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

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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 $TCID_{50}$, 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 NIAID-funded BEI Resources program.

METHODS

The development of WCCM occurred in four phases: Optimization Study 1, Optimization Study 2, Engineering Runs, and Production Runs, as illustrated in Figure 1.

Table 1. *Requirements evaluated for various cell lines to identify conditions for optimal virus production.*

Requirement	Optimization Criteria
Host Cell Line, Seeding Cell Density	Analysis of historically used cell lines and other published cell lines used for virus growth, appropriate cell density (e.g., 80% confluency for monolayers) for timed virus infection.
Optimal MOI Determination	Optimal MOI is determined by testing infectivity over a range of values between 0.1 – 0.00001.
Optimal Time to Harvest Virus	Cytopathic effect (CPE) is monitored daily (e.g., cell rounding, blebbing, syncytia formation, and/or cell death). When CPE is between 75% and 95%, harvest is initiated. Viruses without CPE are monitored daily and harvested on day 3 post-infection and every day thereafter until the cells become unhealthy.
Titer	Virus species dependent, >10e6 by plaque assay and >10e5 $TCID_{50}$/mL by $TCID_{50}$.
NGS for Species, Isolate, and Variant Detection	All stocks, passaged materials and production lots were evaluated for sequence variants.
Sterility Testing	Sterility was tested for 21 days (Harpo's HTYE broth incubated at 37°C and 26°C under aerobic conditions, Trypticase Soy Broth at 37°C and 26°C under aerobic conditions, Sabouraud Broth at 37°C and 26°C under aerobic conditions, Sheep Blood Agar at 37°C under aerobic conditions, Sheep Blood Agar at 37°C under anaerobic conditions, Thioglycollate Broth at 37°C under anaerobic conditions, DMEM with 10% FBS at 37°C under aerobic conditions).
Mycoplasma Testing	Mycoplasma presence was tested by agar and broth culture (14-day incubation at 37°C) and Mycoplasma DNA detection by PCR of extracted virus nucleic acid.
Endotoxin Testing	Endotoxin amount was determined by the Limulus Amoebocyte Lysate assay; ≤ 0.05 EU per mL

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.

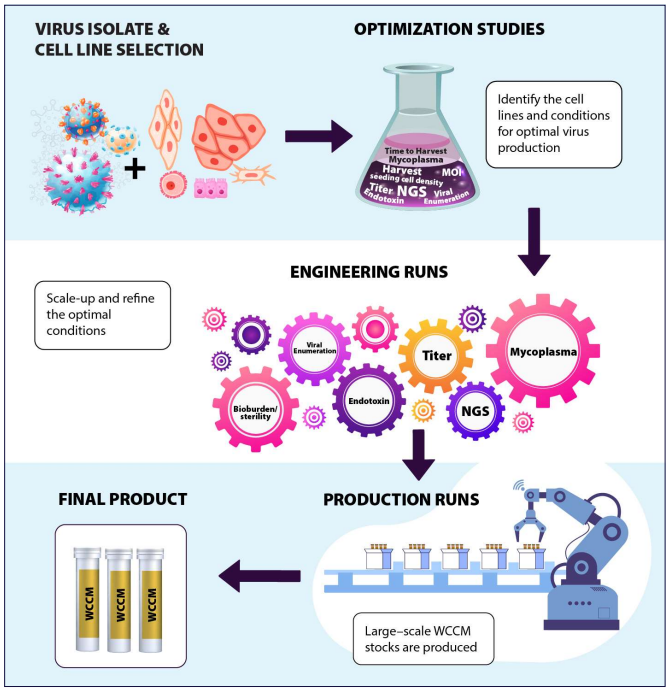
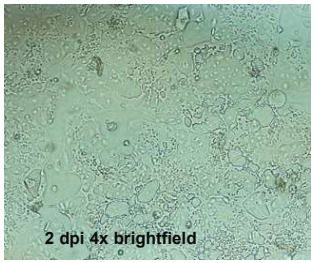


Figure 1. *Flow diagram of the four phases of the production of well characterized challenge material.*



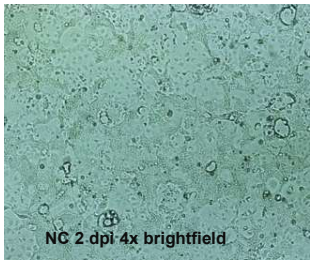


Figure 2. ***CPE/Viral growth pictures for MERS Coronavirus, Jordan 2012.*** *Growth was in Caco-2 cells at a seeding density of 3.50E+04, harvested on day 2 post-inoculation with an MOI of 0.00001 (CPE at harvest was between 60-90%).*

RESULTS

Following production, quality control testing of the virus stocks was performed, including NGS, genome equivalence determination, titer/potency: >10e6 (plaque assay) and >10e5 $TCID_{50}$/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/genome equivalence, titer via plaque assay/$TCID_{50}$, and nucleotide variance by NGS of the virus after one passage in the cell lines chosen for the study. A CoA was created 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 *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.

*WCCM virus stocks are produced in single-use vials and are ready to use for direct exposure for *in vivo* and *in vitro* assays only. They are not available for local expansion.

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