Safety Evaluation of Biotech-derived Pharmaceuticals such as Papillomavirus Recombinant Vaccine Candidate

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A number of recombinant proteins isolated from cell sources are being produced for biopharmaceuticals. Recombinant human papillomavirus (HPV) type 16 L1 virus-like particles (VLPs), recombinant protein produced in Sf9 cell is a HPV subunit vaccine candidate, which has been studied as a preventive vaccine of cervical cancer. However, biopharmaceuticals including biotech-derived recombinant proteins, gene and cell therapeutics and tissue engineered products could be contaminated with impurities including adventitious viruses, oncogenic DNA, mycoplasma, endotoxin etc. To establish the safety evaluation technologies, we have been studing viral clearance validation of insect cell derived recombinant HPV type 16 L1 VLP using Japanese encephalitis virus (JEV) and Bovine viral diarrhea virus (BVDV). The downstream process for the production of recombinant HPV-16 L1 VLPs was sequentially carried out employing detergent lysis (NP-40/PBS), sonication, sucrose cushion centrifugation, and cesium chloride (CsCl) equilibrium density centrifugation. Recombinant HPV-16 L1 capsid protein (56 kDa) expressed in Sf9 cell culture was clearly detected by SDS-PAGE and Western blotting analysis. Each purification step was evaluated to determine reduction factor for viral clearance by infectivity assay and real-time RT-PCR. The results suggest that the purification procedure employed in this study for HPV-16 L1 VLPs produced from recombinant baculovirus-infected Sf9 cells will be effective over 10 log $TCID_{50}$/pool reduction factor in the clearance of enveloped, adventitious viruses with a buoyant density lower than approximately 1.23 g/ml (*Biologicals 2003; JBV 2004 in press*). Residual DNA in recombinant biopharmaceuticals also could be a risk factor and must be evaluated and removed to meet the regulatory guidelines. For example, WHO guideline requires that the residual DNA should be lower than 100 pg/dose. Evaluation could be carried out both for the downstream process and for the final product. This presentation also describes the quantification of residual DNA during purification of recombinant HPV type 16L1 VLPs. We have developed a precise assay based on dot blot hybridization using digoxigenin random primed labeled DNA probes for the detection and quantification of residual DNA in five different process samples and final products. This study shows a safer and more sensitive alternative to radioactive techniques employed for residual DNA quantification and represents method validation data demonstrating precision and reproducibility (*manuscript in prep.*).

Key words: Virus safety evaluation, Virus clearance validation, Recombinant HPV-16 L1, sidual DNA quantification