Sample Application 1R43TR002231-01A1 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: 04/05/2017	Opportunity: PA-16-302	Council: 08/2017
Competition ID: FORMS-D	FOA Title: PHS 2016-02 OMNIBUS SOLICITATION OF THE NIH, CDC, FDA AND ACF FOR SMALL BUSINESS INNOVATION RESEARCH GRANT APPLICATIONS (PARENT SBIR [R43/R44])	
1R43TR002231-01A1	Dual:	Accession Number: 4039883
IPF: 10035919	Organization: MOCKV SOLUTIONS, INC	
Former Number:	Department:	
IRG/SRG: ZRG1 IMST-L (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1:	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	Personal Info	PD/PI
Consultant Info	Consultant Info	Consultant
Subcontractor Info	Subcontractor Info	Other (Specify)-Other Significant Contributor

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

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

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: Mr. First Name*: Personal Info Middle Name: Last Name*: Personal Info Suffix:
Position/Title: Personal Info
Street1*:
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* 10/02/2017
Ending Date* 10/02/2018

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: Mr. 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*
04/05/2017

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: BluePoint Bioscience LLC
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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Funding Opportunity Number: PA-16-302. Received Date: 2017-04-05T14:04:25.000-04:00

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: Proprietary Info
DUNS Number:
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

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 None

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*	MockV_Abstract.pdf
8. Project Narrative*	MockV_Project_Narrative.pdf
9. Bibliography & References Cited	MockV_Bibliography_and_References_Cited_.pdf
10.Facilities & Other Resources	MockV_Facilities_and_Other_Resources.pdf
11.Equipment	MockV_Solutions_Equipment.pdf
12. Other Attachments	MockV_Solutions_SBA_Registration.pdf

Funding Opportunity Number: PA-16-302. Received Date: 2017-04-05T14:04:25.000-04:00

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 techniques in a variety of fields that currently rely on expensive and logistically challenging live viruses. We are currently focused on developing a novel series of assay kits which will enable biopharmaceutical scientists to study the efficacy of manufacturing techniques intended to remove or inactive virus; a contaminant of great concern during the production of biopharmaceuticals. In fact, *Minute Virus of Mice* (MVM) has notoriously contaminated several manufacturing facilities over the past couple decades leading to drug shortages, tremendous costs, and increased regulatory scrutiny. Because of this potential, mammalian derived biopharmaceutics are only approved for clinical or commercial use after their manufacturing processes demonstrate sufficient viral clearance. This is accomplished through small scale "spiking studies" whereby model viruses (ex. MVM) 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 a validation failure. Validation failures lead 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 "MVM-VLP 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 confidentially optimize 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 MVM-VLP Kit would translate into cheaper $/gram manufacturing costs and increase the likelihood of passing regulatory hurdles. Viral clearance is an international regulatory requirement for the ~6,000 mammalian cell derived BP in worldwide development. With the projected cost of a MVM-VLP Kit to be $2,500, we estimate an annual market value of ~$95M/year. To set us on the path toward commercialization, we propose to first develop an Immuno-QPCR assay capable of quantifying MVM-VLP and then utilizing this assay to test the feasibility of accurately predicting live MVM clearance with our non-infectious MVM-VLP surrogate. After successful completion of this Phase I feasibility study we will further optimize and validate the Immuno-QPCR assay. We will then conduct extensive beta testing through a series of Design of Experiments studies, comparing MVM-VLP clearance to live MVM clearance through variety of purification techniques (chromatography, inactivation, filtration) and representative operating conditions.

In this Phase I SBIR study, MockV Solutions plans to test the feasibility of accurately predicting Minute Virus of Mice (MVM) clearance through the use of a non-infectious Virus Like Particle Kit. Using this kit, scientists will be able to develop more efficient biopharmaceutical manufacturing processes and predict viral clearance validation failures prior to expensive regulatory testing. These benefits will reduce the $/gram costs of biopharmaceuticals and ensure the timely regulatory approvals of life-altering therapies.

FACILITIES AND OTHER RESOURCES

MOCKV SOLUTIONS INC (MVS)

MVS, leases office space from BioHealth Innovations in Rockville, MD and has arranged to sub-lease laboratory space for seven months during the course of Specific Aims #1 and #2 from Texcell North America Inc. in Frederick, MD (30 miles from MVS's Rockville facility).

Texcell North America Inc. is a GLP-compliant Viral Clearance / Viral Safety testing site that performs studies in support of biopharma/biotech regulatory applications submitted to the FDA and other international authorities. They conduct routine assay development work and have the ability to lease space within a fully equipped analytical assay development laboratory (Specific Aim #1). In addition, Texcell North America has the ability to lease a BSL-2 viral clearance "suite" for MVM-VLP vs. MVM feasability studies (Specific Aim #2).

Laboratory and Equipment: Texcell North American Inc. maintains a Square Footage facility at Proprietary Info Built in 2012, this facility is designed for analytical assay development and BSL-2/3 viral clearance validation studies and offers clients the proper equipment to perform this work. Equipment available to MVS as part of this project will include: Two BioRad iQ5 real-time qPCR thermocyclers with 12-bit CCD-based optics and analysis software, an ELISA plate washer/reader, Novex SDS PAGE and Western Blot devices, a Molecular Devices ELISA plate reader, a UV/visible spectrophotometers, two 6ft Class BSL-II Biosafety Cabinets with HEPA-filter exhaust, a 4°C, -20°C, and -70°C freezer, two AKTA Explorer 100 systems (GE) with internal pH, conductivity, and UV sensors, HP desktop computer with Unicorn software, a pH and conductivity meter, digital scales, pipettes/micropipettes, pipette/micropipettes, a Millipore purified water system, sources of compressed air, and peristaltic pumps.

Offices and computers: MVS will be given access to a client office space suite during the duration of the lease. This suite has internet access, centralized telephone and voice mail system, access to laser printers, and scanners.

BluePoint Bioscience, LLC

For monoclonal antibody development (part of Specific Aim #1), MVS will contract BluePoint Bioscience, LLC in Ijamsville, Maryland (20 miles from MVS's office in Rockville and 12 miles from Texcell's facility).

BluePoint Bioscience is a full service antibody development and production company. Their stated mission is to develop and produce the highest quality research and diagnostic products in the biotech industry and have all the necessary equipment and resources to engineer, screen, and produce monoclonal antibodies.

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, Liquid nitrogen storage system (4). Vivarium equipment includes; Allentown small rodent cage racks, capacity 60 cages (2) with water bottles, feed hoppers and ID tags.

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.

OTHER RESOURCES

Board of Directors

Personal Info is the founder and Chief Executive Officer of MockV Solutions. Previously, Personal Info was a purification scientist at Human Genome Sciences and later GlaxoSmithKline. This position exposed him to the various efforts involved in developing, optimizing, and testing the virus clearance capabilities of biopharmaceutical manufacturing processes. Personal Info observed inefficiencies, delays, and unnecessary cost overruns stemming from the current viral clearance testing practices. He founded MVS with the objective of providing the industry with novel and effective virus clearance prediction assay kits. His experience as an end-user of virus removal related products and knowledge of the field will help guide the development of a commercially relevant retroviral kit. In addition, his experience as a bench top purification scientist will be utilized while designing and executing Phase I feasability studies.

Personal Info is the Chief Operating Officer of MockV Solutions and is currently an Entrepreneur-in-Residence at Johns Hopkins University and BioHealth Innovation, Inc (BHI, discussed below). Personal Info brings over 20 years of operating and product commercialization experience within 3 three public companies representing the biotechnology, diagnostics and life sciences sector. As COO of Precision Biologics, (a clinical stage oncology company with a Companion Diagnostic) she formulated a virtual business model and negotiated agreements to advance towards Phase 2 clinical trials. During her tenure at Life Technologies [LIFE] she was responsible for the global portfolio as Senior Business Director and managed Joint Ventures and expansion within the Asia-Pacific region. Personal Info provides MVS with translational leadership to advance intellectual property and assists MVS with product development, product strategy, and general business strategy.

Personal Info is the Chief Business Officer of MockV Solutions and a serial entrepreneur who started, grew and successfully sold two life science businesses; Receptor Biology, the first US company that manufactured and sold GPCR reagents to the drug-discovery market and Applied Cell Sciences, a reagents and services company also in the drug discovery market. Receptor Biology was sold to Perkin Elmer Inc. in 2006 while Applied Cell Sciences was sold to ChanTest Corp. in 2008. Personal Info went on to work as VP of business development and reagents and then as VP of Product Services at ChanTest. Throughout these experiences Personal Info has gained knowledge in solving the issues facing new life sciences ventures. Personal Info offers MVS his expertise in crafting sound product development, commercial, and marketing strategies.

Consultant Info is a non-paid, part time employee of MockV Solutions. He is an experienced virologist with years of industry and academ Consultant Info ence creating virus like particles and developing commercially successful biotechnology products. Consultant Info guides MVS on scientific matters regarding VLP production and product development. Most recently, Personal Info has been designing experiments and interacting with MVS's service providers in developing the MMV VLP and quantification assay. For full details on Personal Info please refer to his Biosketch.

Scientific Advisory Board

This board was established to strategically plan the development of MVS's series of viral clearance kits and identify/solve the technical challenges that arise. These relationships are provided through in-kind services, consultant fees, or opportunities to earn equity.

Personal Info see above.

Consultant Info : see above.

Personal Info is a senior process development scientist with Glaxo Smith Kline with over 15 years of experience in the activities associated with virus removal including designing and executing 20 virus removal studies. She is well published on the subject of virus removal and is a member of the PDA and viral safety industry consortium. Personal Info serves as an advisor to MVS on regulatory and bioprocess development and regulatory issues and will be especially helpful in designing proof of concept virus removal studies.

Personal Info is a former downstream process scientist and engineer who co-founded Maryland based biotech company BioFactura Inc. While with BioFactura, Personal Info led the downstream process and analytical development efforts for the company's in-house R&D, federal grant, and commercial client contract

programs. In addition he managed all business operations, finances, legal, and corporate administrative matters. As member of the advisory board Personal Info draws from his experiences as both an end user of viral filtration products and manager of small biotech business operations.

Personal Info is currently the Senior Director of CMC Team Management at MedImmune and the former Senior Director of Purification at Human Genome Sciences. He has led the downstream development, optimization, validation, and tech transfer activities for several FDA approved processes and is well acquainted with the issues of virus removal. As member of the advisory board, Personal Info contributes from the perspective of a downstream process developer as well as from a project oversight manager of a large biopharmaceutical company.

Community Support

In addition to its Board of Directors and Scientific Advisory Board, MVS 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 MVS 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.

MVS is located in Rockville, MD, a hub of scientific research and commercialization. Being so, MVS takes advantage of its proximity to biotechnology corporations and high quality service providers. For example, MVS has recently initiated a proof of concept collaboration with MedImmune, for its first commercial product, the MMV Kit. MVS also enjoys the various Montgomery County and state led initiatives, programs and resources available for the biotechnology community and has received Montgomery County and Maryland State funding ($25,000 and $100,000 respectively).

MockV Solutions Equipment

Through a sublease agreement with Texcell North America Inc., MockV Solutions will have access to the following equipment (located at Texcell, Frederick MD) for Specific Aims #1&2:

- Two AKTA Explorers 100 systems (GE) with internal pH, conductivity, and UV sensors
- HP desktop computer with Unicorn 6.4.2 software
- pH meter
- conductivity meter
- Mettler Toledo digital scale
- Millipore purified water system
- Two 6ft Class BSL-II Biosafety Cabinets with HEPA-filter exhaust
- UV/visible spectrophotometer (Nanodrop 2000c)
- -20c Freezer
- -80c Freezer
- Pipette/micropipettes
- Two BioRad iQ5 real-time PCR detection systems with 12-bit CCD-based optics and analysis software
- BioRad Model 1575 Immunowash Microplate Washer
- Novex SDS PAGE and Western Blot devices
- Molecular Devices ELISA plate reader,
- Humidified CO2 incubators
- Inverted light microscopes
- Low-speed and ultra-speed centrifuges
- sources of compressed air
- peristaltic pumps.

Contact PD/PI: Personal Info

SBIR.gov SBC Registration Control ID Form

SBC CONTROL ID	Proprietary Info

FIRM INFORMATION					
Company	Proprietary Info				
Address					
City		State	Proprietary Info	Zip	Proprietary Info
TIN/EIN		DUNS			
Company URL					
Number of Employees:	# of Employees				
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	No				
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	0%				

SBC CONTROL ID	Proprietary Info

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: Degree Year:

Attach Biographical Sketch*: File Name: Personal Info

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-302 . Received Date: 2017-04-05T14:04:25.000-04:00

PROFILE - Senior/Key Person

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

Position/Title*: Part Time Scientist
Organization Name*: MockV Solutions, Inc.
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: Subcontractor Info First Name*: Subcontractor Info Middle Name Last Name*: Subcontractor Info Suffix:

Position/Title*: Laboratory Director
Organization Name*: BluePoint Bioscience
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*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: 1978 Degree Year: B.S.

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

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

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 SBIR is to demonstrate the feasibility of utilizing non-infectious Minute Virus of Mice – Virus Like Particle (MVM-VLP) as a spiking agent during biopharmaceutical process development "viral clearance" studies. Specifically, we plan to produce a high binding monoclonal antibody against our MVM-VLP and develop a specific and sensitive Immuno-QPCR assay to quantify MVM-VLP in biopharmaceutical solution. We then plan to execute a proof of concept "spiking" study to compare MVM-VLP clearance against live MVM. I have the expertise, leadership, motivation, and support necessary to successfully carry out the proposed work. I posses 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 (MVS) in 2013 after spending six years developing and optimizing purification processes without a cost effective and accurate means of analyzing viral clearance. MVS will provide 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 MVS's mission. As CEO of this venture I have worked with each member of my team in laying the groundwork for our future success. Today, MVS is conducting alpha testing of its lead product candidate (the MVM Kit) with several major biotech companies. We have raised starting capital, been awarded two grants totaling $125,000 (Maryland State and Montgomery County), have completed a collaboration with the FDA (Personal Info) to characterize our lead particle, filed two non-provisional patents, established strategic partnerships with key industry suppliers (Pall Corp, Millipore, Asahi)

and have present at three industry 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, MVS will be relying on the expertise of Personal Info (PD/PI). Throughout the course of this project, Personal Info will provide MVS with world-class expertise in retrovirus like particle engineering, production, and assay development.

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 field 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**. (2016, Aug.) Characterization and Use of a Mock Virus Particle as Spiking Surrogates for Downstream Process Development Studies. Presented at the 8th Annual Cambridge Health Institute Bioprocessing Summit. Boston, MA.

2. Pending 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.

3. Pending Patent: **Cetlin, D**., Dhar, A. 2015. Stock Solution of Retrovirus Like Particles with Method and Kit. Application Number PCT/US16/15620. Filed Jan. 29 2016 (Unpublished),

4. 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*, 1-14.

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

As a non-academic, my 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.

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

Research Support since 2014

Technology Commercialization Fund, TEDCO (Maryland) 02/26/16-Present Role: PI
Commercialization of a Viral Clearance Predication Kit for Biopharmaceutical Process Development.
The goal of this study is to commercialize a kit capable of predicting the removal of Minute Virus of Mice from a biopharmaceutical solution during biopharmaceutical process development through a non-infectious surrogate.
Role: Project Manager

The Life Sciences Impact Grant Program, Montgomery County (Maryland) 02/26/15- 02/26/16 Role: PI
Creation of a Mock Virus Particle Kit for the Biopharmaceutical Process Development Industry
The goal of this study was to initiate the development a non-infectious Retrovirus Like Particle Kit, capable of quantifying the removal of retrovirus from biopharmaceutical solution during biopharmaceutical process development.

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Contact PD/PI: Personal Info

Consultant Info

Contact PD/PI: Personal Info

Subcontractor Info

Contact PD/PI: Personal Info

Subcontractor Info

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

Proprietary Info
ORGANIZATIONAL DUNS*:

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-02-2017 **End Date*:** 10-02-2018 **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 ($)*	Funds Requested ($)*
Personal Info 1.					PD/PI	Institutional Base Salary	Effort			Itemized Cost		
2. Consultant Info					Consultant							

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				Itemized Cost		
# of Employees	Assay Development Scientist	Effort					
	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-16-302 . Received Date: 2017-04-05T14:04:25.000-04:00

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

Proprietary Info

ORGANIZATIONAL DUNS*:

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-02-2017 **End Date*:** 10-02-2018 **Budget Period:** 1

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 1

ORGANIZATIONAL DUNS*: Proprietary Info

Budget Type*: ● Project ○ Subaward/Consortium

Organization: MOCKV SOLUTIONS, INC

Start Date*: 10-02-2017 **End Date*:** 10-02-2018 **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 . Total Direct Costs	Itemized Cost		
Total Indirect Costs			Itemized Cost

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

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

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

K. Budget Justification*	File Name: MockV_Budget_Justification.pdf
	(Only attach one file.)

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

Tracking Number: GRANT12379788 Funding Opportunity Number: PA-16-302 . Received Date: 2017-04-05T14:04:25.000-04:00

RESEARCH AND RELATED BUDGET JUSTIFICATION

MockV Solutions (MockV) is requesting 12 months and total Itemized Cost budget for a Phase I 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 Itemized Cost for Phase 1 over 12 months.

A. Senior/Key Person

1. Personal Info Principal Investigator, (Effort is employed full time by the company. He will be responsible for overall coordination and day-to-day direction of the project. In addition, he will help design, conduct a majority of experiments, and interpret all experiments and will execute the feasibility comparison studies (Specific Aim #2). Total salary requested is Itemized Cost

2. Personal Info Consultant (Effort , is currently a non-paid, part time employee of the company. He will be primarily responsible overseeing the engineering/production of an anti-MVM-VLP monoclonal antibody (Conducted by BluePoint, Specific Aim #1) and the development of an Immuno-qPCR assay. Total salary requested is Itemized Cost

Total Senior/Key Person Itemized Cost

B. Other Personnel

1. **Assay Development Scientist**, (Effort , an individual experienced in Immuno assay development (ex. ELISA) and qPCR. This individual will be responsible for overseeing the development of an Immuno-qPCR assay. S/He will work collaboratively with the PI and Personal Info prepare summary of findings, and assist the PI in managing the laboratory. S/He will be responsible establishing and managing the internal QC/QA program. The salary requested for this position is Itemized Cost

Total Other Personnel Itemized Cost

C. Equipment Description

None requested

Total Equipment Itemized Cost

D. Travel

1. **Domestic Travel costs**

Travel to 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

a. Immuno-assay reagents (ex. microwell plates, HRP-streptavidin, TMB substrate, stop buffer, was buffer, blocking reagents, etc.) Total cost is Itemized Cost
b. qPCR reagents and supplies (TaqMan primers and probe, qPCR Master Mix, tubes, etc.) Total cost is Itemized Cost
c. Protein purification materials (chromatography resin and nanofiltration units). Total cost is Itemized Cost
d. Protein purification hardware (chromatography columns, tubing, AKTA fittings, etc.). Total cost is Itemized Cost
e. Conjugation chemistry chemicals, reagents, and kits. Total cost is Itemized Cost
f. General laboratory consumables, chemicals and reagents. Total cost is Itemized Cost

2. Publication Costs
None

3. Consultant Services
None

4. ADP/Computer Services
None

5. Subawards/Consortium/Contractual Costs

BluePoint Bioscience, LLC (Ijamsville, MD)
BluePoint Bioscience will be contracted to engineer and produce anti-MVM-VLP monoclonal antibodies from mice. The total cost for materials, supplies, personnel, and overhead is Itemized Cost

Texcell North America, Inc. (Frederick, MD)
Texcell North America will be contracted to supply 10L of partially purified biopharmaceutical material and purified stock solution of live MVM for feasibility studies (Specific Aim #2). In addition, Texcell will be contracted to conduct $TCID_{50}$ analysis on

samples generated during feasibility studies. The total cost for materials, supplies, personnel, and overhead is Itemized Cost

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

A rate of Itemized Cost of total direct costs is requested to cover facility, administrative, and insurance costs. MockV is a startup company and is attempting to maintain low overhead costs. Through a partnership with an incubator, BioHealth Innovations, much of these costs are minimized. We will also use these funds to pay for laboratory facilities from Texcell North America Inc. (Frederick, MD). 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 I SBIR proposals when the company does not already have a previously negotiated indirect cost rate (Page I-107 of SBIR/STTR Application Guide.)

H. Fee

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

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		
1. Domestic	Itemized Cost	
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		
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

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

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)

SBIR/STTR Type (select only one)*

● Phase I ○ Phase II ○ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

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

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 e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ○ No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 06/30/2016

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 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 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

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OMB Number: 0925-0001

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1. Human Subjects Section

Clinical Trial? ○ Yes ● No

*Agency-Defined Phase III Clinical Trial? ○ Yes ○ No

2. 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 proved scientific justification

3. *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

4. 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, please 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):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ○ Yes ● No

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

*Previously Reported: ○ Yes ○ No

6. 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:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	MockV_Introduction.pdf
Research Plan Section	
2. Specific Aims	Specific_Aims_MockV.pdf
3. Research Strategy*	MockV_Research_Strategy_.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	MockV_VAS.pdf
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	Letters_of_Commitment_and_Support.pdf
14. Resource Sharing Plan(s)	
15. Authentication of Key Biological and/or Chemical Resources	MockV_AoR.pdf
Appendix	
16. Appendix	

INTRODUCTION TO REVISED APPLICATION

The applicants thank the reviewers for their thorough evaluation of the last Phase I SBIR submission 1 R43 TR002231-01, Demonstrating Utility of a Non-Infectious Minute Virus of Mice – Virus Like Particle for Predicting Viral Clearance during Biopharmaceutical Process Development, and appreciate their encouraging comments regarding the strength of our proposals significance and team. In response to the reviewer's constructive feedback on the proposal weaknesses, several significant revisions have been made. In some cases we have highlighted revisions in the new proposal by bold lettering.

Immuno-qPCR was presented as an innovation, but it is not – This critique was correctly cited by all reviewers. Immuno-qPCR has been utilized in a variety of applications since its introduction by Sano et. al. in 1992. It was not our intent to imply that we were developing a new ultra-sensitive technique for particle analysis. Instead, we were attempting to convey our sentiments that the application of Immuno-qPCR to solve the issue of quantify low levels of non-infectious virus like particles (VLPs) during viral clearance studies was a somewhat unique approach. Nonetheless, the true innovative aspect of our proposal is the utility of VLPs as non-infectious spiking agents to accurately and economically predict live viral clearance. We are proud to note that MockV was recently granted a US patent Notice of Allowance for methods based on this innovation. In the revised proposal we have moved and altered the Innovation section to focus on our utility of VLP. We address the Immuno-qPCR assay in the Approach section.

Sensitivity of the current immune-PCR assay is not reported; only dynamic range – This critique was correctly noted by all reviewers. To address this comment, we have expanded the preliminary study section. Briefly, live MVM assay sensitivities and dynamic ranges typically exceed 10^3 particles/ml and 5 logs respectively. Our commercial MVM-VLP assay benchmark is: a sensitivity of $<10^4$ particles/ml and a dynamic range of > 4 logs. To achieve this, we first developed an ELISA. Although robust, the sensitivity and dynamic range was 10^7 particles/ml and 2 logs respectively. We then developed an Immuno-qPCR assay with MedImmune which increased the sensitivity to ~10^5 particles/ml and extended the dynamic range to ~3 logs.

Not clear if the monoclonal antibody would be able to achieve the desired sensitivity – The reviewers were correct to point out that we failed to provide data or theoretical arguments on why replacing capture pAb with mAb has the potential to reduce assay background and improve sensitivity. We have addressed this in the Rationale subsection for Specific Aim #1 through the inclusion of references and data generated by MedImmune. Moreover, we mention other issues with the current assay and how our approach offers to address them.

The heterobifunctional cross-linking agent could be the current sensitivity problem – To clarify, the current pAb based Immuno-qPCR assay does not contain this cross-linking reagent and therefore it does not currently contribute to assay background or decreased sensitivity. Instead, the current detection biotinylated-pAb is bridged to a biotinylated-oligonucleotide (for qPCR amplification) through NeutrAvidin. We have included a Figure detailing this schematic. However, the reviewers overall point remains valid – we did not adequately provide proof or reasons as to why we believe the pAb contributed to high background/lower sensitivity and why we think a mAb has the potential to improve. As stated above, we address this in the preliminary study section.

Primary and selective assays unclear – To address this, we have clarified the two assays.

A question about how close the VLPs are to real viruses – The physicochemical resemblance of MVM-VLPs to live MVM is essential for accurate MVM prediction during biopharmaceutical processing (ex. chromatography, nanofiltration). For the MVM-VLP Kit to be commercially successful this accuracy must be achieved. To ensure this, we recently conducted and published a characterization and comparison study of MVM-VLP to live MVM, with the FDA. The results are included in the preliminary study section and demonstrate a positive correlation between the two.

Immuno-PCR is thought to amplify the non-specifically bound antibodies - Our current Immuno-qPCR is admittedly complicated and prone to non-specific binding. We detail a streamlined oligo-mAb conjugation approach which will dramatically simplify the Immuno-qPCR assay, reduce the potential for off-target binding, and hopefully increase sensitivity/dynamic range.

Not so innovative because other non-infectious surrogates have been used – Although it is true that previous attempts have been made to solve this issue, no other non-infectious surrogate has been able to accurately mimic the physicochemical properties of the viruses they represent. The novel use of VLP's to solve this issue has recently been awarded a US patent notice of allowance and has already generated much interest from the industry.

Reviewer Comments

RESEARCH STRATEGY

SIGNIFICANCE

This proposal aims to demonstrate the feasibility of predicting *Minute Virus of Mice* (MVM) clearance through the use of a non-infectious surrogate. Viral contamination is an inherent risk during the manufacture of Biopharmaceuticals (BPs)[1-5], a rapidly growing class of therapies which include monoclonal antibodies[6]. Whether introduced endogenously from cell banks or exogenously through manufacturing, unmitigated viral contaminations have led to serious health implications including influenza, acquired immune deficiency syndrome, hepatitis, herpes, measles, and poliomyelitis[7]. International regulatory agencies therefore require BP companies to validate the "viral clearance" efficacy of their manufacturing processes prior to clinical trial or commercial approval[8-19]. Unfortunately, due to the **high costs**[20] **and logistics**[21] associated with viral clearance studies, most companies **delay assessments** and spend considerable up-front resources optimizing manufacturing processes without knowledge of their viral clearance efficacy. This increases the risk of validation failure, forcing companies to invest additional time and money redeveloping process steps. In addition to inefficiencies and cost overruns, often passed on to the consumer, redevelopment can delay the regulatory approvals. It has been estimated that a 6 month delay in regulatory approvals can result in a $100M loss in net product value[22]. This can affect a company's go-to market strategy and cost patients timely access to therapies.

Currently, viral clearance is assessed through **small scale** "spiking studies" whereby specific model mammalian viruses (ex. *Minute Virus of Mice*) are artificially introduced ("spiked") into BP material and subsequent cleared (removed or inactivated) via purification techniques[19,21,23]. These studies require specialized Biological Safety Level laboratories (BSL) and trained personnel resulting in costs that can soar well above $100,000[20]. These hurdles deter companies from analyzing viral clearance during **small scale** process development and lead to the risks described above[20,21]. A **simple, low cost viral clearance assessment kit**, utilized during small scale process development would provide bench-top process development scientists with a unique tool to generate "real time" data on viral clearance. With a "Quality by Design" approach, scientists could confidentially optimize their purification steps to remove virus and determine if process steps are effective before 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, a benefit that dovetails with broader societal aims and regulatory initiatives such as the Affordable Care Act and the Cures Act for Orphan Diseases.

Analytic kits are ubiquitously used throughout small scale process development to determine the removal of other critical BP manufacturing impurities such as Host Cell Proteins, DNA, and Endotoxin. These kits are important low cost tools that help scientists optimize process steps before manufacturing scale-up. However, for over a decade, various attempts to address viral clearance in a similar manner have not resulted in a practical low cost product[21,24-26]. For example, bacteriophage have been assessed as cost effective spiking alternatives, but due mainly to the **physicochemical differences** to mammalian virus models, they do not accurately predict viral clearance[21,24] and have therefore never become widely adopted or commercialized (Table 1).

Method	MVM Prediction Accuracy	Cost/Study*	BSL-I Compatible	Duration	Commercial Availability
Standard Regulatory Validation Method					
Live MVM studies	N/A	> $100,000	No	> 4 weeks	Yes
Current Predictive Methods**					
Bacteriophage	No	N/D	No***	1-3 Days	No
Gold Particles	No	N/D	Yes	1 Day	No
MockV's Proposed Method					
MVM-VLP	Yes	$5,000	Yes	1 Day	Pending

Table 1: Summary of Viral Clearance Methods (*) A study refers to an extensive set of process optimization experiments (~ 20 individual small scale "spiking" experiments). (**) Chinese Hamster Ovary Retro-VLP's have been used for retrovirus prediction but are not utilized as MVM surrogates and are therefore not listed here. (***)Certain strains of bacteriophage are BSL-1 compatible; however, many BP labs choose not to risk introduction of them into their facilities. Other strains are BSL-2.

Innovation

MockV Solutions (MockV) is addressing the problem of accurate and economic prediction of viral clearance through a novel use of Virus Like Particles (VLPs). VLPs are multiprotein structures that mimic the characteristics, organization and conformation of native infectious viruses but are themselves non-infectious[27]. These properties have made VLPs an interesting class of molecules for potential use as vaccines[28]. These features also make them ideal for use as BSL-1 compatible analytical process development tools (Table 1). This proposal focuses on generating the data needed to demonstrate the concept of predicting *Minute Virus of Mice* (MVM) clearance (removal and inactivation) through the use of a non-infectious MVM-VLP surrogate. MVM is among the most common model "spiking" virus cited by international regulatory agencies to demonstrate clearance[29]. The product of this phase I SBIR will be the first BSL-1 compatible viral clearance

assay kit that will allow scientists to accurately and economically predict MVM clearance throughout process development, prior to expensive validation studies. The "MVM-VLP Kit" will include a highly purified and concentrated stock solution of MVM-VLP as a "spiking agent" and reagents for a sensitive and specific Immuno-qPCR (I-qPCR) assay (microwell plate, antibody, primers, probe, etc.). To perform a MVM-VLP spiking study, a scientist would 1) add MVM-VLP stock solution to test material, 2) process material through a purification technique (ex. chromatography), and 3) analyzed MVM-VLP quantity via I-qPCR to determine clearance (Figure 1).

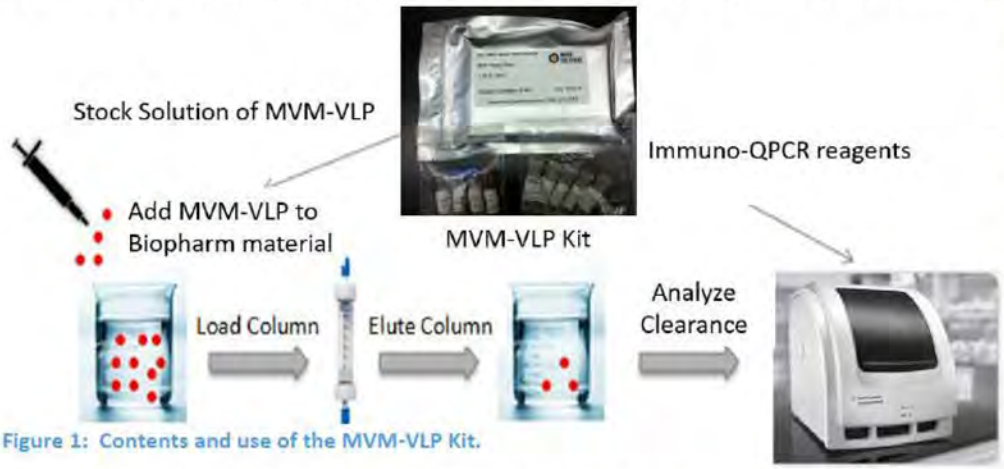


Figure 1: Contents and use of the MVM-VLP Kit.

Intellectual Property: **In March 2017, MockV received a Notice of Allowance from the USPTO for a patent that claims viral clearance methods involving VLPs as spiking agents**[30]. The broad method claims listed in this patent will help MockV establish a strong patent estate surrounding the MVM-VLP Kit and additional kits in the future. MockV is actively pursuing these claims in other countries and has filed a separate provisional application to claim additional concepts surrounding retroviral clearance. MockV believes that Intellectual Property is an essential element of its business strategy and will pursue an aggressive strategy.

Value Proposition: The MVM-VLP kit would provide BP scientists with an economic and practical method for predicting MVM clearance. The value of this kit is ***not*** its use as a replacement to live MVM spiking studies, but rather as an economical and practical tool to develop and optimize manufacturing processes prior to regulatory MVM spiking validation studies.

The **non-infectious** nature of MVM-VLP's and the **rapid turn-around time** of the I-qPCR assay will enable scientists to conduct MVM-MVP Kit evaluations **"in-house"** (BSL-1) in a matter of hours. The "on-site", "on-demand" nature of the MVM-VLP Kit will enable several possibilities not currently conceivable. First, the possibility of applying the FDA concept of **Quality by Design** to viral clearance. The MVM-VLP Kit would allow scientists to analyze viral clearance as an output during **Design of Experiment** studies. Through these types of assessments, scientists could evaluate manufacturing steps (chromatography resins, nanofilters, etc.) and then screen parameters (pH, conductivity, etc.) for optimal MVM clearance. Second, the MVM-VLP Kit would enable rapid **manufacturing quality assurance** analysis to take place in instances where Bulk Drug Substance is quarantined due to quality issues such as after the occurrence of a manufacturing deviation.

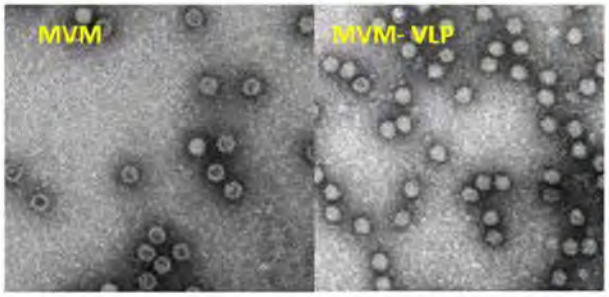
Currently, live MVM "spiking" studies require BSL-2 laboratories which specialize in the propagation and handling of live virus. As most BP companies, large and small, are not equipped to handle such work (see letters of support), studies are only possible through the outsourcing of expensive Contract Research Organizations (CRO's) such as BioReliance who typically require > 4 weeks to generate data sets. These hurdles cause most companies to conduct minimal viral clearance work during process development and risk the consequences of failing regulatory mandated validation studies. Although statistics are not available to cite the number of viral clearance validation failures, it is the experience of the PI that **nearly half** of all validation spiking experiments **fail** to achieve desired viral clearance and **require re-development**. The MVM-VLP kit would be used to **predict the outcomes of regulatory required validation studies**, thereby exposing potential issues ahead of time, minimizing the chance of failure and decreasing the risk of cost-overruns (process redevelopment) and delayed market entries (issues with regulatory applications).

The global market for the MVM-MVP Kit is estimated to be ~ $95M/year. As the popularity of recombinant BP products has increased[6], regulatory attention and international requirements pertaining to viral clearance have increased as well[45]. Today there are an estimated 11,000 BP's at various stages of international pre-clinical and clinical development[6]. Roughly 40-50% of the manufacturing processes that produce these biologic drugs utilize mammalian cell culture[6] and will therefore need to demonstrate viral clearance efficacy. Through a customer market survey (n=31), we have established several key assumptions to base our market model; the projected cost of a kit ($2,500), 6 kits used during process development efforts per BP per year, and a market penetration of 50%. In the near term, we anticipate that VLP kits will be utilized ubiquitously during small scale experimentation (similar to other impurity kits) and shift the paradigm of process development. In the long term, we anticipate that in certain applications, VLP Kits *could* replace live viral spiking studies for regulatory validation.

This revolutionary shift would only be possible after many years of data accumulation, backing from the BP industry and "buy-in" from regulatory agencies. Broader market potentials for viral clearance prediction kits include other applications such as plasma derived therapeutic products and food/water. MockV Solutions focus is to create tools to that solve the unmet needs of scientists as they develop, optimize, and validate products, equipment, and techniques used in various purification industries. Our plan is to grow aggressively after the introduction of the VLP Kit first, followed by development of additional relevant on-demand process analytical technologies applicable to QA/QC of BP manufacturing.

APPROACH

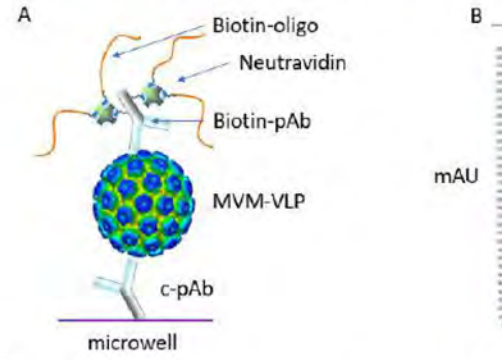
Preliminary Studies: The Particle - MockV has engineered a baculo/Sf9 expression system to produce MVM-VLP. Through a **recently published** collaborative study with the FDA[31], we have determined that the physicochemical properties of MVM-VLP (ex. hydrodynamic radii, surface charge and hydrophobicity) are comparable to live MVM (Figure 2). These results suggest that the removal of MVM-VLP from spiked BP material should be similar to the removal of live MVM.

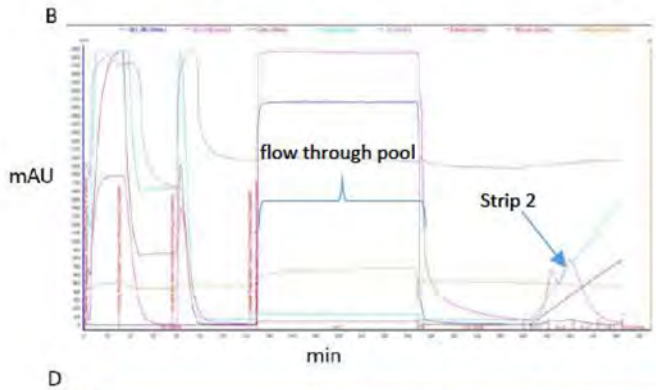


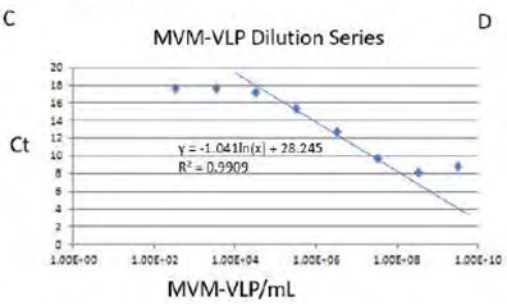
Analysis	Live MVM	MVM-VLP
Hydrodynamic Radii (MALS)	18.4 ± 0.2 nm	17.2 ± 0.1 nm
Diameter (TEM)	24.6 ± 3.6 nm	25.6 ± 3.0 nm
Surface Charge (pI)	5.99	5.81
Relative Hydrophobicity	0.28	0.35

Figure 2: Results from FDA collaboration study. TEM images of MVM (far left) and MVM-VLP (right left) at 110,000x magnification. Summary Table comparison (top). For complete results see Johnson et. al. (31).

To test this hypothesis for chromatography and nanofiltration applications we have initiated collaborations with MedImmune, Texcell (a viral clearance CRO) and Asahi Kasei, a leading nanofiltration supplier (see letters of support). Quantification - For viral clearance analysis, live MVM is quantified through infectivity ($TCID_{50}$) or qPCR assays. These methods provide sensitivities of $<10^3$ particles/ml and dynamic ranges of > 5 logs[32]; however, log reduction values (LRV's) of > 4 logs are universally acknowledged as conveying robust clearance[33], and therefore our commercial assay. benchmark is > 4 logs of dynamic range. Since MVM spiking concentrations are typically ~10^8 particles/ml, our assay sensitivity target is $<10^4$ particles/ml. Due to the inability of utilizing infectivity of qPCR techniques (VLP's being non-infectious and not containing internal nucleic acid), we first developed an Enzyme Linked Immunosorbent Assay (ELISA). Although robust, ELISA sensitivity and dynamic range was 10^7 particles/ml and 2 logs respectively[34]. I-qPCR has successfully been utilized in a variety of applications to increase sensitivity 10^2-10^4 fold over traditional immune-assays[35]. Through a collaboration with MedImmune, we developed an I-qPCR assay which increased MVM-VLP sensitivity to ~10^5 particles/ml and extended the dynamic range to ~3 logs (Figure 3). This assay was utilized by MedImmune to analyze the removal of MVM-VLP from spiked BP







D

	Load + VLP	Pool	Strip 2
Ct	11.8	12.8	10.2
Dilution Factor	600	2	600
Concentration (VLP/ml)	4.6E9	5.9E6*	2E10

* Limit of Quantification

LRV Calculation

$$LRV = \log_{10} \frac{(145.3 \times (4.6 \times 10^9))}{(140.3 \times (5.9 \times 10^6))} = 2.91$$

Figure 3: An Immuno-qPCR utilizing a capture anti-MVM-VLP polyclonal antibody (c-pAb) was developed to quantify MVM-VLP (A). After incubation with oligo-conjugated antibody, microwells are heated and contents are transferred to PCR tubes for Taqman qPCR. MVM-VLP spiked BP material was processed through an AEX membrane (B). Flow through and high salt strip fractions were collected for analysis. A standard curve was generated from a dilution series of MVM-VLP (C) and used to calculate MVM-VLP concentration in the samples collected (D). LRV was then calculated according to standard practice[32]

material via anion exchange chromatography (AEX). As expected, MVM-VLP was completely removed from flow-through material and was recovered during high salt gradient elution.

Specific Aim #1: I-qPCR Assay Development

A) Rationale: The MVM-VLP Kit is currently being tested through separate collaborations with MedImmune and Asahi; however, high I-qPCR background limits assay sensitivity to ~10^5 particles/ml. Based on published studies, I-qPCR sensitivities of 10^2 particles/ml for viral analytes are achievable[36]. MedImmune has determined that the probable source of assay background is the **non-specific binding of the biotin-oligo reagent**. Despite attempts to optimize blocking conditions, wash steps, etc., this reagent remains in the system. Moreover, MedImmune has found that the **removal of pAb** from the system **decreases signal** while the **presence of pAb** itself is **sufficient to generate high signal** (Table 2). This finding is not surprising since pAbs, although generally exhibiting better sensitivities than monoclonal antibodies (mAbs), have a high potential for cross reactivity which can increase non-specific binding and lead to assay background[37,38]. Our approach aims to address both concerns, 1) the non-specific binding of biotin-oligo and 2) the non-specific binding of pAb. To address the later, we will generate highly specific anti-MVM-VLP mAbs, and elucidate an optimal capture/detection pair. Aside from the potential decrease in assay background, a key driver for replacing pAb with mAb is that **mAbs can be generated as a constant and renewable resource**. In contrast, pAbs can differ in specificity and sensitivity from lot to lot since multiple animals are utilized[39]. To address the former, we will replace the biotin-oligo reagent with a directly conjugated oligo-mAb. This will not only address the primary issue of oligo carry over, but will **reduce** the number of detection reagents and assay steps **from 3 to 1** (Figure 4), thereby **reducing assay time, error potential, and commercial production complexity**. Through our S.A. #1 approach, we will develop an I-qPCR assay capable of quantifying ≤ 1x10^4 MVM-VLP's/ml with a dynamic range of ≥ 4 logs.

Reagents					Ct Value
pAb	VLP	b-pAb	N.A.	b-oligo	
Coat	+	+	+	+	13.3
Coat	-	-	-	+	14.0
-	-	-	Coat	+	19.5

Table 2: Microwells were coated with pAb and brought through entire I-qPCR assay (Row 1) or jumped to biotin-oligo addition (Row 2). In either case, high signal (low Ct) was generated. In contrast, coating with NeutrAvidin (N.A.) reduced signal demonstrating that pAb alone contributes to background.

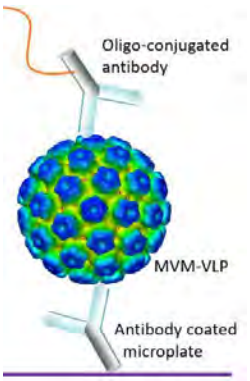


Figure 4: Proposed I-qPCR format. A single oligo-mAb reagent replaces the three step biotin-pAb/NentrAvidin/biotin-oligo detection scheme shown in Figure 3.

B) Experimental Design and Methods: (Performed by MockV and BluePoint Bioscience)

mAb Engineering (BluePoint): Anti-MVM-VLP mAb will be generated in mice and produced according to standard protocols[40]. In short, six female BALB/cAnNHsd mice will be immunized with MVM-VLP. Spleen cells from hyperimmune mice will be fused with NS1 Myeloma cells using standard polyethylene glycol induced fusion methods[41]. After hybridoma screening, subcloning and isotyping, mAb will be produced, in-vitro, using CL1000 Bioreactors (Wheaton Cat#WCL-1000). Cell culture supernatant, containing secreted mAb, will be harvested and clarified before purification through standard Protein-A/G affinity methods. Eluted immunoglobulin will then be pH neutralized, quantified spectrophotometrically, and dialyzed into an appropriate buffer.

Assay Development (MockV): An optimal capture/detection antibody pair will be determined through standard matrix screening protocols[42]. Candidates will include the clonal mAbs generated by BluePoint and pAb used in preliminary studies. In short, microwell plates will be coated with various dilutions of each antibody. Then MVM-VLP will be added to each well at a high, low and zero concentrations. Next, biotinylated detection antibodies (same antibodies screened for capture) will be added to each well at a fixed concentration, followed by Streptavidin-HRP, TMB Substrate and Stop Solution. Absorbance at 450nm (A_{450}) will be determined with a spectrophotometric plate reader. Once identified, the optimal detection antibody will be covalently linked to an oligonucleotide, according to published procedures[43] (same oligo sequence currently used in preliminary studies). In brief, the antibody and a 5' amino-modified DNA oligo will be independently activated with heterobifunctional cross-linking agents and coupled in a single spontaneous reaction before being purified. Next, optimal assay variables and parameters will be elucidated according to published experimental design protocols[40]. Minimal matrix dilution will be accomplished by serially diluting BP material (matrix), spiking MVM-VLP to a known concentration, and determining the percent recovery.

C) Data Analysis and Interpretation:

mAb Engineering: Mouse serum testing and hybridoma screening will be accomplished via "primary" antigen capture ELISA (plate coated with MVM-VLP) and "selective" ELISA (plate coated with irrelevant baculovirus

derived protein). Serum and hybridoma culture supernatant fluid testing positive for the Primary ELISA and negative for the Selective ELISA will signify the production of anti-MVM-VLP antibody. Isotype analysis will be performed via Isotyping Kit (Bioassay Works, Cat# MISOT-010). The static culture antibody production level for each hybridoma cell line will be determined using a mouse immunoglobulin capture ELISA or through Protein-A/G purification followed by spectrophotometric quantification analysis (absorbance 280nm). Static culture antibody production levels of 10 – 30µg/ml will be acceptable while >30µg/ml will be considered good.

Assay Development: A_{450} values from a Molecular Devices ELISA plate reader will be analyzed to determine the optimal capture and detection antibodies. To evaluate oligo-mAb conjugation and optimal assay parameters I-PCR will be conducted utilizing a BioRad iQ5 machine with 12-bit CCD-based optics and analysis software. Fluorescence signal generated from an oligo specific TaqMan probe will be recorded during real-time amplification and normalized with a baseline from initial cycles. Threshold Cycles (‘‘Ct’’) will be calculated at 100 absorbance units. These values represent the number of amplification cycles before a significant signal is generated and are inversely proportional to antigen concentration. Standard curve data will be used to construct precision profiles. This will be accomplished by plotting the coefficient of variation (CV) for the calibrated concentration levels of the replicates of each calibrator versus the nominal MVM-VLP concentration in the calibrator samples. The dynamic range of the calibration curve (quantification limits) will then be defined by the concentrations where the precision profile intersects the 20% CV. All experiments will be carried out in duplicate, standard deviation will be calculated as method error for the double determination.

D) Potential Pitfalls/Alternate Approaches: The proposed assay involves a capture antibody and a conjugated oligo-mAb detection reagent. It is possible that the oligo sequence, although conjugated to a mAb, can bind non-specifically to the capture antibody and contribute to assay background. We will therefore screen several high promising capture antibody candidates against a dilution series of oligo-mAb reagent before selecting our final pair. Aside from non-specific binding, high background signals may persist. If so, parameters such as plate type, blocking buffers and wash buffers will be tested. In addition, alternative conjugation variants of the oligo-mAb reagent can produced by utilizing different molarity rations (ex. 1 oligo:1 mAb:, or 2 oligos:1 mAb:). This strategy may be helpful if addressing low sensitivity (increase oligo:mAb ratio) or high background (decrease oligo:mAb ratio). While determining dynamic range, a significant source of variability in the calibration curves may come from the choice of the statistical model used for the calibration curve. For this reason, several models will be tested. In addition, a curve-fitting method/software that has appropriate weighting methods/options will be utilized. If percent spike recovery falls outside a 50-150% range, or if the precision profile limits are not within the desired working range of 10^6 ± 2 logs MVM/ml, further optimization of the immunoassay is required prior to feasibility study (Specific Aim #2).

E) Expected Outcome: For mAb production we expect to generate up to 18 candidates and produce 2 – 10mg of purified IgG per clone. We expect to develop an I-qPCR assay capable of measuring ≤ $1x10^4$ MVM-VLP’s/ml with a dynamic range of ≥ 4 logs in BP material. We also expect to achieve recovery, accuracy and precision at the limits of quantification and within the measurable range.

Specific Aim #2: Proof of Concept MVM vs. MVM-VLP spiking studies

A) Rationale. For the MVM-VLP Kit to gain commercial acceptance, it must be demonstrated that the clearance of MVM-VLP is comparable to the clearance of live MVM through common manufacturing techniques. AEX, Viral Inactivation from low pH (VI), and Nanofiltration are three standard BP purification techniques. During this Aim, we will conduct spiking studies with these three units of operation to test if MVM-VLP clearance is comparable to MVM. We will also confirm that MVM-VLP’s are non-infectious.

B) Experimental Design and Methods (Performed by MockV)
AEX: A chromatography column will be packed with Q-SFF resin (GE) and qualified to vendor’s recommendations. To establish a baseline performance of the resin without MVM or MVM-VLP, BP material (supplied by Texcell) will be processed through the column using an AKTA explorer (GE) under typical BP processing conditions (pH 7.5, cond. < 10 mS/cm, etc.). For MVM and MVM-VLP spiking experiments, this procedure will be repeated using the same material spiked with either MVM-VLP or MVM (1% v/v). Before and after processing, material will be quantified for MVM-VLP or MVM content. Negative control experiments will be run by increasing the conductivity to 30mS/cm (should lead to less MVM-VLP or MVM removal). To confirm non-infectivity of MVM-VLP, samples will be analyzed by $TCID_{50}$.
VI. BP material will be spiked with either MVM-VLP or MVM (1% v/v), titrated to pH 3.4 with 1M Citric Acid and held for either 15, 45, and 90 minutes before adjusting to pH 6.0 with 1M Tris. Samples taken before Citric Acid addition and after Tris neutralization at each timepoint will be taken and quantified for MVM-VLP or MVM content.
Nanofiltration. An A1HC depth filter will be equilibrated and challenged with 930L/m2 of non-spiked BP solution at 15 psi. Next, the eluent of the filter will be divided into three equivalent fractions. F1, the control, will be

processed through a Vpro nanofilter (Millipore), F2 will receive a 3% (v/v) addition of MVM and will be processed through a separate Vpro filter, and F3 will receive a 3% (v/v) addition of MVM-VLP and will be processed through another Vpro filter. Each fraction will be processed through its respective Vpro filter into a separate collection container at a constant pressure of 30 psi until all material has been passed or until a 90% flux decay has been reached. Before and after Vpro processing samples will be taken for MVM-VLP or MVM quantification analysis.

C) Data Analysis & Interpretation. AEX column performance will be monitored during each experiment by sensors on the AKTA (pH, conductivity, UV, pressure, etc). Graphing these outputs over time will generate a "chromatogram" that will be analyzed using the systems software and allow comparisons among experiments. For nanofiltation, pressure and flux decay vs. time data will be compared among each fraction. For MVM-VLP quantification, the I-qPCR assay developed in Specific Aim #1 will be utilized. For MVM quantification, a traditional $TCID_{50}$ infectivity based assays will be used. To determine MVM-VLP and MVM clearance, LRV will be calculated according to standard equations[32].

D) Potential Pitfalls/Alternate Approaches. The introduction of either MVM or MVM-VLP into BP material may cause protein aggregation leading to a pressure build up over the AEX column or rapid flux decay through the Vpro filter. In this event MVM-VLP or MVM stock solutions could be further purified prior to spiking via ultrafiltration or flow through chromatography. Alternatively, the percent spike may be decreased. It is possible that inactivation techniques may cause fragmentation of MVM-VLP particles and that these fragments may still bind to the capture antibody during I-qPCR. If so, these fragmented particles may generate signal and cause a "false positive" result to be interpreted. To reduce this possibility, samples will be micro-centrifuged with a 100 kDa cut off membrane (Pall Corp, Cat# OD100C33) prior to analysis thereby allowing smaller fragments to pass through while retaining intact particles for analysis. It is possible that the $TCID_{50}$ assay used to quantify live MVM will have lower LOQ than the Immuno-qPCR assay. If this is the case, when complete clearance is achieved, smaller calculated LRV values for MVM-VLP will be expected compared to MVM. This will not be misinterpreted as a failed comparison but an acceptable limitation of the assay so long as the Immuno-qPCR's dynamic range can achieve ≥ 4 logs. Low pH viral inactivation accuracy may be concern; however, this method work through the chemical denaturation of viral surface proteins[44]. Since our proposed I-PCR assay relies on antibodies binding to the MVM-VLP surface, chemical denaturation should reduce binding and diminish or eliminate qPCR signal. Therefore, LRV's of MVM-VLP via inactivation or removal techniques should be comparable to live MVM.

E) Expected Outcome. It is expected that AEX and Vpro performance (ex. pressure) will be similar among MVM and MVM-VLP spiked experiments. For low conductivity AEX spiking experiments it is expected that complete removal of MVM-VLP and MVM will be achieved and assay LOQ's may be reached. During high salt AEX spiking experiments, it is expected and preferable that MVM-VLP and MVM removal will not be complete and assay LOQ's are not achieved. This will allow for direct LRV comparison. In this case, it is expected that removal of MVM-VLP and MVM will be calculated to be within 0.5 LRV of each other. For inactivation experiments, it is expected that increasingly higher LRV's will be calculated as low pH hold time increases (15 -90 min) for both MVM-VLP and MVM. Among time points, it is expected that MVM-VLP and MVM clearance will be calculated to be within 1.0 LRV of each other. It will be expected that MVM-VLP $TCID_{50}$ results will be negative, confirming non-infectivity. Table 2 shows when Aims #1 & 2 will be completed.

Specific Aim Activities		Month											
		1	2	3	4	5	6	7	8	9	10	11	12
S.A. #1	mAb production (BluePoint)												
	Immuno-QPCR Development (MockV)												
S.A. #2	Proof of Concept Studies (MockV)												

In summary, the creation and commercialization of the MVM-VLP Kit will provide bench-top BP process development scientists with a novel tool to generate viral clearance data. By using these simple, low cost kits, scientists would be able to apply a "Quality by Design" approach to process development and confidentially optimize their purification steps before investing significant resources in expensive regulatory-supporting validation studies. The cost savings and de-risking of regulatory validation studies would reduce the $/gram cost of final drug products and would help ensure the approval of therapeutics to in-need patient populations.

In Phase II, we will further optimize and validate our I-qPCR assay. We will then conduct extensive beta testing through a series of Design of Experiments studies, comparing MVM-VLP clearance to live MVM clearance through variety of purification techniques (chromatography, inactivation, filtration) and representative operating conditions. Positive results from these studies will demonstrate the commercial readiness of the technology and will serve as the foundation of a long-term case, to the FDA, for replacing live viral clearance validation studies with non-infectious VLP surrogates.

Vertebrate Animal Section

1. **Description of Procedures**

Description of animals to be used: Mice will be used in to generate anti-MVM-VLP antibody during Specific Aim 1. We anticipate the use of mice four (4) female BALB/cAnNHsd mice which will be immunized with recombinant viral-like particles (VLP) and an additional two (2) female BALB/cAnNHsd mice which will be immunized with VLP conjugated to keyhole limpet hemocyanin (KLH) to ensure hyperimmune response. The mice will be received at 4 – 5 weeks old and housed for >2 weeks to acclimate prior to immunization.

Specific Aim #1: Immuno-QPCR Assay Development

BALB/cAnNHsd mice will be primarily immunized with 300 µL/mouse of purified and concentrated MVM-VLP emulsified in Freund's Complete Adjuvant H37 Ra. At three week intervals, these mice will be injected intraperitoneally with 300 µL/mouse of MVM-VLP emulsified with Freund's Incomplete Adjuvant. Two months after primary injection, serum samples will be obtained from immunized animals via retro-orbital plexus. Selected mice will be euthanized by cervical dislocation and splenectomy will be performed to harvest spleen cells for use in cell fusion.

2. **Justifications**

Mice are the preferred species for hybridoma development using mouse myeloma cell lines of BALB/c origin and required for producing mouse-mouse hybrids.

3. **Minimization of Pain and Distress**

Care will be taken to ensure that the mice experience no unneeded pain or discomfort. The animals will be handled with care and all procedures will be performed by well trained, experienced technicians. Temperature, humidity and lighting are all maintained according to individual species requirements. Vivarium staff perform daily cage maintenance and provide animals with food and water.

4. **Euthanasia**

Euthanasia will be performed, as necessary, consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

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Letters of Support

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Authentication of Key Biological and/or Chemical Resources

MVM-VLP

MockV Solutions (MockV) routinely produces 5L batches of non-infectious MVM-VLP from a baculovirus/Sf9 expression system and conducts gradient centrifugation purification and tangential flow filtration concentration. Purity is confirmed by denaturing polyacrylamide gel electrophoresis, and Western Blot analysis. Concentration is determined by UV-visual light spectroscopy and Transmission Electron Microscopy. MockV will confirm the quality of MVM-VLP used in Specific Aims #1 & 2 of this application via Transmission Electron Microscopy.

Antibodies.

MockV will utilize an anti-MVM-VLP guinea pig polyclonal antibody (pAb) and anti-MVM-VLP mouse monoclonal antibodies (mAb's) during Specific Aim #1 of this application. pAb was produced by MockV in 2015 and has been utilized routinely for research and development purposes. We have properly stored and set aside ample pAb to conduct all Specific Aim # 1 activities. mAb's will be produced by BluePoint Sciences, LLC according to their internal protocols (as budgeted for in this application). All mAb's received will come with specification sheets. We will rely on the quality and specificity of BluePoint for antibody target validation. Lot numbers will be recorded for each antibody upon receipt and use. Vendor recommendations for positive controls will be used to validate targets, Negative controls will be employed in every experiment and the corresponding results will be retained with each dataset.

Mice.

BALB/cAnNHsd mice in this project will be purchased from Envigo, who will provide authentication documents upon delivery.

"In-process" Biopharmaceutical Material

"Spiking studies" will be conducted during Specific Aim #2. This will require the addition of MVM-VLP and MVM into representative Biopharmaceutical Material. We will source this material from Texcell North America, Inc., who has committed to produce 10L of Protein A purified monoclonal antibody derived from Chinese Hamster Ovary cell expression. Texcell will verify the purity and concentration of their mAb via High Pressure Liquid Chromatography and Spectroscopy.

Infectious MVM Stocks

For comparative feasibility studies (MVM-VLP vs. MVM), MockV will acquire high titer MVM stocks from Texcell North America, Inc. Concentration of infectious MVM stock will be analyzed via $TCID_{50}$ assay.