Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Presentation at ESCV 2015: Poster 1

Detection of viral pathogens of the lower respiratory tract using a comprehensive multiplexed PCR-based system in two sample matrices

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Background: Accurately diagnosing the etiologic agents of lower respiratory tract infections (LRTI) requires screening for multiple pathogens including bacteria, viruses, and fungi in complex sample types like sputum, endotracheal aspirates (ETA) and bronchoalveolar lavage (BAL). Many potential LRTI pathogens are opportunistic bacteria and actionable diagnosis requires determining clinically significant levels of these organisms. Co-infections can also add to the diagnostic challenge of pneumonia. To address this unmet need in LRTI diagnostics, BioFire Diagnostics, LLC is developing the FilmArray® LRTI Panel; a multiplexed PCR-based test for use on the FilmArray platform. This system will provide a comprehensive result, including bacteria, viruses and fungi, in approximately 1 h. In this study, a research use only prototype of the FilmArray LRTI panel was used to identify the prevalence of adenovirus, coronavirus, human metapneumovirus, influenza A, influenza B, parainfluenza virus, and respiratory syncytial virus in two sample matrices: sputum-like (sputum and ETA) and BAL-like (BAL, mini-BAL, and bronchial wash). The prevalence of viral detections as well as the incidence of co-detections with bacteria and fungi were assessed for both sample types.

Methods: This study was conducted using residual fresh sputa, ETA, and BAL that were submitted for standard care testing. Specimens were collected at the Medical College of Wisconsin during November 2014–May 2015. All specimens were tested on the prototype FilmArray LRTI panel. A subset of specimens found to be positive for viruses were verified by another molecular method when a physician specified request for a viral test was not submitted to the laboratory. To date, 156 sputum and 130 BAL-like specimens were tested by FilmArray for this study.

Results: Physician requested Nucleic Acid Amplification Tests (NAAT) were only requested in 3.5% (10/286) of all specimens. Of these, all but one NAAT returned negative results. FilmArray confirmed these findings and identified additional viruses in in 18.6% (29/156) of sputum-like and 17% (22/130) of BAL-like specimens. Results from a subset of these viral positive specimens were confirmed by additional PCR assays. Additionally, 62% (18/29) of sputa specimens and 63.6% (14/22) of BAL specimens positive for virus were found to have a co-detection with a bacteria or fungus.

Conclusions: The FilmArray can accurately and efficiently detect viral pathogens in lower respiratory sample matrices. These findings highlight the advantages of the multi-target approach offered by the FilmArray LRTI panel where standard of care testing might fail to accurately report incidence of viral pneumonia.

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Synergistic effect, in human monocytic cell-lines, of Influenza and *Staphylococcus aureus* on inflammation activation and cytotoxicity

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Background: According to WHO, bacterial pneumonia developed during influenza infection is a frequent cause of death. The severity of these co-infections involves a detrimental host innate immune response through the hyper-activation of several cellular immune pathways, including the NF-KB pathway. To explore this, in vitro studies may be valuable tools. This study addressed the specific question of a synergistic impact of influenza and *S. aureus* toxins on pro-inflammatory cytokine production and subsequent cytotoxic effects on human monocytes.

Methods: First, we explored NF-KB pathway activation by *S. aureus* virulence factors on influenza exposed and non-exposed human monocytes. To measure this, we used THP1-XBlue cells, monocytes transfected with a reporter plasmid expressing a secreted embryonic alkaline phosphatase (SEAP) under the control of a promoter inducible by NF-KB. Briefly, THP1-XBlue cells were exposed for 24 h to several concentrations of *S. aureus* virulence factors (Panton Valentine leucocidin (PVL), phenol soluble modulin α 1 and 3 (psmα1, psmα3), α-hemolysin (Hla), cell wall components (heat killed *S. aureus*, HKSA), protein A) and to influenza A/PR8/34 (H1N1). We compared these results with the cytotoxicity of *S. aureus* virulence factors measured on influenza exposed and non-exposed ex-vivo human monocyte cells. Direct cytotoxocity of Influenza A/PR8/34 (H1N1), PVL, protein A, psmα1, psmα3, Hla, and HKSA was also evaluated on the U937 cell line (a human leukemic monocyte lymphoma cell line) by cell quantification and Propidium Iodure (PI) incorporation.

Results: A dose-dependent activation of NF-KB pathway was observed for influenza, PVL, psmα1 and protein A. Protein A was the more potent activator. Psmα1 concentrations needed to activate NF-KB were superior to those produced in vivo by *S. aureus*. A synergistic activation of NF-KB was observed with influenza and PVL (5-fold) or influenza and protein A (3-fold) on activation vs influenza, PVL or protein A alone. A cytotoxic effect was observed at 24 h post-exposition to influenza and a significant increase in monocyte necrosis was induced by co-exposition of monocytes to influenza and PVL (PI incorporation increased by 2–3 fold).

Conclusions: We confirm the synergistic impact of influenza and *S. aureus* toxins on pro-inflammatory cytokine production and cytotoxic effects on human monocytes. The most potent cytotoxic synergy is observed between influenza and PVL whereas we observed only a pro-inflammatory synergy between influenza and protein A. These results will contribute to better understand the physiopathology of influenza-induced bacterial pneumonia.

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