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26.8% end point consolidation. Chest X ray results by age group show that in the 3 months to ⩽1 year group the endpoint consolidation was reduced from 24.6% in the pre-vaccine group to 20% in the vaccine group although it was not statistically significant.. In the 1 to ⩽2 years group it was significantly reduced from 32.9% to 20.7%, 95% CI pre-post (3.5%, 20.9%) (p value <0.01) and in the 2 years to 59 months group from 41.5% to 31.9% (p value <0.02). From the 1504 patients who had an immunization record available, 605 out of 610 (99.2%) in the pre-vaccine group received no PCV7 and 584 of 894 (65.3%) in the vaccine group received at least 1 dose of the vaccine.
Conclusion: There is a marked decrease of endpoint consolidation between the pre-vaccine and the vaccine period, being endpoint consolidation the radiologic finding more related with S pneumoniae pneumonia. There is no decrease in the ED visits and hospital admissions of children with the diagnosis of first-episode of CAP for each of the periods. The increased number of admissions in the vaccine group for first episode of CAP can be due to an increased acuity in the visits to our hospital.

C – Virology I

O15

Application of multiplex PCR and fluid microbead-based assay to investigation of institutional outbreaks of viral respiratory disease

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Objective(s): The aim of this study was to investigate the use of molecular diagnostic assays for the detection of viral pathogens in institutional outbreaks. During the winter months from January to April 2008, specimens from 22 outbreaks of respiratory disease, investigated by public health departments in Saskatchewan, were tested at the Saskatchewan Disease Control Laboratory for viral pathogens.
Methods: 124 specimens were tested (mean 5.6 specimens per outbreak, range 2–12) using conventional virological methods (DFA and tissue culture) in addition to a multiplex PCR-fluid microbead-based assay (using the Abbott RVP kit on the Luminex 200) and real-time PCR assays.
Results: Pathogens were detected by multiplex PCR-fluid microbead-based assay in 79/124 specimens (63.7%). Of these, 60 specimens were positive for pathogens that were detectable by conventional virologic techniques. Viruses were detected in 22 specimens by DFA and in 18 by tissue culture. Multiple viruses were detected in 9 outbreaks. Real-time PCR assays were performed on all specimens from outbreaks in which pathogens were detected by multiplex PCR-fluid microbead-based assay, with the exception of coronaviruses and rhinoviruses/enteroviruses, for which assays were not available. Real-time PCR assays were positive in 56/60 (93%) specimens.
Conclusions: Using conventional techniques (DFA and tissue culture), pathogens were detected from patients in 11 outbreaks. However, a commercial multiplex PCR-fluid microbead-based assay demonstrated pathogens in all 22 outbreaks, of which seven involved only coronaviruses and rhinoviruses/enteroviruses, which were not detectable by conventional methods and for which real-time PCR assays were not available. A combination of conventional methods (DFA) and the multiplex PCR-fluid microbead-based assay would give the most rapid and complete detection of viruses in the outbreaks studied.

O16

Etiological diagnosis of respiratory outbreaks using a combination of antigen and nucleic acid amplification tests

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Objective: In this study, we evaluated the utility of the Luminex xTag™ Respiratory Viral Panel (RVP, Luminex Molecular Diagnostics), in combination with direct fluorescent antigen (DFA) testing and in-house developed nucleic acid amplification tests (NATs), to enhance our diagnosis of epidemiologically linked cases of respiratory infection.
Methods: Specimens from 244 suspected respiratory virus outbreaks were submitted to the Provincial Laboratory for Public Health (ProvLab, Alberta) for diagnostic investigation in 2006/2007 (n = 1108). DFA was performed as an initial screen for influenza virus (IFV) A, IFVB, parainfluenza (PIV) 1–3 and respiratory syncytial virus (RSV). DFA-negative NP samples and throat swabs were subjected to a panel of NATs, according to our routine testing protocol. Individual real-time NATs were directed against IFVA, IFVB, PIV 1–4, RSV, human metapneumovirus (hMPV) and respiratory adenoviruses (ADVs). Nucleic acid extraction was performed using the easyMAG® extractor (bioMérieux). Outbreak samples that gave a negative by DFA and in-house NATs were screened retrospectively by the RVP assay. Additional sequence-based analysis was undertaken for confirmation of samples positive for picornaviruses.
Results: Routine DFA/NAT testing provided an etiological diagnosis in 72.5% of respiratory virus outbreaks in 2006/2007 with 524 (47.3%) of samples having detectable virus using these methods. Outbreaks without an etiological diagnosis occurred predominantly outside of the main respiratory virus season (especially summer and autumn of 2007). Two hundred samples from 51 undiagnosed outbreaks were tested by RVP, resulting in 58 additional positive samples from 30 outbreaks. The majority of viruses identified by RVP were picornaviruses (n = 47), which were confirmed as rhinoviruses by sequencing. RVP also identified samples positive for coronaviruses (n = 9) in previously undiagnosed outbreaks. A combination of DFA, in-house NATs and RVP testing increased the etiological diagnosis to 90.8% for respiratory virus outbreaks in 2006/2007.
Conclusion: The Luminex RVP assay provides enhanced detection of an expanded range of respiratory viral targets when compared to the routine DFA/NAT testing. This is particularly useful in identifying the causes of respiratory virus outbreaks, ensuring appropriate management.

O17

Detection of influenza A virus resistance to oseltamivir by a single nucleotide polymorphism-based assay

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Background: The H1N1 subtype of influenza A virus has recently been recognized to manifest increasing prevalence of the H274Y mutation in its neuraminidase gene (NA) that is associated with resistance to Oseltamivir. An assay capable of rapidly detecting this mutation is essential for the appropriate utilization of Oseltamivir.
Methods: An assay to detect a single nucleotide polymorphism (SNP) in influenza virus in a clinical specimen was developed based on hybridization with 2 probes; one containing the wild type nucleotide and labeled with VIC and the second containing the mutant nucleotide and labeled with FAM that had been designed by ABI. Nucleic acid from clinical samples was extracted using the bioMérieux EasyMag. Samples were tested by real time RT-PCR and real time SNP assays on an ABI 7900. The SNP assays were performed using the Invitrogen Superscript III RT and the Platinum Taq polymerase as a one-step or two-step reaction. A second two-step reaction was carried out using the proprietory ABI polymerase. An aliquot was also subjected to RT-PCR using the Qiagen 1-step RT-PCR reagent followed by cycle sequencing on an ABI 3100.
Results: Aliquots of RNA from a total of 30 nasal swab specimens, collected between Nov 12, 2008 and Jan 7, 2009, in which influenza A/H1N1 virus was detected were sub-typed by the RT-PCR assay and subjected to a one step and two step RT-PCR SNP assays and the nucleotide sequence of the relevant portion of the NA gene was analyzed. When tested by the two-step SNP assay, 29 had the resistant mutation (H274Y) and none were shown to be the sensitive wild type. When tested by the one-step assay, the resistance mutation was