BPMS IBC Protocol Example

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Project Description

1. In lay language, describe your research objectives and hypotheses

Our laboratory investigates the mechanisms of immunity and disease pathogenesis associated with viral infection of the family Exampleviridae. We study innate and adaptive immune responses to help discover disease prevention strategies including therapeutic intervention as well as vaccination. Using cell culture and animal models, we investigate host cell and viral pathways by utilizing genome editing technologies such as RNAi and CRISPR/Cas9.

2. Provide a step by step "walk-through" of your research methodology. Be sure to explain how and why specific agents are used. If there is a connection between this IBC protocol, IRB, ESCRO, and/or IACUC be sure to describe the links.

Our studies will involve 4 major experimental approaches: 1) in vitro infection experiments in cell culture, 2) in vivo infection experiments in animals, 3) creation of recombinant cells, animals, and plants

1) For in vitro infection experiments, our general methodology is begin with the generation of viral stocks. We will obtain lyophilized stocks of multiple types of virus within the Exampleviridae family. These include Human Example viruses 1 and 2 (XV1 and XV2), Mouse Example virus (MXV), and Plant Example Virus (PXV). We will acquire them from the Example Center (website here) and will reconstitute them under BSL2 (XV2, MXV, PXV) and BSL3 (XV1) containment. All in vitro work with any virus will be done in any of the following commonly used laboratory human cell types (Cell A, Cell B), or cells generated from transgenic animals/plants. Viral stocks will be stored in secondary containment and frozen at -80C for use in all future experiments. For ongoing in vitro experiments, regardless of biosafety level (2 or 3), all work will be performed inside a biosafety cabinet (BSC). If any virus or virus-containing cells must be brought out of the BSC, it will be in a secondary container. Centrifugation will be performed with locking gaskets. All work with XV2, MXV, and PXV will be done under BSL2 containment. All work with XV1 will be done under BSL3 containment.

Cell will be transfected with RNAi molecules and are then infected with virus. Supernatant is collected 72 hours post-infection to be quantified for virus via viral plaque assay. Cell monolayer is harvested for total nucleic acid or protein purification from infected cells to determine specific gene expression. Some infected monolayers are fixed (with Z fixative for 20 minutes) and then removed from BSC for immunostaining and further analysis (confocal microscopy and cellomics). We have demonstrated that Z fixative both fixes infected cell monolayers and animal tissue, and inactivates all infective viral particles (Data in SOP in File Cabinet). All other consumables (tubes, plates, etc) will be sterilized with a 1/10 stock bleach solution. This solution will be made fresh daily by pouring one part bleach and nine parts water into a spray bottle.

2) For in vivo experiments, mice will be inoculated with either wildtype virus, recombinant virus, or inactivated virus. Inoculation routes are either intraperitoneally (I.P.) or intracranially (I.C.) at different titers (ranging from 10 plaque forming units (PFU) to 1000 PFU). IACUC protocols linked in Addendum D. Mice will be monitored for the development of clinical signs of disease. Some mice will be used to harvest tissues (brain, spinal cord, lungs, and mediastinal lymph nodes). Tissues will either be fixed for pathological analysis or homogenized for virological

analysis. Fixation will be performed using Z fixative (data for inactivation in SOP in File Cabinet). Homogenization will be performed using Y Homogenizer which still fits inside of the BSC. Pathological analysis includes immunohistochemistry and microscopy. Virological analysis includes plaque assays and RNA isolation for sequencing. All animal work will be performed in a BSC. Infected material for use in pathological studies will be fixed and inactivated prior to removal from the BSC. Infected material for use in virological studies will be placed in primary containment (1 ml tubes). Those tubes will be sprayed/wiped with disinfectant (1/10 bleach) and placed in a locking secondary container. This container will be sprayed/wiped with disinfectant (1/10 bleach) and will be removed from BSC in animal facility and brought to laboratory (Building ABC Room XYZ) and subsequent work (homogenization, plaque assay, RNA isolation) will be performed in the BSC or frozen at -80C.

3) Recombinant DNA technology will be used to create recombinant cell lines, mice, and plants. All work with recombinant DNA will be performed in a BSC with appropriate PPE (labcoat, gloves, eye protection). Cas9 mRNA with multiple single guide RNAs will be directly injected into mouse embryos to generate precise genomic edits at specific loci. Mice developed from these embryos will be genotyped to determine if they carry the desired mutation. We are specifically targeting two genes (Gene Happy and Gene Sad), neither of which are oncogenic or immunoregulatory in nature. Recombinant plants will be generated using CRISPR techonology. Recombinant cell lines will be generated using RNAi and lentiviral systems. Both siRNA and miRNA will be used for transient gene knockdown while lentiviruses will be used to generate stable gene knockouts. A lentivirus containing CRISPR gRNA library will infect both cell types (Cell A and Cell B) to achieve stably knock-out and knock-in cell lines.

The lentivirus packaging system (3rd generation, acquired from this company (website)) is split into 4 total plasmids. One plasmid encodes Rev, one encodes Gag and Pol, one encodes the envelope protein (VSV-G), and the last plasmid encodes the shRNA or cDNA for Cas9. This 4 plasmid system is replication incompetent and having 4 separate plasmids further decreases the possibility of recombination and creation of replication competent particles. Also, the 5' LTR region of the transfer plasmid contains self-inactivation mutations to further decrease possibility of replication competent particle generation.

Cloning of Exampleviridae genes will be performed using E.coli (k12). These genes will be sent for sequencing. There will be no alteration of genes to make the viruses more pathogenic. All mutations we plan to introduce have been shown to attenuate the viral strains we are working with.

Risk Assessment

1. Describe any potential adverse effect(s) to personnel or environment from exposure to the agents/materials involved in this protocol. Include potential routes of exposure.

Risks will include 1) Pathogen risk to human and environment, 2) recombinant DNA risk to human and environment, and 3) RNA isolation from virus

1) Exposure to XV1 and XV2 can result in serious human disease. Accidental laboratory acquired infection could occur through respiratory droplets via aerosol exposure to infected cells, tissue, and/or animals. Exposure to MXV and PXV does not produce human disease, however can cause disease in mice and plants (respectively). We will take all necessary precautions using engineering controls, administration controls, and PPE to mitigate these risks.

2) Exposure to the recombinant technologies that we will be using could potentially cause adverse effects due to genomic integration of lentivirus insert. Also, release of transgenic animals or plants could negatively affect the environment. We will take all necessary biosecurity precautions to prevent any release of such material.

3) Isolating viral RNA for sequencing and subsequent cloning does not pose a serious risk for infection. All RNA isolation will be done in the BSC. Once RNA is obtained, the RNA is not infectious as Exampleviruses are negative sense RNA viruses.

2. How did you determine the appropriate biosafety level for this protocol?

XV1 has been designated as a risk group 3 pathogen to humans by multiple risk group databases (websites). Combining the risk group of this pathogen with the procedures that we will be performing (potentially aerosol generating), the appropriate containment for XV1 would be BSL-3. XV2 has been designated as a risk group 2 pathogen and despite performing similar procedures as XV1 (potentially aerosol generating), the risk of exposure and serious disease occurring from exposure to XV2 is significantly less than exposure to XV1. Thus, we will work with XV2 at BSL-2. While MXV and PXV are not harmful to humans, we will use both at BSL-2 containment to prevent potential contamination and infection of environmental populations of mice and plants.

3a. What is the timeline of your most recent search?

05/1/2019 – 05/14/2019. This was two weeks prior to my submission of this protocol.

3b. Indicate the database used for literature search. Make sure to include "biosafety" and/or "safety" in keyword search.

Google Scholar, PubMed, Scopus

3c. What keywords did you use?

Exampleviridae safety, exampleviridae biosafety, XV1 biosafety, XV1 containment, XV1 symptoms, XV1 human health, PXV plant heatlh, PXV biosafety, MXV mouse heatlh, exampleviridae laboratory practices

3d. Please describe any pertinent safety or hazard analysis find.

The potential hazards and safety requirements of working with these viruses has been well developed and documented. XV1 can pose a serious risk to healthy adult humans, thus why we are taking the containment precautions. MXV and PXV pose risks to the environment if released from the lab, thus why we are working with them at the stated containment despite no risk to human health.

5. Specify from where you receive the materials/agents involved in this protocol. If from a collaborator, please identify name and institution.

Viral stocks will be obtained from ABC Institute from Drs. John and Jane Doe. The viral stocks will come lyophilized. The virus will be propagated in X cells and plaque purified to verify a single population of virus with no contamination.

6. Are there any preexisting patient conditions that may amplify the risks of using this vector/microorganism, etc?

Immunocompromised patients would experience an exacerbated disease progression if exposure and subsequent infection were to occur. It has also been published (reference here) that XV2 may cause adverse fetal response during infection, thus personnel who are pregnant or may become pregnant are potentially at greater risk. All personnel will be advised of these risks.

7. Describe any procedures – with viable biological material – that will be performed outside of primary containment (i.e. outside of a biosafety cabinet). Please provide details about how you will mitigate exposure risks.

Cloning of gene segments of Exampleviridae will be done using E.coli K12 on the benchtop. Standard cloning procedures will be performed outside of primary containment. Appropriate PPE will be worn during such procedures and researchers will be training on minimizing potential spills and aerosols of this biological material. All work with viable viral material will be done inside primary containment. To reiterate, all work done with viral RNA can be safely performed outside of primary containment as the RNA is not infectious.

20. Will work in the protocol involve the use of sharps?

The use of sharps will be avoided in all cases except during animal work. Inoculation of experimental animals with virus will be performed with needs with safety devices in order to minimize the risk of an injury/exposure. Needles will never be recapped. Tissue harvesting will be performed using standard surgical scissors and forceps. Personnel will be carefully trained. All sharps used for in vivo studies will be immediately disposed of into a Regulated Medical Waste sharps container for disposition.

21. Identify and describe the risk(s) to humans/animals/plants associated with the materials used in the experiment and methods that will be taken to prevent exposure to persons and/or the environment.

XV1 and XV2 are a risk to human health, as they cause Example Disease, with symptoms mimicking a classic “flu-like” illness. Fever, joint paint, headache, and nausea. XV2 has been documented to cause adverse fetal responses during infection of pregnant individuals.

Methods to prevent exposure will include containment of all work with infectious samples. Engineering controls include biosafety cabinets for work with infectious samples, fume hoods for work with chemicals, safe sharps, and negative pressure cage racks for infected animals. Administration controls include having all workers trained on written SOPs, safety training, and the laboratory biosafety plan. PPE will be worn when handling any biological material (virus, bacteria, recombinant DNA). PPE includes nitrile gloves, laboratory coats, and eye protection. PPE in the BSL-3 includes a full Tyvek suit, PAPR, double gloves, booties, and a plastic gown.

Addendum A

23. Describe all uses (vectors and inserts) of recombinant material especially viral vectors.

a. These materials will be used to silence, knock-out, or knock-in gene expression in tissue culture, animals, and plants.
b. The Happy and Sad genes encode the Smile and Frown proteins. These proteins may increase or decrease viral replication
c. It has been published (reference here) that inserting these genes in tissue culture and mice does not cause adverse effects on the cell lines we plan to use. The insertion of these genes in plants has been documented (reference here) to cause a shorten growth phase.
d. Target cells are cell lines A and B. Mice will be of the BALB/c, Swiss Webster, and C57BL/6 background. Plants will be of the Plant Example background.
e. The recombinant DNA has not been passaged through any animals or cells that we know of.
f. Lentiviruses are not replication competent. Upon infection of a primary cell, their gene of interest in delivered and no further viral progeny is made.

24. Specifically identify the gene(s) of interest including a description of any associated hazards (i.e. genes that code for toxic, oncogeneic, or otherwise hazardous peptides).

a. The vector will not become more or less infectious.
b. The lentivirus system (purchased from this website) expresses the VSV-G protein as the viral envelope protein which provides the virus a greater cell tropism.
c. This 3rd generation lentivirus can cause infection but cannot replicate.
d. Lentiviruses can integrate into the genome and cause mutagenesis. However, this third generation lentivirus has several safety features that greatly decrease the risk of that happening, including multiple plasmids expressing different genes and SIN (self-inactivating) mutations.

Addendum A-1

Host(s)	Strain(s)	Vector(s)	Gene Targets
E. coli	DH5alpha, TOP10	pCMV-Example pcDNA-Example pcDNA-Example1.0	HAPPY, SAD, intracellular signaling and glycosylation
Cell Line A	addgene # - XXXX	pLENTI-Example pLENTI-Example2.0	HAPPY, SAD, intracellular signaling and glycosylation
Mice	Swiss Webster Balb/c C57BL/6	pLENTI-CRISPR/Cas9	HAPPY, SAD, intracellular signaling and glycosylation
Plants		plantLENTI-CRISPR/Cas9	HAPPY, SAD, intracellular signaling and glycosylation

1. Will an attempt be made to purify any of the foreign gene product(s) encoded by the gene?. If yes, indicate which foreign gene product will be purified and describe the procedure for purification.

Yes, HAPPY and SAD will be purified by affinity chromatography.

2. Will a virus-derived vector system that is engineered to be replication-incompetent be used?

Yes. Company ABC manufactures this 3rd generation lentivirus system. A deletion in the enhancer of the U3 region of the 3' LTR ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. The packaging system is split into two plasmids: one encoding Rev and one encoding Gag and Pol. Tat is eliminated from the 3rd generation system through the addition of a chimeric 5' LTR fused to a heterologous promoter on the transfer plasmid. Expression of the transgene from this promoter is no longer dependent on Tat transactivation.

Addendum A-2

2. If the animal is created by use of viral vectors, describe the vector and promoter. Upload a copy of permit/authorization to the file cabinet.

The lentivirus system used is from this company (website here) to insert genes into animals by the gene editing core. The insert is controlled by the CMV promoter.

3. Provide the gene name and function

The genes are HAPPY and SAD. Both are involved in intracellular signaling and glycosylation.

4. Provide a description of the possible hazards associated with the alteration.

It has been shown (reference here) that knocking out or altering these genes alters susceptibility to infection with Exampleviridae.

Addendum A-3

4.

Construct	Description
Agrobacterium	Immature embryos, callus tissue, or duckweed are transformed by inoculating with Agrobacterium transformed with a vector containing the gene of interest (HAPPY gene described in A-1).
Rhizobium	None
Electroporation	E.coli and Agrobacterium are transformed by electroporation with a plasmid vector containing the gene of interest (SAD gene described in A-1)
Microprojectile bombardment	Callus tissue is transformed by microprojectile bombardment with DNA containing the gene of interest (HAPPY and SAD genes)
Viral directed transformation or viral infection	None
Other	Agrobacterium transformed with a vector plasmid containing the gene of interest are transfected by syringe into Example PLANT to assess transient expression. Also, small RNA oligos are delivered in media to duckweed transiently altering gene expression.

Addendum B

Microorganism : E.coli

Strain : DH5alpha, TOP10

Biosafety Level : BSL1

Risk Group : RG1

Type of Culture : From miniprep (2ml) to maxiprep (500ml) of culture in LB broth

Manipulations : Bacteria will be centrifuged and chemically lysed to purify recombinant DNA

List all Drug resistances to known therapeutics : Ampicillin and kanamycin resistance will be introduced in the recombinant plasmids.

List all known therapeutics that would be used for treatment in the event of an exposure : E.coli is usually found in the human gut and these laboratory strains used for cloning are considered non-pathogenic.

Microorganism : Examplevirus

Strain : XV2 (ref. here)

Biosafety Level : BSL2

Risk Group : RG2

Type of Culture : purified virus, infection of various cell types in volumes between 200ul (96 well plates) to 25ml (183cm flasks), viral supernatant from tissue culture and infected animals

Manipulations : standard pipetting, inoculation into tissue culture and animals, centrifugation, harvesting supernatants, plaque assay, RNA isolation for sequencing

List all Drug resistances to known therapeutics : None known

List all known therapeutics that would be used for treatment in the event of an exposure : This viral strain is susceptible to Drugs X, Y, and Z, which are all commercially available and available at Rutgers Occupational Health.

Microorganism : Examplevirus

Strain : XV1 (ref. here)

Biosafety Level : BSL3

Risk Group : RG3

Type of Culture : purified virus, infection of various cell types in volumes between 200ul (96 well plates) to 25ml (183cm flasks), viral supernatant from tissue culture and infected animals

Manipulations : standard pipetting, inoculation into tissue culture and animals, centrifugation, harvesting supernatants, plaque assay, RNA isolation for sequencing

List all Drug resistances to known therapeutics : Drug Y

List all known therapeutics that would be used for treatment in the event of an exposure : This particular strain has a known resistance to Drug Y, but is still susceptible to drugs X and Z. Occupational health has been notified that in the event of an exposure, these drugs are to be administered to worker. All workers have been made aware of drug resistance and susceptibilities to this virus.

Microorganism : Examplevirus

Strain : MXV (ref. here)

Biosafety Level : BSL2

Risk Group : RG1

Type of Culture : purified virus, infection of various cell types in volumes between 200ul (96 well plates) to 25ml (183cm flasks), viral supernatant from tissue culture and infected animals

Manipulations : standard pipetting, inoculation into tissue culture and animals, centrifugation, harvesting supernatants, plaque assay, RNA isolation for sequencing

List all Drug resistances to known therapeutics : None known

List all known therapeutics that would be used for treatment in the event of an exposure : This virus does not infect humans

Microorganism : Examplevirus

Strain : PXV (ref. here)

Biosafety Level : BSL2

Risk Group : RG1

Type of Culture : purified virus, infection of various cell types in volumes between 200ul (96 well plates) to 25ml (183cm flasks), viral supernatant from tissue culture and infected animals

Manipulations : standard pipetting, inoculation into tissue culture and animals, centrifugation, harvesting supernatants, plaque assay, RNA isolation for sequencing

List all Drug resistances to known therapeutics : None known

List all known therapeutics that would be used for treatment in the event of an exposure : This virus does not infect humans.

Microorganism : Lentivirus

Strain : HIV based, 3rd generation lentivirus system

Biosafety Level : BSL2

Risk Group : RG2

Type of Culture : purified virus, infection of various cell types in volumes between 200ul (96 well plates) to 25ml (183cm flasks), viral supernatant from tissue culture

Manipulations : standard pipetting, inoculation into tissue culture and animals, centrifugation, harvesting supernatants, plaque assay, RNA isolation for sequencing

List all Drug resistances to known therapeutics : N/A

List all known therapeutics that would be used for treatment in the event of an exposure : N/A

Addendum D

Biological Material Used : XV1 virus

Animal Species : mice

Max infectious units/dose : 10^3 plaque forming units per dose

Max dose per animal : 1 dose per animal

Please explain the measures your lab will take to prevent accidental exposure to employees, animal handlers, students, visitors and other animals : All inoculations, handling, and other experimental work with infected mice will take place in a biosafety cabinet. Cages will be clearly marked to alert animal handlers. All animal carcasses and cage bedding/waste will be bagged in a biological safety cabinet and autoclaved for decontamination. Laboratory personnel will receive extensive training in appropriate techniques to minimize risk of exposure.

Describe any human health risk associated with this agent : XV1 can cause serious/severe disease in healthy adult humans. The most common symptoms are usually fever, joint/muscle pain, headache, vomiting. Mortality can be associated if left untreated.

This material is hazardous to : Humans and animals

Biological Material Used : XV2

Animal Species : mice

Max infectious units/dose : 10^3 plaque forming units per dose

Max dose per animal : 1 dose per animals

Please explain the measures your lab will take to prevent accidental exposure to employees, animal handlers, students, visitors and other animals : All inoculations, handling, and other experimental work with infected mice will take place in a biosafety cabinet. Cages will be clearly marked to alert animal handlers. All animal carcasses and cage bedding/waste will be bagged in a biological safety cabinet and autoclaved for decontamination. Laboratory personnel will receive extensive training in appropriate techniques to minimize risk of exposure.

Describe any human health risk associated with this agent : XV2 can cause disease in healthy human adults, but is usually mild and normally lasts for 2-4 days.

This material is hazardous to : Humans and animals

Biological Material Used : MXV

Animal Species : mice

Max infectious units/dose : 10^3 plaque forming units per dose

Max dose per animal : 1 dose per animals

Please explain the measures your lab will take to prevent accidental exposure to employees, animal handlers, students, visitors and other animals : All inoculations, handling, and other experimental work with infected mice will take place in a biosafety cabinet. Cages will be clearly marked to alert animal handlers. All animal carcasses and cage bedding/waste will be bagged in a biological safety cabinet and autoclaved for decontamination. Laboratory personnel will receive extensive training in appropriate techniques to minimize risk of exposure.

Describe any human health risk associated with this agent : This agent is not infectious to humans as it only infects rodents.

This material is hazardous to : animals only

Addendum F

2. Describe the type of work being done with Select Agent(s)/Toxin(s) listed above.

Example Toxin (ET) is added to kill ET-receptor immune cells to observe the effect of specific immune deficiency during infection with Exampleviruses.

4. Describe how adequate training and proficiency testing for personnel working with the Select Agents/Toxins is ensured.

All registered personnel will go through extensive training with the PI on handling the toxin as well as keeping the inventory of ET. All toxin vials will be maintained at a specific concentration. The total toxin amount in the laboratory will never exceed 10 mg (permissible limit is 500 mg).

Addendum G

Biotoxin : Pertussis toxin

Amount Used : stock 10 ug/ml, used 100 ng/ml

Please describe how sharps will be used with this toxin : sharps will not be used with this toxin

1. Describe the reconstitution procedures

It will be reconstituted by adding vehicle (100 mM NaHPO4 / 100mM NaCl; pH 7) to the original ampule in the laboratory chemical fume hood. It will It will then be aliquoted in the hood and stored at 4 degrees C in the refrigerator in room XXX.

2. Describe the safe handling and disposal procedures that will be used for this toxin.

Personnel will follow the procedures in the SOP to dilute the stock 1/100 for use in the assays. The tissue culture supernatant and cells will be inactivated in sodium hypochlorite/bleach for 30 min and disposed of as biomedical waste.

3. Describe the air handling system for the laboratory where work will be performed.

HVAC system in laboratory and a chemical fume hood and biosafety cabinet class B2.

Addendum I

1b. If yes, how often and how is it validated?

The cell sorter is housed in a biological safety cabinet and is validated annually by an authorized company.

1c. If yes, how often and how is it validated?

The aerosol containment unit is validated every 6 months using the Glo-germ method (see attached protocol).

1d. Please describe procedures for decontamination after use.

Sample port is cleaned by applying 10% bleach for 15 min. Sorting chamber is wiped down with paper towel soaked in vesphene solution. All other surfaces that come into contact with samples are also wiped down with paper towel soaked in vesphene solution. The waste container contains bleach with a final concentration of 1-2% bleach. This is allowed to sit overnight before it is discarded.

1e. Please describe procedures and potential risk in the event of a nozzle obstruction.

The instrument has a fail safe in the event of a nozzle obstruction; the same stream is turned off. Additionally the sorting chamber is located behind an aerosol containment unit which applies a negative pressure to collect all aerosols from escaping the chamber. Once the stream turns off, we will wait 2 min for the aerosols to be sucked away. The sorting chamber will be opened and wiped down with vesphene solution. The nozzle will be removed and placed into a 1/10 bleach solution and sonicated for 1 min to remove obstruction. The nozzle will be washed with water and placed back into the instrument and the stream will be turned on again. The nozzle will be cleaned repeated until the obstruction is removed. If the obstruction cannot be removed, a new nozzle will be used.

2. Please provide the manufacturer and model of flow cytometer or cell sorter used.

Company XXXXXX
Model # YYYYYYY

3. Please describe the materials used in these processes.

Lymphocytes isolated from mice will be used in flow cytometric analysis. Human cells isolated from tissue culture that was transduced with lentivirus will be sorted using a cell sorter to isolate cells of interest.

4. Will cells/materials be fixed?

For flow cytometry, all cells will be fixed with 4% paraformaldehyde prior to analysis in the cytomet.