State of Hawaii
Department of Agriculture
Plant Industry Division
Plant Quarantine Branch
Honolulu, Hawaii

November 26, 2024

Board of Agriculture
Honolulu, Hawaii

Subject: Request to: (1) Allow the Importation and Possession of *Monkeypox Virus*, Clade IIb, on the List of Restricted Microorganisms (Part A), for Laboratory Research, By the University of Hawaii; (2) Establish Permit Conditions for the Importation and Possession of *Monkeypox Virus*, Clade IIb, on the List of Restricted Microorganisms (Part A), by Permit, for Laboratory Research, by the University of Hawaii; (3) Determine if the Permit Conditions are Sufficient to Assure the Possession of *Monkeypox Virus* Clade IIb, for Laboratory Research by the University of Hawaii, by Permit, Presents Minimal to No Significant Impact on the Environment.

I. Summary Description of the Request

PQB NOTES: *This Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicant from procedural information and advisory comment, and evaluation presented by PQB. Except for PQB notes, hereafter "PQB NOTES," the information provided in section II from page 3 through page 27 of this submittal is taken directly from the application (Attachment 1) and subsequent written communications provided by the applicant. For instance, the statements on page 25 regarding effects on the environment are the applicant's statements, and not PQB's. This approach promotes greater applicant participation in presenting import requests in order to expeditiously move these requests to the Board of Agriculture (Board), while also distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including Environmental Assessment, Advisory Subcommittee Review, Advisory Committee on Plants and Animals Review, and Proposed Permit Conditions are identified as sections III, IV, V, and IV of the submittal, which begin on pages 27, 28, 31, and 32, respectively.*

COMMODITY: Initial shipment of (1-2 vials of Monkeypox Virus, (hMPXV/USA/MA001/2022, Lineage B.1, Clade IIb).

SHIPPER: BEI resources,10801 University Boulevard, Manassas, VA 20110

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CATEGORY: *Monkeypox Virus* is on the List of Restricted Microorganisms (Part A). Pursuant to section150A-6.3, Hawaii Revised Statutes and chapter 4-71A, Hawaii Administrative Rules (HAR), microorganism species on the List of Restricted Microorganisms (Part A) are categorized as high-risk microorganisms, which may be allowed into the State under a permit approved by the Board.

PQB NOTES: *Although Monkeypox Virus is on the List of Restricted Microorganisms (Part A), the specific virus, Monkeypox Virus, Clade IIb, has been classified as a Biosafety Level 2 (BSL2) pathogen by the CDC. The Personal Protective Equipment (PPE) required for BSL2 are:*
Long pants or long garments that cover legs entirely, shoes that cover the entire foot. Long hair must be pulled back. Wrap-around back open gowns must be worn. Disposable nitrile gloves are to be used always and while handling infectious cultures secondary pair of gloves should be worn over the primary glove. An N95 mask for respiratory protection and eyeglasses for eye protection are required. Face shields or splash goggles are required when splash risks are present.

Additional PPE proposed for MPXV: Although not recommended for BSL2, a white manipulation sleeve must be worn over the gown during the handling of infectious cultures. Sleeves should be disposed of after every use.

II. Information Provided By the Applicant in Support of the Application

PROJECT: Establishing Human 3D Organoid Systems to Elucidate Monkeypox Virus Tropism and Pathogenic Mechanisms

PURPOSE: Human monkeypox is an emerging zoonotic disease caused by a monkeypox virus (MPXV). The recent outbreak of MPXV clade IIb with ~35,000 cases in the USA led World Health Organization (WHO) to declare this outbreak as a Public Health Emergency of International

Concern[1]. Approximately 99% MPXV cases are in men[2]. As of Feb 2024, there have been a total 46 MPXV cases reported in the state of Hawaii[5] (32 cases in Honolulu, 4 cases each in the Big Island of Hawaii and Kauai, 2 in Maui, and the rest out of state). This outbreak of MPXV is still ongoing and is different from past outbreaks because it is more readily spread through sexual contact. So far there are 93,030 cases reported with 176 deaths in more than 20 countries primarily in immune-compromised individuals[3]. There are two clades of monkeypox: Clade I – which is classified as a select agent due to higher mortality; and Clade II which is classified as a BSL2 pathogen by the CDC due to lower mortality (<1%)[4]. Clade II comprises two subclades—clade IIa and clade IIb, with the latter being the circulating variant during the 2022 global outbreak. The order of disease severity of different clades is clade I > clade IIa > clade IIb (more details in attachment 2).

Currently, drugs and vaccines designed to treat smallpox are the only options to treat MPXV clade II infections. Several unknowns hamper our ability to control virus spread and develop novel testing tools, therapeutics, and vaccines. There is an urgent need to understand both, the basic biology of MPXV and its pathogenesis mechanisms. We propose to conduct experiments to:

- Understand the target of this virus and pathogenesis mechanisms including how it causes damage to skin cells and other cells including the testis and immune cells.
- Understand the mechanisms by which the MPXV virus evades or modulates the host immune response. Mainly, to identify specific pathways involved in disease pathogenesis, which can aid in developing targeted antiviral strategies.
- Develop relevant tools using human cells to test different drug candidates.

We are seeking authorization to import and study only the non-select agent variant of monkeypox, i.e., Monkeypox Clade II. The CDC has classified MPXV clade II as BSL-2 pathogen[4] and as per their guidelines, we propose to work with this virus in the BSL-2+ suites at JABSOM. However, some experiments that involve handling a larger volume of virus will be conducted in the BSL3 (detailed description of virus handling in BSL2+ and BSL3 lab provided in later sections). BSL2+ suites follow BSL3 practices in the BSL2 laboratory (more details on BSL2+ lab in section 3). As per CDC guidelines, diagnostic samples of non-select MPXV can be handled in the BSL2, cell culture-based work can be done in BSL2+ labs if only one non-select MPXV is being manipulated and BSL3 lab has to be used if more than one poxviruses are manipulated (BMBL 6th edition handbook, pages 268-

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270, more details in later sections). This endeavor aims to enhance our scientific knowledge and research capabilities, benefiting the global population. This research is an exceptional opportunity to position UH's research at the forefront of the emerging pathogens and antiviral therapeutic development field. For the current permit, we only plan to import MPXV clade II to conduct cell culture studies (no other poxviruses and no animal model work using clade II MPXV is planned).

OBJECTIVE: Newly re-emerged monkeypox (MPXV clade II) was responsible for the ~30,000 infections in the US in 2020-2022 and since then has led to global efforts to understand the pathogenesis and transmission trends of different variants of this virus. The infections reported during the 2022–2023 outbreak in the USA are attributed to Clade II. The clinical spectrum of MPXV ranges from fever, muscle aches, and mild to severe rashes on the body. The symptoms generally resolve within weeks but the risk factors for severe disease include immunocompromised conditions. There are several unknowns regarding the transmission and cell-type-specific response of the virus in humans. We will begin our research with different in vitro cell culture systems. Transmission of MPXV occurs primarily through close or intimate contact, involving direct skin-to-skin and sexual contact with an infected person. Cell types that support virus infection are not clear and recent reports show that the virus can persist in the seminal fluid even after clearing from the skin scabs thus suggesting its potential to be a sexually transmitted pathogen. However, testicular infections of MPXV are not yet well characterized and are critical to understanding disease transmission kinetics and long-term effects on males, especially men who have sex with men (MSM), an underrepresented group in biomedical research. Similarly, immune response to virus infection in the cells in skin and lungs, another organ affected by MPXV, is not clear. We propose to compare the MPXV clade II infection in different skin, lung and testicular cells to understand why the virus can persist longer in certain cells. Further, we propose to understand the immune response to this virus in different skin and testicular cells to correlate with pathogenesis and transmission.

We will use 2D human skin epithelial cells, lung epithelial and testicular epithelial cells (Sertoli cells), and 3D multicellular organoids (we have established collaboration to receive 3D organoids of lungs and testis from Wake Forest Institute, Winston Salem, NC) to model MPXV infection in humans. We will infect these cells and organoids with clade II MPXV with a low dose of virus (between MOI 0.1 to 1) and define, virus replication kinetics, and analyze host antiviral and inflammatory

response. Once these novel models are established to study MPXV, they can be used to identify potential diagnostic biomarker candidates and evaluate the efficacy of promising therapeutic candidate compounds to timely clear MPXV infection.

Benefit to people in Hawaii: As of Feb 2024, there have been a total 46 MPXV cases reported in the state of Hawaii (32 cases in Honolulu, 4 cases each in the Big Island of Hawaii and Kauai, 2 in Maui, and the rest out of state). The outbreak of MPXV is still ongoing and is different from past outbreaks because it is more readily spread through sexual contact. Males, particularly men who have sex with men (MSM), an underrepresented group in biomedical research, are more susceptible to having MPXV disease. There is limited knowledge on the cell targets of this virus, if it can establish long-term persistence in any tissues and what are the immune mechanisms that help timely virus clearance. New research to understand virus transmission kinetics and immune response is needed to develop proper guidelines to prevent virus transmission and how long to monitor infected individuals. Therefore, the importation of this virus and subsequent research will answer some of these questions and provide valuable insights about the evolving virus and transmission kinetics to both the local community including MSM as well as the clinicians of Hawaii.

PROCEDURE: MPXV will be handled in both the BSL2+ (BSL2 lab with BSL3 practices) and BSL3 labs depending on the amount of virus handled in each experiment. A list of experiments conducted in BSL3 and BSL2+ is provided below (more details on each experiment in later sections).

BSL3 lab: Any experiment where a larger volume of stock virus is handled (more than 10mL stock virus).

i. Preparation of virus stock (detailed SOP provided below)

BSL2+ lab (Virus stock volume handled is expected to be between 200ul to 1mL depending on each experiment):

i. Virus infection of different human cell types
ii. Virus quantitation assays
iii. Cell viability assays
iv. RNA and DNA extraction
v. Protein extraction from infected cells

The Standard Operating Procedures (SOP) of virus handling in the BSL2+ are described below:

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SOP 1: General Lab Safety requirements when entering the BSL-2+ laboratory.

General Lab Safety requirements:

- Eating, drinking, or any behavior that could result in self-contamination is prohibited.
- Loose hair will be kept away from the face.
- Long pants and covered shoes must be worn.
- All trash will be placed in the red autoclave bags and sterilized prior to disposal – no black trash cans in lab.
- All work in the biosafety cabinet (BSC) will be done OVER absorbent paper.
- All items moving into or out of the biosafety cabinet will be sprayed with 70% ethanol.
- 10% bleach will be used to decontaminate all items that come in contact with the virus.
- All pipet tips are considered sharps, and following decontamination, will be placed in a rigid plastic bottle for steam sterilization.
- All pipets will be decontaminated and then placed in red autoclave bags.
- All infectious fluid will be decontaminated prior to disposal.
- All plasticware will be steam-sterilized prior to disposal.
- Plasticware not decontaminated prior to disposal must be steam-sterilized immediately.

PPE requirement:
Non-infectious culture manipulation:
Wrap-around gown.
Black nitrile gloves
Surgical mask

Infectious culture manipulation:
Wrap-around gown.
Black nitrile gloves
White manipulation sleeve
Second pair of gloves – make sure the glove is OVER the manipulation sleeve.
Hair bonnet
Safety eye glasses
N95 mask

Entrance into the BSL-2+ laboratory
Please note: Do not bring personal items into the MPXV suite. NO BACKPACKS, NO PERSONAL LISTENING DEVICES, CELL PHONES, LAPTOPS, BOOKS, ETC. Cardboard is not allowed in the BSL-2+ facility. If using a cardboard box to transport items, transfer items and dispose of the cardboard immediately.

1. Place keycard on the biometric reader.

2. **NO PIGGYBACK POLICY ENFORCED**. Each person must use their own keycard to enter the BSL-2+ lab.
3. Put on the gown.
4. Immediately don the first layer of gloves and N95 mask.
5. Don secondary gloves, sleeves and safety glasses.
6. Set up the hood to start MPXV work

SOP 2: EXITING THE BSL-2+ LABORATORY.

1. Clean BSC with 10% bleach (made fresh daily), dry and wipe down with 70% alcohol.
2. Spray down and decontaminate PPE with 70% ethanol.
3. Switch on the UV and leave it on for 20 min to decontaminate the BSC.
4. Remove the mask and store it in a brown bag in a drawer.
5. Remove sleeves and gloves and place in waste container.
6. Hang the gown at the designated hooks.
7. Wash hands with soap and water for minimum 30 sec.
8. Now exit from MPXV BSL-2+suite.

SOP 3: WORKING WITH MPXV CLADE II IN THE BSL-2+ SUITES.

Setting up the hood

1. Clean BSC with 70% alcohol
2. Dry it completely and spread the versi dry.
3. Place supplies into the BSC
4. Make fresh 10% bleach in a small plastic beaker and place in the BSC
5. Place a covered trash pan, tissue culture plates, tips, media etc in the BSC
6. Arrange all items in good configuration so that air flow will not be obstructed.
7. The personnel, *BSC and the suites are now ready for the following virus manipulations.*

SOP 4: MPXV CLADE II INFECTION AND CELL HARVESTING IN THE BSL-2+

Before working with the MPXV, the lab worker must:

- *have the approval of the PI who shall provide specific training according to this SOP*
- *complete UH lab safety training and JABSOM Kaka'ako hazardous waste generator training prior to working with any chemicals;*
- *sign this SOP as documentation that he/she understands the hazards and has been trained in how to complete the following tasks safely.*

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- *All handling of MPXV-related cultures will be in certified BSC (inspected and certified every year)*

Statement of Understanding and Compliance

I confirm that I have read and understand this SOP and will comply with the procedures and policies.

Name: **Signature:** **Date:**

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PPE Requirements & Special Practices: In addition to standard BSL-2+ Manipulation Suite PPE (wrap-around blue gown, black primary gloves, manipulation sleeves, and secondary grey gloves, bonnet), N95 mask and safety glasses shall be worn when working with the MPXV as per the entry SOP.

Virus infection procedures:

1. Carefully remove the vial of virus stock from the -80 freezer and place in secondary containment.
2. Take the vial in secondary containment into the BSL2+ suite and immediately place in BSC.
3. Carefully remove the virus vial from the secondary container and place it in a rack in one corner of the BSC.
4. Prepare the dilution of the stock virus as needed and keep it ready for infection.
5. Spray your hands and area with 70% ethanol and dry for a min.
6. Then carefully bring the tissue culture plates of cells from the incubator and place in the BSC. Cells are grown in either 96-, 48- or 24-well plates depending on experiment design.
7. Per the experiment design, infect the cells with the appropriate volume of diluted virus and return the plates to the incubator located in the suite. Infected tissue cultures will be cultured in the assigned incubator in the BSL-2+ laboratory.
8. Discard any remaining virus in the vial into the fresh 10% bleach solution and let sit for at least 20 minutes.
9. After infection, wash the cells in the tissue culture plate with 2X PBS.
10. Remove PBS wash with a pipet and carefully discard all the waste into the bleach solution.

11. Add fresh media over the cells and return to the incubator. Media volume for 96-, 48- and 24-well lates is 100uL, 400uL and 800uL per well.
12. Determine if the virus waste has been in the bleach solution for at least 20 minutes. After the 20 minutes decontamination time, the virus is totally inactivated, i.e., not viable as per CDC.
13. Use a pH strip (located near the sink) to check the pH to ensure the solution is in the acceptable range of 5.5 and 9.5. If necessary, add water until the pH is within the acceptable range.
14. Pour the media waste down the sink drain followed with water for at least 1 minute.
15. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP*.

Procedure of cell and supernatant harvesting:

1. At different time points after infection, carefully take the plate out of the incubator and place it in the BSC hood.
2. Remove approximately 100-400ul media from each well and store in an Eppendorf tube. Label it with date, amount, virus details and store it in a 96-well cardboard box.
3. Carefully wash the cells with PBS and carefully discard all the waste into the bleach solution.
4. Now add cell lysis reagent in the wells and collect the lysate in the Eppendorf tubes. This lysate inactivates the virus and will be used for DNA and RNA extraction.
5. Wash plates with 10% bleach solution once and discard all the waste into the bleach solution container.
6. Carefully place the supernatant and lysate tubes in a 96-well box. Place this box in a secured secondary container and store the samples in the -80°C freezer.
7. Determine if the virus waste has been in the bleach solution for at least 20 minutes. After the 20 minutes decontamination time, the virus is totally inactivated, i.e., not viable.
8. Use a pH strip (located near the sink) to check the pH to ensure the solution is in the acceptable range of 5.5 and 9.5. If necessary, add water until the pH is within the acceptable range.
9. Pour the media waste down the sink drain followed with water for at least 1 minute.
10. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP*.

SOP 5: VIABILITY ASSAY OF MPXV INFECTED CELLS

Before working with the MPXV, the lab worker must:

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- *have the approval of the PI who shall provide specific training according to this SOP.*
- *complete UH lab safety training and JABSOM Kaka'ako hazardous waste generator training prior to working with any chemicals.*
- *sign this SOP as documentation that he/she understands the hazards and has been trained in how to complete the following tasks safely.*

Statement of Understanding and Compliance

I confirm that I have read and understand this SOP and will comply with the procedures and policies.

Name: **Signature:** **Date:**

PPE Requirements & Special Practices: In addition to standard BSL-2+ Manipulation Suite PPE (wrap-around blue gown, black primary gloves, manipulation sleeves, and secondary grey gloves, bonnet), N95 mask and safety glasses shall be worn when working with the MPXV as per the entry SOP.

Cell Viability assay (conducted in 96-well plates):

1. Set up the hood as described in SOP 3.
2. Carefully remove the tissue culture plates of MPXV-infected cells from the incubator and place in the BSC.
3. Add 20ul of One stop cell viability solution in each well and carefully return the plates in the incubator.
4. After 2hrs, take the plate out from the incubator, place it in a secondary container and take it to the plate reader.
5. Carefully place the plate in the plate reader and read the viability as per company's protocol settings.
6. Remove the plate after reading and place it in the secondary container. Carry the secondary container to the BSC.
7. Carefully discard all the waste from the plates into the bleach solution.
8. Determine if the waste has been in the bleach solution for at least 20 minutes. After the 20 minutes decontamination time, the virus is totally inactivated, i.e. not viable.
9. Use a pH strip (located near the sink) to check the pH to ensure the solution is in the acceptable range of 5.5 and 9.5. If necessary, add water until the pH is within the acceptable range.
10. Pour the media waste down the sink drain followed with water for at least 1 minute.
11. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP*.

SOP 6: MPXV PLAQUE ASSAY

Before working with the MPXV, the lab worker must:

- *have the approval of the PI who shall provide specific training according to this SOP.*
- *complete UH lab safety training and JABSOM Kaka'ako hazardous waste generator training prior to working with any chemicals.*
- *sign this SOP as documentation that he/she understands the hazards and has been trained in how to complete the following tasks safely.*

Statement of Understanding and Compliance

I confirm that I have read and understand this SOP and will comply with the procedures and policies.

Name: **Signature:** **Date:**

PPE Requirements & Special Practices: In addition to standard BSL-2+ Manipulation Suite PPE (wrap-around blue gown, black primary gloves, manipulation sleeves, and secondary grey gloves, bonnet), N95 mask and safety glasses shall be worn when working with the MPXV as per the entry SOP.

Virus infection procedures for plaque assay:

1. Set up the hood as described in SOP 3.
2. Carefully prepare ten-fold dilutions of the supernatant samples.
3. Take the vials in secondary containment and immediately place them into the BSC.
4. Carefully remove the 12-well tissue culture plates of Vero cells from the incubator and place them in the BSC, remove most of the culture media by aspiration leaving about 300ul of media.
5. Add 1 dilution (100µL) to each well (do *not disturb cell layer* when pipetting in the sample).
6. Discard any remaining virus dilutions in the tubes into freshly prepared 10% bleach solution and let sit for at least 20 minutes.
7. Let the plates sit in the incubator for an hour and then add 3ml of the overlay (containing DMEM and 0.5% agarose) over the cells in each well and swirl gently.
8. Return the plates to the incubator after the agarose is solidified.

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9. Determine if the virus waste has been in the bleach solution for at least 20 minutes.
10. Pour the media waste down the sink drain followed by water for at least 1 minute.
11. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP.*
12. The plates will be incubated at 37°C for up to 96 hours.
13. The plates will be removed from the incubator and the cells will be fixed with 10% buffered formalin for 10 min.
14. The overlay will be removed gently and stained with 0.2% crystal violet to visualize plaque forming units (pfu).

Plaque reading: The plaques will be read using a plaque reader lightbox. Assay plates will be discarded into biohazard waste bags and decontaminated by autoclaving

SOP 7: DAILY DECONTAMINATION PROCEDURE FOR LIQUID WASTE

No liquid waste is to be discharged into sinks without prior decontamination.

1. All Liquid effluent waste in the containment area will be collected daily and decontaminated with fresh 10 % sodium hypochlorite solution.
2. Waste is treated for a minimum of 15 minutes before being neutralized with water.
3. Neutralized waste is discharged in the sink with additional copious amounts of water.
4. A 50 ml solution of Rocal disinfectant (quaternary ammonium) or a similar type of disinfectant will be added to the sink trap before and after each use.

Other SOPs like spill clean-up etc. are provided in the attachment 5.

The SOPs of virus handling in the BSL3 are described below:
All handling of MPXV-related cultures will be in certified BSC in the BSL3 (inspected and certified every year)

SOP 1: ENTRY INTO THE BSL-3 LABORTORY

Entrance into the BSL-3 Anteroom

1. Pass the keycard over the reader at the hallway door and touch your finger on the biometric sensor.
2. Enter the hallway and proceed to the end of the hall.
3. Before entry into the BSL-3 anteroom, check the Magnahelic gauge for a reading of -0.05 or greater (indicating increased *negative* pressure). Enter your name, date, time of entry, purpose of entry, and the current reading

of the Magnahelic. Note that the pressure was appropriately negative and is a suitable range for entry. If the reading is not sufficiently negative, you must call your PI and/or JBF Supervisor (Eileen Nakano 692-1612) and inform them of the insufficient pressure and obtain approval and further instructions before entering.

4. Ring the bell twice and wait for 15 sec. Make sure that no one is exiting the BSL-3 prep suite before attempting to enter.
5. Place the keycard on the biometric reader.
6. Place finger or thumb on top of biometric surface and wait for green light to appear.
7. Once again, be aware of persons that may be coming out of the BSL-3 prep suite. Do not open the door if you see persons exiting the BSL-3 prep suite.
8. Open the door and proceed to the anteroom.
9. Make sure that the door is closed.
10. **NO PIGGYBACK POLICY ENFORCED**. Each person must use their own keycard to enter the BSL-3 lab.

Please note: Do not bring personal items into the anteroom. NO BACKPACKS, NO PERSONAL LISTENING DEVICES, CELL PHONES, LAPTOPS, BOOKS, ETC. Cardboard is not allowed in the BSL3 facility. If using a cardboard box to transport items, transfer items and dispose of the cardboard immediately.

Entrance into the BSL-3 Prep room

1. Put on gown and shoe covers/designated shoes.
2. Check the Magnahelic which monitors the pressure of the Prep Room, making sure that airflow is negative (-0.020 to -0.05). Sign Entry Log Sheet: enter your initials, date, time, and pressure reading.
3. Place the keycard next to the Biometric Palm reader.
4. Place your palm on the Biometric reader to gain access into the preparation room.
5. Open the door and enter the preparation room area.
6. Immediately don the first layer of gloves.
7. Survey the room for any irregularities and lab cleanliness.
8. Check on autoclave, ultra-cold freezer (-80°C), refrigerator, and tissue culture incubator readings.
9. If you are the first BSL-3 user of the day, pour 50 mL disinfectant into the drains of the Prep Room sinks.
10. After finishing assigned tasks in the Prep Room, place items to be moved into your assigned Manipulation Suite into a closed carrier container or the Transport Cart.

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11. Move tissue cultures or virus stock into an assigned Manipulation Suite where work is to be performed (refer to the SOP- *Movement of virus between Manipulation Suite and Prep Room*).

Entrance into the BSL-3 Manipulation Suite for MPXV

1. Check signage at the entrance of your assigned Manipulation Suite for agent specific information.
2. Check Magnahelic to confirm negative pressure (approx) 0.05 inside the Manipulation Suite. Note initials, date, pressure, and time of entrance in the Manipulation Suite Logsheet, then enter the selected suite.
3. Place Keycard near the biometric reader. NO PIGGYBACKING!
4. Enter Suite and place the carrier container on to the counter. If using a Transport Cart, move through the door with the cart and place near the end of the counter.
5. Immediately don secondary gloves and disposable sleeves over your gown once fully inside the Manipulation Suite.
6. Immediately wear an N95 mask and bonnet.
7. Work can begin adhering to the guidelines of the Working in the BSL-3 Manipulation Suite SOP.

SOP 2: EXITING THE BSL-3 LABORTORY

1. Clean BSC with 10% bleach (made fresh daily), dry and wipe down with 70% alcohol.
2. Switch on the UV and leave on for 20 min to decontaminate the BSC.
3. Add 1% bleach to the sink to fill the trap.
4. Remove N95 masks.
5. Discard the N95 mask if doing high-risk procedures. Save N95 mask appropriately if doing low-risk procedures. There is a shortage of masks and PPE. Consult your supervisor.
6. Remove sleeves and outer gloves and place in waste container.
7. Now exit from MPXV suite into the preparation room
8. Remove inner gloves and booties. Wash hands with soap and water for minimum 30 sec.
9. Exit preparation room
10. Remove gown in the anteroom
11. Sanitize hands with waterless hand sanitizer just before exiting the BSL-3 anteroom
12. Exit BSL-3 anteroom.
13. Wash hands with soap and water in the IBR corridor rest room.
14. Exit hallway in the BSB lobby

SOP 3: WORKING WITH MPXV IN THE BSL-3 SUITES

1. Clean BSC with 70% alcohol
2. Dry it completely and spread the versi dry.
3. Place supplies into the BSC; a covered trash pan, small beaker with freshly prepared 10% bleach, tissue culture plate, media etc.
4. Arrange all items in good configuration so that air flow will not be obstructed.
5. Wear N95 mask and bonnet.
6. The personnel, BSC and the suites are now ready for the following virus manipulations.

SOP 4: MPXV STOCK PREPARATION IN THE BSL3

Before working with the MPXV, the lab worker must:

- *have the approval of the PI who shall provide specific training according to this SOP*
- *complete UH lab safety training and JABSOM Kaka'ako hazardous waste generator training prior to working with any chemicals;*
- *sign this SOP as documentation that he/she understands the hazards and has been trained in how to complete the following tasks safely.*

Statement of Understanding and Compliance

I confirm that I have read and understand this SOP and will comply with the procedures and policies.

Name: **Signature:** **Date:**

PPE Requirements & Special Practices: In addition to standard BSL3 Manipulation Suite PPE (booties, wrap-around blue gown, black primary gloves, manipulation sleeves, and secondary grey gloves), N95 mask and safety glasses shall be worn when working with the MPXV as per the entry SOP

Stock virus preparation procedures:

1. In the prep room, carefully remove the original vial of virus from the -80 freezer and place in secondary containment.
2. Take the vial in secondary containment into the suite and immediately place in BSC.
3. Carefully remove the T75 or T125 flask with Vero cells from the incubator and place it in the BSC.

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4. Infect T75flask (10mL media) with 100uL and T125flask (25mL media) with 200uL of thawed virus and return the flask to the incubator located in the suite. INFECTED TISSUE CULTURES WILL BE INCUBATED ONLY IN THE ASSIGNED INCUBATOR FOR MPXV IN THE SUITE. NO INCUBATIONS OF INFECTED CULTURES WILL BE HOUSED IN THE PREP ROOM.
5. After 2 hrs of infection, add 10mL of fresh media in the flask and return to the incubator. For stock preparation, washing cells after 2 hrs is not needed.
6. Determine if the virus waste and tips etc. has been in the bleach solution for at least 20 minutes. After the 20-minute decontamination time, the virus is totally inactivated, i.e. not viable as per CDC.
7. Use a pH strip (located near the sink) to check the pH to ensure the solution is in the acceptable range of 5.5 and 9.5. If necessary, add water until the pH is within the acceptable range.
8. Pour the waste down the sink drain followed with water for at least 1 minute.
9. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP*.

Procedure of harvesting supernatant as virus stock:

1. After 4 days carefully take the flask out of the incubator and place it in the BSC hood.
2. Remove the media and place in a 50mL falcon tube (~20mL for T75 and 35mL for T125 flask).
3. Bring the rotor of the centrifuge inside the BSC hood. Carefully place the tubes in the rotor and screw the rotor cover completely. Spray the rotor with 70% ethanol and wipe it well from the outside.
4. Carefully carry the rotor to the centrifuge (located just behind the BSC) and spin the virus supernatant for 15 min at 7,000 rpm to remove any cell debris. Bring the rotor back to the BSC and carefully remove the falcon tubes out of the rotor.
5. Bring the rack of freezer cryovials already labeled with all required details (virus information, date, amount, etc.) in the BSC.
6. Carefully aliquot approximately 200 or 500ul virus supernatant in each vial.
7. Wash the flask and tubes with 10% bleach solution once and discard leftover waste into the bleach solution container.
8. Carefully place the vials in a 96-well box. Place this box in a secured secondary container and move it out to the prep room to store the stock virus vials in the -80°C freezer.
9. Determine if the infectious waste has been in the bleach solution for at least 20 minutes. After the 20-minute decontamination time, the virus is totally inactivated, i.e. not viable.
10. Use a pH strip (located near the sink) to check the pH to ensure the solution is in the acceptable range of 5.5 and 9.5. If necessary, add water until the pH is within the acceptable range.
11. Decontaminate the BSC and work surfaces as described in the *Clean-up SOP*.

Other SOPs like spill clean-up and emergency response are provided in the attachment 5.

DISCUSSION:

1. Person Responsible:

Saguna Verma, Ph.D.
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Dr. Verma, a formally trained virologist and immunologist, conducts research on the pathogenesis of different viruses including West Nile virus, dengue virus, and Zika virus, rVSV-Ebola virus, and SARS-CoV-2 virus. She has more than 20 years of experience working with these pathogens in BSL2, BSL2+, and BSL3 laboratories and delineating several pathways associated with disease pathogenesis. She has used both cell culture models and mouse models to understand mechanisms associated with virus pathogenesis. Her laboratory has optimized virus infections in cutting-edge 2D and 3D organoid models (studies funded as Principal Investigator by NIH). She has also trained several students, technicians, and post-doc fellows in both BSL2+ and BSL3 laboratories. She is currently actively working with SARS-CoV-2 in the BSL3 laboratory. She is trained in classical and molecular microbiological techniques, as documented in her publications, and has a track record of successful grant funding from local and national agencies. She has completed all the necessary training including Blood Borne Pathogens (8/18/2023), Biosafety (8/18/2023).

2. Safeguard Facility and Practices:

Facility of virus storage and safeguard: JABSOM BSB is a secured facility and only approved personnel with proper training have access to secured labs. MPXV clade II will be used and stored at the JABSOM, Biosciences Building in a -80°C freezer dedicated to storing BSL2+ level viruses (more details on the facility and security below and in the attachments 3 and 4).

BSL2+ suite location: BSL2+ suite is located on the third floor of the BSB building. Location is Room 332, Third Floor, 651, Ilalo Street, Honolulu, Hawaii 96813. The facility was inspected by the UH Biosafety officer in October 2023.

BSL3 suite location: BSL3 suite is located on the first floor of the BSB building, JABSOM, 651 Ilalo Street, Honolulu, Hawaii 96813. BSL-3 laboratory will be used to propagate the virus to make stock as described in later sections. This facility was inspected by UH Biosafety Officer in January 2024.

Virus storage room: The original MPXV clade II virus vial obtained from BEI resources will be stored in the BSL3 -80°C freezer in the prep room. This vial will be used to make the virus stock in the BSL3 suite for subsequent experiments. The virus stock after propagation will be stored in screwcap vials in the -80°C freezer in Room# 331, BSB, 651, Ilalo Street, Honolulu, Hawaii 96813. Each vial will have 200-500ul of stock. This freezer is locked and only personnel approved to work with MPXV will have access to the freezer lock key.

Lab contact information: Saguna Verma, Ph.D., (application PI) Ph: 808-692-1662 and Vivek R Nerurkar, Ph.D., (JBF Director) Ph: 808-692-1668

BSL2+ facility specifics: As per the CDC, the BSL-2+ facility involves moderate to high-risk agents and therefore requires a strict adherence to BSL-2 containment with BSL-3 work practices and procedures. Importantly, BSL-2+ laboratories are not open to the public and only trained personnel as described below are allowed access.

Biosecurity:
BSL2+ and BSL3 Biosafety practices:

All viral stocks in the Department of Tropical Medicine, Medical Microbiology and Pharmacology, are under the supervision of the Lab Director. MPXV will be only grown in closed-lid cell culture vessels in CO_2 incubators, in secured BSL-3 lab that is only accessible by trained laboratory personnel. Culture vessels will be only opened inside the Class II Biosafety Cabinet (BSC), using an aseptic technique. Virus stocks will be transported from the BSL3 storage freezer to the BSL2+ MPXV suite in small batches (10-20 vials, each containing 200-500uL virus) in a secured secondary container. These vials will be stored in designated freezer in the room 331 and will be used for subsequent experiments. Typically, one vial (200-50uL) of virus in a secured secondary container is transported at a time for viral manipulations. All procedural manipulations of cell culture and virus assays will be done inside a BSC (refer to attached SOPs for details of PPE and lab practices, Attachment 5).

All manipulations will be done by skilled and trained researchers. Personnel will undergo rigorous training and proficiency testing before getting access to the BSL2+ and BSL3 labs. Each investigator will have had prior experience working with BSL-2 pathogens and will be able to understand proper handling techniques of the virus and will comply with the safety regulations set forth by UH Biosafety Program and Office Research Compliance (refer to attached SOPs for details of PPE and lab practices, Attachment 5). All personnel will attend regular risk assessment and new SOP training as and when planned by the Lab Director.

Researchers never conduct work with exposed skin surfaces on hands and arms and are discouraged from working with any deep wounds or cuts, no matter how well bandaged. Proper personal protection equipment practically eliminates any potential lab infection. Activities involving the use of sharps are avoided whenever possible. In the laboratory, virus will be presumed to be present in all materials derived from MPXV culture, and in/on all equipment and devices coming into direct contact with any of these materials. Laboratory personnel will take great care in decontaminating all of the above items as per the SOPs.

MPXV clade II is transmitted through skin-to-skin contact directly. In the laboratory setting, clade II MPXV infection from human-to-human or stock vial to human is not possible. The only plausible cause of infection in the laboratory setting is if a technician, during active manipulation of the virus, accidentally pokes himself deep into the skin with a contaminated sharp object, providing direct access of the virus to the bloodstream. These risks are mitigated in cell culture experiments where sharp objects, like needles, are not used. We also minimize the use any glass products like slides eliminating the associated risks. Media is also stored in high-quality plasticware. In some cases, glass flasks are used to prepare culture media with agarose for plaque assay, but this media is always non-infectious. In the cell culture setting, the possible risk of infection can be during protocols that generate aerosols (very unlikely for MPXV clade II as it is not an airborne pathogen). However, sonication is mostly used for processing tissues. We do not plan to work with mice at this stage and sonication is not needed when handling cell cultures. In case, we plan to work with mice, we will apply for a fresh permit later. Thus, we have designed our protocols and procedures to avoid any sonication procedures and eliminate the need for sharps. However, strict SOPs and the use of PPE during handling of any type of virus cultures will be in place to protect against any potential exposure. All wastes will be treated with chemical neutralizing agents such as sodium hypochlorite solution or quaternary ammonium compound solution (refer to the SOPs for more details).

With proper precautions in place, training of personnel, and strict adherence to SOP, not only is the risk of laboratory infection extremely unlikely, but accidental release is also nearly impossible. Experiments and procedures are designed to limit the amount and concentration of virus. Stocks are aliquoted in small amounts and the virus is contained at all time points except for during direct manipulation. If an accidental spill were to occur in the lab, due to the limited quantity and concentration, the risk of virus spread would be minimal. Only inactivated samples like RNA and fixed cells are allowed to leave the BSL2+/BSL3 suites. Moreover, research technicians are trained to respond to isolate the spill immediately and chemically neutralize the spill, virtually eliminating all possibility of adverse environmental and health effects. They attend yearly risk assessment training and drills.

Practices to minimize theft: Only personnel approved by the JBF Director will be authorized to enter BSL-2+ and BSL3 laboratories. There are already protocols in place

to monitor entry and exit of personnel in the BSL-2+ and BSL3 laboratories. In addition, proper inventories will be kept and closely monitored and just as with other permits, all annual inventories will be submitted yearly to the Hawaii Department of Agriculture.

Equipment use for research: Equipment commonly used for live virus cultures are described below. Only inactive virus samples containing virus DNA or cellular RNA are used for PCRs and other assays.

Basic equipment for live virus culture

- Cell culture hood (i.e., laminar-flow hood or biosafety cabinet)
- Incubator (humid CO2 incubator)
- Centrifuge
- Refrigerator and freezer (–20°C and -80°C)
- Inverted microscope
- Plate reader for cell viability assays.
- Autoclave to decontaminate waste.

Procedures

i. **Virus stock preparation:** Vero cells will be grown in a T125 flask (with 30mL media) and infected with original MPXV clade II from BEI. After 4 days, the supernatant will be centrifuged to remove cell debris. Then, 10% FBS will be added and virus stock will be aliquoted in freezer vials in the volume of either 200 or 500uL/vial. The expected titer of the stock is ~10^7 PFU/mL based on other studies.

ii. **Virus infection of different human cell types:** Different human cells will be cultured in 48- or 24-well plates and grown to 80% confluency. The cells will then be infected with diluted MPXV clade II and following the infection at different time points, supernatant will be collected, and cells will be washed with PBS and used to extract DNA and proteins for measuring different host responses. These infection experiments will take between 2-10 days for sample collection before the plates are discarded. For an MOI of 1 or 0.1 infection, a maximum of 200uL of virus stock will be needed per plate.

iii. **Virus measurement using plaque assay:** Plaque assay using Vero cells is a widely used method to measure infectious virus in the supernatant. Vero cells grown in 12- or 6-well plates will be incubated with the supernatant from infected cells at different time points and overlaid with 1% agarose as described in the attached SOP (Attachment 5). The colonies will be counted after 3-4 days[6].

iv. **Cell viability assays:** Death of cells caused by the virus at different days after infection will be measured using a widely used cell viability kit as described in our previous studies[7]. The plate will be read in the plate reader. This experiment takes total 2-4 hrs.

v. **Host response studies:** Infected cells will be washed and DNA/RNA will be extracted using commercially available kits and used to run PCR assays to

profile host immune and inflammatory genes as described in our previous studies[7].

CDC recommendation for vaccination for MPXV researchers: The CDC recommends vaccination with JYNNEOS (smallpox vaccine) to all personnel working with MPXV. This should protect the researcher from severe symptoms. Further, the possibility of LAI (laboratory-acquired illness) in cell culture research for MPXV clade II is unlikely, however, if suspected, a detailed post-exposure plan is developed and is attached separately (Attachment 5). More details on the MPXV vaccine recommendation and treatment options are in section# 5 below.

3. Method of Disposition: As per CDC, the most accepted method to inactivate waste generated in poxvirus studies is autoclave of solid waste and treatment with 10% sodium hypochlorite for liquid waste [8]. All liquid waste such as excess viral stock in volumes of less than 1 mL, dilute, working virus inoculum and infected virus cultures will be chemically neutralized with 10% sodium hypochlorite solution for a minimum of 15 minutes, then diluted to a suitable neutral pH and disposed of down the drain. Solid wastes like plastic wares associated with the viral agent will be chemically neutralized with 10% sodium hypochlorite solution for a minimum duration of 15 minutes, placed in red bags, and then autoclaved. These methods will completely destroy the virus [8]. Autoclaving conditions are: 15 pounds/square inch pressure/250 F,/60 minutes. Live MPXV will never have direct contact with the environment.

4. Abstract of Organism:

Monkeypox (MPXV)

Virus History: Monkeypox disease is caused by a sylvatic, double-stranded DNA zoonotic monkeypox virus (MPXV), an orthopoxvirus that belongs to the same Poxviridae family as the variola virus responsible for causing smallpox. Although MPXV exhibits symptoms akin to smallpox, they are generally less severe, and instances of fatality are infrequent with 93,030 cases worldwide and only 176 deaths. The discovery of MPXV dates to 1958, following two outbreaks of a pox-like disease observed in colonies of research monkeys. Prior to the 2022 outbreak, MPXV had been reported in individuals across various central and western African nations. The virus was endemic to Central Africa regions and most MPXV cases outside of Africa were linked to international travel or through the importation of animals. The 2022 outbreak is concerning because of large number of cases found in non-endemic regions like US.

There are two distinct types of the MPXV virus: **Clade I and Clade II.** The Clade I variant, which is designated as a select agent, carries a fatality rate of approximately 10% and the Clade II variant with less than 0.2% (CDC description of clades I and II is attached, Attachment 2). The infections reported during the 2022–2023 outbreak in the

C22

USA is attributed to Clade II, specifically Clade IIb. Infections associated with Clade II are rarely fatal, with over 99% of affected individuals likely to recover.

Clade II variant is designated as BSL2 pathogen by the CDC due to lower disease severity and mortality[3,4,9]. Handling of MPXV clade II clinical samples tissue samples is recommended in BSL2 (CDC description attached, Attachment 2), however, cell culture work is recommended in BSL2 lab with BSL3 practices (BSL2+) if working with only one type of non-select poxvirus. As per CDC guidelines, we will work with the clade II variant of MPXV only in the BSL-2+ lab (except for virus stock preparation experiments which will be done in the BSL3 lab). For further details, see: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition (poxviruses pages 268-270).

Copy and paste from page 270 of BMBL 6th Edition book:
BSL-2 facilities with BSL-3 practices are advised if vaccinated personnel perform laboratory work with monkeypox virus. BSL-2 and ABSL-2 containment plus vaccination are recommended for work with vaccinia and other human pathogenic poxviruses. The lowering of containment to BSL-1 for the manipulation of attenuated poxviruses and vectors (e.g., modified virus Ankara [MVA], NYVAC, TROVAC, and ALVAC) in areas where no other human orthopoxviruses are being used may be considered. However, **higher levels of containment are recommended if these strains are used in work areas where other orthopoxviruses are manipulated.** Vaccination is not required for individuals working only in laboratories where no other orthopoxviruses or recombinants are handled.

Based on these guidelines, we will require vaccination for all personnel working with the Clade II MPXV to work in the BSL2+ laboratory. Additionally, we do not plan to work with any other orthopoxviruses, therefore handling MPXV clade II in the BSL2+ suite falls under the guidelines of the CDC. However, for extra precaution, we will conduct virus stock preparation experiments in the BSL3. Virus stock will be aliquoted in freezer vials in small volumes (200-500uL per vial) and a small batch of stock will be stored in the BSL2+ lab for other experiments as described in the SOP and other sections.

About MPXV and its life cycle
The MPXV genome has more than 200 kilobase pairs and is approximately seven times the size of severe acute respiratory coronavirus 2 (SARS-CoV-2). However, MPXV has a much less serious threat of a massive global pandemic. Unlike COVID-19, MPXV does not transmit human to human very easily through the air like COVID-19, and is not contagious until the MPXV case becomes symptomatic, which makes it much easier to isolate and prevent the spread of MPX cases. Reported cases in recent outbreaks have mainly but not exclusively been identified among men who have sex with men (MSM).

Virus targets and replication: MPXV uses several receptors to enter cells including many surface glycoproteins. MPXV can infect many cell types including skin keratinocytes, fibroblasts and macrophages. Once it reaches lymphoid tissues via infected immune cells, it can spread to other organs like lungs, spleen and liver. Through blood stream, the virus reaches to distant organs like gonads. Recently, MPXV

was isolated from the semen of infected individuals, highlighting the possibility of sexual transmission.

MPXV initially attaches to host cell receptors through interactions between viral surface proteins and host cell receptors, including heparan sulfate proteoglycans and integrins. After attachment, the virus enters the host cell. Once inside the host cell, the virus undergoes uncoating, where the viral outer membrane is removed, releasing the viral core into the cytoplasm. The viral DNA encodes proteins involved in viral replication and evasion of host immune responses. The viral DNA replicates in the nucleus using host cell machinery, producing new copies of viral DNA. Viral structural proteins and newly replicated viral DNA assemble in the cytoplasm to form mature virions. Released virions can infect neighboring cells, restarting the infection cycle. Early detection of MPXV antigen in both monocytes and neutrophils has been suggested to be critical in preventing virus spread but the virus has evolved several strategies to block immune response.

MPXV Clade IIb Symptoms

MPXV manifests with distinctive symptoms. Affected individuals often develop a rash, appearing on various parts of the body such as the hands, feet, chest, face, mouth, or in proximity to the genital area. The incubation period spans from 3 to 17 days, during which the infected person may be asymptomatic and feel generally well. The rash undergoes several stages, including scabbing, before eventual healing. Initially resembling pimples or blisters, the rash can be accompanied by pain or itching. Additional symptoms of MPXV encompass:

- Fever
- Chills
- Swollen lymph nodes
- Exhaustion
- Muscle aches and backache
- Headache
- Respiratory symptoms like sore throat, nasal congestion, or cough

Individuals may experience all or only a subset of these symptoms. Vigilance for MPXV symptoms is advised for 21 days following the last exposure. If symptoms, particularly a rash persists, seeking medical attention is crucial. Onset of MPXV symptoms typically occurs within 3 weeks of exposure, with flu-like symptoms often preceding the rash by 1-4 days. However, individuals with compromised immune systems, those with a history of eczema, and pregnant or breastfeeding individuals may face a higher risk of severe illness or fatality.

MPXV Clade IIb Transmission

Infected individuals can transmit the virus from symptom onset until the rash completely heals, and a new layer of skin forms[10]. Recent data indicates that some individuals can transmit MPXV to others 1-4 days before symptoms emerge[11], though the extent of this phenomenon during the current outbreak remains unclear. Importantly, there is no

current evidence suggesting that individuals who never develop symptoms can transmit the virus to others. Transmission of MPXV occurs primarily through close or intimate contact, involving direct skin-to-skin or sexual contact with an infected person. This includes contact with the MPXV rash and scabs, as well as exposure to their saliva, upper respiratory secretions (snot, mucus), and areas around the genitalia[10,11].

Additionally, MPXV can be transmitted through indirect means, though less likely. This includes touching objects, fabrics, or surfaces that have been used by someone with MPXV and haven't been properly disinfected[10,11]. These objects may include clothing, bedding, towels, etc. MPXV can also pose a risk during pregnancy, potentially spreading to the fetus or the newborn through close contact during and after birth[11]. Animals, particularly wild ones such as small mammals like squirrels, rats, and mice in areas where MPXV is naturally found, can carry and transmit the virus to humans through close contact if they touch a rash, scab, crust, saliva from infected animal[10,11]. Regarding waterborne transmission, there is no evidence linking MPXV to water in pools, hot tubs, or splash pads. The MPXV virus is effectively neutralized in water with chlorine levels recommended for disinfection in recreational water venues, as specified by the CDC and mandated by U.S. jurisdictions. Similarly, MPXV is not considered as an airborne pathogen.

Diagnosis
Monkeypox disease is diagnosed by PCR test for the MPXV (both clades) on a viral swab taken from one or more skin scabs or ulcers. MPXV is not easily detected in the blood. Skin lesions, when present, are presumed to be the primary source of viral shedding and testing. Detecting antibodies against the virus in the blood is not a standard diagnostic test for MPXV.

Treatment
Currently, there are no antiviral drugs or vaccines developed specifically for both Monkeypox Clade I and II. However, an antiviral that was developed to treat smallpox (tecovirimat) was approved by the European Medicines Agency in January 2022 for the treatment of MPXV under exceptional circumstances[9]. Four smallpox vaccines developed to eradicate smallpox are also effective against monkeypox. Namely, these are JYNNEOS, MVA-BN, LC16 and OrthopoxVac, however, although mass vaccination campaigns have not been initiated[9], these vaccines are available for people who work with MPXV in the US including in Hawaii. Over-the-counter medicines to alleviate fever, pain and other symptoms are mainly used to mitigate lifestyle impact.

The **CDC recommends that laboratory personnel working with monkeypox virus should receive prophylactic, pre-exposure vaccination against smallpox (JYNNEOS vaccine)**[12]. To date, the **Hawaii Department of Health (DOH) has administered 4,400 doses of the JYNNEOS vaccine allocated by the Strategic National Stockpile and is available to healthcare workers and post-exposure prophylaxis** of individuals who came in close contact with monkeypox infected

individuals[13]. The JABSOM laboratory personnel who will be working with the virus will follow the CDC recommendations.

Lab Acquired Infections (LAIs)
In the scientific literature, there has been no reported cases of monkeypox LAI in a laboratory setting. For the current permit, we do not plan to infect animals with MPXV clade II virus. Laboratory-acquired exposures to Orthopoxviruses and subsequent infections are occasionally reported among laboratory personnel. In the United States, a total 14 Orthopoxvirus infections were reported during 2004–2014 among personnel working in diagnostic and research facilities. Most of these LAI are attributed to needle pricks in researchers working with mice.
(https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5715a3.htm).

5. Effects on the Environment:

Potential impact on the environment of Hawaii
Monkeypox Clade IIb cases in Hawaii: As of Feb 2024, there have been a total 46 MPXV cases reported in the state of Hawaii. Specifically, 32 cases in Honolulu, 4 cases each in the Big Island of Hawaii and Kauai, 2 cases in Maui, and the rest were out of state.

Impact on plants: Regarding plants, there isn't any known direct relationship between monkeypox and infection in plants. The virus has never been reported to be transmitted from humans to plants, therefore there will be no impact on the Hawaiian plant life.

Impact on wildlife: The likelihood of monkeypox impacting wildlife in Hawaii is extremely low. MPXV is primarily endemic to Central and West Africa and primarily circulates among specific rodent species. Animals shown to be infected with MPXV are rope and sun squirrels, giant-pouched rats, Chinchillas, and prairie dogs (a kind of squirrel found in 10 US states and Hawaii is not one of them) in those regions. After the MPXV cases increased in the US in 2022, attempts have been made to identify species that can get infected with MPXV. As per the CDC website, we do not know if reptiles, amphibians, fish, or birds can get MPXV. It is unlikely since these animals have not been found to be infected with other orthopoxviruses. It is also not known if pets like dogs and cats can be infected with MPXV. According to CDC, no pets or other animals were confirmed to have the virus during the global MPXV outbreak that began in 2022.
(https://www.cdc.gov/poxvirus/mpox/veterinarian/mpox-in-animals.html).

The rodent species that can harbor MPXV as described above are not native to Hawaii and are not typically found in the state of Hawaii. Therefore, conditions necessary for the virus to establish itself in Hawaiian wildlife are highly improbable. Hawaii's geographic isolation, stringent biosecurity measures, and lack of natural reservoir hosts for monkeypox significantly mitigate the risk of introduction and transmission within local

wildlife populations. For the current permit, we do not plan to infect animals with MPXV clade II virus thus further reducing the risk to Hawaii wildlife.

In summary, the transmission of monkeypox between rodents and humans is highly improbable for several reasons. Given the safe laboratory practices it is highly unlikely that MPXV will escape biocontainment in the BSL-2+ or BSL3 labs (refer to all SOPs for virus handling details). Firstly, while rodents can serve as reservoir hosts for monkeypox, the virus typically circulates within specific rodent species in endemic regions of Ce0ntral and West Africa, and these species are not native to Hawaii. Secondly, even in regions where monkeypox is endemic, direct transmission between rodents is not well established and is reported in rare cases without any conclusive evidence of direct transmission. Furthermore, the likelihood of infected rodents transmitting the virus to humans is minimized by the low frequency of human-rodent interactions in controlled environments and the implementation of effective vector control measures. Consequently, the probability of rodents getting infected with monkeypox and then transmitting the virus to each other or humans, particularly in regions like Hawaii with stringent biosecurity measures, is exceedingly remote.

Virus release during emergency scenarios such as tsunamis is also extremely remote. Should a tsunami or hurricane warning occur, we have very specific SOPs in place to wind up MPXV experiments and decontaminate the infectious cultures to prevent spread into the environment (attached with the application, Attachment 5).

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III. Environmental Assessment (EA):

Pursuant to a May 2008 Hawaii Intermediate Court of Appeals decision (Ohana Pale Ke Ao v. Board of Agriculture, 118 Haw. 247 (Haw. App. 2008), the Department of Agriculture's (Department's) import permit process is subject to the requirements of the Hawaii Environmental Protection Act, chapter 343, Hawaii Revised Statutes (HRS). Under this decision, the requirement for an EA as a condition of the import permit or related authorization applies in those circumstances where the underlying permit activity for the importation initiates a "program or project" and where the use of state or county funds or state or county lands is involved. When those circumstances are present, as they appear to be when a new organism is used at University of Hawaii (state lands), an EA is required to determine whether the proposed project or program is likely to have a significant impact on the environment. However, certain activities may be eligible for "exemption" from an EA under provisions established through the Environmental Council, State Office of Planning and Sustainable Development's Environmental Review Program (ERP)(formerly, the Office of Environmental Quality Control (OEQC)), provided that the project or program is determined to have little or no impact on the environment.

Exemption from EA: In September 2008, the Department obtained the concurrence of the former OEQC Environmental Council for exemption from EA for those Plant Quarantine Branch import permits and related authorizations that satisfy certain criteria, including conditions to minimize risk to agriculture, horticulture, the environment, or animal or public health. The exemption from EA for microorganisms applies to the import of microorganisms for various purposes according to their placement on lists maintained by the Board of Agriculture (Board) and subject to permit conditions appropriate to eliminate or minimize risks associated with the microorganisms and their use. (See Exemption Class #10., item 3.a of the Department's exemptions, under the links for exemption lists for state agencies at: http://hawaii.gov/health/environmental/oeqc/index.html/). Permit conditions address matters such as health requirements, special precautions, and safeguarding from escape, theft, or release. Under the exemption, purposes for importation of microorganisms include, but are not limited to, food and beverage processing; clinical laboratory diagnostics or quality control testing; medical or scientific research by qualified entities and universities in standard research settings; classroom instruction at

universities or high schools; microbial products; algae research or algae cultivation and production for food, feed, or export for processing for uses such as cosmetics, food supplements, and pharmaceuticals. The exemptions from EA are only applicable when a project or program will probably have minimal or no significant effect on the environment. Under OEQC's rules and the Department's exemption list, exemptions are inapplicable when the cumulative impact of planned successive actions in the same place, over time, is significant, or when an action that is normally insignificant in its impact on the environment may be significant in a particularly sensitive environment.

PQB NOTES: *UH will be issuing the applicant an EA exemption to satisfy the requirement for the Environmental review.*

IV. ADVISORY SUBCOMMITTEE REVIEW

This request was submitted to the Advisory Subcommittee on Viruses for their review. Their recommendations and comments are as follows:

1. I recommend approval ___/___ disapproval to allow the importation of the Monkeypox Virus, Clade IIb, on the List of Restricted Microorganisms (Part A), for Laboratory Research, by the University of Hawaii.

Dr. Edward Desmond: No response.

Mr. David Clements: Recommends approval.

Comments: None.

Dr. A. Christian Whelen: No Response.

Dr. Raquel Wong: Recommends approval.

Comments: None.

Dr. Hongwei Li: Recommends approval.

Comments: "Monkeypox Virus Clade IIb is designated as a BSL2 pathogen, and has minimal effects on the environment."

Dr. Grieg Steward: Recommends approval.

Comments: None.

2. I recommend approval___/___ disapproval to Establish Permit Conditions for the Importation of Monkeypox Virus, Clade IIb, a Virus on the List of Restricted Microorganisms (Part A), by Permit, for Laboratory Research, by the University of Hawaii;

Dr. Edward Desmond: No response.

Mr. David Clements: Recommends approval.

Comments: None.

Dr. A. Christian Whelen: No Response.

Dr. Raquel Wong: Recommends approval.

Comments: None.

Dr. Hongwei Li: Recommends approval.

Comments: "My approval is based on BSL2+ facility that will be used for the virus, high security of the JABSOM building, and the applicant's working experience in virology."

Dr. Grieg Steward: Recommends approval.

Comments: None.

3. Are the Proposed Permit Conditions sufficient to assure that the Laboratory Research using Monkeypox Virus, Clade IIb, a Virus on the List of Restricted Microorganisms (Part A), by the University of Hawaii, Presents Probably Minimal or No Significant Effects on the Environment;

___probably minimal or no significant effects on the environment.
___other (If "other," please explain.)

Dr. Edward Desmond: No response.

Mr. David Clements: Probably minimal or no significant effects on the environment.

Comments: None.

Dr. A. Christian Whelen: No Response.

Dr. Raquel Wong: Probably minimal or no significant effects on the environment.

Comments: "Safeguards practiced in the laboratory are sufficient to prevent significant effect on the environment."

Dr. Hongwei Li: Probably minimal or no significant effects on the environment.
Comments: None.

Dr. Grieg Steward: Probably minimal or no significant effects on the environment.

Comments: "BEI product information indicates BSL-3, but CDC recommends BSL-2 with BSL-3 practices and personnel vaccination, which is as proposed in the application."

4. Are the Proposed Permit Conditions sufficient to assure that the item requested for import presents probably minimal or no significant effects on the environment should it be accidentally released?
___Yes
___No (If "No," please explain and suggest appropriate conditions.)

Dr. Edward Desmond: No response.

Mr. David Clements: Yes.

Comments: None.

Dr. A. Christian Whelen: No Response.

Dr. Raquel Wong: Yes.

Comments: "Laboratory safeguards practiced are sufficient to prevent release, therefore will have no impact to the environment.".

Dr. Hongwei Li: Yes.

Comments: None

Dr. Grieg Steward: Yes.

Comments: None.

V. ADVISORY COMMITTEE ON PLANTS AND ANIMALS REVIEW

This request was submitted to the Advisory Committee on Plants and Animals (Committee) at its meeting on October 11, 2024. Acting PQB Microorganism Specialist Shelby Ching provided a synopsis of the request.

After Ms. Ching presented a synopsis of the request, Chairperson Takeshima asked if there were testimonies from the public, there were none.

Chairperson Takeshima asked if there were any questions from the Committee. Committee Member Thomas Eisen asked about the exemption for an Environmental Assessment (EA). In response, Ms. Ching stated that UH has the ability to issue their own exemption. Committee Member Eisen asked if the EA Exemption had been issued or if it was still pending.

Committee Member Joshua Fisher asked if there were contingency plans in the SOPs addressing tsunamis and hurricanes, and how prolonged power outages would affect the laboratory. The applicant, Dr. Saguna Verma stated there is backup power that will run for three days. During a hurricane warning two months prior, they stopped experiments, decontaminated and bleached everything. Mr. Fisher wanted to ensure procedures are in place if there is an impending storm. Dr. Verma said they have procedures for cell cultures and animal experiments in the event of a natural disaster. In the past, hey have sacrificed animals during a warning of a power outage or storm. These precautions are taken very seriously.

Committee Member Rob Hauff asked why all research activities would not be done under BSL3 conditions. Dr. Verma responded they wanted to do everything in BSL2+ because it was more than Centers for Disease Control and Prevention (CDC) requires for working with monkeypox virus, which is a BSL2 pathogen. Everyday experiments will be working with 100 microliters of virus and the risk for that is very low. However, when they are making the stock virus or when they do one big experiment, they will work in BSL3, where the security and backup power is better. They will then bring maybe 5 or 10 small vials of virus up to the BSL2 lab where student training is easier and smaller amounts of virus are less risky. It is also a good idea to have samples of the virus in multiple labs.

Committee Member Gracelda Simmons asked if there was a reason there was no response from the Advisory Subcommittee Members Dr. Desmond, Department of Health State Lab Director and prior Lab Director Dr. Whelen. Ms. Ching was unsure of the reasons for the non-response from the respective Subcommittee Members.

PQB NOTES: *This request was sent out to the HDOA Subcommittee on Virus for comments on April 17, 2024, with a deadline for a response by May 1, 2024. No responses were received by Dr. Desmond or Dr. Whelen during that time.*

Hearing no other comments from the Committee, Chair Takeshima called for a motion to approve. Motion was approved by Committee Member Sam Gon and seconded by Committee Member Maria Haws.

Chair Takeshima called for the vote.

Recommend Approval: 7/0/1 (**Yes:** Takeshima, Hauff, Haws, Gon, Fisher, Mizuno, Eisen; **Abstain:** Simmons)

Motion passed.

VI. Proposed Permit Conditions:

1. The restricted article(s), Monkeypox Virus Clade IIb, shall be used for laboratory research, a purpose approved by the Board of Agriculture (Board), and shall not be sold, given, or transferred in Hawaii, except as approved by the Board. Release of the restricted article(s) into the environment is prohibited.

2. The permittee, Saguna Verma, Ph.D., University of Hawaii, Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, 651 Ilalo Street, BSB 320E, Honolulu, Hawaii 96813, shall be responsible and accountable for all restricted article(s) imported, from the time of their arrival to their final disposition.

3. The restricted article(s) are subject to the pre-entry requirements of section 4-71A-8, Hawaii Administrative Rules (HAR), and the inspection requirements of section 4-71A-9, HAR.

4. Each shipment shall be accompanied by a copy of the Plant Quarantine Branch (PQB) permit for the restricted article(s) and an invoice, packing list, or other similar PQB approved document listing the scientific and common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee for the restricted article(s).

5. The restricted article(s) shall be safeguarded at the University of Hawaii at Manoa, 651 Ilalo Street, JABSOM, Biosciences Building, BSL 3 suite and BSL2+ suite, Honolulu, Hawaii 96813, sites inspected and approved by the PQB prior to importation. Removal of the restricted article(s) to another site or room shall require site inspection and approval by the PQB Chief prior to movement.

6. The restricted article(s) shall be maintained by the responsible person, Saguna Verma, Ph.D., University of Hawaii, Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine, 651 Ilalo Street, BSB 320E, Honolulu, Hawaii 96813, or by trained or certified personnel designated by the permittee.

7. The permittee shall adhere to the use, facility, equipment, procedures, and safeguards proposed and described in the permit application, as approved by the PQB Chief and Board.

8. The approved site(s), restricted article(s), and records pertaining to the restricted article(s) under permit shall be subject to post-entry inspections pursuant to section 4-71A-16, HAR. The permittee shall make the site(s), restricted article(s) and records pertaining to the restricted article(s) available for inspection upon request by a PQB inspector.

9. The permittee shall submit an annual report of all the restricted article(s) imported or possessed for the calendar year by January 31st of the following year. The report shall include:

   a. The permit number, scientific name, and quantity of the restricted article(s) imported or possessed;

   b. The status of use and possession of the restricted article(s);

   c. A summary of any significant changes to the permittee's operation, personnel, and/or procedures; and

   d. Any significant events that occurred at the permittee's site.

10. The permittee shall have available a procedural or biosafety manual for review and approval by the PQB at the time of initial site inspection and any subsequent post-entry inspection(s), which identifies the hazards that will or may be encountered, and which specifies practices and procedures designed to minimize or eliminate risks of theft, exposure, or contamination, and/or accidental release of the restricted article(s), including the risk of introduction and spread of pests that may be associated with the restricted article(s) into the environment. The permittee shall adhere to all practices and procedures as stated in this biosecurity manual.

11. It is the responsibility of the permittee to comply with any applicable requirements of municipal, state, or federal law pertaining to the restricted article(s). The permittee shall also comply with University of Hawaii Institutional Biosafety Committee instructions.

12. The permittee shall submit to the PQB chief a copy of all valid licenses, permits, certificates or other similar documents required by other agencies for the restricted article(s) and operation of the facility where the restricted article(s) are safeguarded. The permittee shall immediately notify the PQB Chief in writing when any of the required documents are suspended, revoked, or terminated. This permit may be amended, suspended, or cancelled by the PQB Chief upon suspension, revocation, or termination of any license, permit, certificate, or other similar document required for the restricted article(s) or operation of the facility safeguarding the restricted article(s).

13. Upon completion or termination of the use of the restricted article(s), the restricted article(s) shall be destroyed by autoclaving. In the event autoclaving is not possible, the permittee shall obtain written authorization from the PQB chief for an appropriate alternate method of destruction.

14. The permittee shall immediately notify the PQB chief verbally and in writing under the following circumstances:

    a. Any changes to the approved sites, facilities, procedures, or equipment used to contain the restricted article(s). Any such changes must be approved by the PQB and in compliance with permit conditions prior to implementation.

    b. If any theft, accidental release, disease outbreaks outside of containment, or other exposure and/or pest emergence involving or suspected to involve the restricted article(s) under this permit, occurs.

    c. If a shipment of the restricted article(s) is delivered to the permittee without a PQB "Passed" stamp, tag or label affixed to the article, container or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State. Under this circumstance, the permittee shall not open or tamper with the shipment. Additionally, the permittee shall secure all restricted article(s), shipping containers, shipping documents and packing materials for the PQB.

    d. If the permittee will no longer import and/or possess the restricted article(s) authorized under this permit. Under this circumstance, the permittee shall inform the PQB Chief of the final disposition for the restricted article(s), submit a final report on the method of destruction of

the restricted article(s) to the PQB chief within 30 days of completion or termination of the use of the restricted article(s), and the permit will be cancelled.

15. Any violation of the permit conditions may result in citation, permit cancellation, and enforcement of any or all penalties set forth in HRS §150A-14.

16. A cancelled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancellation, any restricted article(s) imported may be moved, seized, treated, quarantined, destroyed, or sent out of state at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee.

17. The permit conditions are subject to cancellation or amendment at any time due to changes in statute or administrative rules restricting or disallowing import of the microorganisms or due to Board action disallowing a previously permitted use of the restricted article(s).

18. The permittee is responsible for all costs, charges, or expenses incident to the inspection, treatment or destruction of the restricted article(s), as provided in Act 173, Session Laws of Hawaii 2010, Section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

19. The permittee shall comply with the appropriate Centers for Disease Control and Prevention and National Institutes of Health Biosafety Level guidelines for laboratory facility for safety equipment, standard microbiological practices and special practices as found in the current edition of the *Biosafety in Microbiological and Biomedical Laboratories*, as described in the permittee's procedural or biosafety manual.

20. These permit conditions are subject to amendment by the PQB Chief to conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

21. The permittee shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents, employees, and the Board of Agriculture members for any and all claims against the State of Hawaii, its officers, agents, employees or Board of Agriculture members that may arise from or be attributable to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the federal or state employee is a permittee in the employee's official capacity.

Monkeypox Virus, Clade IIb
Saguna Verma, University of Hawaii at Manoa
November 26, 2024

Board

STAFF RECOMMENDATION: Based upon internal review, the recommendations and comments of the Advisory Subcommittee on Virus, and the Advisory Committee on Plants and Animals' recommendation to approve this request, the Plant Quarantine Branch recommends approval of this request with the proposed import permit conditions.

Respectfully Submitted,

JONATHAN HO
Manager, Plant Quarantine Branch

CONCURRED:

GREG TAKESHIMA
Acting Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

SHARON HURD
Chairperson, Board of Agriculture