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Posters III: Sexually transmitted infections

PIII-1
Frequency and genotyping of human papillomavirus in pap smear samples

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Human papillomavirus (HPV) is associated with pre-cancerous cervical lesions. In this study, we determined the frequency and genotyping of HPV in pap smear samples dating from the January 2006 to June 2009. We used PCR method to detect HPV DNA using MY09/11 L1 consensus primers and to genotype using type 6, 11, 16, 18, 31, 33, 35, 45, 52, 58 specific primers. 80 (40.82%) of the 196 samples were positive for HPV DNA. Of total HPV DNA positive samples, 37 (46.25%) were found single type, 35 (43.75) were found multiple type for HPV and 8 (10%) were found any type with existing primers. Distrubution of the positivity of HPV genotypes were found as follows: for HPV 6, 33.75% (n = 27); HPV 16, 23.75% (n = 14); HPV 11, 11.50% (n = 19); HPV 18, 3.75% (n = 3); HPV 31, 3.75% (n = 3); HPV 33, 2.5% (n = 2); HPV 35, 5% (n = 4); HPV 45, 3.75% (n = 3); HPV 52, 7.5% (n = 6); HPV 58, 1.25% (n = 1). In conclusion, this study provided information on the frequency of genital HPV infection of women in patients admitted to our hospital. However, further studies including large number of populations are needed for determination of real prevelance of genital HPV infections in our country.

PIII-2
Diagnosing human papillomavirus and human papillomavirus type 16 by real-time PCR in patient undergone to colposcopy and significance of the diagnosis

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Human papilloma virus (HPV) is a health problem as a sexually transmitted disease and as a critical factor in the pathogenesis of cervical cancer. The aim of this study is to detect HPV and HPV type 16 by Real-Time PCR from Colposcopy Patients in Obstetrics and Gynecology Clinic in our hospital. DNA extraction was performed by phenol-chloroform-isoamylalcohol method from smear samples. MY09/11 primer set was used for PCR amplifications, Real time nested amplifications of MY09/11 products were done by GP5+/GP6+ primers and Cyanine-5 labeled HPV DNA specific probe. Real time PCR product analysis was done by melting curve analysis on LightCycler Software version 3.5.3 (LC 2.0 Roche Diagnostics, Germany). Melting peaks of 78–82ºC showed the detection of HPV DNA in the sample. HPV positive MY09/11 amplicons were sequenced by OpenGene® automated DNA sequencing system. Eventually, a statistically significant difference determined between positivity HPV and HPV 16 in patients that positive in result of colposcopic observation. Also, no statistically significant difference could be detected among of pregnancy number, result of PAP smear test, marital status and age in patients (X^2=8.43, p = 0.014, X^2=0.21, p = 0.899, X^2=0.75, p = 0.687, respectively). Diagnosis of HPV is now of high importance, which is recognized among most significant factors of cervical cancer. In our study, a statistically significant relation was found between colposcopic findings and prevalence of HPV. As a result detection of HPV and HPV type 16 is very important in follow up of colposcopy patients.

Posters IV: Respiratory viruses infections

PIV-1
Respifinderplus for the diagnostics of respiratory infections with a focus on the 2009 A/H1N1 pandemic

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Objectives: We evaluated RespifinderPLUS (RF), a commercial assay detecting Influenza-A/B, Influenza-H5N1, RSV-A/B, Parainfluenza-1–4, Coronavirus-OC43/229E/NL63, Rhinovirus, Adenovirus, Metapneumovirus, Chlamydia pneumoniae, Mycoplasma pneumoniae, Legionella pneumophila and Bordetella pertussis.
Methods: We tested 283 samples from external quality control (QC) panels (n = 79), prospectively collected patients samples (n = 118) from winter 2007/08 analyzed with isolation (ISO), real-time PCR or antigen testing (AG), as well as samples from suspected "swine flu" A/H1N1 cases (n = 86) collected since April 2009. Turnaround time was 30 h; hands-on time was 3 h.
Results: Of 79 QC samples covering all 19 targets, 72 (91%) gave correct results. False negative results were noticed for Legionella QC samples below 30,000 CFU/ml.
Of the 118 patient samples, 22 were positive with both RF and conventional assays; 20 were positive with RF only; 76 were negative. Compared to specific RSV and Influenza-PCR (103 samples), RF had a specificity of 98% and a sensitivity of 95%. In this collective, RF significantly increased the proportion of identified pathogens compared to classical methods alone (36% vs. 19% positive samples).
In the 86 samples of suspected new Influenza A/H1N1, the proportion of positive samples was higher (65%): 31 Rhinovirus, 7 Influenza-A (all A/H1N1), 4 Metapneumovirus, 3 Influenza-B, 2 Parainfluenza, 2 Coronavirus, 1 RSV-B, 1 Mycoplasma pneumonia, 30 negative.
Conclusion: Despite limitations due to the lower sensitivity for Legionella, RF is a great asset to the laboratory diagnostics of respiratory infections, with high specificity, sensitivity comparable to PCR, broad coverage of pathogens, as well as limited hands-on and turnaround times.

PIV-2
The Ibis T5000 for high-throughput identification and discrimination of the pandemic H1N1 influenza virus in Chicago

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The emerging pandemic influenza virus (pH1N1) necessitates a rapid, accurate, high-throughput assay for diagnosis. We began seeing influenza-like disease in late April, some with non-typeable influenza A by in-house commercial multiplex PCR assays. Department of Public Health (DPH) testing resulted in delays in confirmation. We sought to demonstrate the ability of Ibis T5000 technology to detect pH1N1, determine when the virus began circulating in Chicago and characterize the epidemiology of the virus in the area. The Ibis T5000 couples nucleic acid amplification by PCR with electrospray ionization mass spectrometry.
Our study identified samples positive for influenza between 18/12/2008–19/5/2009 by two PCR assays, PF (Proflu-1, Prodesse) and RVP (Respiratory Viral Panel, Luminex). Random selection of negative samples were run to calculate sensitivity and specificity. The comparative gold standard for influenza A diagnosis was one positive commercial PCR assay, and for pH1N1 additional DPH confirmation was needed. The T5000 assay was run in blinded fashion.
A total of 177 patient samples were assessed (PF=145, RVP=31, RVP+DPH=3). The earliest detected case was on 29/4/2009. The sensitivity and specificity for detected influenza A were 95% and 91% against PF, and 91% and 100% against RVP. The assay was 100% sensitive and specific in the identification of pH1N1. Thirty-eight unique pH1N1 strains were identified.
The T5000 is a highly sensitive and specific technology for identifying influenza A, in general, and pH1N1, in particular. The rapid, high-throughput format further underscores its use for routine epidemiologic surveillance of seasonal influenza and for monitoring of the current pandemic.