+ sample yielding a unique subtype Tm intermediate between the H1N1 and H3N2 reference Tm was noted. This Tm value matched that predicted from the sequence of the 2009 Novel Influenza A H1N1 (swine) virus. This and subsequent samples were sent to the IDPH and confirmed as Novel H1N1 using the CDC assay performed at IDPH. From 4/22/09 to 05/23/09, 1,917 specimens were tested, and 362 (18.9%) were identified as probable Novel H1N1 using the subtyping assay; same-day diagnoses of Novel H1N1 infections were possible. **Conclusions:** Influenza A subtyping via sequence variations in the matrix protein gene was useful in guiding clinicians to appropriate antiviral therapies, and also in rapidly identifying the Novel Influenza A H1N1 strain in the community.