**Virus Stocks Serving as Ready-to-Use Well Characterized Challenge Material (WCCM) for Animal Challenge Studies**

Ashley Castens, Heather Couch, Sujatha Rashid, and Rebecca Bradford

**ABSTRACT**

The National Institute of Allergy and Infectious Diseases (NIAID) supports the development of novel therapeutics and biological inhibitors as part of its mission to combat infectious diseases. The NIAID In-Vitro Assessment of Antimicrobial Activity (IVAAA) program supports this mission by providing antiviral screening assays and seeks to improve production capacity while ensuring genomic homogeneity through Well Characterized Challenge Material (WCCM) for use in therapeutic product development. ATCC Federal Solutions has supported NIAID through the development of methodologies to produce and characterize WCCM that met stringent quality specifications. These high-quality virus stocks have shown a decreased number of induced changes in cellular function which are known to affect assay reproducibility. ATCC has optimized production methods through analyses of multiple cell lines and the selection of those that maintain the quality of progeny virus. Other optimization parameters include cell seeding density, the multiplicity of infection (MOI), time to harvest, genome equivalents determination, genome sequencing, variant analysis by next-generation sequencing (NGS), titer by TCID50, and/or plaque assay, endotoxin content, sterility, and mycoplasma detection. The WCCM stocks (e.g., Nipah, Lassa, MERs-CoV viruses) will be made available to qualified researchers through the BEI-funded BEI Resources program.

**METHODS (cont.)**

1) **Optimization Study 1:** Up to five or six cell lines, originating from both human and non-human primates (NHPs), were evaluated for permissiveness to viral infection and performance characteristics for the production of WCCM. The requirements in Table 1 were evaluated for each cell line and used to identify the cell line and condition that resulted in optimal virus production, including those least likely to generate impactful mutations.

2) **Optimization Study 2:** Optimal conditions were selected based on the results from optimization study 1. Additional optimization studies would have occurred if the optimal conditions could not be narrowed down.

3) **Engineering Runs:** The optimal cell line was scaled up, simulating the final production runs while refining the optimized conditions.

4) **Production Runs:** Large-scale WCCM virus stocks (>1L per production run) were produced. Figure 2 shows CPE/Viral growth pictures of a production run of MERs Coronavirus, Jordan 2012 WCCM.

**RESULTS**

Following production, quality control testing of the virus stocks was performed, including NGS, genome equivalence determination, titer/potency: >10^6 (plaque assay) and >10^5 TCID50/mL, sterility, endotoxin, and mycoplasma detection. ATCC performed data analyses and statistics on each engineering and production run for all lots of WCCM produced. QC tests were performed using WCCM specifications as test criteria. A Certificate of Analysis (CoA) was provided for each lot produced, and WCCM stocks are available upon request for use directly in vitro and in vivo experiments. In addition to test specifications, sequences and variants identified through bioinformatics are provided on every CoA. The final WCCM product produced by ATCC was deposited into the BEI Resources Catalog.

**SUMMARY**

Large-scale WCCM virus stocks were produced under conditions determined through optimization studies. Optimization was measured through infectivity via CPE, titer by plaque assay/TCID50, and nucleotide variants by NGS after one passage in the cell lines chosen for the study. A CoA was generated for all lots produced and included a description of the tests, specifications, and results for infectivity, genome variance, sterility, and mycoplasma. WCCM stocks are available upon request for use in vitro and in vivo experiments. To date, ATCC has produced, authenticated, and distributed the BSL-3 WCCM of MERs Coronavirus, Jordan 2012, and over 120 variants of SARS-CoV-2, and managed the production and distribution of the BSL-4 WCCM of Nipah Virus Bangladesh, Lassa Virus Josiah, and Marburgvirus Angola.

**ACKNOWLEDGEMENTS**

BEI Resources is funded under contract HHSN27220160013C by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services. The views expressed herein neither imply review nor endorsement by HHS nor by the U.S. Government.

© ATCC 2019. The ATCC trademark, trade name, any and all ATCC catalog numbers listed in this publication are trademarks of the American Type Culture Collection unless indicated otherwise.

© 2023 American Type Culture Collection. The ATCC trademark and trade name, and any other trademarks listed in this publication are trademarks owned by the American Type Culture Collection unless indicated otherwise.