Advanced techniques for detection and identification of viral contaminants using the Ibis Universal Biosensor

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Background:
Adventitious viral agents represent a major risk associated with the use of cell-substrate derived biologicals, including vaccines and antibodies. Current practices for definitive detection of viral agents rely on culture or antigen-specific antibody testing, which are time-consuming. We demonstrate the use of Electrospray Ionization Mass Spectrometry (ESI-MS), which relies on base composition signatures obtained from PCR amplification of broadly conserved regions of viral genomes, as an innovative approach to identification of common and emerging viruses.

Methods*:*
Ibis has developed PCR assays targeting over 50 different RNA and DNA viral families. These provide varying degrees of detailed characterization for each group of targeted viruses. We will describe the use of these assays for the identification of viruses from blinded specimens obtained from collaborators in the biopharmaceutical industry. Samples included characterized viral isolates spiked into various cell substrate matrix (e.g. CHO, Vero, MDCK, MRC-5) or in plasma. We will also demonstrate the use of this assay for identifying a previously unknown "mystery virus" that had been extremely difficult to characterize by existing methods.

Results*:*
We tested all samples against the broad viral primer panels described above. Results were analyzed using Ibis' genomic signature analysis software and visualization tools. The blinded study included viral species from four different groups: Parvovirus (MMV), Retrovirus (X-MuLV), Cardiovirus (EMCV) and Reovirus (ReoIII), at two spike levels (20,000 and 200,000 copes/ml) in three different cell matrices (Vero, CHO and MRC-5). PCR/ESI-MS correctly identified MMV when present at both dilutions. In contrast, there was no detection of X-MuLV in any of the samples. Remaining two viral species in the blinded panel EMCV and ReoIII were outside the purview of the original assay. A secondary panel that included Cardiovirus and Reovirus targets was later used to confirm detection of EMCV and Reovirus in these samples. Analysis of the sample containing the "mystery virus" revealed presence of Bluetongue virus. The control samples with or without a cell-line background showed no viral detections. These findings were later independently confirmed, demonstrating the utility of the technology in virus discovery.

Conclusion*:*
The innovative ESI-MS technology rapidly and accurately detects viruses using a broad virus detection assay. This technology has previously been tested extensively in a variety of applications for mycoplasma, bacterial and viral detection where we have demonstrated successful detection of low abundance pathogens in complex nucleic acid backgrounds. The utility of this technology to detect adventitious agents to ensure safety of biological products warrants further evaluation.

Key Objectives
Description of a novel technology platform with broad utility in the biotechnology industry.
Take Home Benefits:
Significant advancement in the area of viral detection that can be applied to virus safety testing.
Target Audience:
Pathogen Safety, Virology, Virus discovery, Regulatory, Process Development, Quality Assurance, Quality Control.