Sample Application 2R44TR002231-02A1 Redacted

NCATS is pleased to offer this valuable resource of sample applications to the small business community. Seeing how successful applicants have presented their ideas brings a wholly new perspective to our own application preparation. In that spirit, with the gracious permission of successful grant applicants, NCATS and other Institutes at NIH provide below samples of funded applications, and summary statements, data or resource sharing plans, leadership plans and more.

Always follow your funding opportunity's instructions for application format. Although these applications demonstrate good grantsmanship, time has passed since these grant recipients applied. The samples may not reflect the latest format or rules.

Electronic Cover Sheet		
PI: Personal Info	Title: Demonstrating Utility of a Non-Infectious Minute Virus of Mice Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development	
Received: 04/05/2019	Opportunity: PA-18-574 Clinical Trial:Not Allowed	Council: 10/2019
Competition ID: FORMS-E	FOA Title: PHS 2018-02 Omnibus Solicitation of the NIH, CDC, and FDA for Small Business Innovation Research Grant Applications (Parent SBIR [R43/R44] Clinical Trial Not Allowed)	
Personal Info	Dual:	Accession Number: 4293860
IPF: 10035919	Organization: MOCKV SOLUTIONS, INC	
Former Number:	Department:	
IRG/SRG: ZRG1 IMST-F (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 2: Itemized Cost Year 3:	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Personal Info		PD/PI
Consultant Info		Consultant
		Consultant
		Consultant
Personal Info		Other (Specify)-Scientist
Subcontractor Info		Other (Specify)-Subaward Project Leader
		Technician
		Technician

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
TR002231

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT12768120

5. APPLICANT INFORMATION

Organizational DUNS*: Proprietary Info

Legal Name*: MOCKV SOLUTIONS, INC
Department:
Division:
Street1*: Proprietary Info
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix:
Position/Title:
Street1*: Personal Info
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: Personal Info Fax Number: Email: Personal Info

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** Personal Info

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Demonstrating Utility of a Non-Infectious Minute Virus of Mice Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development

12. PROPOSED PROJECT
Start Date* Ending Date*
10/01/2019 09/30/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MD-008

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix:

Position/Title: CEO

Organization Name*: MOCKV SOLUTIONS, INC

Department:

Division:

Street1*: Personal Info

Street2:

City*:

County:

State*:

Province:

Country*:

ZIP / Postal Code*:

Phone Number*: Personal Info Fax Number: Email*: Personal Info

15. ESTIMATED PROJECT FUNDING

	Itemized Cost
a. Total Federal Funds Requested*	
b. Total Non-Federal Funds*	
c. Total Federal & Non-Federal Funds*	
d. Estimated Program Income*	

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix:

Position/Title*: CEO

Organization Name*: MockV Solutions, Inc.

Department:

Division:

Street1*: Personal Info

Street2:

City*:

County:

State*:

Province:

Country*:

ZIP / Postal Code*:

Phone Number*: Personal Info Fax Number: Email*: Personal Info

Signature of Authorized Representative*
Personal Info

Date Signed*
04/05/2019

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: MOCKV SOLUTIONS, INC
Duns Number: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Proprietary Info

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Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Chromatan Corporation
DUNS Number: Subcontractor Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Subcontractor Info

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Funding Opportunity Number: PA-18-574. Received Date: 2019-04-05T15:30:56.000-04:00

Contact PD/PI: Personal Info

Project/Performance Site Location 2

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: REGENXBIO Inc.
DUNS Number: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Proprietary Info

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Project/Performance Site Location 3

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: WuXi Advanced Therapies
DUNS Number: Proprietary Info
Street1*:
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:
Project/Performance Site Congressional District*: Proprietary Info

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Additional Location(s) File Name:

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	PROJECT_SUMMARY.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	RS_and_CP_references.pdf
10.Facilities & Other Resources	MockV_Facilities_and_Other_Resources.pdf
11.Equipment	MockV_Solutions_Equipment.pdf

Funding Opportunity Number: PA-18-574. Received Date: 2019-04-05T15:30:56.000-04:00

PROJECT SUMMARY/ABSTRACT

MockV Solutions, Inc. (MockV) is an early stage company creating viral-surrogate tools that solve the unmet needs of scientists as they develop and optimize products and techniques in a variety of industries that currently rely on expensive and logistically challenging live virus analysis. Virus is a contaminant of great concern during biopharmaceutical production. Because of this concern, international regulatory agencies require proof that manufacturing steps such as chromatography or nanofiltration can effectively remove virus. This is accomplished through small scale “spiking studies” whereby model viruses (ex. Minute Virus of Mice) are artificially introduced into biopharmaceutical material and removed or inactivated by purification techniques. These studies require specialized Biological Safety Level laboratories (BSL) and trained personnel resulting in costs that can soar well above $100,000. Due to these hurdles, most companies delay assessments, thereby increasing the risk of failure leading to cost over-runs and delayed regulatory approval which can cost a company valuable revenue/patent life and cost patients timely access to therapies. MockV is developing a BSL-1 compatible, low cost MVM clearance prediction kit based on the novel use of Virus Like Particles (VLP’s) as non-infectious surrogates to live infectious MVM. Utilized during manufacturing process development, the “MVP Kit” would provide scientists with a unique tool to generate “real time” data on MVM clearance. With a “Quality by Design” approach, these scientists could more efficiently engineer their manufacturing process to clear MVM and determine the efficacy of process steps before investing significant resources in costly validation studies. The time and resources saved by the MVP Kit would translate into manufacturing cost of goods and increase the likelihood of passing regulatory hurdles. This project will fund efforts to advance the MVP Kit toward commercialization through product development and applications testing efforts. First, we will optimize MVP production through small scale cell expression screening experiments and downstream chromatography process development efforts. We will also establish analytical MVP Quality Control metrics. After scale-up, we will produce MVP to support applications testing. In this phase of the project, we will be collaborating with REGENX BIO and Chromatan, to assess the utility of MVP for gene therapy and continuous processing applications. During this phase, spiking studies will be conducted to compare the removal of MVP vs live MVM. The data generated could support the use of the MVP Kit in these two emerging and important markets. Finally, we will develop a second-generation proximity ligation assay for quantifying MVP in high throughput screening applications. After successful completion of this Phase II study we will conduct extensive beta testing prior to commercialization.

In this Phase 2 SBIR study, MockV Solutions plans to demonstrate the ability of its MVP Kit to predict viral clearance during gene therapy and continuous processing applications. Using this kit, scientists will be able to engineer more efficient biopharmaceutical manufacturing processes and predict viral clearance validation failures prior to expensive regulatory testing. These benefits will reduce biopharmaceutical manufacturing costs of goods and help ensure the timely regulatory approvals for life-altering therapies.

FACILITIES AND OTHER RESOURCES

The Specific Aims of this project will be centered around two main clusters –the greater Washington D.C. area (MockV and REGENXBIO) and the greater Philadelphia (ChromaTan, WuXi and Teva). Travel to the Washington D.C. area by ChromaTan, WuXi and Teva will not be necessary. Travel to Philadelphia will be necessary for REGENXBIO when conducting MVM spiking studies at WuXi; however it is common for biopharmaceutical companies to travel when performing CDMO studies. ChromaTan's Phase #3 milestones are funded through a Subaward and therefore day to day activities will be managed by ChromaTan personnel. Teva biopharmaceutical material will be delivered to ChromaTan's site, so no consistent travel on behalf of Teva to ChromaTan is required. MockV will visit the ChromaTan site on several occasions during Specific Aim #3 to maintain project focus and direction. In addition, bi-weekly teleconferences will be scheduled.

MockV, leases office space from BioHealth Innovations in Rockville, MD and lab space from BluePoint Bioscience in Ijamsville, MD. During the 2-year course of Specific Aims #1-4, MockV will continue to sub-lease laboratory space from BluePoint Bioscience LLC in Ijamsville, MD (20 miles from MockV's Rockville facility).

BluePoint Bioscience operates as a contract research laboratory that specializes in antibody development and recombinant protein production. The facility is fully equipped with the necessary resources to support the objectives outlined in Specific Aims #1-4, including: small scale MVP screening, scale-up of MVP production, MVP characterization, I-qPCR analysis, and PLA assay development. **BluePoint is operated by a single employee who has agreed to continue leasing space and providing access to all equipment for the duration of this project (see letter of commitment). To alleviate any concerns regarding competition for lab space and equipment, it should be pointed out that over the past year and half in which MockV has subleased, there has never been in issue with space or competition for shared equipment. As MockV grows, it is probable that a larger facility will be required. As lab space is plentiful along the I-270 corridor, this should not be perceived as an issue.**

Laboratory and Equipment: BluePoint Bioscience. LLC., maintains a Square Footage facility at Proprietary Info built in 1984. The facility is designed to support Research and Development and complies with the Frederick County requirements for occupancy. Certificate of Occupancy building permit number 51298, issued 05/07/2007. Available equipment includes:

- Laminar flow tissue culture hoods, 4 ft. (3)
- SterilCult Incubators (3)
- Reach-in Environmental Chamber
- Novex SDS PAGE and Western Blot devices (4)
- BioRad SDS PAGE and Western Blot devices (4)
- Molecular Devices ELISA plate reader
- Spectrophotometer
- Shaker/Incubator
- Eppendorf microfuge (2)
- Sorvall RT6000D swing bucket centrifuge
- Nikon inverted phase microscope (2)
- Olympus inverted phase microscope
- Nikon vertical fluorescence microscope
- Olympus BX-40 vertical fluorescence microscope
- Bio-Rad Biologic LP chromatography system, Chromatography cabinet
- -20C Freezer and Liquid nitrogen storage system

Offices and computer: BluePoint Bioscience's laboratory is equipped with Wifi internet access, centralized telephone and voice mail system, computers, laser printers, photocopiers and fax machines. They are connected by a Linux local area network (LAN) with a dedicated server, router, and network modem.

During Specific Aim #2 of this application, significant portions of work will be conducted at REGENXBIO, free of charge (see letter of commitment). REGENXBIO has leased Square Footage of space on the first and second floors of Proprietary Info located within the Shady Grove Life Sciences Center in Montgomery County, Maryland **(5 miles from MockV's office and 17 miles from the laboratory)**. This building is a state-of-the-art

facility that houses RegenX's laboratory facilities which include R&D, bioanalytical and small-scale production laboratories for Adeno-Associated Virus (AAV) work. To support the scientific work being done for Specific Aim 2, RegenX will utilize its upstream AAV production and downstream AAV purification capabilities.

Upstream AAV Production Lab

To generate AAV material for MVM vs. MVP spiking studies, large batches of AAV will be produced. This activity will be completed within the recently reconstructed upstream AAV production lab at REGENXBIO. Key capabilities, facilities and equipment include:

- Multiple single-use stirred-tank bioreactors, including: Sartorius BIOSTAT CultiBag STR Plus 2 x 50L and 1 x 200L, GE Xcellerex XDR 50L, Sartorius Biostat RM rockers
- Benchtop controllers, Sartorius biostat b-dcu II and Sartoirus Biostat B
- Stackable incubation shakers, Infors HT multitron pro shaker (6)
- Thermo Scientific Heracell CO_2 incubators
- Biological safety cabinets and chemical hoods: Nuaire labgard class II type A2 (5), PHCBI class II type A (3), Kewanunee supreme air (2)
- Analytical instrumentation, including: Nova Biomedical bioprofile flex2 osmo20 (2), Roche CedeX Bio HT, Beckman Coulter Vicell XR (4), mettler Toledo multiparameter pH/Cond (3)
- Refrigerators and -30/-80c freezers: Thermo Forma 4c (5), Thermo Revco -30c (5), Thermo TSX series -80c and PHCBI twin guard -80c.
- central RODI system for lab water
- central lab gas manifold system to include: Medical grade compressed air, Vacuum, Nitrogen, Oxygen, C02, O2
- centrifuges, scales, pumps, autoclave, sterilizer, acid neutralization tank and a monitoring system for temperature controlled environments.

Downstream AAV Purification Lab

To prepare cell culture AAV material for spiking studies and to conduct small scale MVP spiking studies, REGENXBIO will utilize their downstream purification laboratory. Key capabilities, facilities and equipment include:

- Cell culture harvesting equipment, including: Millipore MilliQ qPod depth filtration system (4)
- Resin and membrane chromatography systems, including: GE AKTA Avant (6), AKTA Pure (2)
- Tangential Flow Filtration systems, including: Spectrum Krosflow TFF system
- Analytical instrumentation to track step yield and product quality, including: Capillary Gel Electrophoresis - Beckman Coulter PA800, spectrophotometer – Agilent Technologies Cary 60 UV-Vis, Dynamic Light Scattering Wyatt Technologies Dynastar Pro Nanostar, ddPCR – Biorad qx200 droplet reader with c1000 touch thermal cycler and automated droplet generator

REGENXBIO MVM spiking studies will be conducted at WuXi AppTec in Philadelphia. It is common for biopharmaceutical companies to conduct CDMO work such as viral clearance studies away from their primary base of operations.

During Specific Aim #3, the MVP Kit will be evaluated for continuous processing applications. To accomplish this, MockV has subawarded Chromataon Corporation (see letter of commitment). Chromatan is a leading developer of continuous processing equipment and has leased Square Footage f laboratory space at Proprietary Info **(35 miles from Teva and 33 miles from WuXi)**. Within this space, two counter current tangential flow chromatography skids will be dedicated for testing purposes. In addition, the following capabilities and equipment will be made available:

- Steris Autoclave
- Akta Pure Chromatography System
- UV-Vis Spectrometer – Spectronic 1001+ (Milton-Roy)
- Swing-bucket centrifuge - GT-2 (VWR)
- RO water system APEC water systems

- Type 1 water system - Milli-Q+ (Millipore)
- Precision balances (Myweigh, AdamEquipment)
- pH and conductivity meter (Sper Scientific)
- Two chemical fume hoods (Labconco)
- Deep freezer (Model# 5704 VWR)
- Epoch microplate spectrophotometer (BioTek)
- A microplate spectrophotometer and HPLC is available for kinetics experiments and analytical chromatography measurements.
- Masterflex and Watson Marlow pumps and Pendotech pressure gauges are available for critical flux experiments.

In addition, Teva Pharmaceuticals USA, Inc. 145 Proprietary Info **(35 miles from ChromaTan and 31 miles from WuXi)** has agreed to provide in-process monoclonal antibody material in support of Specific Aim #3, free of charge (see letter of support).

To execute live viral clearance MVM spiking studies as part of Specific Aims # 2 and #3, facilities and laboratories at WuXi App Tec will be utilized, free of charge (see letter of commitment). WuXi App Tec has leased Square Footage of space at Proprietary Info (33 miles from ChromaTan and 31 miles from Teva). This building is a state-of-the-art facility that houses WuXi's laboratory facilities for performance of Early Phase Clinical Manufacturing and Testing. The testing facilities include laboratories for: Analytical Development, Cell Line Characterization, Lot Release, Materials Characterization, and Viral Clearance. To support the scientific work being done for Specific Aims 2 and 3 (in conjunction with RegenX and Chromatan), WuXi will utilize the capabilities of its Virus Purification and Viral Clearance Suites.

<u>Virus Purification Lab</u>
This space has been developed and outfitted to support the ongoing activities of the Viral Clearance Group. This lab is equipped for the downstream purification of multiple types and grades of virus stock that are used to carryout virus spiking studies in the Viral Clearance Suites. As such, the capabilities of and equipment contained within this lab include:

- Tangential Flow Filtration
- Column Chromatography using multiple modes of separation
- High Purity Water (Nano Pure D11901)
- Biological Safety Cabinets (Baker SterilGard III)
- pH Meters (Sartorius pHBasic+)
- Conductivity Meters (Orion Star A112)
- Chromatography Columns (GE Healthcare Lifesciences BPG 100, GE Healthcare Lifesciences XK50, Millipore Vantage)
- Tangential Flow Filtration Housings (Millipore)
- Ultra Low Temperature Freezers (Forma Scientific)
- FPLC Chromatography Skids (GE Healthcare Lifesciences, AKTA® Avant® 150 and AKTA Explorer® 100.)

<u>Viral Clearance Execution Suite(s)</u>
This space has been developed and outfitted to perform the ongoing activities of the Viral Clearance Group and Remote Viral Clearance Team. This lab is equipped for the performance of multiple, simultaneous, chromatographic steps and nanofiltration steps to carryout virus spiking studies. As such, the capabilities of and equipment contained within this lab include:

- Nanofiltration
- Column Chromatography with multiple modes of separation
- High Purity Water (Nano Pure D11901)
- Biological Safety Cabinets (Baker SterilGard III)
- pH Meters (Sartorius pHBasic+)
- Conductivity Meters (Orion Star A112)
- Ultra Low Temperature Freezers (Forma Scientific)
- FPLC Chromatography Skids (GE Healthcare Lifesciences, AKTA® Avant® 150 and AKTA Explorer® 100.)

- Nanofiltration pressure vessels (Asahi-Kasei)
- Refrigerated Cold Room
- Water Bath (Brinkman Ecoline)

Viral Clearance Analysis Suite(s)

This space has been developed and outfitted to perform the analysis of samples that are generated by the Viral Clearance Group and Remote Viral Clearance Team during the execution of Viral Clearance Studies. This lab is equipped for the performance of plaque assays and Quantitative Polymerase Chain Reaction (qPCR) analysis. As such, the capabilities of and equipment contained within this lab include:

- Plaque assays
- qPCR analysis
- High Purity Water (Nano Pure D11901)
- Biological Safety Cabinets (Baker SterilGard III)
- pH Meters (Sartorius pHBasic+)
- Ultra Low Temperature Freezers (Forma Scientific)
- Water Bath (Brinkman Ecoline)
- 2-8º C Refrigerators (Sanyo LabCool)
- 37º C Incubators (Thermo Steri-Cult)
- PCR System (ABI 7500 Fast)
- UV Spectrophotometer (NanoDrop 2000)
- Sonic Disruptor (Fisher Scientific Model 500)

OTHER RESOURCES

MockV is now a member and sponsor of the Membrane Science, Engineering and Technology Center, a NSF Industry/University Cooperative Research initiative comprised of academia and industry groups that work to provide effective solutions to real industrial problems through cutting edge research in the area of membrane science, engineering and technology. Through this organization, MockV will have the ability to submit grant applications that could further its progress toward commercialization and present data to other members - which include many of the strategic partners that MockV has engaged with for business development.

Board of Directors

Personal Info **– CEO**: Personal Info has over 10 years of experience in bioprocess development and project management. He founded MockV Solutions after spending seven years as a purification scientist at Human Genome Sciences where he participated in the manufacturing process development, characterization, validation, and technical transfer activities for monoclonal antibodies.

Personal Info **– COO**: Personal Info has over 20 years of operating and product commercialization experience within 3 three public companies representing the biotechnology, diagnostics and life sciences sector. During her tenure at Life Technologies she was responsible for the global portfolio as Senior Business Director and managed Joint Ventures and expansion within the Asia-Pacific region. Along with her role as Chief Operating Officer of MockV, Personal Info serves as an Entrepreneur in Residence at Johns Hopkins University and BioHealth Innovation, a position focused on translating research technologies to market. Personal Info holds a PhD from the University of Maryland and served as a Staff Fellow at the National Institutes of Health. She holds a Certificate of Financial Management from Cornell University and is a graduate of the Program on Leadership and Strategy in Pharmaceuticals and Biotech at the Harvard Business School.

Consultant Info **- Lead Scientific Advisor**: Consultant Info has 20 years of biotechnology and molecular virology experience. He is currently the director of the Aquaculture Pathology Laboratory at the University of Arizona. Consultant Info has spent much of his professional career working on developing diagnostics, oral vaccines and therapies, and genetic markers for viral disease resistance. He began working with MockV in 2013 and successfully led the scientific efforts to produce Mock Virus Particles.

Personal Info **- Director Chemistry Manufacturing and Controls**: has 40 years of industry experience and expertise relating to hybridoma development, animal sensitizations and maintenance, cell fusion, cloning, subtyping, production and purification of recombinant proteins, cell line cryopreservation, assay development and diagnostic kit manufacturing. As a manager for 15 years at Chemicon International, Inc. (now part of Merck Millipore), Personal Info created the Antibody Development Department involved in the production and characterization of over 4000 monoclonal and polyclonal antibodies and created the “Light Diagnostics” diagnostics division.

Personal Info **- Research Analyst**: Personal Info supports the Entrepreneurs-in-Residence program in evaluating the commercialization potential of new technologies and works with BHI's client companies to achieve strategic business development goals. He holds a B.S in Biomedical Engineering from Case Western and an M.S in Supply Chain Management from the University of Maryland.

SCIENTIFIC AND COMMERCIAL ADVISORY BOARD

Personal Info **- Scientific and Commercial Advisory Board**: Personal Info has gained over 20 years of experience in the development, characterization and validation of purification processes from early development through licensure while at Genentech, Human Genome Sciences, GlaxoSmithKline and MedImmune. While at MedImmune, Personal Info led a department of CMC Team Project Managers, driving the strategy and delivery of CMC plans for all molecules in the MedImmune portfolio. He currently serves as the Vice President, BioPharmaceutical Development at MacroGenics

Personal Info **- Scientific and Commercial Advisory Board**: Personal Info has over 25 years of biotechnology experience. He is currently the Chief Operating Officer of Adaptive Phage Therapeutics, a leading company in bacteriophage treatment for multi-drug resistant bacterial infections. Previously, Personal Info founded, grew and successfully sold two life science business. The first, Receptor Biology (sold to Perkin Elmer, Inc.), was the first US company that manufactured and sold GPCR reagents to the drug-discovery market. The second, Applied Cell Sciences (sold to ChanTest Corp), was a reagents and services company within the drug discovery market.

Personal Info **- Scientific and Commercial Advisory Board**: Personal Info has over 25 years biotechnology experience. He is currently the Scientific Director of Texcell N.A., a viral clearance CRO that conducts live viral spiking studies for the biopharmaceutical industry. Prior to this position, Personal Info served as a Scientist at SAIC/NCI and as a Project Manager for HIV Antiviral Drug Development at the Southern Research Institute.

Personal Info **Scientific and Commercial Advisory Board** Personal Info has over 20 years of biotechnology experience. He is currently the Director and Head of CMC Partnership at Viela Bio, a recent spin-out from MedImmune. Prior to this position, Personal Info served as Associate Director of R&D at MedImmune where he led a commercial purification process optimization group and managed viral clearance activities for all stages of project support.

In addition to its Board of Directors and Scientific and Commercial Advisory Board, MockV benefits from a partnership with **BioHealth Innovations, (BHI)**, a non-profit, private-public partnership catalyzing biotechnology growth in the Maryland region. Through their agreement, BHI offers MockV substantial business-related support at subsidized or no cost. Support includes, business, marketing legal, IP, and IT consulting, office space, accounting and banking support, and access to investor and professional networks.

MockV is located in Rockville, MD, a hub of scientific research and commercialization. Being so, MockV takes advantage of its proximity to biotechnology corporations and high-quality service providers. For example, MockV has conducted proof of concept collaborations with MedImmune (Gaithersburg), the NIH-VRC (Gaithersburg) and GSK (Rockville). MockV also enjoys the various Montgomery County and state led initiatives, programs and resources available for the biotechnology community including Montgomery County’s SBIR/STTR Matching Grant Program – the first such program in the country.

MockV Solutions Equipment

- Applied Biosystems 7500 Fast Real Time-PCR system with analysis software
- GE AKTA Explorer with Unicorn Software
- Pendotech PMAT3 Sensor Monitor
- Gilson micropipettes

Through a sublease agreement with BluePoint Bioscience, LLC, MockV Solutions will have access to the following equipment (located at BluePoint Biosciencel, Ijamsville MD) for Specific Aims #1-4:

- Laminar flow tissue culture hoods, 4 ft. (3)
- SterilCult Incubators (3)
- Reach-in Environmental Chamber
- Novex SDS PAGE and Western Blot devices (4)
- BioRad SDS PAGE and Western Blot devices (4)
- Molecular Devices ELISA plate reader
- Spectrophotometer
- Shaker/Incubator
- Eppendorf microfuge (2)
- Sorvall RT6000D swing bucket centrifuge
- Nikon inverted phase microscope (2)
- Olympus inverted phase microscope,
- Nikon vertical fluorescence microscope
- Olympus BX-40 vertical fluorescence microscope
- Bio-Rad Biologic LP chromatography system
- Chromatography cabinet
- -20°C Freezer
- Liquid nitrogen storage system (4)
- Low-speed and ultra-speed centrifuges

For Specific Aim #2, REGENXBIO will have access to the following equipment:

- Sartorius BIOSTAT CultiBag STR Plus 2 x 50L and 1 x 200L,
- GE Xcellerex XDR 50L
- Sartorius Biostat RM rockers
- Sartorius biostat b-dcu II and Sartoirus Biostat B
- Infors HT multitron pro shaker (6)
- Thermo Scientific Heracell CO_2 incubators
- Nuaire labgard class II type A2 (5)
- PHCBI class II type A (3)
- Kewanunee supreme air (2)
- Nova Biomedical bioprofile flex2 osmo20 (2),
- Roche CedeX Bio HT
- Beckman Coulter Vicell XR (4)
- Mettler Toledo multiparameter pH/Cond (3)
- Thermo Forma 4c (5),
- Thermo Revco -30c (5)
- Thermo TSX series -80c
- PHCBI twin guard -80c.

- Millipore MilliQ qPod depth filtration system (4)
- GE AKTA Avant (6)
- AKTA Pure (2)
- Spectrum Krosflow TFF system
- Capillary Gel Electrophoresis - Beckman Coulter PA800
- Agilent Technologies Cary 60 UV-Vis
- Dynamic Light Scattering Wyatt Technologies Dynastar Pro Nanostar
- ddPCR – Biorad qx200 droplet reader with c1000 touch thermal cycler and automated droplet generator

For Specific Aim #3, Chromatan Corp will have access to the following equipment:

- Steris Autoclave
- Akta Pure Chromatography System
- UV-Vis Spectrometer – Spectronic 1001+ (Milton-Roy)
- Swing-bucket centrifuge - GT-2 (VWR)
- Type 1 water system - Milli-Q+ (Millipore)
- Precision balances (Myweigh, AdamEquipment)
- pH and conductivity meter (Sper Scientific)
- Two chemical fume hoods (Labconco)
- Deep freezer (Model# 5704 VWR)
- Epoch microplate spectrophotometer (BioTek)
- Masterflex and Watson Marlow pumps and Pendotech pressure gauges

For Specific Aim #2,3, WuXi App Tec will have access to the following equipment:

- High Purity Water (Nano Pure D11901)
- Biological Safety Cabinets (Baker SterilGard III)
- pH Meters (Sartorius pHBasic+)
- Conductivity Meters (Orion Star A112)
- Chromatography Columns (GE Healthcare Lifesciences BPG 100, GE Healthcare Lifesciences XK50, Millipore Vantage)
- Tangential Flow Filtration Housings (Millipore)
- Ultra Low Temperature Freezers (Forma Scientific)
- FPLC Chromatography Skids (GE Healthcare Lifesciences, AKTA® Avant® 150 and AKTA Explorer® 100.)
- Biological Safety Cabinets (Baker SterilGard III)
- Nanofiltration pressure vessels (Asahi-Kasei)
- Water Bath (Brinkman Ecoline)
- PCR System (ABI 7500 Fast)
- UV Spectrophotometer (NanoDrop 2000)
- Sonic Disruptor (Fisher Scientific Model 500)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Personal Info Middle Name Last Name*: Personal Info Suffix:

Position/Title*: CEO
Organization Name*: MOCKV SOLUTIONS, INC
Department:
Division:
Street1*: Personal Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Personal Info Fax Number:

E-Mail*: Personal Info

Credential, e.g., agency login: eRA Commons Username

Project Role*: PD/PI Other Project Role Category:

Degree Type: MS,BS Degree Year: 2011,2006

Attach Biographical Sketch*: File Name: Personal Info

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-18-574 . Received Date: 2019-04-05T15:30:56.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Personal Info Middle Name Last Name*: Personal Info Suffix:

Position/Title*: Senior Scientist
Organization Name*: MockV Solutions, Inc.
Department:
Division:
Street1*: Personal Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Personal Info Fax Number:

E-Mail*: Personal Info

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Scientist

Degree Type: BS Degree Year: 1978

Attach Biographical Sketch*: File Name: Personal Info

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Consultant Info First Name*: Consultant Info Middle Name Consultant Info Last Name*: Consultant Info Suffix:

Position/Title*: Consultant
Organization Name*: The University of Arizona
Department:
Division:
Street1*: Consultant Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Consultant Info Fax Number:

E-Mail*: Consultant Info

Credential, e.g., agency login: eRA Commons Username

Project Role*: Consultant Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1992

Attach Biographical Sketch*: File Name: Consultant Info

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Consultant Info First Name*: Consultant Info Middle Name Last Name*: Consultant Info Suffix:

Position/Title*: Professor of Chemical Engineering
Organization Name*: The Pennsylvania State University
Department: Chemical Engineering
Division:
Street1*: Consultant Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Consultant Info Fax Number:
E-Mail*: Consultant Info

Credential, e.g., agency login: eRA Commons Username

Project Role*: Consultant Other Project Role Category:

Degree Type: Ph.D. Degree Year: 1985

Attach Biographical Sketch*: File Name: Consultant Info
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Subcontractor Info Middle Name Last Name*: Subcontractor Info Suffix:

Position/Title*: CEO
Organization Name*: Chromatan Corporation
Department:
Division:
Street1*: Subcontractor Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Subcontractor Info Fax Number:
E-Mail*: Subcontractor Info

Credential, e.g., agency login: eRA Commons Username

Project Role*: Other (Specify) Other Project Role Category: Subaward Project Leader

Degree Type: BS Degree Year: 2000

Attach Biographical Sketch*: File Name: Subcontractor Info
Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Consultant Info Middle Name Last Name*: Consultant Info Suffix:

Position/Title*: Consultant
Organization Name*: Self Consulting Company
Department:
Division:
Street1*: Consultant Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Consultant Info Fax Number:
E-Mail*: Consultant Info

Credential, e.g., agency login:

Project Role*: Consultant Other Project Role Category:

Degree Type: MS Degree Year: 2012

Attach Biographical Sketch*: File Name: Consultant Info

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Subcontractor Info Middle Name Last Name*: Subcontractor Info Suffix:

Position/Title*: VP of Engineering
Organization Name*: Chromatan Corporation
Department:
Division:
Street1*: Subcontractor Info
Street2:
City*:
County:
State*:
Province:
Country*:
Zip / Postal Code*:

Phone Number*: Subcontractor Info Fax Number:
E-Mail*: Subcontractor Info

Credential, e.g., agency login:

Project Role*: Technician Other Project Role Category:

Degree Type: BS Degree Year: 2002

Attach Biographical Sketch*: File Name: Subcontractor Info

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person				
Prefix:	First Name*: Subcontractor Info	Middle Name	Last Name*: Subcontractor Info	Suffix:
Position/Title*:	Associate Director of Process Sciences			
Organization Name*:	Chromatan Corporation			
Department:				
Division:				
Street1*:	Subcontractor Info			
Street2:				
City*:				
County:				
State*:				
Province:				
Country*:				
Zip / Postal Code*:				
Phone Number*: Subcontractor Info		Fax Number:		
E-Mail*: Subcontractor Info				
Credential, e.g., agency login:				
Project Role*: Technician		Other Project Role Category:		
Degree Type: BS		Degree Year: 2012		
Attach Biographical Sketch*:	File Name: Subcontractor Info			
Attach Current & Pending Support:	File Name:			

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Personal Info

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons Username

POSITION TITLE: PD/PI

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Maryland, College Park	B.S.	05/06	Cellular, Molecular Biology and Genetics
Johns Hopkins University	M.S.	05/11	Biotechnology

A. Personal Statement

The goal of this Phase 2 SBIR project is to advance the first Biosafety Level 1-compatible viral clearance kit toward commercialization through product development and applications testing efforts. The resulting “MVP Kit” would serve as non-infectious alternative to viral clearance studies during biopharmaceutical process development. We plan to optimize MVP production, test the utility of this approach for gene therapy and continuous processing applications and develop a second-generation quantification assay. This work builds well from the successful completion of our Phase 1 project, in which I served as PI. I have the expertise, leadership, motivation, and support necessary to successfully carry out the proposed work. I possess a fundamental knowledge base in protein purification and industrial biopharmaceutical process development. As a Purification Scientist at Human Genome Sciences and GlaxoSmithKline, I frequently designed, executed, and analyzed experiments aimed at developing, optimizing, validating or transferring our downstream biopharmaceutical purification processes. My role as a viral clearance specialist grew from my interest and participation in viral clearance studies and scale up. During these efforts I was frequently involved in project leadership roles and collaborations with contract research organizations, academic institutions and industry leaders. From 2010-2013 I served as the downstream Bulk Drug Substance representative for our companies lead commercial compound, Benlysta – which became the first approved therapy for treating Lupus in over 50 years. In this role I was responsible for strategically planning the cross function activities required to manufacture final drug product.

I founded MockV Solutions (MockV) in 2013 after spending six years developing and optimizing purification processes without a cost effective and accurate means of analyzing viral clearance. MockV is providing scientists with novel assay kits enabling them to easily study viral clearance efficacy accurately and economically during process development. I have organized a team of experts in virology, business, process development, and commercialization who understand the value of our mission. As CEO of this venture I have worked with each member of my team in laying the groundwork for our success. We have raised starting capital, been awarded a US Patent, received four grants (totaling $525,000), formed collaborations with leading biotech companies and federal agencies, published results in scientific journals, established strategic partnerships with key industry suppliers (e.g. Thermo, Sartorius, Asahi) and routinely present at major industry conferences. As a result of these experiences, I am aware of the importance of frequent communication among project members and the construction of realistic research plans, timelines, and budgets. This current SBIR application builds logically on the work I am now undertaking; however, to fill a gap in scientific knowledge, we will be relying on the expertise of several industry leaders and professionals: Consultant Info , Consultant Info and Consultant Info .

In summary, I have a fundamental understanding of the process development issue this project will address. I have a demonstrated record of successful project management and productive research within the fields of process development and viral clearance. My expertise and experience have prepared me to lead the proposed project and I highly value my team of collaborators who will be relied upon to guide this project to success.

1. **Cetlin, D.**, et al. (2018) "Use of a noninfectious surrogate to predict minute virus of mice removal during nanofiltration." *Biotechnology progress* 34.5: 1213-1220.

2. Johnson, S., Brorson, K. A., Frey, D. D., Dhar, A. K., & **Cetlin, D. A.** (2017). "Characterization of Non-Infectious Virus-Like Particle Surrogates for Viral Clearance Applications". *Applied biochemistry and biotechnology*, *183*(1), 318-331.

3. US Patent: **Cetlin, D**., Dhar, A. (2013). Methods and kits for quantifying the removal of mock virus particles from a purified solution. Publication Number US20150072339 A1. Filed Sep 9, 2014. Published Mar 12, 2015.

4. **Cetlin, D**. (2018). *Predicting MVM Clearance by Nanofiltration and Anion Exchange Chromatography Using a Non-Infectious Mock Virus Particle.* Presented at the 10th Annual Bioprocessing Summit, Boston, MA.

B. Positions and Honors

2006-2007	Senior Lab Technician, Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD
2007 -2012	Bioprocess Associate I-III, Department of Purification Sciences, Human Genome Sciences, Rockville, MD
2012-2013	Scientist II, Department of Purification Sciences, Glaxo Smith Kline, Rockville, MD
2013- Present	Founder and C.E.O. of MockV Solutions Inc.

C. Contributions to Science

My early scientific contributions at Human Genome Sciences, and later Glaxo Smith Kline, were primarily geared toward practical internal developments, not necessarily research intended for external publications. Nonetheless, I frequently assumed leadership roles in projects which resulted in industry wide improvements or novel techniques which were later published or presented on by adopters of the technology.

1. I was the Downstream Process Development project leader for a collaborative effort between GlaxoSmithKline and the University of Colorado which investigated the mechanisms of increased flux decay associated with nanofiltration. Through the course of this study, we found that freezing and thawing of a biopharmaceutical (typically employed for viral clearance validation) caused a substantial decrease in nanofilter performance. The impact of decreased nanofiltration performance adds significant costs to the manufacturing of biopharmaceuticals as nanofiltration is the second most expensive step in a typical process. Freezing and thawing also caused formation of aggregates and particles across a broad size range, including particles that could be detected by microflow imaging (≥1 μm in size). However, removal of these particles offered little protection against flow rate decline during viral filtration. Further investigation revealed that trace amounts of aggregates (ca. 10^{-6} of the total mass of protein in solution) approximately 20–40 nm in size were primarily responsible for the observed membrane fouling. These observations are valuable insights that could be used in viral clearance validation planning to maximize nanofiltration performance, ultimately leading to $/gram cost reductions for biopharmaceuticals.

    - Barnard, J., Kahn, D., Cetlin, D., Randolph, T., Carpenter, J., (2014). Investigations into the Fouling Mechanism of Parvovirus Filters During Filtration of Freeze-Thawed Monoclonal Antibody Drug Substance Solutions. Journal of Pharmaceutical Sciences, 103, 890-899.

2. Quality by Design, an approach to biopharmaceutical process optimization which utilizes risk assessments, design of experiments (DOE), and statistical data analysis, has gained traction within the biopharmaceutical industry since its introduction around 2008. In 2012 I was the Downstream Process Development project lead for a Quality by Design study aimed at defining optimal process parameters for nanofiltration operation. Through this study, I was able to significantly reduce the surface area of our manufacturing scale nanofiltration membrane (second most expensive step in a typical biopharmaceutical process), reducing the $/gram costs of the therapeutic. In addition, my Quality by Design approach to nanofiltration was useful to others in the industry who planned on executing similar strategies to reduce $/gram costs.

    - Cetlin, D. (2012, May.) Optimization of the Viresolve Pro in a mAb Purification Process: A Quality by Design Approach. Presented at the 1th Annual Millipore Viresolve Pro User Conference. Bethesda, MD.

3. I was the Downstream Process Development project leader for developing a Single Pass Tangential Flow Filtration (SP-TFF) step for a commercial biopharmaceutical manufacturing process. This effort was in collaboration with Pall Corporation and resulted in the first ever successful implementation of a SP-TFF step into a commercial manufacturing process. This technology allowed direct flow-through concentration of our biopharmaceutical with no recirculation of the product, enabling previously un-attainable high concentrations of our antibodies to >200 grams/liter. As a result, we were able to manufacture our therapeutic in a subcutaneous formulation for at-home injectable patient use. The patient benefits of self applied subcutaneous injections (as opposed to intravenous delivery in a hospital setting) are enormous. Since this project, and the emergence of this pioneered technology, many other therapeutics, previously delivered as an intravenous medication, have been being formulated for subcutaneous use.

4. More recently, the contributions of MockV's basic approach to viral clearance – the use of non-infectious surrogates for the prediction of model viruses have been recognized by the industry. In 2018, the results from two separate MockV collaborations were presented at industry conferences. These presentations won accolades from the scientists and industry leaders in attendance:

    - 2018 Recovery Conference (Ashville, NC) – Award for best poster
      Joshua D. Orchard, David Cetlin, Melanie Pallansch, Bill Kerns, Sara Hapip, Robert Barlow, Jon Borman, Luke Pallansch, Matthew Dickson. (2018) Using A Non-Infectious MVM Surrogate for Assessing Viral Clearance During Downstream Process Development. Recovery Conference. Ashville, N.C.

    - Viral Clearance Week - 21st Planova Workshop (San Francisco, CA) – Voted best Presentation
      Cetlin, David. Use of A Non-Infectious Surrogate to Predict Minute Virus of Mice Removal During Nanofiltration. Viral Clearance Week - 21st Planova Workshop. San Francisco, CA

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1R43FD006472-01 Personal Info PI) 09/01/18-08/31/19
Demonstrating Feasibility of Producing a Highly Purified and Concentrated Stock Solution of Retrovirus Like Particles for Biopharmaceutical Process Development Viral Clearance Studies.

Completed Research Support

1R43TR002231-01A1 Personal Info (PI) 12/01/17-11/30/17
Demonstrating Utility of a Non-Infectious Minute Virus of Mice-Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Personal Info

eRA COMMONS USER NAME (credential, e.g., agency login): NA

POSITION TITLE: Senior Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Southern Illinois University	B.S.	06/1978	Zoology

A. Personal Statement

I have the technical expertise and motivation to lead several of the efforts laid out in MockV Solution's Phase 2 SBIR application. Specific Aim #1 involves the optimization of a scale-able MVP production process and the establishment of quality systems and protocols**.** For close to 40 years I've worked with many cell-based expression systems including E. Coli and Sf9. As the laboratory manager at Chemicon International, Inc and lead scientist at Invitrogen Federal Systems, Inc I led several efforts to optimized protein production in these systems. While working at Biotech Research Laboratories, and subsequently at Granbio, Inc., I helped develop and produce Immunofluorescence assays for detection of serum immunoglobulin to Epstein-Barr virus proteins. These tests were FDA registered (510k) in-vitro diagnostic products that required FDA pre-market submission and production under FDA compliance. I was responsible for creating supporting documentation required to comply with FDA standards as indicated in the Code of Federal Regulations, Title 21, and specific for Medical Devices and Quality System Regulation. These types of skills that I've developed and utilized for decades will lend themselves well to the efforts required to optimize MVP production and build a commercial platform for Kit production.

I possess a fundamental understanding of quality systems and possess a track record of successful implementation efforts. During my employment at Chemicon International, Inc., I initiated development of Good Manufacturing Practice (GMP) methods for product manufacture to expand our product line into the infectious disease diagnostic market. I was directly responsible for the introduction of Chemicon's in-vitro diagnostic products that required manufacture under FDA compliance. This quality system evolved into Chemicon's ISO13485 certified quality system. BluePoint Bioscience, LLC. was formed to develop and manufacture in-vitro diagnostic products. This goal inspired us to start the business using a quality system at the core of our procedures. We follow many of the guidelines of the CFR Title 21 quality system regulation. We operate the laboratory under documentation consistent with good manufacturing practices such as: Material Specifications, Manufacturing Procedures, Standard Operating Procedures, Quality Control Procedures, Equipment calibration and monitoring, Lot numbering and document control, Labeling control. MockV Solutions has recognized the importance of designing quality into the early stages of commercial Kit production and I have recently accepted a role with the company as Director of Chemistry, Manufacturing and Controls. The duties of this role overlap well with the responsibilities inherent in Specific Aim #1 of this application.

I have a broad background in biology and bioengineering. After receiving a B.S. from Southern Illinois University, I was employed for 7 years at Biotech Research Laboratories, Inc. in Rockville, Maryland. In this role I served as the Supervisor for the company's product manufacturing division. I was directly responsible for the production of Epstein-Barr Virus (EBV) immunofluorescent diagnostics, EBV and HTLV-I enzyme immunosorbent diagnostic kits and nonbiologic product lines. In addition, I established and managed the company's hybridoma development department. I then spent 3 years at Granbio, Inc. Temecula, California where I was responsible

for operations of the EBV diagnostic production facility and initiated hybridoma development projects for the production of monoclonal antibody to various EBV antigens. I then spent 15 years at Chemicon International, Inc. Temecula, California (now Merck Millipore) where I created the Antibody Development Department involved in the production and characterization of over 4000 monoclonal and polyclonal antibodies and created the "Light Diagnostics" diagnostics division. In this role I directly managed diverse team of 15 scientists. After returning to the east coast, I spent 2 years at Invitrogen Federal Systems, Inc. Frederick, Maryland managing Defense Threat Reduction Agency sponsored development of a handheld, six-analyte, pathogen detection device before establishing my current company, BluePoint Bioscience LLC in Ijamsville, Maryland in 2007. BluePoint Bioscience is a full service antibody development and production business. My mission, as Laboratory Director and CEO, has been to develop and produce the highest quality research and diagnostic products in the biotech industry. We have worked with and advised dozens of clients with various service needs including; cell culture, antibody development and production, protein purification and modification, antibody fragmentation, enzyme and fluorescence labeling, immunoassay development and Diagnostic Kit development and production.

In 2015, I began to assist MockV Solutions in their efforts to characterize a non-infectious MVM particle ("MVP"). Eventually this relationship led Bluepoint to generate anti-MVP antibody hybridoma cell lines which were used as part of MockV's Phase 1 SBIR grant efforts to quantify MVP. Because of my scientific interest in the work and the commercialization potential for their technology, I agreed to remain involved in the Phase 1 project as an assay development scientist and helped lead the successful effort to increase the dynamic range of MockV's Immuno-qPCR assay. The specific aims in store for this Phase 2 application constitute a natural extension of this prior work. I am eager to continue this work and progress the MVP Kit toward commercialization.

B. Positions and Honors

1979-1986	Hybridoma Lab Manager/Production Manager, Biotech Research Laboratories, Inc. Rockville, MD
1986-1989	Laboratory / Production Manager, Granbio, Inc. Temecula, California
1989 -2004	Laboratory Manager, Chemicon International, Inc. Temecula, California
2005 -2006	Lead Scientist and Manager, Invitrogen Federal Systems, Inc. Frederick, Maryland
2007 -	CEO and Laboratory Director, BluePoint Bioscience, LLC, Ijamsville, Maryland
2018-	Director of Chemistry, Manufacturing and Controls, MockV Solutions, Inc. Rockville, MD

C. Contributions to Science

I developed a monoclonal antibody to the immediate early antigen of cytomegalovirus (CMV) that I used to produce an in-vitro diagnostic kit for the detection of CMV. The use of this antibody allowed for pre-cytopathic effect (CPE) results reducing the cell culture incubation period from >96 hours to <48 hours, with significant correlation at 24 hours post-inoculation. This kit continues to be marketed by Merck Millipore and may represent the industry standard for cell-culture confirmation of CMV infection.

I developed a monoclonal antibody to fluorescein isothiocyanate (FITC) that allowed me to develop an ultra-sensitive enzyme-linked immunoassay (ELISA). Utilizing the ability to conjugate FITC to a monoclonal antibody at a relatively high level, I was able to complex anti-FITC labeled alkaline phosphatase to the FITC labeled antibody and confirm assay sensitivity that exceeded a biotin/streptavidin model.

After joining Chemicon International Inc., a start-up antibody brokering company, I created the tissue culture and hybridoma development laboratories and continued efforts to optimize antibody process development procedures to improve production capacity and product quality. I was responsible for the installation and operation of high output hollow-fiber bio-reactors to produce gram quantities of monoclonal antibodies. I was also responsible for implementing static-culture perfusion bio-reactors in the production of sub-gram quantities of monoclonal antibody in-vitro. This effort resulted in substantial reduction of in-vivo production requirements as well as ensuring antibody quality and reproducibility. As manager of the Antibody Development Department it was my team's goal to develop monoclonal antibodies to support the market needs determined by our business development group. We were responsible for all aspects associated with monoclonal antibody development including: animal immunization and surgical procedures, carrier protein conjugations, cell fusion, cloning, development of EIA, western blot and IFA/ICC screening assays, isotyping, antibody production,

purification and quantitation, and conjugation of enzyme, biotin and fluorescent labels. The success of this initiative enabled us to produce > 100 polyclonal / year and > 30 monoclonal /year. This not only transformed Chemicon into a full service biotech company which was later bought by Serologicals (Merck Millipore) for $95M, but the techniques which I pioneered became the standard model adopted by others in the industry. Several notable monoclonal antibody products which my techniques brought to fruition include:

a) Anti-Connexin 43; research in gap junction function
b) Anti-L-glutamate decarboxylase (GAD) 67; for use in research of diabetes and nervous system disorders
c) Anti-MMP 1, 2, 9, and 26; for use in cancer metastasis research
d) Development of hundreds of clones producing antibody to hundreds of target analytes

Also at Chemicon, I Created the “Light Diagnostics” division focusing on our infectious disease, In-Vitro Diagnostic (IVD) products, which include:

a) Respiratory Panel 1 Viral Screening & Identifcation Kit; An indirect immunofluorescence assay for the qualitative identification of Adenovirus, Influenza A and B, Parainfluenza 1, 2, and 3 and Respiratory Synctial Virus (RSV)
b) CMV Immunofluorescence Assay; Cytomegalovirus Pre-CPE Culture Identification Test
c) CMV CMV Direct Immunofluorescence Assay
d) SimulFluor HSV/VZV Immunofluorescence Assay; for the simultaneous detection of Herpes simplex virus and Varicella zoster virus in direct specimens and culture confirmation
e) CMV pp65 Antigenemia Immunofluorescence Assay; for the detection of CMV pp65 matrix protein in peripheral blood leukocytes
f) SimulFluor CMV/Adeno Immunofluorescence Assay for the simultaneous detection of CMV and Adenovirus
g) HSV DFA; for detection of Herpes Simplex Virus 1 and 2 in direct specimens and culture confirmation
h) Varicella-zoster Virus Direct Immunofluorescence Assay; for detection of VZV in direct specimens
I) Measles IgM Capture Enzyme Immunoassay; for the qualitative detection of Measles specific serum IgM

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

1R43FD006472-01 Personal Info PI) 09/01/18-08/31/19
Demonstrating Feasibility of Producing a Highly Purified and Concentrated Stock Solution of Retrovirus Like Particles for Biopharmaceutical Process Development Viral Clearance Studies.
Role: Lab Technician

Completed Research Support

R43TR002231 Personal Info (PI) 12/01/17-11/30/18
Demonstrating Utility of a Non-Infectious Minute Virus of Mice – Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development
The goal of this current grant is to test the feasibility of accurately predicting Minute Virus of Mice (MVM) clearance through the use of a non-infectious Virus Like Particle Kit.
Role: Assay Development Scientist

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Subcontractor Info

Biosketches

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Biosketches

Contact PD/PI: Personal Info

Subcontractor Info

Biosketches

Contact PD/PI: Personal Info

Subcontractor Info

Biosketches

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Personal Info				PD/PI	Institutional Base Salary	Effort			Itemized Cost		
2.		Personal Info				Senior Scientist		"			"		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** Itemized Cost

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
# of Employees	Molecular Biologist	Effort			Itemized Cost		
	Total Number Other Personnel					**Total Other Personnel**	Itemized Cost
						Total Salary, Wages and Fringe Benefits (A+B)	

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-574 . Received Date: 2019-04-05T15:30:56.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)*** Itemized Cost
1 . Beckman VI-Cell XR Cell Viability Analyzer	
2 . Nova Biomedical Bioprofile Flex 2 cell culture analyzer	
3 . Masterflex Precision Pump	
4 . GE WAVE Bioreactor 20/50EHT	
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel

	Funds Requested ($)* Itemized Cost
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)* Itemized Cost
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*:

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2019 **End Date*:** 09-30-2020 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)*** Itemized Cost
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Technical Assistance	
Total Other Direct Costs	

G. Direct Costs	**Funds Requested ($)*** Itemized Cost
Total Direct Costs (A thru F)	

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)** Itemized Cost	**Funds Requested ($)*** Itemized Cost
1 . None - will negotiate			
Total Indirect Costs			

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): None.

I. Total Direct and Indirect Costs	**Funds Requested ($)*** Itemized Cost
Total Direct and Indirect Institutional Costs (G + H)	

J. Fee	**Funds Requested ($)*** Fee

K. Total Costs and Fee	**Funds Requested ($)*** Itemized Cost

L. Budget Justification*	File Name: Budget_Justification_05Apr19.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12831646 Funding Opportunity Number: PA-18-574 . Received Date: 2019-04-05T15:30:56.000-04:00

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Personal Info				PD/PI	Institutional Base Salary	Effort			Itemized Costs		
2.		Personal Info				Senior Scientist							

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person Itemized Cost

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
# of Employees	Molecular Biologist	Effort			Itemized Cost		
	Total Number Other Personnel				**Total Other Personnel**	Itemized Cost	

Total Salary, Wages and Fringe Benefits (A+B)

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item **Funds Requested ($)***
Itemized Cost

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name:

D. Travel **Funds Requested ($)***
Itemized Cost

1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

Total Travel Cost

E. Participant/Trainee Support Costs **Funds Requested ($)***
Itemized Cost

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

Number of Participants/Trainees **Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Contact PD/PI: Personal Info

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-01-2020 **End Date*:** 09-30-2021 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	Itemized Cost
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Technical Assistance	
Total Other Direct Costs	

G. Direct Costs	**Funds Requested ($)***
Total Direct Costs (A thru F)	Itemized Cost

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . None - will negotiate		Itemized Cost	
Total Indirect Costs			Itemized Cost

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) None.

I. Total Direct and Indirect Costs	**Funds Requested ($)***
Total Direct and Indirect Institutional Costs (G + H)	Itemized Cost

J. Fee	**Funds Requested ($)***
	Fee

K. Total Costs and Fee	**Funds Requested ($)***
	Itemized Cost

L. Budget Justification* File Name: Budget_Justification_05Apr19.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH AND RELATED BUDGET JUSTIFICATION

MockV Solutions (MockV) is requesting 24 months and total Itemized Cost budget for a Phase II SBIR study. MockV has spoken with the NCATS program coordinator Personal Info about the scope and aims of this project, which make it eligible for an increased budget cap and timelines under the waiver topic - "Accelerate bioengineering approaches to the development and clinical application of biomedical material, devices, therapeutics, and or/diagnostics". Hence, we are requesting this amount for Phase 2 over 24 months.

A. Senior/Key Person

1. Personal Info , Principal Investigator, is Chief Executive Officer and a full time employee of the company. He will work on the project at Effort direct commitment and be responsible for the overall coordination and day-to-day direction of the project. In addition, he will help design, conduct chromatography and nanofiltration experiments, interpret all results and execute MVP clearance studies for Specific Aim's # 2 and 3. Personal Info has 11 years of experience investigating viral clearance through biopharmaceutical separation techniques. Total salary requested over the course of two years, including fringe benefits, is Itemized Cost
2. Personal Info Senior Scientist, is Director of Chemistry Manufacturing and Controls and a full time employee of the company. He will work on the project at Effort direct commitment and be responsible for oversight of MVP and antibody production, chemical conjugation procedures and quality control/assurance. Personal Info has 30+ years' experience with recombinant cellular expression systems, immunological techniques and quality management systems. Total salary requested, over the course of two years, including fringe benefits, is Itemized Cost

Total Senior/Key Person Itemized Cost

B. Other Personnel

1. **Senior Scientist**, A suitable individual for this position will be hired full-time by the company on receipt of award. She/he will work on the project at Effort direct commitment and will be responsible for conducting many of the optimization experiments during Specific Aim #1 and performing I-qPCR analysis during Specific Aims #'s 1-3. In addition, this individual will help in developing Proximity Ligation techniques during Specific Aim #4. The salary requested, over the course of two years, including fringe benefits, is Itemized Cost

Total Other Personnel Itemized Cost

C. Equipment Description

a) Cole-Parmer Masterflex® L/S® Stainless-Steel IP65 Precision Pump System. This pump will be needed to deliver media and feed additions during large scale MVP production. Total Cost: Itemized Cost

b) GE WAVE Bioreactor 20/50EHT Rocker Warmer System with Controllers (used). This bioreactor will be needed to produce marge amounts of MVP in support of Specific Aims #2 and #3. This bioreactor will also enable MockV to scale up MVP production for future commercial applications. Total Cost: Itemized Cost

c) Beckman Vi-Cell XR Cell Viability Analyzer (used). This analyzer will be used to measure viable cell density, cell viability and cell diameter during small scale Sf9 optimization of MVP production. This analyzer will also enable MockV to scale up MVP production for future commercial applications. Total Cost: Itemized Cost

d) Nova Biomedical BioProfile FLEX2 automated cell culture analyzer. This analyzer will be used to measure pH, Dissolved Oxygen, Glucose, Lactate, Glutamine and $NH4^+$ during MVP production. This analyzer will also enable MockV to scale up MVP production for future commercial applications. Total Cost: Itemized Cost

Total Equipment Itemized Cost

D. Travel

1. Domestic Travel costs

Travel to and from Chromatan and WuXi sites in PA and industry conferences for presentation of MockV technology and networking with experts in the field is request. Total amount requested is Itemized Cost

2. Foreign Travel Costs

None

Total Travel Costs Itemized Cost

E. Participant/Trainee Support Costs

None

Total Participant/Trainee Support Itemized Cost

F. Other Direct Costs

1. Materials and supplies

Item or Category	Provider	Unit Price	Quantity	Total Price
Sf-900™ III SF Media	Gibco	Itemized Cost		
Express Five™ SF Media	Gibco			
STERILE TUBING WELDER, MODEL SCD 312	TERUMO			

		Itemized Cost
I-PER™ Insect Cell Protein Extraction Reagent	Thermo Scientific	
Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)	Thermo Scientific	
20L Cellbag (Perfusion Version)	GE	
AminoLink™ Coupling Resin	Thermo Scientific	
Generik® FPLC Empty Columns, (0.66 x 25)	Sepax	
Generik® FPLC Empty Columns, (1 x 25)	Sepax	
Generik® FPLC Empty Columns, (2.5 x 25)	Sepax	
HiPrep 16/60 Sephacryl S-500 HR	GE	
TaqMan™ Fast Advanced Master Mix	Applied Biosystems	
NuPAGE™ 12% Bis-Tris Protein Gels, 1.0 mm, 1-well	Invitrogen	
TMB One Component HRP Membrane Substrate	Surmodics	
Sf9 2G HCP ELISA Kit (F840)	Cygnus	
BacPAK™ Baculovirus Rapid Titer Kit	Takara	
TEM Analysis	EMBS	
Q Sepharose Fast Flow, 300 mL	GE	
POROS™ CaptureSelect™ AAV9 Affinity Resin	ThermoFisher	
POROS™ CaptureSelect™ AAVX Affinity Resin	ThermoFisher	
CIMmultus™ QA-8 Advanced Composite Column	BIA Separation	
DNA ligase	Invitrogen	
TaqMan™ Protein Assays Oligo Probe Kit	Applied Biosystems	
TaqMan™ Protein Assays Buffer Kit	Applied Biosystems	
TaqMan™ Protein Assays Core Reagents Base Kit	Applied Biosystems	
TaqMan™ Protein Assays Fast Master Mix	Applied Biosystems	
Protein G Sepharose™ 4 Fast Flow	GE	
CELLine 1000 Package of 3	Wheaton	
Fetal Bovine Serum, certified, US origin	Gibco	
RPMI 1640 Medium, GlutaMAX™ Supplement, HEPES	Gibco	
Insulin from bovine pancreas	Sigma aldrich	
Molecular Biology reagents and kits (ex. template nucleic acid, plasmids, restriction enzymes)		
General laboratory consumables, chemicals and reagents.		
qPCR reagents and supplies excluding Master Mix (TaqMan primers and probe, plastics, etc.)		
Immunoreagents (antibodies, etc.)		

RegenX (AAV Production)		Itemized Cost
WuXi (Viral Clearance testing)		
Teva (mAb production)		
Total		

2. **Publication Costs**
   None

3. **Consultant Services**

Consultant Info Consultant will be a non-employee consultant during the duration of Specific Aim #3. He will work on the project at an agreed to rate of Itemized Cost and will dedicate Effort during the two-year duration of this project. He will be responsible for guiding the scientific efforts relating to counter current tangential flow chromatography and viral clearance (Specific Aim #3). Consultant Info has 35 years of experience working on bioprocessing systems and is a member of the Virus Filtration FMEA Task Force of the Parenteral Drug Association. Total salary requested is Itemized Cost

Consultant Info Consultant, is the Lead Scientific Advisor to MockV Solutions and will serve as a consultant during the duration of the project. He will work on the project at an agreed to rate of Itemized Cost and will dedicate Effort during the two-year duration of this project. He will be primarily responsible guiding the overall scientific approaches of Specific Aims #1 and 4. Consultant Info has extensive experience as a Molecular Virologist. Total salary requested is Itemized Cost

Consultant Info Consultant, will be a non-employee consultant during the duration of Specific Aim #1. He will work on the project at an agreed to rate of Itemized Cost and will dedicate Effort during the first 12-months of this project. He will be responsible for guiding the scientific efforts of optimizing MVP production (Specific Aim #1). Consultant Info has 18 years of experience working with insect and mammalian expression systems. Total salary requested is Itemized Cost

Total Requested: Itemized Cost

4. **ADP/Computer Services**
   None

5. **Subawards/Consortium/Contractual Costs**

ChromaTan (Philadelphia, PA)
ChromaTan is an industry leader within the continuous processing space for biopharmaceuticals. Their expertise and knowledge is required in order to test the concept of predicting viral clearance with a non-infectious surrogate through their counter current tangential flow chromatography continuous processing system – the

CCTC system. The total cost for materials, supplies, personnel, and overhead over a two year period is: Itemized Cost

6. Equipment or Facility User Rental Fees

None

7. Alterations and renovations

None

8–10. Technical Assistance

MockV will utilize the technical assistance award to support business development consultant costs.

Total Requested: Itemized Cost

Total Other Direct Costs Itemized Cost

G. Indirect Costs

A rate of Itemized Cost of total direct costs is requested to cover facility, administrative, insurance, corporate legal, accounting, information technology, and website costs. Through a partnership with an incubator, BioHealth Innovations, BioHealth Innovation (BHI) will provide commercial assistance to support the commercial development pathway to advance the product. BHI's team of professionals will assist in the refinement of the go-to-market strategy and identify the ultimate commercial of the opportunity through achieving feedback from potential customers, funding partners, and strategic partners. Indirect costs will support these initiatives. We will also use these funds to pay for laboratory facilities from Bluepoint Bioscience. (Ijamsville, MD) and employee bonuses. We will employ standard accounting and administrative services to comply with the award.

The total amount requested is Itemized Cost This amount is appropriate to cover the company's current projected indirect costs and is consistent with NIH's policy for Phase II SBIR proposals when the company does not already have a previously negotiated indirect cost rate.

H. Fee

A fee of Fee of total costs (direct and indirect) is requested for a total amount of Fee

For price listings on equipment and significant material costs, please refer to our original application budget justification.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	Itemized Cost
Section B, Other Personnel	# of Employees
Total Number Other Personnel	
Total Salary, Wages and Fringe Benefits (A+B)	Itemized Cost
Section C, Equipment	
Section D, Travel	Itemized Cost
1. Domestic	
2. Foreign	Itemized Cost
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	Itemized Cost
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	0
Section F, Other Direct Costs	Itemized Cost
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	Itemized Cost
Section H, Indirect Costs	
Section I, Total Direct and Indirect Costs (G + H)	
Section J, Fee	Fee
Section K, Total Costs and Fee (I + J)	Itemized Cost

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ○ Project ● Subaward/Consortium

Enter name of Organization: Chromatan

Start Date*: 10-01-2019 **End Date*:** 09-30-2021 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Subcontractor Info				Subaward Project Lead	Institutional Base Salary	Effort			Itemized Cost		
2.		Subcontractor Info				Technician		"			"		
3.		Subcontractor Info				Technician		"			"		

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person Itemized Cost

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
# of Employees	Associate Scientist	Effort			Itemized Cost		
	Associate Scientist						
	Total Number Other Personnel				**Total Other Personnel**		Itemized Cost
					Total Salary, Wages and Fringe Benefits (A+B)		

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ○ Project ● Subaward/Consortium

Organization: Chromatan

Start Date*: 10-01-2019 **End Date*:** 09-30-2021 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)*** Itemized Cost
1 . Counter Cuurent Tangential Chromatography Skid 1 Rental 5 months	
2 . Counter Cuurent Tangential Chromatography Skid 2 Rental 2 months	
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel

	Funds Requested ($)* Itemized Cost
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)* Itemized Cost
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: Proprietary nfo

Budget Type*: ○ Project ● Subaward/Consortium

Organization: Chromatan

Start Date*: 10-01-2019 **End Date*:** 09-30-2021 **Budget Period:** 1

F. Other Direct Costs	**Funds Requested ($)***
1. Materials and Supplies	Itemized Cost
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	

G. Direct Costs	**Funds Requested ($)***
	Itemized Cost
Total Direct Costs (A thru F)	

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	**Funds Requested ($)***
1 . None- will negotiate	Itemized Cost		
Total Indirect Costs			Itemized Cost

Cognizant Federal Agency None.
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)***
	Itemized Cost
Total Direct and Indirect Institutional Costs (G + H)	

J. Fee	**Funds Requested ($)***

K. Total Costs and Fee	**Funds Requested ($)***
	Itemized Cost

L. Budget Justification* File Name: Subaward_07Jan19.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH AND RELATED BUDGET JUSTIFICATION

Chromatan is an industry leader within the continuous processing space for biopharmaceuticals. Their expertise and knowledge are required in order to test the concept of predicting viral clearance with a non-infectious surrogate through their counter current tangential flow chromatography continuous processing system – the CCTC system. The budget period shown is for the two year duration of the project.

A. Senior/Key Person

1. Subcontractor Info is Chief Executive Officer of Chromatan Corporation and a full-time employee of the company. He will work on the project at Effort time direct commitment and will manage the overall project deliverables and serve as the point person for all cross functional communications between the teams. Total salary requested for two years, excluding fringe benefits, is Itemized Cost
2. Subcontractor Info is VP of Engineering of Chromatan Corporation and a full-time employee of the company. He will work on the project at Effort time direct commitment and will oversee the manufacturing, delivery and quality control of the CCTC systems which will be used for this project. Total salary requested for two years, excluding fringe benefits, is Itemized Cost
3. Subcontractor Info s Associate Director of Process Sciences at Chromatan Corporation. and a full-time employee of the company. He will work on the project at Effort ime direct commitment and will manage the applications testing and will be responsible for aggregating and writing up data analysis. Total salary requested for two years, excluding fringe benefits, is Itemized Cost

Total Senior/Key Person Itemized Cost

B. Other Personnel

1. **Associate Scientist II,** will be hired as a full-time employee of the company. S/he will work on the project at Effort time direct commitment and will conduct laboratory experiments with guidance from Subcontractor Info Total salary requested for two years, excluding fringe benefits, is Itemized Cost

2. **Associate Scientist II,** will be hired as a full-time employee of the company. S/he will work on the project at Effort -time direct commitment and will conduct laboratory experiments with guidance from Personal Info and Personal Info Total salary requested for two years, excluding fringe benefits, is Itemized Cost

Total Other Personnel Itemized Cost

C. Equipment Description

Equipment	Source	Cost
CCTC system I - rental for 5 months (40% discount)	Chromatan Corporation	Itemized Cost
CCTC system II - rental for 2 months (40% discount)	Chromatan Corporation	
Numera inline sampling system [Free of cost]	Chromatan Corporation	
UPLC system [Free of cost]	Chromatan Corporation	

Total Equipment Itemized Cost

D. Travel

1. Domestic Travel costs

Travel to meeting with multiple team members to Rockville, MD and Navy Yard in Philadelphia (Wuxi location). Total amount requested is Itemized Cost

2. Foreign Travel Costs

None

Total Travel Costs Itemized Cost

E. Participant/Trainee Support Costs

None

Total Participant/Trainee Support **$0**

F. Other Direct Costs

1. Materials and supplies

Material	Model number - Provider	Amount	Unit price ($)	Total price ($)
CCTC Single-use Assemblies (40% discount) for ProA	CHROM-ProA-10001	Itemized Cost		
CCTC Single-use Assemblies (40% discount) for AEX	CHROM-AEX-20001			
Protein A resin (Donated by Chromatan)	82080 - Thermo Fischer Scientific			
AEX resin (Donated by ChromaTan)	1255907-Thermo Fischer Scientific			
POROS A Analytical column ($1,200, Donated by ChromaTan)				
YMC SEC analytical column ($2,000, Donated by ChromaTan)				
HCP ELISA Kit	F105 - Cyngus Tech			
DNA ELISA Kit	Quest 5-hmC- Zymo Research			
Chemicals	Buffer, salts, acid, base - Various sources			
Total				

CCTC Single-Use Assemblies will be used to execute the CCTC runs (1 run for individual steps and 2 runs for integrated design)

Protein A and AEX resins will be donated by Chromatan Corporation.

HCP, DNA, and leached protein A ELISA kit will be purchased to enable sample analysis for impurity contents in the purified product.

Chemicals like salts, acids, bases and other lab supplies will be purchased to support making all the buffers Itemized Cost

Total Material and supplies: Itemized Cost

2. **Publication Costs**
   None

3. **Consultant Services**
   None

4. **ADP/Computer Services**
   None

5. **Subawards/Consortium/Contractual Costs**
   NA

6. **Equipment or Facility User Rental Fees**
   None

7. **Alterations and renovations**
   None

8–10. Technical Assistance
None

Total Other Direct Costs Itemized Cost

G. Indirect Costs
Chromatan will use the suggested NIH indirect cost rate of Itemized Cost
Total indirect costs: Itemized Cost

H. Fee
None

TOTAL DIRECT/INDIRECT: Itemized Cost

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
		Itemized Cost
Section A, Senior/Key Person		
Section B, Other Personnel	# of Employees	
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		Itemized Cost
Section C, Equipment		
Section D, Travel	Itemized Cost	
1. Domestic		
2. Foreign		Itemized Cost
Section E, Participant/Trainee Support Costs	Itemized Cost	
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		Itemized Cost
1. Materials and Supplies	Itemized Cost	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		Itemized Cost
Section G, Direct Costs (A thru F)		
Section H, Indirect Costs		
Section I, Total Direct and Indirect Costs (G + H)		
Section J, Fee		
Section K, Total Costs and Fee (I + J)		

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	Itemized Cost					

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

Agency to which you are applying (select only one)*

○ DOE ● HHS ○ USDA ○ Other:

SBC Control ID:* Proprietary Info

Program Type (select only one)*

● SBIR ○ STTR

○ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

Application Type (select only one)*

○ Phase I ● Phase II ○ Fast-Track ○ Direct Phase II ○ Phase IIA ○ Phase IIB

○ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ○ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* # of Employees

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ○ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ○ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ○ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ○ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ○ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ○ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ○ No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:* CP_05Apr19.pdf

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ○ Yes ● No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ○ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ○ Yes ○ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ○ Yes ○ No

12. Provide DUNS Number of non-profit research partner for STTR.*

A. ***Value of the SBIR/STTR Project, Expected Outcomes, and Impact.***

Value of Project

The goal of this Phase 2 SBIR project is to advance the first BioSafety Level 1-compatible viral clearance kit toward commercialization through product development and applications testing efforts. Small molecule therapeutics have substantially increased human life expectancy and quality of life in the last century; however, the knowledge gained from decoding the human genome combined with innovative technologies and drug-discovery tools has enabled the development of an entirely new category of drugs – biopharmaceuticals ("BP's"). Recombinant antibodies, the first major class of BPs, have become the standard treatment of care for many diseases. Building on the success of antibody therapies, BP companies are now expanding their pipelines to additional classes of drugs, namely cell and gene therapies[1]. Compared to the manufacturing of small molecule drugs, which typically involve relatively simple, controlled chemical reactions, BP's are produced through complex, time consuming and expensive biologic processes. One of the critical concerns during BP manufacturing is the potential for viral contamination[2-6].

Although rare, viral contamination events do occur - 26 have been reported since Genentech introduced recombinant human insulin in 1982[7]. The effects of a contamination event can impact many aspects of a business, including: production shutdown, delays in product approvals, increased regulatory scrutiny, lost product sales, changes in public perception and exposure to lawsuits[7]. More importantly, viral contamination events impact the lives of patients as the safety and availability of their therapeutics could be compromised[8-10]. For this reason, international regulatory agencies require BP companies to demonstrate the "viral clearance" efficacies of their manufacturing process steps prior to awarding clinical trial or commercial approval[11-13]. Currently, this is accomplished through **small scale** "spiking studies" whereby specific model mammalian viruses are artificially introduced ("spiked") into BP material and subsequently removed via process purification steps such as chromatography and filtration[14,15]. These studies require specialized BioSafety Level (BSL)-2 laboratories and trained personnel resulting in costs that can soar well above $100,000[16,17]. These hurdles **deter** companies from analyzing viral clearance during the years of small-scale **process development**. Instead, companies spend considerable resources optimizing these steps **before gaining any knowledge** of their actual viral clearance efficacy. Unfortunately, this increases the risk of regulatory failure, forcing companies to invest additional time and money redeveloping process steps, which in turn, could postpone regulatory approvals. This can affect a company's go-to-market strategy and delay patients' timely access to therapies.

Viral clearance not only presents challenges to current BP process development efforts, it is also critical to the successful advancement of two highly impactful new trends in the biopharmaceutical industry: **gene therapy** and **continuous processing**[18-20]. Recent advancements in gene delivery and manufacturing have spurred tremendous efforts to develop and commercialize gene therapies as the next class of potent therapeutics[1]. These products promise to address diverse medical conditions from ultra-rare orphan diseases to the leading causes of human illness[1]. Concurrently, technology platforms which enable the "continuous processing" of BP's have emerged as viable strategies to increase efficiency and reduce manufacturing costs for a host of BP moieties[21-23]. Although the benefits of these exciting fields dovetail with broader societal aims and initiatives such as the Affordable Care Act and the Cures Act for Orphan Diseases, their rapid onset has left many questions surrounding manufacturing and validation un-addressed. In the case of gene therapy, the vectors which are utilized to deliver therapeutic payload, such as adeno-associated virus (AAV), are similar in size to the model parvovirus used for spiking studies[24,25]. This precludes the use of virus filters in gene therapy processes, a cornerstone of mAb processes and viral clearance validation. As a result, the burden of viral clearance will fall to chromatographic methods of separation which will require much optimization. In the case of continuous processing, the operation of linked units with more complex flow paths have created unique challenges in both guaranteeing the required level of viral clearance and properly validating the virus removal capabilities of the overall process[19,20]. Experimental trial and error is the natural approach, but the costs associated with viral clearance present a major hurdle to companies considering the adoption of these innovative technologies.

An economical and accurate alternative to live virus spiking studies would provide tremendous value to the BP industry. MockV Solutions (MockV) was founded to unlock this value by commercializing a series of non-infectious "Mock Virus Particle (MVP)" Kits – engineered to generate predictive viral clearance data at a fraction of the current costs and time. The **non-infectious** nature of MVP's and the **rapid turn-around time** of analysis will enable scientists to conduct evaluations **"in-house"** (BSL-1) in a matter of hours, as opposed to the BSL-2 environments and weeks required to conduct a live virus study.

Technology Objectives and Expected Outcomes

In this Phase II SBIR project, MockV will further advance its lead viral clearance prediction kit – the "MVP Kit" - toward commercialization through product development and applications testing efforts. Specifically, our technology objectives will include the optimization and internalization of Mock Virus Particle (MVP) production, an effort that will result in enhanced yields, decreased costs and increased control over the most expensive and logistically challenging element of kit production. An additional objective will be to optimize a "high-throughput" MVP quantification assay. This effort builds from the assay optimization success of our Phase 1 project and will result in a second-generation product, enabling end-users to integrate sample analysis into automatic liquid handling systems and robotics. Finally, through committed collaborations with key industry partners, gene therapy and continuous processing applications for the MVP Kit will be tested. In each application, the clearance of non-infectious MVP will be compared to live Minute Virus of Mice (MVM) through parallel spiking studies. It will be expected that the data generated through these efforts will demonstrate the utility of non-infectious MVP as an accurate predictor of MVM clearance for each emerging application. Overall, this project will prepare MockV for a successful MVP Kit launch in Phase III and will expand its commercial potential into these new and growing BP manufacturing markets.

Impact

A **simple, low cost, BSL-1 compatible viral clearance assessment kit**, utilized during small-scale process development, would provide scientists with a unique and practical tool to generate "real-time" data on viral clearance efficacy. With a "Quality by Design" approach, scientists could confidently optimize the virus removal capabilities of purification steps in continuous and/or conventional processes prior to investing significant resources in regulatory-supporting viral clearance validation spiking studies. The time and resources saved by utilizing such a tool would translate into reduced cost of good (COGS) for a variety of therapeutic modalities and greatly reduce the existing barriers to process innovation in the fields of gene therapy and continuous processing.

While the use of antibodies (mAb's) have become a standard treatment for many diseases, the next wave of industry changing therapies are coming in the form of cell and gene therapies[26]. These complex macro-molecules have the potential to revolutionize treatment of care, helping many patients who do not respond to traditional small molecules or mAb's[1]. Viral vector-based gene therapies are predicted to be a large part of the pharmaceutical industry in the future but require enabling technologies[26]. With clinical translation in mind, significant investment is being made in the more applied aspects of gene therapy, such as optimizing the protocols required for manufacture of good-manufacturing practice grade vector stocks at the scale required for treating human subjects and ensuring that novel vectors are suitable for commercial manufacturing methods[27]. More so than for small molecule manufacturing, BP processing must be streamlined in order to increase access to patients through the reduction of costs and standardization of processes. The paradigm of BP manufacturing is quickly changing in order to produce safer and cheaper therapies. For antibody production, companies have already begun to introduce continuous manufacturing techniques into process development efforts which will enable streamlined antibody production workflows[21-23]; however, testing for viral clearance efficacy is a crucial barrier for widespread implementation of this strategy[19,20].

MockV's MVP kit will allow BP companies to de-risk viral clearance approaches and quickly develop production methods that will enable innovative therapies to be developed and manufactured at lower costs. The current "wait and see" approach to viral clearance is too risky as manufacturing processes may be established that ultimately fail to achieve benchmarks. This jeopardizes regulatory approvals and can potentially result in the set-back of clinical trials or commercial launch for months or years, costing a company patent life and/or sales revenue. More importantly, in some instances, failed viral clearance validations may cause a company to abandon development of a drug altogether, leaving patients without potentially lifesaving therapeutics.

B. Company.

MockV Solutions Inc. was founded in 2013 by Personal Info to commercialize a series of patent protected research kits that lower the cost and complexities associated with viral clearance during biopharmaceutical process development/characterization. After spending years as a process development scientist at Human Genome Sciences Personal Info was unsatisfied with the issues that arose from the industry norm of delaying viral clearance assessments until late in the product development cycle. He often witnessed situations in which tremendous resources were spent up-front, optimizing process steps that fell short of viral clearance expectations just prior to BLA submissions – resulting in situations in which process re-development would be required under intense timelines and scrutiny. As Personal Info grew to understand from conversing with colleagues at other companies, this widespread scenario led to the validation of sub-optimal manufacturing processes which locked in place years of unnecessarily higher drug production costs. Personal Info realized that the utilization of non-

infectious viral surrogates that mimicked the physicochemical characteristics of model spiking viruses would alleviate these issues and offer a breakthrough solution to the bioprocess industry.

Headquartered in Rockville, MD, a hub of scientific research and commercialization, MockV entered into a long term partnership with BioHealth Innovations (BHI), a public-private partnership that has provided operational and business support along with marketing, legal, IP, accounting and IT consulting. Since 2013, MockV has received several grants and awards, including:

- Non-Federal Funding Info
-
-

- Phase 1 SBIR, NCATS/NIH, 1R43TR002231-01A1: Demonstrating Utility of a Non-Infectious Minute Virus of Mice-Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development. 12/01/2017-11/30/2017.
- Phase 1 SBIR, CDER/FDA, 1R43FD006472-01: Demonstrating Feasibility of Producing a Highly Purified and Concentrated Stock Solution of Retrovirus Like Particles for Biopharmaceutical Process Development Viral Clearance Studies. 09/01-2018-08/31/2019.

Upon awarding of an NIH SBIR Phase I Grant 1R43TR002231-01A1, MockV began transitioning from a 100% virtual company to a self-sufficient operation, having sub-leased Square Footage of laboratory space at Bluepoint BioScience in Ijamsville, MD. This space, which is fully furnished with equipment dedicated for cell culture manufacturing, immuno-assay development and molecular biology, will enable us to execute our immediate and mid-term lab-dependent milestones, including:

- Specific Aims 1-4 of this Phase II application
- The complete internalization/scale-up of MVP Kit manufacturing with quality control.
- The development of additional prediction Kits (ex. RVLP Kit - FDA SBIR funded)

MockV is managed by founder and Chief Executive Officer Personal Info . Daily scientific activities are overseen by Personal Info and Personal Info Director of Chemistry Manufacturing and Controls. Consultant Info Lead Scientific Advisor, consults and guides these activities on a weekly basis. To help carry out the ultimate mission of sustaining a business that commercializes the first series of non-infectious viral clearance research kits, MockV draws from the business expertise of Personal Info as Chief Operating Officer and Personal Info Research Analyst. To supplement our team, MockV has established a Scientific and Commercial Advisory Board, comprised of scientific and product-minded experts from industry and academia. To fulfill the lab-based milestones discussed above, we anticipate hiring an additional employee, to serve as a senior scientist and lab manager. Two additional consultants will guide Phase II activities – Consultant Info an industry expert on recombinant protein expression and scale up, and Consultant Info a world renown bioprocess scientist with expertise in viral clearance and continuous processing applications.

Team

Personal Info **– CEO**: Personal Info has over 10 years of experience in bioprocess development and project management. He founded MockV Solutions after spending seven years as a purification scientist at Human Genome Sciences where he participated in the manufacturing process development, characterization, validation, and technical transfer activities for monoclonal antibodies.

Personal Info **– COO**: Personal Info has over 20 years of operating and product commercialization experience within 3 three public companies representing the biotechnology, diagnostics and life sciences sector. During her tenure at Life Technologies she was responsible for the global portfolio as Senior Business Director and managed Joint Ventures and expansion within the Asia-Pacific region. Along with her role as Chief Operating Officer of MockV, Personal Info serves as an Entrepreneur in Residence at Johns Hopkins University and BioHealth Innovation, a position focused on translating research technologies to market. Personal Info holds a PhD from the University of Maryland and served as a Staff Fellow at the National Institutes of Health. She holds a Certificate of Financial Management from Cornell University and is a graduate of the Program on Leadership and Strategy in Pharmaceuticals and Biotech at the Harvard Business School.

- Lead Scientific Advisor: Consultant Info has 20 years of biotechnology and molecular virology experience. He is currently the director of the Aquaculture Pathology Laboratory at the University of Arizona. Personal Info has spent much of his professional career working on developing diagnostics, oral vaccines and therapies, and genetic markers for viral disease resistance. He began working with MockV in 2013 and successfully led the scientific efforts to produce Mock Virus Particles.

- Director Chemistry Manufacturing and Controls: Personal Info has 40 years of industry experience and expertise relating to hybridoma development, animal sensitizations and maintenance, cell fusion, cloning, subtyping, production and purification of recombinant proteins, cell line cryopreservation, assay development and diagnostic kit manufacturing. As a manager for 15 years at Chemicon International, Inc. (now part of Merck Millipore), Personal Info created the Antibody Development Department involved in the production and characterization of over 4000 monoclonal and polyclonal antibodies and created the “Light Diagnostics” diagnostics division.

- Research Analyst: Personal Info supports the Entrepreneurs-in-Residence program in evaluating the commercialization potential of new technologies and works with BHI's client companies to achieve strategic business development goals. He holds a B.S in Biomedical Engineering from Case Western and an M.S in Supply Chain Management from the University of Maryland.

- Scientific and Commercial Advisory Board: Personal Info has gained over 20 years of experience in the development, characterization and validation of purification processes from early development through licensure while at Genentech, Human Genome Sciences, GlaxoSmithKline and MedImmune. While at MedImmune Personal Info led a department of CMC Team Project Managers, driving the strategy and delivery of CMC plans for all molecules in the MedImmune portfolio. He has extensive knowledge of IND and BLA regulatory processes. He currently serves as the Vice President, BioPharmaceutical Development at MacroGenics.

- Scientific and Commercial Advisory Board: Personal Info has over 25 years of biotechnology experience. He is currently the Chief Operating Officer of Adaptive Phage Therapeutics, a leading company in bacteriophage treatment for multi-drug resistant bacterial infections. Previously, Personal Info founded, grew and successfully sold two life science business. The first, Receptor Biology (sold to Perkin Elmer, Inc.), was the first US company that manufactured and sold GPCR reagents to the drug-discovery market. The second, Applied Cell Sciences (sold to ChanTest Corp), was a reagents and services company within the drug discovery market.

- Scientific and Commercial Advisory Board: Personal Info has over 25 years biotechnology experience. He is currently the Scientific Director of Texcell N.A., a viral clearance CRO that conducts live viral spiking studies for the biopharmaceutical industry. Prior to this position, Personal Info served as a Scientist at SAIC/NCI and as a Project Manager for HIV Antiviral Drug Development at the Southern Research Institute.

Scientific and Commercial Advisory Board: Personal Info has over 20 years of biotechnology experience. He is currently the Director and Head of CMC Partnership at Viela Bio, a recent spin-out from MedImmune. Prior to this position, Personal Info served as Associate Director of R&D at MedImmune where he led a commercial purification process optimization group and managed viral clearance activities for all stages of project support. He has extensive knowledge of IND and BLA regulatory processes.

Corporate vision

MockV was founded on a fundamental scientific premise: utilize non-infectious surrogates for predicting viral clearance. We have successfully leveraged this concept into a patentable invention and have demonstrated proof of concept with several prominent industry and agency partners (see Table 1). Since the dissemination of our data through publications in leading scientific journals[28,29] and presentations at conferences, demand for our future product line has been high. Due to this demand, the company made its “MVP Prototype Kit” available for purchase in March of 2018. This Kit is based on a non I-qPCR optimized, pre-Phase 1 assay and underperforms relative to the improvements we’ve made through Phase 1 efforts. Although we maintain full transparency regarding the limited capabilities and expectations of this Prototype Kit, orders from biopharmaceutical companies based in the US, Canada, EU, China, Japan and Australia (including repeat orders) have netted > $100,000 to date. Attention has been paid from several major biopharmaceutical industry vendors, including some who have sent representatives of their respective investment groups to meet with us and tour our facilities (see letters of interest). We believe that forming a strategic equity partnership with one these industry vendors would enable us to rapidly reach the maximum number of customers with our kits. An equity investment from a

strategic partner may also set the stage for an eventual acquisition of the company, an exit strategy that would be seriously considered.

C. ***Market, Customer, and Competition.***

The worldwide biopharmaceutical (BP) industry is an active, profitable and growing entity. Approved drugs take in over $275 billion/year while growing at a worldwide rate of 27%/year[30]. Despite this growth, companies are aggressively looking for opportunities to cut costs and increase efficiency. At the same time, manufacturing planning and decision-making are becoming more complex as an ever-increasing diversity of processing options become available including; modified cell lines, new forms of process equipment, alternative chromatography methods and materials. Increasingly, companies are forced to make difficult and costly strategic decisions about commercial manufacturing early in product development, generally setting pipeline product processes by the end of Phase II clinical trials. Effective planning is required to avoid problems down the road while scientific data are needed to provide the framework to make critical decisions.

Table 1: MockV Collaborations

Collaborator	Brief Description	Status	Notes
FDA (CDER)	Compare physicochemical properties of MVP vs. MVM.	Completed (Published in Applied Biochemistry and Biotechnology)	
Asahi Kasei	Compare MVP vs. MVM clearance and flux decay for Planova and BioEX	Completed (Published in Biotechnology Progress)	Voted best presentation at Planova User Conference 2018 (S.F.)
MedImmune	Compare MVP vs. MVM clearance for virus filtration and AEX	Completed (pending publication)	Voted best poster at 2018 Recovery Conference (Ashville, N.C.)
NIH (VRC)	Screen resins for MVP clearance in High Throughput Screening format (Tecan).	Completed (pending publication)	
GSK	Compare MVP vs. MVM clearance for AEX (DOE)	Completed (pending publication)	Funded through Phase 1 SBIR (NIH - NCATS)
ThermoFisher	Screen HIC resins for MVP vs. MVM Clearance	Completed (BPI Webinar – 24Apr19)	
Amgen	Compare MVP vs. MVM clearance for mixed mode chromatography DOE	Anticipated completion by Q2 2019	
RegenX BIO	Compare MVP vs. MVM clearance for gene therapy process	Pending Phase II SBIR (NIH - NCATS)	
Teva Pharmaceuticals	Compare MVP vs. MVM clearance for continuous processing	Pending Phase II SBIR NIH - NCATS)	
Just Biotherapeutics	Compare RVLP VS. XMulV clearance	Anticipated completion by mid 2019	Funded through Phase 1 SBIR (FDA)

In this era of disruption, companies are continuing to increase spending and investment in new manufacturing process technologies and innovations that promise to increase downstream manufacturing productivity and efficiency. According, to a survey conducted by BioPlan Associates, Inc., the change in 2017 vs. 2018 budgetary spending for “new technologies that improve efficacies/costs for downstream production” increased by 7% and for “process development” by 6.8%, two of the 4 highest increases by category[30]. In fact, budgetary increases for downstream production were at a notably high rate. Despite demand for improvements, downstream purification remains the most challenging part of drug development. The industry and its vendors (e.g. Millipore, Pall, etc.) are actively seeking to address this demand through a range of technology innovations, generally focused on the development of cheaper, more effective purification steps. MockV Solutions is focused on providing the industry with a novel series of analytical tools to assess these innovative options. There are currently an estimated 5,224 biopharmaceuticals in various stages of development and an additional 1,211 already marketed (Figure 1)[31]. This diverse group of compounds includes recombinant therapeutics, vaccines, blood derivate, allergenics, cell immunotherapies, stem cell therapies, RNAi gene therapies and others. 2017 was a record year for FDA biopharmaceutical approvals, including a record number of recombinant protein/monoclonal antibody (mAb) approvals[30]. This likely reflects an increasing number and percentage of biopharmaceuticals vs. pharmaceuticals in worldwide drug development pipelines.

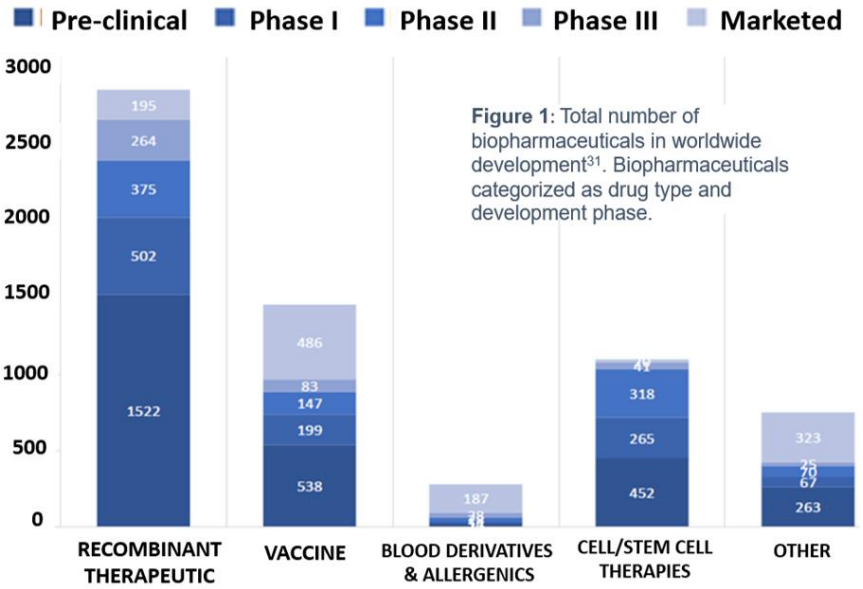


Figure 1: Total number of biopharmaceuticals in worldwide development[31]. Biopharmaceuticals categorized as drug type and development phase.

Monoclonal Antibodies and Gene Therapy Market Sectors

BP companies that utilize mammalian cell lines to generate mAb's are a primary market for MockV's MVP kits. Mammalian cell culture systems continue to dominate over alternative expression systems such as bacterial or yeast. In a recent survey, nearly 80% of respondents reported their organization utilizes mammalian manufacturing, up from 54% in 2003[30]. Much of the growth in the global biopharmaceutical pipeline is due to an increasing number of recombinant mAb's, now a greater than $75 billion market and accounting for more than 90% of the world's mammalian cell culture capacity[30]. MockV will target the estimated 2,108 mAb manufacturing processes in current development. The following characteristics demonstrate why mAb manufacturers are a primary focus: 1) mAb manufacturing places high demands on bioprocessing in terms of technical complexity, 2) mAb manufacturing requires multiple process steps and orthogonal approaches to viral clearance, 3) mAb manufacturing involves a high cost for purification, 4) there is a clearly defined and accessible customer segment, enabling a targeted marketing strategy and direct sales through a modestly sized sales team.

Another primary market for MockV's MVP Kit is the ever-expanding number of BP companies engaged in gene therapy activities. The increasing potential of gene therapy to effectively cure human diseases has created an unprecedented interest in developing these technologies. To date, three viral vector-based therapies have been approved by the FDA - two cell therapies and one gene therapy[32]. Gene therapies in development are centered on rare orphan diseases, mainly cancers and inherited monogenic diseases that typically have significant unmet needs[26]. Beyond the initial indications, there has also been growing interest in treatment of cardiovascular and infectious diseases which would greatly increase the number of patients eligible for gene therapies[33]. Increased confidence in gene therapy for clinical medicine has created intense competition in the biopharmaceutical space to develop life changing drugs for numerous disease indications. The treatment of the coagulation factor disorders haemophilia A and B using AAV vectors are examples where at least 10 separate companies have initiated or announced intended trials[34].

The current pipeline for gene therapies includes 351 drugs in clinical trials with 316 in preclinical development[32,35-37]. Many of these candidates are expected to come to market successfully in the next few years[33]; however, this widespread success is contingent on the maturation and standardization of process development and manufacturing activities. As manufacturing standards catch up to those of more established therapeutic modalities, process validation remains a crucial step. Viral clearance is one of these key validation issues that has been standardized for other BP moieties, but not fully implemented for gene therapy. Recently, the FDA cleared the first gene therapy in the US: Luxturna. Although Luxturna does not include viral clearance data as part of its validation package[38], new international guidelines regarding viral clearance have been drafted and future validation regulations are expected[27]. Applying our MVP kit to the gene therapy market will allow MockV to gain ground floor access to this quickly growing market.

Continuous Processing Market opportunity

There is significant pressure from patients and regulatory agencies to bring down costs of medicines and help launch life-savings products to market in a faster manner. Solving these problems requires innovation in manufacturing technologies to bring down costs and increase manufacturing flexibility. BP companies are currently using column chromatography — an expensive, outdated, and inefficient technology for purifying biologics. This is causing significant bottlenecks in manufacturing resulting in poor facility utilization, high costs, and long lead times for scaling up production. In addition, these bottlenecks and inefficiencies result in significant demand for capital for drug innovator companies to manufacture Phase I clinical material (>$5 Million). At large scale, column chromatography requires up to $17 Million in capital investments and has significant scale-up limitations. These include challenging packing procedures, cleaning validation, storage, and large floorspace requirements. Antibody purification is especially costly because of the high cost of Protein A media, which ranges from $10,000-$15,000 per liter. Continuous processing has now emerged as a viable alternative to inefficient batch purification methods[39]. Platforms such as ChromaTan's CCRC offer an attractive alternative to batch and multi-column processes. One significant hurdle hindering widespread adoption of CCTC, and continuous processes in general, are the inherent risks and logistical challenges of conducting viral clearance validation spiking studies. Especially relevant is the question of viral validation of integrated continuous platforms and whether performing the studies on the well characterized individual operations in batch mode would translate well to the fully integrated continuous process. The MVP market opportunity in this space will be significant - there are currently dozens of companies developing continuous processing platforms[40] with no process development/viral clearance solutions in mind.

Overall MVP Market Opportunity

Through a customer survey, MockV has identified five phases throughout a BP lifetime, from pre-clinical development through post-market approval, where the MVP kit would be utilized (Figure 2). These findings indicate the demand for viral clearance data during all stages of process development. A breakdown of the numbers revealed that over 90% of scientists surveyed expect that they will use at least 1 MVP Kit during process development activities while 100% would use at least 1 Kit during characterization activities. ~20% and ~50% indicated they would use more than 6 Kits during process development and characterization phases, respectively. Over 90% indicated that they would use at least 1 Kit before filing an IND and 50% said they would use at least 1 Kit after an NDA/BLA approval. Validation usage was overall lower than expected but this was likely a result of faulty questionnaire wording implying that MVP Kits could be used as an actual Validation method as opposed to a pre-Validation process check-up. Another important observation is the value that researchers placed on the MVP Kit – responders indicated that they would pay up to $5,000 per Kit.

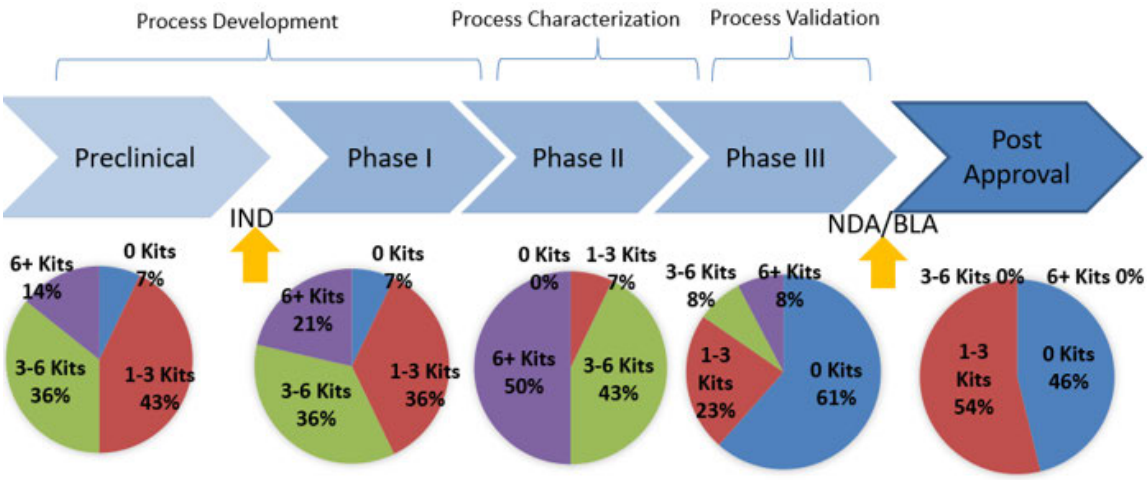


Figure 2: Market survey results (n=28) for MVP Kit usage during various clinical phases and process stages for biopharmaceuticals. Market data diagramed as pie graphs below each subsequent clinical phase

Based on these survey results, and the total number of mAb's and gene therapy products in various stages of development, we calculated the current addressable market for the MVP Kit to be $61M, while the projected addressable market is $204M (Table 2). Some key assumptions used to generate this model include: 10% and 20% growth estimates for the mAb and gene therapy pipelines, respectively (based on historical trends[35,41]) and an MVP Kit cost of $5,000. Not captured in this market estimation are potential revenues from non-mAb/gene therapy products such as recombinant proteins, blood or plasma derivates and vaccines. In addition, these market estimations are based solely on our lead MVP Kit which address parvovirus clearance. Our product line will include additional Kits that will cover retrovirus, rhabdovirus and other virus types that are of particular concern for certain industry sectors (e.g. HIV for blood products). Key market drivers that will sustain growth include: the continued growth of the global biopharmaceutical pipeline, the continued investment in process development and technologies to increase manufacturing processing efficiency (e.g. continuous manufacturing), the continued growth of the viral clearance industry and regulatory requirements surrounding viral clearance, the application of specific MVP Kits to new markets (e.g. water, food).

Current MVP Kit Market					
	Preclinical	Phase I	Phase II	Phase III	**Total Addressable Market**
mAb	2155	210	290	250	
Gene Therapy	316	114	204	33	
Expected Kits	3.32	3.6	5.075	1.3	
Total Kits	8205	1166	2507	368	**12,246**
Cost per Kit	$5,000	$5,000	$5,000	$5,000	
Total Revenue	$41,024,576	$5,832,000	$12,535,250	$1,839,500	**$61,231,326**
Projected 2027 MVP Kit Market					
	Preclinical	Phase I	Phase II	Phase III	**Total Addressable Market**
mAb	5590	545	752	648	
Gene Therapy	1957	706	1263	204	
Expected Kits	3.32	3.6	5.075	1.3	
Total Kits	25056	4502	10228	1109	**40,894**
Cost per Kit	$5,000	$5,000	$5,000	$5,000	
Total Revenue	$125,280,822	$22,509,790	$51,138,226	$5,542,959	**$204,471,796**

Table 2: Current and Projected mAb and gene therapy market for the MVP Kit. Expected Kit values based on market survey results. Post approval products not included.

Target Customer

MockV's MVP Kit offers a practical solution for generating viral clearance data at a minimal cost. Among the target markets that could benefit from such a tool, the BP industry stands out for their need to generate low cost data, early in drug development. Alternative target markets include the water and food industries which need to analyze virus removal for a variety of safety reasons. From within the BP market, several target customer sectors exist. The main customer sectors are: 1) process development scientists employed by BP companies or Contract Development and Manufacturing Organizations (CDMOs), 2) Viral Clearance Contract Research Organizations (CRO's) that offer live virus spiking studies, 3) product development scientists who work for industry vendors that supply BP companies with products and equipment to purify BP.

Process Development Scientists: BP process development scientists are employed to establish efficient and safe manufacturing processes for their therapeutic candidates. On average, these scientists spend 6 years and $50-100M developing, optimizing and validating manufacturing processes[42]. A major focus is the assessment of impurity removal, originating from the upstream cell culture process. To accomplish this, commercial kits are utilized to quantify the amounts of these impurities. MockV's MVP kit will be the first commercial kit to addresses viral clearance (Figure 3). By nature, process development is an experimental, data-driven discipline. The scientists who steer the industry are encouraged to explore novel products or techniques that could improve their processes' safety profile or production costs. A survey found that process development is one of the top areas in which both industry suppliers and end-users are developing and evaluating new technologies[30]. This sentiment was echoed in a market survey conducted by MockV which indicated a strong demand for multiple products within this space (partial data shown in Figure 2). These results are also supported by all members of MockV's scientific advisory board who have spent considerable portions of their careers in the process development aspect of the industry and have intimate knowledge of the subject.

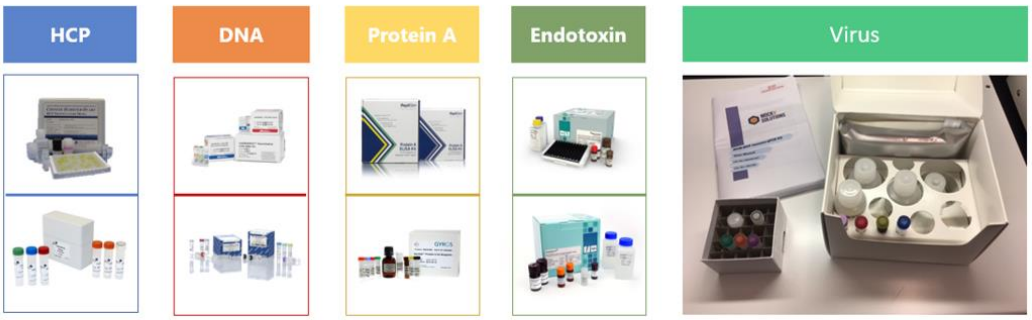


Figure 3: Commercial Kits available for Host Cell Protein (HCP), DNA, residual Protein A and endotoxin have helped process development scientists optimize BP manufacturing for decades. MockV's MVP Kit (left) will be the first Kit to address viral clearance.

Process development scientists employed by CDMO's are an important and increasing market focus. Current trends in BP process production reveal an increased reliance on CDMOs as one-stop shops for large molecule process development and manufacturing The CDMO model allows for specialization in manufacturing and scale up. CDMOs have also heavily invested in gene therapy capabilities. This has also led to major partnerships and agreements by both traditional and new CDMOs. As an example, Lonza has entered into 6 major manufacturing partnerships with gene therapy companies in the last year and has built a dedicated viral therapy facility. The top 5 CDMOs hold 47% of the market share: Lonza, Boehringer Ingelheim, Patheon, Samsung Biologics and Catalent[43]. Developing relationships with these companies will be important; but, up and coming CDMO's focused on gene therapy, continuous processing and other innovative strategies such as AGC Biologics, Fujifilm Diosynth, WuXi Biologics, Brammer Bio and Paragon may be more likely to adopt new technologies and provide a competitive advantage to their customers over established companies.

CROs: Another market segment that could benefit from MockV's products are the CRO's who conduct live virus spiking studies. By offering a cheap alternative to their expensive and logistically challenging validation studies, CRO's could 1) attract customers earlier in product cycles, 2) market their service throughout process development and characterization, and 3) establish business relationships that would later translate into expensive validation studies. Texcell N.A. has realized the value of this approach and has partnered with MockV on several projects (see letter of support).

Product Development Scientists: Industry vendors such as ThermoFisher, Sartorius-Stedim, Millipore, Pall Corporation and Asahi Kasei employ thousands of product development scientists whose task it is to develop and commercialize tools for BP manufacturing. Chromatography resins, nanofilters and Tangential Flow Filtration membranes are a few examples of the technologies that are incorporated into a BP process. Just like Process Development Scientists, *Product* Development Scientists are concerned with the viral clearance efficacy of their products. Also similar to process scientists, the hurdles that these product scientists face in testing viral clearance is a major barrier to innovation and product development. MockV's MVP Kit will allow these developers

to screen materials, configurations, flow paths, etc. during product development and ultimately deliver high quality, effective products to the market (see letters of support).

Alternative Approaches

The current method for obtaining viral clearance data is through live viral clearance "spiking studies". Due to the complexities and threats that live viruses pose, studies can range from $100,000-$500,000[16-17], depending on scope. Additionally, these studies involve specialized (BSL-2/3) CRO's and take months to design, execute, and analyze. Because of this, spiking studies are generally performed only prior to a regulatory filing and data is often absent during the years of process development and characterization. MockV's MVP Kit will allow for the economic and accurate prediction of virus clearance to be executed in-house, under BSL-1 conditions. This will enable scientists to build confidence into their process, increasing their chance of achieving necessary levels of viral clearance during validation. Prior to MockV, several approaches had been introduced to address this issue (Table 3); however, none of these attempts has resulted in an accurate and dependable commercial option.

Method	Prediction Accuracy to MVM			Cost/Study[1]	BSL-I Compatible	Duration	Commercial Availability
	Size	Charge	Hydrophobicity				
Standard Regulatory Method							
Live Virus Study	N/A	NA	NA	> $50,000	No	> 4 weeks	Yes
Predictive Methods							
Bacteriophage	Yes	No	No	No Data	No[2]	1-3 Days	No
CHO-RVLP[3]	No	No	No	No Data	Yes	1 Day	No
MVP	Yes	Yes	Yes	$5,000	Yes	1 Day	Pending

Table 3: Summary of Viral Clearance testing options (1) For the sake of comparison, a "Study" refers to ~ 10 individual small scale "spiking" experiments. (2) Certain bacteriophage are BSL-1 compatible; however, many BP labs choose not to risk introduction of them into their facilities. Other strains are BSL-2. (3) Chinese Hamster Ovary – Retrovirus Like Particles. These particles are only useful for retrovirus prediction.

Bacteriophages: For the reasons mentioned above, BP manufacturing process development scientists have attempted to use bacteriophages as viral surrogates during filtration and chromatography spiking studies[44-46]. The advantages of using bacteriophages as surrogates to mammalian viruses during viral clearance studies include their size similarity to viruses, their ability to be easily cultivated and assayed (compared to live virus), and their non-infectious nature, which allows them to be BSL-1 classified (true for some, not all). Although some evidence supports the use of bacteriophages as a virus surrogate during viral filtration studies, the question of whether bacteriophages can accurately represent mammalian viruses has been and remains controversial[44,45]. The common bacteriophages used in typical manufacturing process development experiments exhibit different physicochemical properties to the live viruses they replace[44]. During physicochemical based separation (i.e. ion exchange chromatography), these differences may translate into vastly different clearance results. Thus, inaccurate data generated by the substitution of bacteriophage may lead to sub-optimal or potentially faulty process development decisions. MockV Solutions use of MVPs as viral surrogates overcomes the risks associated with bacteriophages by assembling MVP's from the same proteins which comprise the actual virus it represents. In addition, MVPs are quantifiable through non-infectivity-based methods, which are quicker, easier and more robust than the plaque assays currently used to quantify bacteriophage.

CHO-RVLPs: Recently, scientists have used Chinese Hamster Ovary – Retrovirus Like Particles (CHO RVLPs) as surrogates to retrovirus during spiking studies[47]. The benefits of using these particles include their physicochemical similarities to retrovirus, and their non-infectious nature, allowing them to be BSL-1 classified. However, compared to MockV's MVP-based surrogate kits, these benefits are diminished. First and foremost, RVLPs are similar in size and surface charge only to large enveloped virus such as Xenotropic Murine Leukemia Virus and are therefore not an appropriate substitute for other regulatory associated viruses such as MVM (the target MVP of this Phase 2 application and lead commercial product). Furthermore, CHO-RVLP methods involve the spiking of impure contaminants into purified biopharmaceutical material. This practice may introduce undesired impurities into solution such as host cell proteins, DNA, etc. During clearance experiments, the presence of these impurities may lead to questionable results. MockV's MVP spiking solution will be highly purified and standardized and will thus not contribute impurities to an experiment. In fact, MockV's awarded Phase 1 FDA (NCATS) SBIR grant is aimed to generate and demonstrate the concept of utilizing highly purified and concentrated solution of CHO-RVLP.

Barriers to Adoption

The main alternative to adopting MockV's strategy is the status-quo – **not performing any viral clearance related work during the years leading up to validation.** MockV believes that enabling scientists to quickly obtain accurate, low-cost viral clearance data throughout the development of a BP manufacturing process will overcome the added expense not currently incurred and lead to widespread adoption. Commercially available impurity kits are ubiquitous during process development (e.g. HCP, see Figure 3). The MVP Kit will enter a mature and fluid market in which established workflows are in alignment with its use – the barrier to entry is low. To accomplish this optimistic outlook, the MVP Kit will need to demonstrate high reproducibility and accuracy to its customers. While this endeavor is being undertaken by MockV for mAb process development through a series of collaborations (see Table 1), further establishing the capabilities of the MVP Kit for gene therapy and continuous bioprocessing will allow integration of this product at this nascent stage. Accomplishing this, MockV will become an industry leader in viral clearance process development technology. A SWOT analysis is provided in Figure 4.

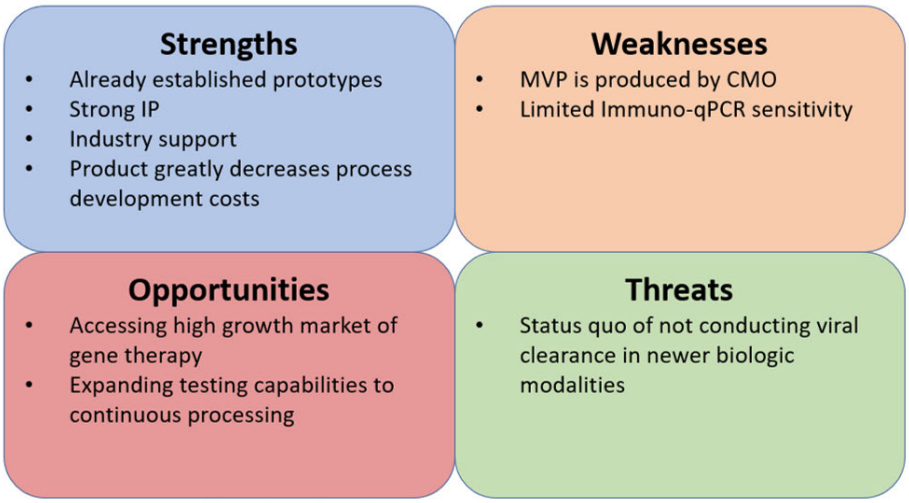


Figure 4: SWOT analysis of MockV Solutions

Competition

The MVP Kit will facilitate the development of more robust and efficient BP manufacturing processes, decrease failed live viral clearance studies, decrease regulatory delays, and a decrease in unnecessary and costly re-development efforts. As such, competitors would be attracted to the market. MockV will address this first and foremost through the enforcement of its IP (see below). Second, with the head start we have built and the future leverage provided by a large strategic partner (global distribution and marketing platforms) we will aspire to saturate the market with our brand before competition can become a major factor. Until such a strategic partnership is formed, MockV has the advantage of remaining small and quick relative to the most foreseeable competitors (ex. Cygnus, ThermoFisher). This promotes innovation at the expense of lacking comparative resources. A major reason why the awarding of this Phase II grant is so critical at this time is that it allows us to remain small and agile will providing us with the resources that our competition would have at their disposal. Note – our strategy, which we are successfully implementing, is to form collaborations with these competitors so that one eventually becomes the key strategic partner or outright buyer of the technology instead of its competition.

D. ***Intellectual Property (IP) Protection***.

MockV believes that IP is an essential element of its business strategy and is pursuing a careful yet aggressive strategy to protects its interests. To this end, we were awarded US Patent Patent Info in 2017 with claims covering non-infectious methods for predicting viral clearance. These broad method claims serve as the foundation in the US for establishing a strong patent estate protecting a series of MVP-based commercial kits. **In 2018 we filed a divisional application to pursue kit claims in the US**. Moreover, in December 2018, the EPO allowed claims similar to those that have issued in the US, which are expected to issue in Europe by the Summer/Fall of 2019. The same scope of claims are pending in Japan, China and Australia. MockV has also pursued additional, newly filed applications to claim method/kit improvements to retroviral clearance.

E. ***Finance Plan.***

Upon inception, MockV was funded through founder and private investments. Starting in 2015, MockV was able to secure external resources through a series of county, state and federal awards (see above). This capital has recently enabled MockV to develop and test a prototype version of its first potential commercial product - the MVP Kit. Starting in mid-2018, industry demand for this prototype has enabled MockV to generate a modest revenue stream which, aside from demonstrating the market potential of this product line, enables the daily operation of the company and maintains a funding source for all current industry collaborations (see Table 1, above). Assuming a steady stream of revenue from prototype sales, MockV estimates that an additional $3.5M in capital will be required to advance the critical activities of the company. Therefore, the company will seek federal funding, equity investment, or partnerships (or a combination thereof) to: 1) Optimize and commercialize

the MVP Kit, 2) Expand application scenario testing (e.g. Gene therapy, continuous processing), 3) Internalize the Kit production capabilities, including MVP production, 4) Strengthen and maintain IP and, 5) Expand the product line (e.g. Retrovirus Kit).

Based on the appeal of our proof of concept datasets, generated throughout our Phase 1 SBIR project and published collaborations, we have entered into discussions with potential industry partners and investors to further the goals outlined above. Leaders from these large and internationally established process development vendor companies have provided MockV with letters of endorsement for the optimization of the MVP Kit and have expressed promising outlooks for our overall commercial potential. Recently, MockV has conducted site visits of with two of these leaders who, on-behalf of their companies, expressed serious interest in a strategic investment. Several of these companies were able to provide letters of support to this submission (e.g. ThermoFisher, Asahi Kasei Bioprocess America, Cygnus Technologies, and Sartorius-Stedim).

While we will rigorously continue to seek all types of funding outlined above, it is deemed preferable to advance the bulk of our immediate aims through non-dilutive SBIR funding while offering an introductory equity stake in MockV to a well established strategic industry partner. Ideally, after successful completion of a Phase 2 project, this partnership would provide a faster and more cost-effective way to bring the MVP Kit to market. With the existence of an established, multinational marketing and distribution operation, the MVP Kit, and follow-on products, would reach the maximum set of hands within the bioprocess development community. These scientists will then utilize these kits to optimize safe modes of biopharmaceutical manufacturing and decrease the overall costs of production.

F. ***Production and Marketing Plan.***

Production

A goal of this Phase 2 SBIR is to pursue the optimization efforts of the first commercially viable, BSL-1 compatible viral clearance kit. This “MVP Kit” will include a purified and concentrated stock solution of Mock Virus Particle “MVP” as a spiking agent and quantification reagents to perform a highly sensitive and specific companion assay. The systematic production of each kit component will occur at MockV Solutions, after the internalization of several key processes that are currently outsourced to contract manufacturing labs, namely MVP production. The decision to fully internalize Kit production operations is primarily driven by the long-term cost saving benefits derived from the ability to scale-up MVP production and eliminate the overhead costs associated with outsourcing. Currently, a contract lab based in Maryland produces small batches (5L) of MVP while utilizing an antiquated purification process (density gradient centrifugation). Although reliable, improved yields and lower $/particle batches can be achieved through the implementation of a chromatography-based purification approach. MockV has recently demonstrated proof of concept of purifying crude extracts of cell lysate containing MVP through a custom-made affinity chromatography resin, made by coupling its proprietary anti-MVP monoclonal antibody to a base matrix. This approach is a promising first step in the technical transfer of the MVP producing process to its facility. With complete independence over Kit production, MockV will be able to quickly and efficiently manufacture all components, reference internal documents and provide technical support to customers. An additional factor in the decision to internalize production was the recently accumulated expertise brought into MockV through the appointment of Personal Info to Director of Chemistry Manufacturing and Controls (CMC, see Biosketch and Letter of Commitment). In addition to his experience as an assay development scientist, Personal Info brings a proven system of documentation and good manufacturing methodologies to MockV, which include: raw material specification control, manufacturing procedures, quality control testing and quality assurance review.

After the optimization of the MVP Kit, some of the components currently utilized will become obsolete while others that are currently outsourced will transition toward internal production. The predicted impact of optimization and internalization on production costs is shown in Table 4. As seen, the total cost of producing the commercial MVP Kit is projected to decrease 49% from $2,777 to $1,412. This decrease is mainly a result of A) assay optimization leading to reductions in Kit components and B) the internalization and scale up of the MVP production process. While production costs will decrease, the sale price of the Kit will increase to $5,000. This increase is acceptable according to market survey (see Overall MVP Market Opportunity section) and is justified due to the improvements made to the quantification assay - increasing dynamic range and enabling

Process	Cost/Kit ($)	Sale Cost ($)	Gross Margin (%)
Current (CMO)	$2,777	$3,000	7%
Commercial (Internal)	$1,412	$5,000	72%

Table 4: MVP Kit production costs, sale price and gross margins. Current (prototype) vs. projected commercial.

Log Reduction Values of > 4.0 while decreasing assay time in half. Through these efforts, we anticipate the expected gross margin of the MVP Kit to be approximately 72%.

Marketing Plan

Initially, MockV has promoted its work through the publication of collaborative projects in scientific journals and through posters/presentations at industry conferences. In the following years, we will embark on an active marketing approach through social media networks, direct networking, and more traditional media like mailings and print materials. MockV Solutions will continue to exhibit at vendor fairs and bioprocessing conferences. In addition, strategic advertisements will be placed on relevant websites and in leading trade journals such as GEN and Bioprocess International. In fact, MockV exposure generated through articles published in these journals[48,49] has already driven considerable traffic. MVP Kits will be sold directly through the MockV website (www.mockvsolutions.com). Distribution will be managed through a sales and marketing team. While this team will travel and offer on-site laboratory demonstrations of the kit, travel time and expense will be limited to new prospects or new teams at existing customer locations. To increase market reach, and for non-US sales, MockV will sign distribution agreements with one or more major downstream process development companies or CROs.

G. ***Revenue Stream.***

MockV is developing and commercializing a series of analytical kits for the biopharmaceutical manufacturing process development industry. As we move toward full commercialization, demand for our un-optimized pre-Phase 1 MVP Kit Prototype has steadily increased (33% Y/Y growth projected for 2019 based on Q1 sales). This indicates commercial interest in the product and helps to build partnerships with key industry partners. Table 5 is a 5 year Pro Forma. In 2019 and in 2020, Kit sales are based on the Prototype model ($3,000/Kit) and growth projections of 33% and 50% Y/Y for 2019 and 2020 respectively. For 1H 2021, Kit sales are still based on the Prototype model but projections are based on 100% growth from 2H 2020 to 1H 2021. In Q3 2021 we plan to fully launch the MVP Kit ($5,000/Kit) and assume a 100% increase in sales during that quarter. In subsequent quarters we expect 50% growth (Q/Q). Some other key assumptions used to generate the forecast include: 1) A total infusion of $3.5M (grant + investment from 2019-2021), 2) An incremental COG decrease throughout 2020 and 2021 until 72% margins are accomplished in Q1 2022, 3) the expansion of sales into innovative market segments like gene therapy and continuous processing in 2021 to fuel growth.

MVP kit for mAbs

The application of the MVP Kit to mAb customers will be the initial revenue stream for the company. Revenue is dependent on the establishment of the commercial MVP Kit. MockV will sell direct, and through strategic partners. One particular customer focus will be CDMOs. As increased development is conducted through CDMOs, establishing relationships with them will be a useful approach.

	2019	2020	2021	2022	2023
Grants	$ 1,000,000	$ 1,000,000	$ -	$ -	$ -
Investment	$ -	$ -	$ 1,500,000	$ -	$ -
Kit Sales	$ 120,000	$ 180,000	$ 930,000	$ 5,484,375	$ 27,764,648
Revenue	$ 1,120,000	$ 1,180,000	$ 2,430,000	$ 5,484,375	$ 27,764,648
COGS	$ 111,084	$ 143,875	$ 360,225	$ 1,548,788	$ 7,840,737
Facilities & Operation	$ 15,000	$ 15,000	$ 46,000	$ 76,000	$ 150,000
Capital Equipment	$ 7,000	$ 50,000	$ 200,000	$ 500,000	$ 1,000,000
Head Count	3	3	5	8	15
Salaries	$ 270,000	$ 270,000	$ 427,000	$ 679,700	$ 1,277,670
R&D	$ 448,725	$ 448,725	$ 673,088	$ 1,009,632	$ 1,514,448
Legal/Patent	$ 50,000	$ 75,000	$ 100,000	$ 150,000	$ 200,000
M&S	$ 5,000	$ 5,000	$ 200,000	$ 400,000	$ 800,000
G&A	$ 250,489	$ 250,489	$ 375,734	$ 450,880	$ 541,057
Expense	$ 1,157,299	$ 1,258,089	$ 2,382,047	$ 4,815,000	$ 13,323,912
Net Profit	**$ (37,299)**	**$ (78,089)**	**$ 47,953**	**$ 669,375**	**$ 14,440,737**

Table 5: 5 Year Pro Forma

MVP kit for Gene Therapies

The application of the MVP Kit to gene therapy customers is a promising extension of MockV's core technology. The revenue stream for gene therapies will be behind that of the mAbs because additional proof of concept testing will be required. Otherwise, the revenue growth will be similar to that of the mAb market.

From gene therapy to monoclonal antibodies, from platform to continuous processing, the innovation that MockV is bringing to the market is highly valued and anticipated. In summary, the commercialization of the MVP Kit will provide bench-top BP process development scientists with a novel tool to generate quick, economic and accurate viral clearance data.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12831646 Funding Opportunity Number: PA-18-574 . Received Date: 2019-04-05T15:30:56.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	Introduction_30Mar19.pdf
Research Plan Section	
2. Specific Aims	Specific_Aims_04Apr19.pdf
3. Research Strategy*	Research_Strategy_5Apr19.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Consortium_and_Contractual_Arrangements.pdf
9. Letters of Support	Merged_File.pdf
10. Resource Sharing Plan(s)	Data_Sharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	MockV_AoR.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12831646
Funding Opportunity Number: PA-18-574. Received Date: 2019-04-05T15:30:56.000-04:00

Reviewer Comments

SPECIFIC AIMS

Introduction: MockV Solutions, Inc. (MockV) is an early stage company creating viral-surrogate tools that solve the unmet needs of scientists as they develop and optimize products and techniques in a variety of industries that currently rely on expensive and logistically challenging live virus analysis. We are currently focused on developing a novel series of analytical assay kits which will enable biopharmaceutical (BP) process development scientists to study the efficacy of manufacturing techniques intended to remove or inactive virus, a contaminant of great concern during the manufacturing of BPs. The goal of this Phase 2 SBIR project is to advance the first Biosafety Level 1-compatible viral clearance kit toward commercialization through product development and applications testing efforts.

Significance: Viral contamination is an inherent risk during the manufacture of BPs. International regulatory agencies require "viral clearance" validation studies prior to approving BP's for clinical trials or commercial use. This is accomplished through small scale "spiking studies" whereby model viruses (ex. *Minute Virus of Mice, "MVM"*) are artificially introduced into BP material and subsequent removed or inactivated via purification techniques. These studies require specialized Biological Safety Level -2 laboratories (BSL-2) and trained personnel resulting in costs that can soar well above $100,000. Due to the high costs and logistics associated with viral clearance studies, most companies delay assessments during process development, increasing the risk of a validation failure. Failures force companies to re-develop their manufacturing process. In addition to in-efficiencies and cost over-runs, re-development can delay the regulatory approval of BP's. It has been estimated that a 6 month delay in approvals can result in a $100M loss in net product value. This can affect a company's market strategy and cost patients timely access to therapies. A BSL-1 compatible, low cost viral clearance prediction kit, utilized during small scale process development would provide bench-top scientists with a unique tool to generate "real time" data on viral clearance. With a "Quality by Design" approach, these scientists could confidentially optimize conventional or continuous manufacturing process to clear virus and determine if process steps are effective before investing significant resources in regulatory-supporting validation spiking studies. The time and resources saved by utilizing such a tool would translate into cheaper $/gram manufacturing costs and increase the likelihood of passing regulatory hurdles.

Product: The product of this Phase 2 SBIR will be a BSL-1 compatible assay kit, which will allow scientists to accurately and economically predict MVM clearance throughout process development, prior to expensive validation studies. This "MVP Kit" will include a highly purified and concentrated stock solution of MVP for use as a "spiking agent" and reagents for a sensitive and specific Immuno-qPCR assay (microwell plate, antibody, primers, probe, etc.). We anticipate that the MVP Kits will be utilized ubiquitously during small scale activities and shift the paradigm of BP process development.

Long Term Goal: In the long term, we anticipate that in certain applications, the MVP Kit could replace live MVM spiking studies and become an accepted validation practice. This revolutionary shift will only be possible after many years of data accumulation and "buy-in" from regulatory agencies. In the meantime, the MVP Kit will become a standard process development tool that will increase the approval rates of BP's and decrease their manufacturing costs.

Phase 2 Objective: MockV's Phase 2 objective is to advance the MVP Kit toward commercialization through production optimization and test its utility in gene therapy and continuous process applications. We aim to prove that our surrogate will accurately predict MVM removal throughout a downstream gene therapy process and demonstrate its value as a tool for developing continuous processing techniques. To accomplish these objectives, the following aims will be achieved:

Aim 1: Optimize scalable MVP production, establish high quality manufacturing systems and protocols.

Aim 2: Validate the use of MVP as an MVM surrogate for gene therapy applications.

Aim 3: Validate the use of MVP as an MVM surrogate for continuous processing applications.

Aim 4: Optimize a second generation high throughput MVP quantification assay

Expected Outcomes: For Aim 1, MockV expects to optimize a scalable process and reduce MVP production costs by at least 50%. For Aims 2&3, we expect our non-infectious MVP to accurate predict MVM removal during gene therapy and continuous processing applications. For Aim 4, a second-generation assay will be established that increases MVP quantification dynamic range and assay robustness.

Plans for Phase 3: We will continue to internalize the production of Kit components while adhering to the quality control standards and methods established during Phase 2. We will then conduct extensive beta testing of the MVP Kit through industry collaborators and partners prior to commercial launch.

Commercial Application: Viral clearance is an international regulatory requirement for the ~5,000 BP in worldwide development. With the projected cost of an MVP Kit to be $5,000, we calculated the current addressable market to be $61M, while the projected addressable market is $204M. We are currently generating data through collaborative studies with leading industry partners and are gaining exposure through the strategic dissemination of data. An established sales team will conduct in-lab product demonstrations while Kits will be sold through our website and international distribution partners.

RESEARCH STRATEGY

The goal of this Phase 2 SBIR project is to advance the first Biosafety Level 1-compatible viral clearance kit toward commercialization through product development and applications testing efforts.

SIGNIFICANCE

Viral safety is a critical aspect of manufacturing biopharmaceuticals (BPs)[1-5]. Although well characterized mammalian cell lines such as CHO have been employed for decades, endogenous expression of retroviral like particles and exogenous contamination events from viruses such as Minute Virus of Mice (MVM) warrant continued vigilance[6,7]. International regulatory agencies require BP manufacturers to validate the "viral clearance" efficacy of their downstream manufacturing process steps prior to awarding clinical trial or commercial approval[8-10]. Currently, this is accomplished through **small scale** "spiking studies" whereby specific model mammalian viruses (ex. MVM) are artificially introduced ("spiked") into BP material and subsequently removed via downstream purification steps such as chromatography and virus filtration[11,12]. Spiking studies require specialized Biological Safety Level (BSL)-2 laboratories and trained personnel resulting in costs that can soar well above $100,000[13,14]. These hurdles **deter** companies from analyzing viral clearance during the years of small-scale **process development** leading to validation. Instead, companies spend considerable resources optimizing manufacturing processes **before gaining any knowledge** of their viral clearance efficacy. Unfortunately, this increases the risk of validation failure, forcing companies to invest additional time and money redeveloping process steps, which in turn, could postpone regulatory approvals. This can affect a company's go-to-market strategy and delay patients' timely access to therapies.

Viral clearance not only presents challenges to current BP process development efforts, it is also critical to the successful advance of two highly impactful new trends in the biopharmaceutical industry: **gene therapy** and **continuous processing**[15-17]. In the case of gene therapy, the vectors which are utilized to deliver therapeutic payload, such as adeno-associated viral (AAV), are similar in size to MVM, the parvovirus used internationally as a model "spiking" virus for sized based separations[18,19].This precludes the use of parvo-grade virus filters in gene therapy processes, a cornerstone of mAb processes and viral clearance validation. As a result, *the burden of viral clearance will fall to chromatographic methods of separation*. Affinity and anion exchange chromatography (AEX), are common methods within gene therapy purification processes[20,21], but the optimization of these steps to remove virus will incur large efforts and costs. In the case of continuous processing, complex flow paths, significant variation among the available systems and the nature of operating in a continuous mode unto itself have created unique challenges from a viral clearance validation perspective[16,17]. Experimental trial and error is the natural approach, but the costs associated with viral clearance present a major hurdle to companies considering the adoption of these innovative technologies.

A **simple, low cost, BSL-1 compatible viral clearance assessment kit**, utilized during small scale process development, would provide scientists with a unique and practical tool to generate "real time" data on viral clearance efficacy. With a "Quality by Design" approach, scientists could confidently optimize the virus removal capability of purification steps in continuous or conventional processes prior to investing significant resources in regulatory-supporting viral clearance validation spiking studies. The time and resources saved by utilizing such a tool would translate into cheaper $/gram manufacturing costs for a variety of therapeutic modalities and greatly reduce the existing barriers to process innovation in the fields of gene therapy and continuous processing.

Innovation: MockV Solutions (MockV) is addressing the barriers imposed by the cost and logistics associated with viral clearance through a novel and US patented use of Virus Like Particles (VLPs). VLPs are multiprotein structures that mimic the morphology and surface characteristics of native infectious viruses but are themselves non-infectious[22]. These properties have made VLPs an interesting class of molecules for potential use as vaccines[23], but these same features also make VLPs ideal for use as BSL-1-compatible analytical process development tools. Realizing this, MockV decided to express the major capsid protein of MVM in a recombinant expression system to form MVM-VLP's, referred to here as Mock Virus Particles, "MVP's". **These purified MVP's have been shown to be physicochemically similar to MVM[24] and during Phase 1, the feasibility of predicting MVM clearance through the use of MVP's was demonstrated**. The commercial product to be developed and tested in this phase 2 SBIR will be the first BSL-1 compatible, viral clearance assay kit. The "MVP Kit" will include a highly purified and concentrated stock solution of MVP as a "spiking agent" and reagents for a sensitive and specific Immuno-qPCR (I-qPCR) assay. To perform an MVP spiking study, a scientist would: 1) add MVP stock solution to test material, 2) process material through a purification technique (e.g. chromatography), and 3) analyze MVP quantity via I-qPCR to determine clearance (see Fig. 1, original application). The ease, rapidity and price-point at which viral clearance data could be accumulated has the potential to shift the paradigm of BP process development as it relates to viral clearance (See letters of support from industry, academia and institutions). Aside from the innovative application of VLP's to BP process

development, the MVP Kit will be a catalyst to screen and adapt other innovative technologies and techniques in the field of BP manufacturing. For example, continuous processing has been cited as having the ability to overcome many of the challenges in current batch manufacturing by providing full automation, shorter processing times, higher efficiencies and lower costs[25-27] (also see letters of support). Although several companies have introduced systems, viral clearance in continuous operation remains a significant unsolved barrier to widespread adoption[16,17]. MockV is in a unique position to address this issue with a low cost viral surrogate approach that would enable exploration within this vibrant space. Similarly, the MVP Kit can lower the viral clearance exploration barrier to other innovative technologies or products such as High Throughout Resin Screening and universal isotype AAV Affinity resins (see data from NIH Collaboration and Letter of Support from ThermoFisher).

Value Proposition: The MVP Kit would provide downstream scientists with the most economic, accurate and practical method for predicting MVM clearance. The **non-infectious** nature of MVP's and the **rapid turn-around time** of analysis will enable scientists to conduct evaluations **"in-house"** (BSL-1) in a matter of hours, as opposed to the weeks required to plan and conduct viral clearance studies. The physicochemical resemblance of MVP to MVM enables the accurate prediction of MVM clearance based on size, charge and hydrophobic modes of separation[24,28]. Although long-term potential exists to utilize MVP's for validation purposes, the value of this technology ***is not as a replacement to live viral spiking studies, but to enable viral clearance measurements as part of process development, process characterization and pre-validation efforts*** (see Figure 2, Commercialization Plan). A wide range of therapeutic modalities including AAV and mAbs would benefit from its use as would those implementing traditional or continuous processing techniques. For a more thorough discussion, please see our Phase 1 application and Phase 2 Commercialization Plan.

B. Approach

1. Preliminary Data and Phase 1 Project Report

Prior to its Phase 1 SBIR award, MockV had demonstrated the physicochemical similarities between its non-infectious, recombinantly produced MVP and live infectious MVM through a collaboration with Personal Info lab at the Center for Drug Research and Evaluation, FDA, Silver Spring, MD. Published results from this collaboration suggested that the MVP could be used as an accurate predictor of MVM clearance for downstream bioprocess applications[24]. To test this hypothesis for chromatography and virus filtration, a number of collaborations with leading industry vendors and biopharmaceutical companies have been undertaken (Commercialization Plan, Table 1). In a published collaboration with Asahi Kasei, flux decay profiles and Log Reduction Values (LRV's) were found to be similar between MVM and MVP[28]. More recently, in a completed study with MedImmune, the practical aspects of utilizing MVP in a typical process work-flow were assessed along with MVM predictability for Anion Exchange Chromatography (AEX) applications (summarized in original submission, Figure 2). This data was submitted for publication and presented at the 2018 Recovery of Biological Products Conference (Asheville, NC) where it received an "award for best poster" by the attendees. In these initial collaborations, a polyclonal based (pAb) Immuno-qPCR (I-qPCR) assay was utilized to quantify MVP. Although robust and more sensitive than a previously established ELISA, the high background limited LRV determinations to ~3.0. Because of this issue, and the long duration of the assay (~ 8 hours), the NIH- VRC, in a recently completed collaboration, chose to utilize a MesoScale Discovery analytical platform for MVP analysis. Although this platform provided a rapid assay capable of providing LRV's ≥ 4.0, the MVP spiking requirements had to be increased by 100-fold. Nonetheless, this assay was utilized in conjunction with a High Throughput Resin Screening study for the NIH's vaccine production platform. During this study, 8 separate AEX resins were rapidly screened over a variety of pH values and conductivities for MVP clearance. The results (manuscript in preparation) led the NIH to select several resins for further vaccine production development.

During Phase 1, the following objective was proposed and achieved: Improve the current I-qPCR assay to enable the calculation of higher LRV's and reduce assay time/complexity. The strategy consisted of replacing an anti-MVP capture pAb with a monoclonal antibody (mAb) and directly conjugating it to a nucleic acid, forming a detector probe, as opposed to relying on a series of Biotin-Neutravidin interactions (see Figure 3, original application). Having successfully produced hybridoma cell lines expressing anti-MVP mAb just prior to funding, Phase 1 assay development commenced by selecting highly specific and sensitive clones. From ELISA and Western blot screening experiments, an IgG1k mAb was identified and used for subsequent assay development. First, the optimal concentration of "capture" mAb was determined through ELISA experimentation in which decreasing concentrations of mAb were coated onto microtiter plates. The results demonstrated that a mAb concentration of 1 µg/mL was required for optimal MVP capture. Next, several coupling strategies were attempted to conjugate chemically-modified Target DNA (same amplicon sequence as pre-Phase 1) to the mAb, including primary amine/NHS ester and thiol/disulfide interactions. This effort led to a variety of different

conjugation products with different mAb:DNA ratios. These products were then purified by extensive dialysis and systematically screened by binary I-qPCR experiments (+ MVP/-MVP) to yield MVP-specific conjugates with low prevalence of non-specific interactions. The result of this work led to the selection of several high performing candidates which were further screened by conducting I-qPCR experiments while varying the concentration of each conjugate. These results led to the selection of yet another smaller batch of high performers which were screened in conjunction with assay additives and blocking agents, including: Bovine Serum Albumin, detergents, sheared salmon-sperm DNA, Non-fat dry milk and gelatin. These elements were incorporated into plate-blocking solutions, wash buffers, component-diluents or combinations thereof. The results from these experiments led to the final selection of components, concentrations, and conditions that were ultimately incorporated in subsequent proof of concept studies. Further assay optimization experiments were undertaken to reduce process time and sources of error. These efforts led to a decrease in step-incubation time from 60 to 30 minutes and the replacement of a cumbersome 95˚C pre-qPCR dissociation step with a more easily manageable low-pH dissociation step. **Once finalized, intra-assay and inter-assay testing was performed according to FDA guidance[50].** Results are shown in Figures 1 and 2. For intra-assay verification, a 10-fold titration series of MVP was analyzed (n=9). The average Ct values, standard deviations (σ) and coefficients of variation (CV) demonstrated acceptable precision (CV ≤ 5.0%) at each point tested. A regression line of best fit demonstrates acceptable linearity over the range tested (r^2 =0.98). To determine the statistical difference between assay background (0 MVP/mL) and dilute MVP data points (1E5, 1E6 MVP/mL), a Students T Test was performed. The results demonstrate a statistical difference between background and 1E6 MVP/mL (p=0.001). Also demonstrated was a statistical difference between each titration series point, including 1E5 and 1E6 MVP/mL (p=0.008). These results enabled us to set a lower limit of quantification (LLOQ) of 1E6 MVP/mL. For inter-assay assessment, testing was performed at several collaborative locations (Asahi, Amgen and GSK). The results from each testing site demonstrated acceptable precision at each titration point tested (CV's ≤ 5.0%). In addition, a comparison of the regression lines generated from each titration series demonstrates the consistent performance of the assay among operators/sites. T Tests were performed between assay background and dilute MVP data points assessment, as described above. The results demonstrate statistical differences between background and 5E5 MVP/mL (p≤0.05), enabling a **dynamic range of 4.5 $\log_{10}$** to be determined when the starting titration is 1E10 MVP/mL**- a ~30-fold improvement over the pre-Phase 1 assay**.

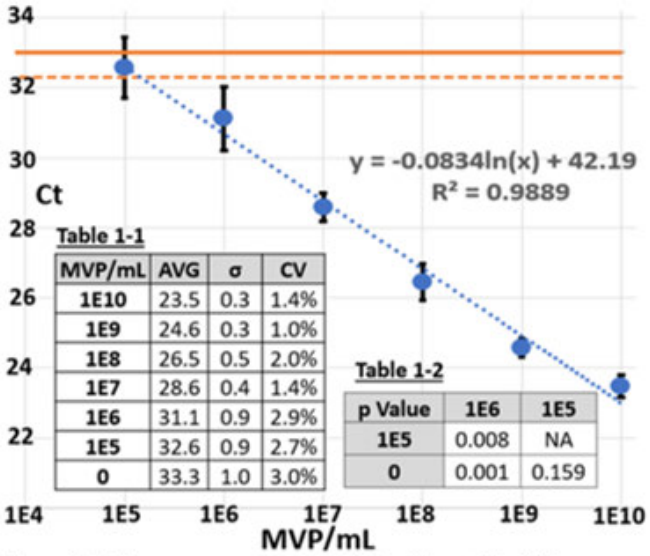


Table 1-1

MVP/mL	AVG	σ	CV
1E10	23.5	0.3	1.4%
1E9	24.6	0.3	1.0%
1E8	26.5	0.5	2.0%
1E7	28.6	0.4	1.4%
1E6	31.1	0.9	2.9%
1E5	32.6	0.9	2.7%
0	33.3	1.0	3.0%

Table 1-2

p Value	1E6	1E5
1E5	0.008	NA
0	0.001	0.159

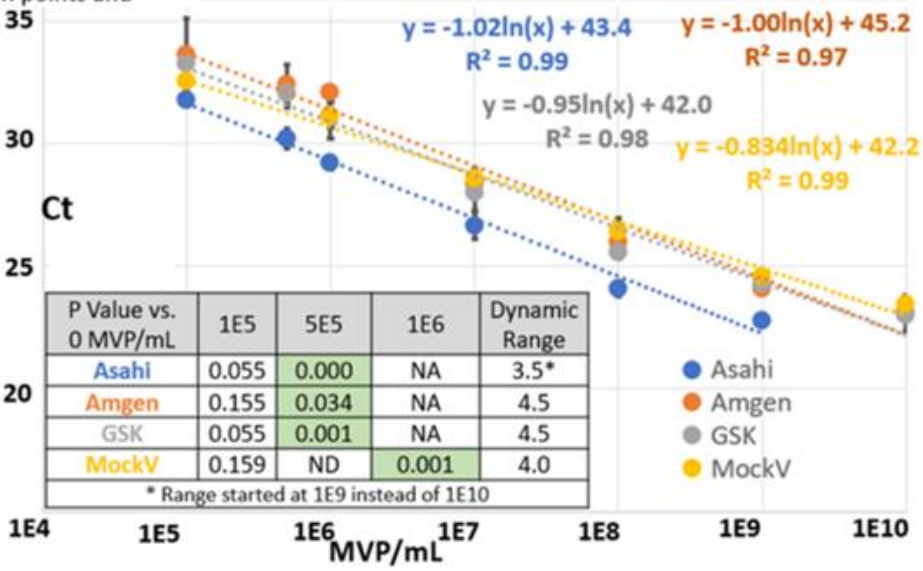
Figure 1: Intra-assay assessment of the Phase 1 I-qPCR assay. Table 1-1 depicts 10-fold titration series results (n=9). The average Ct of each titration is graphed against titration concentration. Error bars are included at each point (1σ) and a regression line is included between points. A solid horizontal line (orange) depicts the background signal (33.3) while the dotted line depicts 1σ below (32.3). Table 1-2 depicts the P value results from a T Test between points indicated.

Through a collaboration with GSK, a full factorial Design of Experiments (DOE) with a response surface modelling central composite face centered design was constructed to analyze the impacts of pH and Conductivity (Cond.) on AEX column chromatography viral clearance (Figure 5, original application). First, to test for potential assay interference, in-process samples from a GSK mAb process were conditioned according to the pH and Cond. described in Figure 5 (original application), spiked to a concentration of 8.0 $\log_{10}$ particles of MVP/mL and tested "neat" and diluted 1:2 and 1:10 via I-qPCR. The results indicated that 1:10 dilutions of each load preparation would yield nearly 100% recovery. Moving forward, all analyses were conducted with load samples diluted 1:10. Next, roughly 100 mL of each conditioned load was spiked to 10 $\log_{10}$ MVP/mL. After sampling, this spiked material was processed through Q-SFF packed columns (GE) with an AKTA Pure (GE). For Runs 1-6, parallel conditioned loads were challenged with 8.0 $\log_{10}$ $TCID_{50}$/mL of **live MVM** and processed accordingly. Samples from MVP (n=3) and MVM spiked runs (n=6) were analyzed by I-qPCR or $TCID_{50}$ infectivity assay, respectively. From these results, LRV's were determined and a direct comparison between particles was made (Figure 3). At pH 7.0, Cond. 3.0, LRV's of ~4.0 for both particle types indicate MVP to be an accurate surrogate while at higher Cond., MVP was able to predict the poorer clearance level of MVM. At centerpoint conditions

(pH 7.5, Cond. 8.0), both particle types were shown to be completely removed by the AEX resin. Complete removal of both particle types was seen again at pH 8.0, Cond. 3.0; however, at a higher conductivity only MVP was completely removed – residual MVM was detected by infectivity assay resulting in an LRV of 3.2. Overall, these results suggest that in 4 out of the 5 conditions tested, MVP served as an accurate indicator for MVM removal. To utilize the MVP as a tool for understanding parameter interactions and mapping process design space, results from the 10-run MVP Central Composite Design results were modeled with respect to the conductivity and pH, using a backward stepwise regression method. The resulting model's statistical significance was then tested using analysis of variance (ANOVA) and effects tests. Each test was performed at a significance level of 0.05. The quality of the model was then evaluated using a lack of fit test and an "actual by predicted" plot. A statistically significant and valid model was built from the data set (r^2 =0.92, p <0.01, no lack of fit). A 2D response surface graph and interaction plot (Figure 6B, original application) were constructed to show the general trend of LRV outcomes when operating at various load pH and conductivity parameters. The results demonstrate that load pH and load conductivity both have a strong impact on LRV. At a pH of 8, the conductivity range of 3 to 13 mS/cm had little effect on viral clearance, but at a pH of 7, conductivity has a high impact on viral clearance. The surface plot diagram shows that higher conductivities and lower pH's resulted in worse viral clearance - results that aligned with our prior expectations for MVM clearance via AEX. These data, obtained through the power of DOE and MockV's MVP surrogate would have required at least 10 small scale MVM spiking runs, costing at least $50,000 at a CRO and requiring at least a month. With the projected cost of the MVP Kit, this same study would cost no more than $5,000 and could be fully executed in-house in no more than a few days. This study unequivocally demonstrates the

Figure 2: Inter-assay comparability was assessed at multiple sites. CV's from the average Ct values derived from each MVP dilution series are depicted in the table to the right. The average Ct values were graphed vs. MVP concentration (below table). Error bars are included at each point (1σ). In most cases the error was too small to see through excel software. Regression lines are included between points and equations are shown on the top left of the graph. Insert table depicts the P value results from a T Test between 0 MVP/mL and the points indicated. Dynamic range achieved at each site is also included.

	Asahi (n=3)		Amgen (n=3)		GSK (n=5)		MockV (n=9)	
MVP/mL	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)	AVG	CV (%)
1E10	ND	ND	23.1	1.1	23.0	3.2	23.5	1.4
1E9	22.8	1.3	24.1	0.3	24.3	1.9	24.6	1.0
1E8	24.1	2.2	26.0	2.3	25.6	1.3	26.5	2.0
1E7	26.7	1.1	28.4	0.7	28.0	2.8	28.6	1.4
1E6	29.2	1.4	32.1	0.7	31.2	1.8	31.1	2.9
5E5	30.2	1.0	32.4	2.5	32.1	2.0	ND	ND
1E5	31.8	1.5	33.7	4.3	33.3	1.4	32.6	2.7
0	32.9	0.3	36.2	4.1	33.9	1.0	33.3	3.0



P Value vs. 0 MVP/mL	1E5	5E5	1E6	Dynamic Range
Asahi	0.055	0.000	NA	3.5*
Amgen	0.155	0.034	NA	4.5
GSK	0.055	0.001	NA	4.5
MockV	0.159	ND	0.001	4.0

* Range started at 1E9 instead of 1E10

	LRV	
	MVP (n=3)	MVM (n=6)
Run 1 (pH 7.0, 3 mS/cm)	4.31 ± 0.24	4.11 ± 0.22
Run 2 (pH 7, 13 mS/cm)	1.46 ± 0.53	2.43 ± 0.26
Run 3 (pH 7.5, 8 mS/cm)	≥ 4.5 ± 0.00	≥ 6.14 ± 0.59
Run 4 (pH 7.5, 8 mS/cm), 20 cm	≥ 4.41 ± 0.11	4.27 ± 0.30
Run 5 (pH 8.0, 3 mS/cm)	≥ 4.30 ± 0.04	≥ 5.99 ± 0.64
Run 6 (pH 8, 13 mS/cm)	≥ 4.47 ± 0.08	3.24 ± 0.30

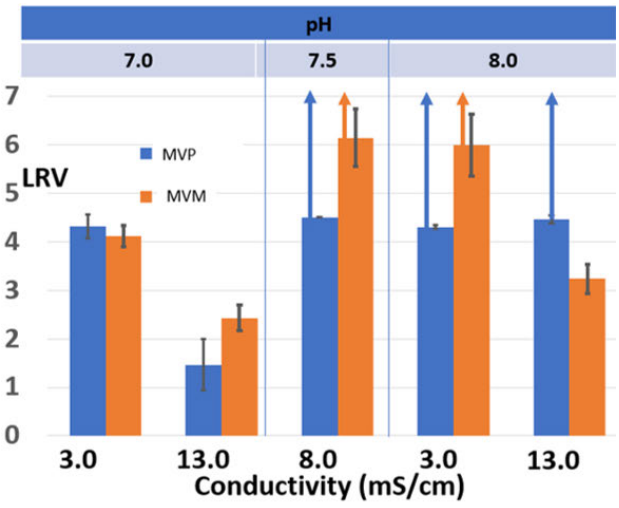


Figure 3: Average LRV values obtained from I-qPCR or $TCID_{50}$ analysis of load/pool samples from each experiment (above). A graphical representation of the LRV's grouped by pH and Conductivity is shown at right. For pH 7.5, Cond. 8 mS, only Run 3 is shown. Error bars represent 95% confidence intervals. Upward arrows depict that complete clearance was achieved (LLOQ determines LRV)

feasibility and value of utilizing MockV's MVP technology as a cost-effective, accurate, and rapid means to accelerate the process development efforts of biopharmaceuticals.

2. Specific Aims (S.A.) of Phase 2 Project

S.A. #1: Optimize scalable MVP production, establish quality manufacturing systems and protocols.

A. Rationale: The goal of this phase 2 SBIR is to advance the first BSL-1 compatible viral clearance kit toward commercialization. This "MVP Kit" will include a purified and concentrated stock solution of MVP. Currently, a contract lab produces small batches (5L) of MVP with an un-optimized baculovirus/Sf9 process. Purity is achieved through density gradient centrifugation, a scale-limiting approach. Improved yields and lower $/particle batches can be achieved through scale-up optimization of baculovirus/Sf9 production and the implementation of a chromatography-based purification strategy. Recently, MockV has demonstrated that crude extracts of Sf9 lysate containing MVP can be purified through a custom-made affinity chromatography resin (Figure 7, original application). This approach is a promising first step in the technical transfer of the MVP producing process. With complete independence over Kit production, MockV will be able to quickly and efficiently manufacture all components, reference internal documents and provide technical support to customers. Establishing quality systems of manufacturing and control from the start is imperative for a successful and sustainable Kit business model -the opportunity to initiate this priority will be undertaken as part of this S.A.

B. Experimental Design and Methods: ***Small scale expression optimization:*** Sf9 cell vials will be thawed, seeded into 125 mL shaker flasks (working volume ~ 30 mL) and cultured in a shaker incubator set to appropriate settings (ex. 27 ºC and 120 rpm). Cells will then be scaled into a larger shake flask and used to seed a series of 125 mL flasks at $0.5x10^6$ - $1.0x10^6$ cells/mL for optimization experiments. After overnight incubation, cells will be infected with a titration series of baculovirus stock, enabling a range of multiplicity of infection (MOI) to be tested. After infection, cell viability and cell diameter will be monitored on a daily basis. To monitor virus replication and MVP production, an immunofluorescence assay (IFA) will be established by screening a panel of mouse anti-MVP mAb's (MockV) and using FITC or Dylight™ 488-labeled secondary anti-mouse antibodies (KPL, Thermo) as detectors. Analysis will be conducted with a vertical fluorescence microscope after fixing cells with acetone and/or methanol. Through this technique we will be able to optimize expression by monitoring virus replication and MVP production throughout a time-course analysis. Based on the data, an optimal MOI will be established. The relationship between cell density and MOI will then be determined through additional small-scale studies. Virus replication and MVP production will be monitored according to IFA. Based on this data, the harvest time points for top performing conditions will be established. Cell culture from these top performing conditions will then be centrifuged. From there, two MVP extraction/purification methods can be compared. The first method involves re-suspension of cell pellet in PBS followed by multiple freeze/thaw cycles and centrifugation at speeds up to 24,000 g. The second involves the use of cell lysis reagent (I-PER™, Thermo) with protease inhibitor, according to manufacturer protocol – a technique that may be more amenable to large scale production[29].

Large scale Production in Wave Bioreactors: Large scale production in wave bioreactors will be carried out with the optimal conditions discovered from the small scale shake flask screening experiments. A vial of frozen cell stock will be thawed into a ~30 mL working volume shake flask with the appropriate media. Cells will be sub-cultured every 2-3 days to keep the cell density between $0.2\text{-}5x10^6$ cells/mL with viabilities above 95%. These cells will also be expanded using the same shake flasks and incubator settings outlined in the small-scale experiment above (27 ºC and 120 rpm). Once cells are scaled up to 2 x 600 mL working volume shake flasks at a density near $5.0x10^6$ cells/mL, 10 L of culture will be seeded at $0.5x10^6$ cells/mL in a 20 L wave bioreactor, allowing for efficient gas exchange. The bioreactor will be maintained at a speed of 22-25 rpm with an ~8º angle. Temperature will be kept at 27 ºC with a constant air/oxygen mixture supplied at a rate of 0.1 VVM to the head space (inlet filter) on the wave bioreactor. Viable Cell Density and cell diameter will be monitored daily on the VICELL XR. Infection will occur at the optimal cell density based on the small-scale experiment results ($1.0\text{-}2.0x10^6$ cells/mL) and similarly, harvest will occur according to small-scale results.

MVP Purification: Custom MVP Affinity resin will be generated according to vendor protocols (Aminolink, ThermoFisher) and packed into a small-scale chromatography column. To determine dynamic binding capacity (DBC), clarified Sf9 lysate containing MVP will be processed through the equilibrated column via an AKTA explorer (GE) and flow through fractions will be collected for breakthrough analysis. During a subsequent "standard" run, the loading capacity will be set to 50% of the volume required to achieve DBC. After wash and elution of MVP from the column during this standard run, purity will be determined as described below. Depending on these results, chromatography parameters will be adjusted and/or additional modes of purification will be attempted downstream of the affinity step (see Alternate approach). Once sufficiently purified, MVP will be concentrated and formulated according to a previously established Ultrafiltration/Diafiltration (UF/DF) Tangential Flow Filtration (TFF) method. In order to establish Quality Control (QC), the resulting MVP will be

analyzed according to the techniques detailed in Table 1 (see below). MVP retains from previous outsourced productions (utilized in Phase 1 and collaborations) will be used as a gold standard to benchmark the results.

C. Data Analysis and Interpretation: During small scale screening experiments, virus replication and MVM-VP2 expression will be tracked by an immunofluorescence assay (IFA). Through this technique we will also be able to monitor MVP production as a function of positive fluorescence. Cell viability and diameter will be measured using a VICELL XR automated cell counter (Beckman Coulter). During large scale production, pH, Dissolved Oxygen (DO), Glucose, Lactate, Glutamine and NH_4^+ will be monitored daily via Nova BioFlex, FLEX2. Modifications to the Wave settings (rocker speed and angle) will be made based on DO level (target of 40 ± 10%). Glucose will be maintained at 2 g/L while L-glutamine will be maintained at ≥ 2 mM for the duration of the production. To determine Affinity chromatography DBC, samples will be analyzed via I-qPCR. TEM, SDS-PAGE/WB and Sf9 Host Cell Protein (Cygnus, Cat# F840) analyses will be used to monitor the purity and concentration of MVP throughout the purification development efforts. Baculovirus (BV) removal will be determined through TEM analysis and a commercially available titration assay (Clontech, Cat# 631406).

D. Potential Pitfalls/Alternate Approaches: Glucose and L-glutamine levels require monitoring to ensure that cells have enough nutrients prior to infection. Additions of these components may be required; however, it is expected that the basal media used should have ample amounts to support cell growth up to the point of infection and therefore additional feeds would likely only occur after infection. Although a one-step, affinity-based method of MVP purification has already demonstrated promise, residual levels of HCP and baculovirus/debris may necessitate additional modes of purification. In this event, several polishing strategies may be implemented including AEX and/or size based (gel filtration) chromatography[30,31]. If evidence of baculovirus persists, Triton X-100 could be added to disrupt its lipid envelope while maintaining the integrity of MVP[32].

E. Expected Outcomes: It is expected that the top conditions elucidated during screening will have a 25-50% increase in relative fluorescence. Once scaled into Wave bioreactors, the increased infection rate seen in the small scale should also translate to increased production levels of high purity MVP (see Table 1 for expected quality attributes and purity criteria). Although the percentage increase in infected cells and production levels of MVP are not expected to correlate directly, it is safe to assume that a 50% increase in the number of infected cells should equate to at least a 25% increase in MVP production. If all other production associated costs remain fixed, this 25% increase in productivity would reduce the cost of the concentrated stock solution of MVP by approximately 25%. It will also be expected that downstream purification efforts will enable a high yielding and scalable process. Effective scale-up will translate into process yield improvements of 25-50%, as compared to the current density gradient centrifugation process. a minimum, the combined efforts of S.A. #1 will reduce the costs of producing MVP by ~ 50%, enabling future commercial production.

Table 1

Attribute	Test	Specification
Morphology	TEM	Spherical, ≥80% intact
Size	TEM	20-30nm
Concentration	TEM	≥ 1E13 particles/mL
Purity	SDS-PAGE	90% pure
	Sf9 HCP	≤ 1 ng/mL
	BV HCP	0 PFU/mL (LOD)

S.A. #2: Validate the use of MVP as an MVM surrogate for gene therapy applications

A. Rationale: A low cost MVM surrogate would enable gene therapy process scientists to develop and optimize effective manufacturing process steps capable of demonstrating robust MVM clearance. As seen from the data generated by our pre-Phase 1 collaborations and Phase 1 feasibility study with GSK, our MVP can be used as an economic and accurate model to predict MVM removal for a variety of separation techniques utilized for monoclonal antibody (mAb) manufacturing processes. In S.A. #2 of this Phase 2 application, we will validate that our MVP can likewise serve as a predictive marker of MVM clearance for real-world gene therapy manufacturing process steps. This will be made possible through a collaboration with REGENXBIO Inc. (see letter of commitment), a leading clinical-stage biotechnology company seeking to improve lives through the curative potential of gene therapy. REGENXBIO is currently in clinical Phase I/II for three orphan drug adeno-associated virus (AAV) viral vector gene therapy candidates and has developed Affinity and anion exchange chromatography (AEX) modes of purification as part of their manufacturing processes. For future Biologics License Applications (BLAs) to the FDA, gene therapy companies will be required to validate the ability of their processes to clear endogenous and exogenous (adventitious) viruses[33,34]. Therefore, we will conduct parallel MVP and live MVM spiking studies (see letter of commitment from WuXi AppTec) on REGENXBIO's affinity and AEX steps with the two serotypes, AAV8 and AAV9, used in their gene therapy product candidates. Upon comparison of clearance results, these tests will reveal: 1) if the removal of MVP is comparable to MVM, 2) how effective gene therapy affinity and anion exchange process steps are at removing MVM.

B. Experimental Design and Methods: ***Affinity Chromatography:*** Two 1 x 20 cm Omnifit™ EZ columns (Diba Industries, Inc) will be packed with serotype-specific POROS™ CaptureSelect™ AAV affinity resins (Thermo Scientific™), one with AAVX resin for AAV8 and one with AAV9 resin for AAV9. Each column will be qualified through 2% volume 1M NaCl spike. To establish baseline performance of the resins (no MVM or MVP spike), AAV harvest supernatants (concentrated, buffer exchanged and clarified) will be processed through their respective columns using center-point pH, conductivity and loading conditions. Processing will be performed on an AKTA Avant (GE Healthcare) and consist of: 3 column volumes (CV) of equilibration, loading of the clarified supernatant, a 6 CV wash step, a 3 CV elution step in which a low pH buffer will be utilized to elute bound AAV with set UV criteria, a 3 CV column regeneration (strip) step and a 3 CV column storage step. For MVP and MVM spiking experiments, three center-point runs will be conducted, as described above, with load material spiked with either MVP (10 $\log_{10}$ MVP particles/mL) or MVM (~8.5 $\log_{10}$ $TCID_{50}$/mL). In addition, for each serotype, three MVP and MVM spiked worst-case runs will be conducted, as described above, at maximum load. Samples collected during each run, including column load and pool, will be analyzed in triplicate to quantify particle concentration and statistically determine MVP/MVM LRV (See Section C).

AEX: CIMmultus™ QA AEX monoliths (BIA Separations) will be processed with un-spiked load material (AAVX and AAV9 elution pools) at center-point conditions to establish baseline performance. Processing will be performed on an AKTA Avant and consist of: 3 CV's of equilibration, loading of AAV material while collecting the flow through of the product with set UV criteria, a 3 CV buffer chase step, a 3CV column regeneration (strip) step and a 3 CV column storage step. For MVP and MVM spiking experiments, three center-point and three worst-case conditions (pH of column equilibration and AAV load material will be increased to 0.5 pH units higher than centerpoint) will be conducted in which load material will be spiked with either MVP (10 $\log_{10}$ MVP particles/mL) or MVM (~8.5 $\log_{10}$ $TCID_{50}$/mL). Samples collected during each run, including column load and flow-through pool, will be analyzed in triplicate to quantify particle concentration and statistically determine MVP/MVM LRV (See Section C). A summary of all Affinity and AEX experiments is listed in Table 2 (above).

AAV Serotype	Process Step	Baseline Runs	Center-point Runs		Worst-Case Runs	
		No Virus Spike	MVM Spike	MVP Spike	MVM Spike	MVP Spike
AAV8	Affinity (AAVX)	N=1	N=3	N=3	N=3	N=1
	AEX	N=1	N=3	N=3	N=3	N=1
AAV9	Affinity (AAV9)	N=1	N=3	N=3	N=3	N=1
	AEX	N=1	N=3	N=3	N=3	N=1

C. Data Analysis and Interpretation: The chromatographic performance of each experiment will be monitored by in-line pH, conductivity, UV, and pressure sensors present on the AKTA Avant. Unicorn 7.0 Software (GE Healthcare) will graph these outputs over time generating a "chromatogram" which enable comparisons among experiments. In addition, the Unicorn software will mathematically evaluate the quality of affinity resin packs through Asymmetry and Height Equivalent to a Theoretical Plate (HETP) results. AAV step yield for each run will be determined by a digital droplet PCR method established by REGENXBIO. For MVP quantification, samples will be analyzed in triplicate by the improved I-qPCR method detailed in the preliminary section. Briefly, the Threshold Cycle (Ct) values from each sample will be back-calculated to a corresponding MVP concentration based on a standard curve equation generated from a 10-fold dilution series of MVP standards. MVP concentration data from each sample will only be considered valid if the CV's among triplicates are equal to or below 5%. For MVM spiking experiments, MVM titer within samples will be analyzed via a traditional $TCID_{50}$/mL infectivity-based assay (performed at WuXi). In brief, samples will be serially diluted and monolayers of 324K cells will be infected using at least eight 0.25 ml replicates of the appropriate dilution of sample. Observation for Cytopathic Effects will be conducted after 10–12 hr of incubation at 37°C. Virus titers will be calculated by the Spearman-Karber method[53]. LRV for each chromatography experiment will be calculated according to known protocols[14] via the following equation: Whereas C*l* and C*p* are the MVP or MVM particle concentrations of the load and pool samples, respectively from a single chromatography experiment, while V*l* and V*p* are the volumes of those respective samples.

$$LRV = log_{10}\left\{\frac{(Cl\ x\ Vl)}{(Cp\ x\ Vp)}\right\}$$

D. Potential Pitfalls/Alternate Approaches: In-process AAV material contains host cell proteins, DNA, and other impurities along with the gene therapy product of interest. It is possible that elements of this heterologous mixture could interfere with the assays utilized to quantify MVP. To alleviate this concern, spike/recovery analysis will be conducted prior to MVP spiking studies and minimal required dilutions will be determined. In short, Affinity and AEX loads will be spiked with a known quantity of MVP "neat" and diluted 1:2 and 1:10. The results will inform us prior to spiking studies if sample dilutions are necessary. A separate potential issue - the introduction of either particle type into AAV material may cause a pressure build up over the column or monolith,

resulting in the stoppage of those experiments. In this event MVP or MVM stock solutions could be further purified via ultrafiltration or flow-through chromatography prior to spiking. Alternatively, the % spike may be decreased. Finally, it is possible that the $TCID_{50}$ assay used to quantify live MVM will have a lower LLOQ than the I-qPCR assay used to quantify MVP. If this is the case, smaller LRV values for MVP will be expected than MVM when complete clearance of each particle is achieved. This will not be misinterpreted as a failed comparison but an acceptable limitation of the I-qPCR assay so long as the reportable LRV's for MVP are ≥ 4.0.

E. Expected Outcomes: It is expected that packed affinity columns will be qualified to specifications of 0.5-2.0 for Asymmetry and < 0.1 cm HETP prior to experimentation. For MVP analysis via I-qPCR it is expected that the r^2 value of the standard curve equation, generated from the 10-fold dilution series of MVP Standard will be ≥ 0.95. It is also expected that the assay's lower limit of quantification (LLOQ) is ≤ 5.7 log_{10}, enabling LRV's of ≥ 4.5 to be determined. The MVM titer infectivity assay will provide acceptable accuracy and precision over a dilution range of 9.0 log_{10} with a LLOQ of ~1.0 $TCID_{50}$/ml. For center-point affinity chromatography runs, comparable process performance, as depicted by chromatogram overlays will be expected between MVM and MVP spiked runs. Likewise, comparable process performance between MVM and MVP will be expected when utilizing worst-case parameters. Step yields of ≥ 90% will be anticipated for center-point runs while yields of ≥ 80% will be anticipated for worst-case conditions. For center-point affinity chromatography, robust clearance (LRV ≥ 4.0) of both MVM and MVP are expected while a modest decrease in removal is anticipated for worst case run parameters (LRV of 2.0-3.0). For center-point MVM and MVP spiked AEX experiments, comparable process performance will be expected. Likewise, comparable process performance will be expected between the two particle types when utilizing worst-case parameters. Step yields of greater than 90% will be anticipated for center-point runs while yields of ≥ 80% will be anticipated for worst-case. For center-point AEX chromatography, moderate clearance of both MVM and MVP (LRV of 2.0-3.0) are expected while poor removal is anticipated when operating under worst case parameters (LRV of 0.0-2.0). A summary of the expected outcomes is depicted in Table 3.

Table 3	Affinity Chromatography				AEX			
	Center-Point		Worst-case		Center-Point		Worst-case	
	MVM	MVM	MVM	MVM	MVM	MVM	MVM	MVM
Performance	Comparable		Comparable		Comparable		Comparable	
Step Yield	≥ 90%		≥ 80%		≥ 90%		≥ 80%	
LRV	≥ 4.0[1]		2.0-3.0[1]		2.0-3.0[1]		0.0-2.0[1]	

1 More critical than achieving a certain LRV range for each condition is that LRV's achieved for MVP will be ± 1.0 to those achieved for MVM.

S.A. #3: Validate the use of MVP as an MVM surrogate for continuous processing applications.

A. Rationale: Conventional packed-bed column chromatography has been used to purify a variety of biopharmaceutical compounds from complex cell culture fluid. However, conventional column chromatography has multiple drawbacks including relatively high pressure, low productivity, and large capital costs. In addition, column chromatography is inherently a batch process, which necessitates innovation for the development of integrated continuous processes for lower cost and flexible bio-manufacturing. A number of continuous column chromatography systems, such as periodic countercurrent column chromatography (PCC)[35] and simulated moving bed (SMB)[36], have been developed, but they produce cyclic steady state and thus the product concentration, impurity profile, and elution pH all vary with time[37]. A column-free Continuous Countercurrent Tangential Chromatography (CCTC) system has been used successfully for initial capture and subsequent polishing of mAbs[38,39]. A schematic of the CCTC system and the principles of its operation can be found in our original submission and in previous publications[38,39]. One significant hurdle hindering widespread adoption of CCTC, and continuous processes in general, are the inherent risks and logistical challenges of conducting viral clearance validation spiking studies[16,17]. Understanding the impact of key operating parameters on viral clearance efficacy would be highly valued, if not critical, before companies utilizing this technology were to conduct expensive viral clearance validation studies. Likewise, the continuous mode of operation presents a logistical challenge to the act of "spiking" virus into the process. Several methods have been previously reported for virus clearance in continuous processes, but given the large variability between available continuous systems, no industry standard has been established[16,40,41,]. A low cost, viral surrogate would enable scientists to conduct process development studies on continuous systems and explore the impacts of process parameters on viral clearance efficacy prior to performing any expensive virus validation studies. A low cost surrogate would also allow scientists to **experiment with different modes of introducing virus into the continuous system**, a benefit that could lead towards establishing best practices for validating virus clearance in continuous processes.

B. Experimental Design and Methods: ***Designing the CCTC system:*** Before conducting MVP or MVM spiking studies, Protein A and Mixed Mode Chromatography (MMC) CCTC steps will be developed as previously described[38,39]. Briefly, Protein A static binding capacity experiments will first be conducted by mixing 5 mL of

equilibrated Praesto™ Protein A (Purolite) resin (25% slurry) with various amounts of clarified mAb harvest feedstock (Teva Pharmaceuticals, see letter of commitment). The resulting mixtures will be stirred continuously for 30 min. 500 µL samples of the supernatant will then be obtained and filtered through 0.22 µm syringe filters. Filtrate will be analyzed via Protein A HPLC for mAb concentration. For MMC, the same procedure will be followed with the following modifications: Capto™ Adhere (GE) resin, Protein A-purified/neutralized mAb feedstock and UV-A_{280} for determining mAb concentration. Next, binding kinetics experiments will be conducted in which 5 mL of each resin will be added to 100 mL beakers (independently) along with 20 mL's of mAb material. During a two-hour period under constant mixing on a shaker, 1.5 mL samples of supernatant will be collected and filtered through 0.22 µm syringe filters. Filtrate will then be analyzed for mAb concentration by HPLC or UV-A_{280}. Next, critical flux experiments will take place in order to ensure stable operation. To determine critical flux for each resin, slurry will be circulated through a hollow fiber module (Spectrum Labs) in total recycle mode, with the permeate flow rate controlled using a Masterflex® peristaltic pump (Cole Parmer). Transmembrane pressure (TMP) data will be collected via in-line pressure transducers (Pendotech) with the permeate and retentate flow rates incrementally increased every 20 mins while maintaining a constant ratio of permeate to retentate flow rate. The critical flux will be identified as the permeate flux at which the TMP becomes unstable (increasing with time during constant flux operation). CCTC experiments will then be conducted for each resin using a hollow fiber membrane area that ensures filtration flux will be ≤ 20% of the critical flux. For Protein A CCTC operation, six chromatographic steps will be operated while five will be operated for MMC. Table 4 summarizes these steps and the proposed buffers. For each CCTC experiment, the system will be filled with buffers for their corresponding steps. Each step is made of multiple stages arranged in a countercurrent configuration to maximize performance. A typical 3-stage step will be employed. Resin will be introduced once system pressures stabilize, with the outflow from the equilibration stage will be diverted to waste until the resin concentration is at least 95% of the slurry concentration entering the system. At this point, the outflow from the equilibration stage will be recycled directly to the inlet of the binding stage and mAb material will be introduced. Permeate samples collected throughout operation from all stages will be analyzed for step yield.

Table 4: Summary of CCTC Step parameters

Protein A	Buffer Composition	pH
Binding	20 mM Na_2HPO_4	7.2
Wash-1	20 mM Na_2HPO_4, 500 mM NaCl	7.2
Wash-2	20 mM Na_2HPO_4	7.2
Elution	Acetic acid	2.6
Stripping	HCl	2.0
Equilibration	20 mM Na2HPO4	7.2
MMC	**Buffer Composition**	**pH**
Binding	60 mM NaOAc	5.3
Wash	20 mM NaOAc + 100 mM NaCl	5.4
Elution	76 mM NaOAc + 50 mM NaCl	4.1
Strip	120 mm NaOAc + 500 mM NaCl	2.8
Equilibration	60 mM NaOAc	5.3

Testing MVM and MVP Clearance in packed column and CCTC mode: To provide baseline MVP and MVM clearance results, Praesto™ Protein A and Capto Adhere MMC experiments will first be performed in packed-bed columns (1 x 20 cm Omnifit columns). Non-spiked control runs will be performed on an AKTA Pure system (GE) as follows: Columns will be equilibrated with 5 CV's of respective equilibration buffer (see Table 4 for buffer compositions and pH). mAb load material will then be added to 75% saturation (see Section C, below). For Protein A, 3 CV's each of Wash buffer 1 and 2 will precede elution while a single wash step will be employed for MMC. Eluted mAb will be collected according to in-line A_{280} (1.0 AU/cm front peak to 2.0 AU/cm back peak) and analyzed along with load samples to determine step yield. Protein A runs will be performed at a linear velocity of 230 cm/h while MMC will be run at 300 cm/hr. MVP and MVM spiking studies will be performed identically except mAb loads will be spiked to either 10 $\log_{10}$ MVP/mL or 8.0 MVM $TCID_{50}$/mL. Load and elution samples from spiked runs will be analyzed for MVP or MVM concentration via I-qPCR or $TCID_{50}$ infectivity, respectively as described in Section C of S.A. #2. LRV's will also be determined as previously described. After designing the CCTC systems for Protein A and MMC processing, each will be utilized independently to test MVM and MVP removal. **Two separate CCTC spiking approaches will be tested with MVP**. During a "batch-spike" approach, MVP will be spiked to a concentration of 10 $\log_{10}$ MVP/mL in Protein A or MMC load and processed through the respective system according to the optimized parameters established above. In this manner, the introduction of MVP will occur at the onset of the first binding stage. Permeate samples will be collected from all stages throughout the experiment and analyzed for mAb and MVP concentration. For an "in-line spike" approach, each system will first be allowed to reach steady-state, as indicated by a steady elution outlet mAb concentration profile, and then 10 $\log_{10}$ total particles of MVP will be injected at three time points: 20, 140 and 260 mins for Protein A and 20, 120 and 220 min for MMC. These intervals were chosen to correlate with the 20 min CCTC cycle time and providing a gap of six or five cycles between different MVP injections for Protein A and MMC, respectively. The actual time required for each resin type to travel from the binding step to the elution step will be calculated based on the design of the CCTC system to ensure that sufficient residence time is provided to fully process each MVP spike. Samples will be collected from all stages during the window in between spikes and analyzed via I-qPCR for MVP concentration. LRV's and mass balance determinations will be made.

Because of the large load volumes required to run either Protein A or MMC CCTC systems in continuous mode and the large/dilute permeate samples taken during each experiment for analysis, the "batch-spike" approach for MVM would be logistically and economically challenging to perform at a CRO. Therefore, to compare MVP clearance to MVM in CCTC mode of operation, the "in-line spike" approach will be attempted with MVM (performed at WuXi). This will be accomplished by the same procedure described above for MVP aside from analysis being performed via Infectivity assay as described in Section C of S.A. #2.

C. Data Analysis and Interpretation: CCTC process development (PD) for Protein A and MMC operations will be performed on bench scale to determine the key parameters. To determine the binding characteristics of Teva's mAb for both Protein A and MMC resins, equilibrium binding isotherms will be evaluated as detailed above. The bound concentration of mAb to resin (Q) will be measured using the following equation: $Q = [(C_0 - C_f)V_S]/ V_R\phi$ where (C_0) is the initial mAb concentration after mixing with the resin slurry, (C_f) is the final concentration of mAb in solution, (V_S) is the total volume of the fluid phase in the final solution, (V_R) is the volume of the resin slurry, and (ϕ) is the resin concentration determined from the settled resin volume. Based on this data, an operating Q of 75% saturation will be utilized to ensure negligible product loss. The ratio of the slurry flow rate to the feed flow rate (γ) will also be determined from the target Q and the respective resin concentration through the equation: $\gamma = C_{feed}/(\phi Q)$. The required residence times in the static mixers and binding steps will then be determined based on the data from the binding kinetics experiments. Analysis of the binding data will be performed using a lumped parameter model fit utilizing piece-wise values of the rate constants as described in the literature[38,49]. Data from critical flux experiments will be used to determine the hollow fiber membrane area (A) by applying the equation: $A = q_p/J$ where q_p is the required permeate flow rate through the hollow fiber (determined from the required conversion), and J is the filtrate flux (set to 80% of critical flux to provide a margin of safety). The analysis of these data will form the basis to build detailed process models which will enable Chromatan (subaward) to design proper CCTC flow paths which will be incorporated into fully functioning CTTC PD skids. During a run, the CCTC PD system will collect system pressures and pH data for each step and UV from elution permeate. Constant signals will be used to verify the stability of the system. mAb step yields will be determined from elution samples generated throughout processing via off-line analysis (Protein A HPLC for Protein A, and A_{280} for MMC). For MVP spiked runs, the MVP concentration of all load and elution samples will be quantified via I-qPCR, as described in Section C of S.A. #2. LRV determinations will be calculated based on the results. Comparisons between the two spiking approaches (batch and in-line) will be made and will influence the way in which CCTC spiking experiments are done in the future. For MVM spiked runs, load and elution titers will be determined through the $TCID_{50}$/mL infectivity assay described in Section C of S.A.#2. LRV's will be determined and compared to the in-line MVP LRV results. This comparison will enable us to assess the accuracy of predicting MVM removal via MVP for Protein A and MMC in a continuous CCTC format.

D. Potential Pitfalls/Alternate Approaches: Designing the CCTC system to operate efficiently with representative in-process material will be achieved through the experiments detailed in above. However, since detailing the complexity of CCTC design surpasses the limits of this application it should suffice to say that the expertise ChromaTan (subaward) has accumulated in CCTC development will enable us to rectify possible issues that may arise throughout this S.A. For example, if Protein A mAb step yield is found to be lower than expected, the number of wash step stages or buffer flow rate could be increased. Similarly, for MMC separation, if MVP removal is lower than expected based on packed column results, the number of stages in the bind and wash steps or buffer flow rates could be increased**.** In addition, the CCTC system could be used for in-line experiments with buffer composition. A previous publication has shown the effectiveness of varying in-line buffer conditions during the elution step to influence product quality and yield[39]. MVP and MVM spiking studies will follow similar operation strategies as discussed for non-spiking runs. One point of consideration is the number of cycles or "aging" that each resin will experience throughout the study. Published literature has demonstrated that the number of resin cycles should not affect particle removal[42]; however, if a considerable (>10%) difference is determined between the first and last collection points, follow-up experiments will be performed using only "used" Protein A and MMC resins. It is possible that low MVP and/or MVM LRV values are achieved for packed bed Protein A and MMC experiments. This is not entirely unexpected since viral clearance via Protein A chromatography is considered to be a variable unit of operation[43] and a complex dynamic of hydrophobicity and ionic interaction governs MMC clearance. If LRV's of < 1.0 are achieved, we will first attempt to alter bind and elute conditions to reduce virus particle breakthrough during elution (the ability to use MVP to accomplish this task directly highlights its value). If thse efforts fail, alternative resins such as MabSelect (GE) or Nuvia aPrime (Biorad). The current plan is to injected 10 $\log_{10}$ total MVP during each in-line injection. If required, the concentration of MVP can be increased to overcome potential issues with quantifying dilute samples. Alternatively, concentrating samples via centrifugal filtration units prior to analysis could be attempted.

E. Expected Outcomes: Optimized parameters for Protein A and MMC CCTC operation will be determined empirically but expectations based on previously reported results[38,39] are summarized in Table 5. Similarly, previous experiments can be used to form a basis for expecting that ≥ 90% mAb yields can be achieved throughout steady state operation of Protein A and MMC. It is expected that TMP for each system will remain constant during each step (ex. binding, wash, etc.) and deviations will all be within 10% of baseline. For packed column Protein A experiments, it will be expected that non-robust removal of MVM and MVP will be achieved (LRV's of 1.0-3.0). It will also be expected that the MVP LRV's will be ± 1.0 to MVM LRV's, thereby demonstrating that MVP is an accurate and dependable surrogate for MVM. For packed column MMC experiments, it will be expected that LRV of ≥ 3.0 will be achieved. Again, it will be expected that MVP LRV will be ± 1.0 to MVM LRV. The packed column LRV achieved for each resin type will serve as benchmarks for CCTC LRV which are expected to be ± 1.0 for their respective resin/particle comparison. For example, if a Protein A packed column spiked with MVP achieves an LRV of 2.0, the Protein A CCTC MVP LRV is expected to be 2.0 ± 1.0. Finally, we expect to achieve similar results (LRV ± 0.5) between the two methods of introducing MVP into the CCTC system (batch vs. in-line spike); although, due to a potential dilution effect, the concentration of MVP contained within each sample may be below the lower LOQ of the I-qPCR assay, thereby impeding the calculation of comparable LRV. As discussed in Section D above, this may be mediated through pre-assay sample concentration.

Table 5: Expected Operating Parameters for CCTC

Parameter	Protein A	MMC
Resin Capacity (mg/mL)	30-35	18-30
mAb Flow Rate (mL/min)	10	13
Slurry Flow Rate (mL/min)	15-25	
Buffer Flow Rate (mL/min)	15-60	

S.A. #4: Optimize a second generation high throughput MVP quantification assay

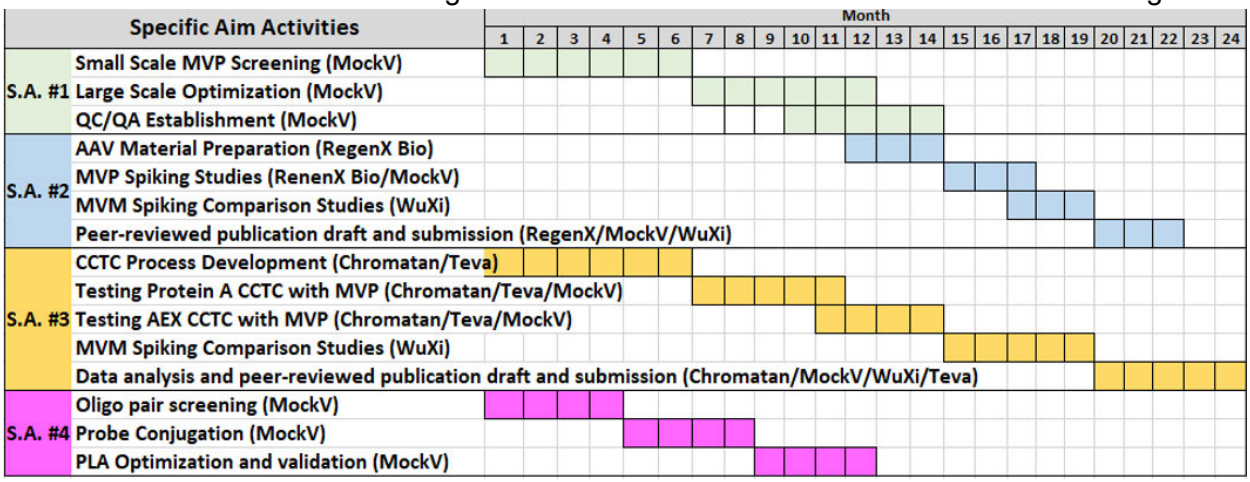
A. Rationale: The ability to quantify MVP in bioprocess solution is a critical step in determining clearance. As these particles are non-infectious and do not contain internal nucleic acid, it is impossible to determine their quantity through standard infectivity plaque assays or traditional qPCR. Our Phase 1 efforts led to the establishment of a sensitive and specific I-qPCR assay, enabling us to calculate LRV of 4.5, an improvement of > 1.0 from our pre-Phase 1 assay. These improvements allow us to prove the overall concept of predicting MVM clearance through an accurate and practical non-infectious surrogate. Despite this success and the ability to fully commercialize a product that meets the expectations of the industry, it was apparent that further improvements to a quantification assay *could* be achieved. First, residual assay background is still a challenge that limits expansion of the dynamic range. In retrospect, this finding was not surprising considering the nature of immunosorbent assays and the extreme sensitivity of qPCR (in our case, > 99.99% of oligo-conjugated mAb was effectively washed out of the system, yet the minute quantities non-specifically bound, led to background - even in the presence of blocking reagents). Second, the manual hands-on time required to perform the assay (4 hours) still creates bottlenecks for the end-user during high throughput process development screening experiments. This fact was highlighted during a recent collaboration with the NIH in which an alternative quantification strategy was adopted due to its high throughput potential (see above). During Phase 1, a Proximity Ligation Assay (PLA) was attempted to address these two issues. Although far from being optimized, preliminary PLA results demonstrated a 4.0 $\log_{10}$ LRV potential (Figure 9). It is our belief from the experiences gained during Phase 1 that LRV of > 5.0 $\log_{10}$ could be achieved through assay optimization. Furthermore, PLA eliminates the need to "capture" analyte via an antibody or wash unbound components[44,45]. All binding and ligation reactions occur in a single tube. This not only reduces background and increases assay precision but enables the incorporation of automatic liquid handling – a modality that lends itself well to high throughput workflows.

B. Experimental Design and Methods: Pairs of complimentary PLA oligo's from published sources will be screened for activity according to known protocols[46-48]. In brief, biotinylated anti-MVP mAb will be incubated at 37°C for 60 minutes with each oligo pair (streptavidin conjugated) and then treated with a reaction buffer containing DNA Ligase for 10 minutes at 37°C. Ligated product will then be added to qPCR strips where a PCR mix, containing primers and probe spanning the ligated product, will be added. qPCR will then be performed according to standard cycling parameters. After selecting an optimal pair, the two individual oligo's will each be directly conjugated to anti-MVP mAb according to known methods, forming two "Assay Probes". In brief, the antibody and each 5' amino-modified DNA oligo will be activated with heterobifunctional cross-linking agents and coupled in a single spontaneous reaction before purifying away excess oligo via dialysis. To test the dynamic range and sensitivity of the PLA, a 10-fold dilution series of MVP will be prepared and aliquoted into a qPCR plate. A solution containing equal amounts of each conjugated Assay Probe will be administered into each well and allowed to incubate with the MVP for 60 minutes at 37°C. A DNA Ligase containing reaction buffer will be added to each well and incubated for 10 minutes at 37°C. Sample will be drawn from each well and added to a separate qPCR plate containing PCR mix (comprising primers and probe spanning the ligated product).

C. Data Analysis and Interpretation: For PLA oligo pair screening, qPCR amplification will only occur when both streptavidin conjugated oligos bind to the same biotinylated anti-MVP mAb and are therefore in close proximity to ligate. When this occurs, average threshold cycle (Ct) values will be determined by the qPCR system software. When performing these screening experiments, ΔCt values for each oligo pair will be calculated by subtracting a pair's Avg Ct value from its control Ct value (the value produced when omitting the biotinylated MVP mAb from the reaction). Large ΔCt values (typically > 8.5) indicate that each oligo in a pair are efficiently bound in close proximity to the same target analyte and are able to form a ligation product that is effectively quantifiable through Taqman qPCR. The larger the ΔCt value, the better the oligo pair. By comparing these values, an optimal oligo pair will be selected. When performing the PLA assay with the optimal Assay Probes, the Avg Ct values of a 10-fold MVP dilution series will first be determined. Typically, when diluting an analyte 10-fold, Ct shifts of ~3.3 Ct's are obtained when 100% qPCR efficiency is achieved. This value will serve as a benchmark when interpreting results. Upper and lower assay limits of quantification will be calculated according to appropriate statistical methods, thereby establishing the assay's dynamic range. To determine intra-assay precision, coefficients of variation (CV's) will be calculated among replicates of each concentration point in a dilution series. To determine inter-assay precision, CV's will be calculated from each concentration point among several independent runs. In each case, CV's below 5% will indicate acceptable precision.

D. Potential Pitfalls/Alternate Approaches: When performing the PLA oligo pair screening experiments, ΔCt values < 8.5 might result. This might indicate that excess free biotin or streptavidin is present in the preparation. In this event, the oligo pair may bind to the free biotin instead of the biotinylated antibodies, leading to a decrease in signal; or inversely, free streptavidin might bind to the biotinylated MVP mAb – occupying sites that would otherwise be free for oligo binding and signal generation. In either case, a repeated dialysis of the biotinylated antibody and streptavidin oligo's should remedy the situation. Another potential issue may be if the Avg Ct values diluent-only samples (no MVP) are lower than expected. This "background" signal may be caused by excess Assay Probes or ligation components and may be remedied by adjusting the concentrations or volumes of each. Alternatively, a high background signal may have been caused by excessive DNA Ligase activity prior to qPCR cycling. In this case, a protease step may be included just after the 10 minute ligation incubation, prior to qPCR. Should PLA fail to generate the dynamic range, precision and automation potential we desire, an entirely separate approach could be attempted. This approach would attempt to internalize a known nucleic acid target sequence into MVP during production and would thereby eliminate the "Immuno portion" of the I-qPCR assay (antibodies, plate binding, washing, etc. - the direct source of background). With internalized target nucleic acid, MVP particles could be quantified directly through qPCR after denaturing the capsid superstructure.

E. Expected Outcomes: It will be expected that an optimized PLA will yield a dynamic range of ≥ 5.0 $\log_{10}$, enabling LRV's of ≥ 5.0 to be achieved during spiking experiments. It is also expected that this optimized assay will improve inter/intra assay precision and lend itself to liquid handling automation equipment, thereby reducing hands-on assay time to < 2 hours. S.A. #1-4 will be accomplished over a 24-month period (Gantt). MockV will be solely responsible for executing all S.A. #1 and #4 activities while S.A. #2 will be a collaborative effort involving REGENX BIO and WuXi App Tec. S.A. #3 is mainly subawarded to ChromaTan who will develop and execute continuous processing experiments, with Teva material, at their facility before transferring to WuXi App Tec for viral clearance. The commitments obtained from all parties involved in this project reflects the industry's interest in pursuing an accurate and economical BSL-1 viral clearance surrogate for process development purposes. In Phase 2B or 3, we will continue to internalize the production of Kit components while adhering to the quality control standards and methods established during Phase 2. We will then conduct extensive beta testing of the MVP Kit through industry collaborators and partners prior to commercial launch.



PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

Research Strategy & Commercialization Plan

Research Strategy Bibliography and References Cited

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Consortium and Contractual Arrangements

This project is in support of MockV Solutions Inc. (MockV) as it advances it's core technology toward commercialization. As such, MockV will lead the scientific efforts through the appointment of its CEO – Personal Info as PD/PI of the project. Most work and analysis will be conducted by MockV; but, due to the nature and scope of the work proposed, several additional companies will play important roles.

Chromatan is an industry leader within the continuous processing space for biopharmaceuticals. Their expertise and knowledge are required in order to test the concept of predicting viral clearance with a non-infectious surrogate through their counter current tangential flow chromatography continuous processing system – the CCTC system. Chromatan will conduct these activities as a sub-award outlined in research strategy and budget justification.

REGENXBIO is a leading clinical-stage gene therapy company currently in clinical Phase I/II for three orphan drug adeno-associated virus (AAV) viral vector gene therapies. The company has developed Affinity and anion exchange chromatography (AEX) modes of purification as part of their manufacturing processes. REGENEXBIO will provide material and space for affinity and AEX steps with the two serotypes, AAV8 and AAV9, used in their gene therapy product candidates. REGENEXBIO's expertise in gene therapy production will be crucial for completing specific aim 2 focusing on gene therapy. REGENXBIO has agreed to participate in this study at no cost.

WuXi is contract development and manufacturing organization with experience conducting viral clearance testing. The company will be providing viral clearance testing for specific aims 2 and 3. WuXi's provides access to BSL-2/3 facilities for necessary validation testing of viral clearance prediction of MockV's kits. BSL-2/3 facilities are highly specialized and require MockV to outsource this part of the project. WuXi has agreed to participate in this study at no cost.

Teva Pharmaceuticals is leading generic pharmaceutical company. Teva manufactures many monoclonal antibody therapeutics. Teva will be supplying monoclonal antibody material for testing viral clearance in continuous processing supporting specific aim #3. This material will be utilized in Chromatan's CCTC system for validation and application development. This biologic material is a necessary component for testing the application and cannot be produced in house. Teva has agreed to participate in this study at no cost.

All parties have agreed that the overall scientific leadership of the project will be from Personal Info (see letters of support). **All parties have agreed that any IP developed during the course of the study that pertains to MockV's technology will be the sole property of MockV Solutions.** All parties have also agreed to publish and share data once accumulated through these efforts.

Letters of Commitment and Support Contents:

Letters of Support

MockV supports NIH's policy that data sharing is "essential for expedited translation of research results into knowledge, products and procedures to improve human health." We agree that all data generated which could be useful to the bioprocess community will be published, presented or otherwise made available to the public.

Authentication of Key Biological and/or Chemical Resources

Antibodies.

MockV will utilize mouse anti-MVP monoclonal antibodies (mAb) during this project. mAb hybridoma's were produced in 2017 by BluePoint Bioscience for MockV (MockV owns all rights to the hybridoma cell lines) and have been properly stored and maintained according to BluePoint Science's internal protocols. All mAb produced from MockV's hybridomas by Bluepoint, such as those used during Phase 1 studies, are tested for quality and specificity according to BluePoint's validation protocols which include positive and negative controls. Results from each target validation are retained electronically and lot numbers are assigned to each batch prior to receipt by MockV. Specification sheets also accompany the transfer of material. Resource pending, MockV will sequence the antibody genes from several of its high performing hybridomas.

MVP (Minute Virus of Mice - Virus Like Particle)

MockV Solutions (MockV) routinely contracts the production of MVP from Ascentgene Inc. (Gaithersubur, MD). SDS-PAGE, TEM, ELISA and I-qPCR are utilized upon receipt to verify physicochemical characterization. As MockV transitions to produce MVP in-house, it will incorporate these characterization methods into a batch release criterion.

"In-process" Biopharmaceutical Material

"Spiking studies" will be conducted during Specific Aim #2 and 3. This will require the addition of MVP and MVM into representative Biopharmaceutical Material. We have collaborated with REGENXBIO and Teva Pharmaceuticals to source this material. They each are committed to produce their respective in-process material through their batch record operating procedures and to 1) check the critical quality attributes of the material produced and 2) the process attributes of generating the material prior to use. Critical quality attributes include, but are not limited to, purity (by SEC-HPLC, IEX-HPLC), impurity profile (HCP, DNA, SDS-PAGE) and concentration (A280, ddPCR). Process attributes include chromatogram review and step yield determination.

Infectious MVM Stocks

For comparative feasibility studies (MVP vs. MVM), MockV will collaborate with WuXi App Tec – a leading CRO that produces high titer MVM stocks. To ensure the accurate quantitation of virus in samples, representative samples of process materials will be evaluated in the relevant test systems for toxicity to indicator cells and interference with virus quantitation in the infectivity assay. Sample dilution will be used to mitigate any observed effect. For these evaluations, a positive control with a known amount of virus in diluent will be utilized and for a negative control, diluent will be evaluated.