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Viral metagenomic analysis to complement the viral risk assessment and adventitious agent testing of live virus vaccines

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NGS-based virus metagenomic testing of our live virus vaccines has been performed at the characterization level for regulatory filings. These studies further inform the viral risk assessment for rapid development of accelerated programs, complement traditional *in vivo* and *in vitro* testing for contaminating viruses, and supplement late-stage adventitious virus agent testing.

Bioinformatic analysis for metagenomic studies was executed using the High-performance integrated virtual environment (HIVE). Recent efforts to automate analysis applicable to GMP studies led to the development of an internal application, ViruScreen, with customized workflows for short nucleic acid sequencing reads. ViruScreen provides user-friendly solutions for complex computations. Analysts perform basic curation of the reference viral database prior to execution of read alignments. Further, ViruScreen has built-in capability for *de novo* assembly to align longer contigs to the viral database. In addition to a graphic user interface, a downloadable report is generated.

We used *in silico* datasets and nonGMP Illumina sequencing data of the dengue virus vaccine to compare the HIVE and ViruScreen pipelines. Datasets were used to assess detection of the WHO viral reference panel available for adventitious virus detection studies. Results of short read alignments demonstrated similar specificity and sensitivity between HIVE and ViruScreen indicating that our internal application is suitable to perform bioinformatic analysis to identify potential contaminants.

Because bioinformatic analysis varies depending on the specific study needs, ViruScreen includes an additional option of *de novo* assembly on the sequence reads. ViruScreen data indicate that using both short read and contig alignments provides comprehensive analysis for detection of potential low-level adventitious viruses and increases the specificity of detection. Taken together, the present study charts progress to implement a robust virus detection analysis for products such as live virus vaccines as health authorities and new guidelines support the use of molecular technologies for regulatory filings.