NOTICE **SUBMIT TO uhpermit@hawaii.edu** NOTICE

PQ-7 (01/04)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

Attachment 1

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No._____ Date: _____

☐ Disapprove ☐ Other _____

Processed by: _____ Date: _____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: 03/6/2024

In accordance with the provision of Chapter 4-71, Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Attachment 1

Please type or print clearly.

Quantity Various or #,vol, wt.	Commodity Bacteria/Virus/Protozoan/Plasmid/DNA/Cell Line Catalog Number if applicable	Scientific Name SPELL OUT !! NO ABBREVIATIONS
2-3 x 1 ml Vial	Monkeypox Virus (MPXV) clade II from BEI Resources	Monkeypox Clade IIb (hMPXV/USA/MA001/2022, Lineage B.1, Clade IIb)
	Product sheet attached	

Name and address of shipper: BEI resources,10801 University Boulevard, Manassas, VA 20110

(Mainland or Foreign address)

Approximate date of arrival: May 1, 2024

Mode of Shipment: ☐ Mail ☑ Air Freight ☐ Boat

Type of Permit:

--- Import
☐ one time only ☑ multi-shipments

--- Intrastate shipment
☐ one time only ☐ multi-shipments

☐ Possession

Object of importation:

☐ Kept caged at all time
☑ Used for propagation
☐ Imported for exhibition
☐ Imported for liberation
☐ Other purposes - specify LABORATORY RESEARCH

Please type or print clearly.

Applicant's Name Saguna Verma

Company Name University of Hawaii at Manoa, JABSOM
(if applicable)

Hawaii Mailing Address Dept of Tropical medicine, BSB 320, 651 Ilalo Street, JABSOM, Honolulu, HI, 96813

Telephone number 808-692-1662

Facsimile number saguna@hawaii.edu

Fee Amount Enclosed (cash, check or mail order) $ 250
For expedited review

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or 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 WHO to declare this outbreak. as a Public Health Emergency of International Concern. Currently, drugs and vaccines designed to treat smallpox are the only options to treat MPXV infections. Several unknowns hamper our ability to control virus transmission and develop novel therapeutics, and vaccines. There is an urgent need to understand both, the basic biology of MPXV and its pathogenesis mechanisms. Our objective is to join the global effort to combat MPXV disease. (more details attached)

2. Person responsible for the organism (include name, address and phone number).

   Saguna Verma, PhD
   Professor and Graduate Co-Chair,
   Department of Tropical Medicine, Medical Microbiology and Pharmacology
   John A. Burns School of Medicine
   University of Hawaii at Manoa
   651 Ilalo Street, BSB 320E
   Honolulu, HI 96813

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

   Facility of virus storage and safeguard: MPXV clade II will be stored at two locations at the JABSOM- in a -80oC freezer dedicated to store viruses in the BSL2+ (stock virus in small quantiity for experiments) and BSL3 (storage of stock virus vials). Location of use: BSL2+ suite on the 3rd Floor, and BSL3 suite on the first floor, BSB, 651, Ilalo Street, Honolulu, Hawaii

4. Method of disposition.

   As per CDC, the most accepted method to inactivate waste generated in MPXV clade II studies is autoclave of solid waste and treatment with 10% sodium hypochlorite for liquid waste. All liquid waste 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.
   More details attached

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

   MPXV clade II does not infect plants. MPXV has been reported in some rodent species in endemic regions like Central Africa but those rodents are not found in Hawaii. 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. This virus will be confined to the aforementioned laboratories, which are high security locations. All personnel undergo rigorous training prior to entering the laboratories, and wearing of appropriate PPE is enforced. Therefore, the likelyhood of MPXV clade II impacting wild life In hawaii is extremely low to none. More details attached. Emergency response measures are included in the attachments.

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I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature _____ **Date** 3-6-2024

(Applicant)

List of attached documents in PDF

1. CV of the PI
2. PDF of CDC link for the classification of MPXV clade I and II and biosafety level and procedures. This link refers to follow guidelines from BMBL book, chapter VIII-E Viral Agents for culture work.
3. Pages 269-270, Chapter VIII-E Viral Agents on MPXV from BMBL 6th edition book that explains working with MPXV and other pox viruses in different biosafety levels.
4. Printout of CDC information on monitoring and risk assessment of MPXV disease
5. Product sheet of MPXV clade II

Curriculum Vitae

Name: **Saguna Verma, Ph.D.**

Current title & department: Professor and Graduate Co-Chair, Department of Tropical Medicine, Medical Microbiology and Pharmacology, John A. Burns School of Medicine

Current Address: 651 Ilalo Street, BSB 320-E, Honolulu, Hawaii 96813
Telephone: 808-692-1662, Fax: 808-692-1984
E-mail: saguna@hawaii.edu

Professional Training and History

Education

1982-85	B.S.	Zoology, Chemistry, Botany; Devi Ahilya University, Indore, India
1985-87	M.S.	Biochemistry; Devi Ahilya University, Indore, India
1989-93	Ph.D.	Life Sciences (Concentration: Endocrine Biochemistry); Devi Ahilya University, Indore, India

Professional Positions Held

1989-1991	Junior Research Fellow supported by Council of Scientific & Industrial Research (CSIR) Fellowship, School of Life Sciences, Indore, India
1992-1993	Senior Research Fellow supported by CSIR, School of Life Sciences, Indore, India
1994-1999	Project Scientist, Genes and Proteins Laboratory, National Institute of Immunology, New Delhi, India
1999-2002	Break in career to raise two kids
2003-2005	Junior Researcher, Retrovirology Research Laboratory, Department of Tropical Medicine and Medical Microbiology (DTMMMP), John A. Burns School of Medicine (JABSOM), University of Hawaii at Manoa (UHM), Honolulu, Hawaii
2005-2010	Assistant Researcher, DTMMMP, JABSOM, UHM, Honolulu, Hawaii
2010-2014	Assistant Professor (tenure-track), DTMMMP, JABSOM, UHM, Honolulu, Hawaii
2014-2016	Associate Professor (tenure-track), DTMMMP, JABSOM, UHM, Honolulu, Hawaii
2016-2020	Associate Professor (tenured), DTMMMP, JABSOM, UHM, Honolulu, Hawaii
2020-	Professor, DTMMMP, JABSOM, UHM, Honolulu, Hawaii
2019-	Graduate Co-Chair, DTMMMP, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, Hawaii

Awards and Honors

1985	Merit Scholarship in B.S. by the Devi Ahilya University, Indore, India
1989-1993	University Grants Commission-Council of Science and Industrial Research Fellowship (CSIR), India
1992	Madhya Pradesh Council of Science and Technology, India YOUNG SCIENTIST, Merit certificate in 7th Young Scientist conference organized by Council of Science and Technology, India
2005	Hawaii State-Biomedical Research Infrastructure Network (BRIN) Travel Award to attend the Symposium on Kawasaki Disease, San Diego, California
2011	J. Immunology paper selected for *'In this Issue'* highlighting top papers in February issue, Journal of Immunology
2012	Invited speaker, Keystone Symposium '*Innate Immunity: Sensing the Microbes and Damage Signals*' in Keystone, Colorado

2013	Invited speaker at the `*Block symposium*' of the Annual Meeting of American Association of Immunologists
2015	Recipient of the `*Careers in Immunology Fellowship'* award by the American Association of the Immunologists
2017	Invited speaker at the '*NIH/NIAID Symposium: Molecular Mechanisms and Immune Consequences of Pathogen Reservoirs'* at Rockville, Maryland
2018	*J. Virology* 2017 paper on the Zika virus infection and host response in human testicular cells was highlighted in the Editors news story in the Jan 2018 issue of *Nature Medicine*
2019	Recipient of American Association of Immunologists Laboratory Travel grant to cover the cost of PI and a student to attend AAI annual meeting in San Diego
2019	Keynote speaker at the prestigious joint meeting of North American Testis Workshop/American Socciety of Andrology held on April 3-6, 2019 in Chicago
2020	Invited speaker at the meeting 'Viruses' at Barcelona, Spain, February 2020
2021	Invited speaker at the annual meeting of Society of Study of Reproduction, December 2021

Society Memberships

2008-present	American Society of Microbiology
2009-present	American Society of Virology
2013-present	American Association of Immunologists

Teaching and Mentoring

Instructional Activities

Courses taught for the Department of Tropical Medicine, Medical Microbiology and Pharmacology as Instructor

2006	TRMD 699, `*Oxidative stress and human diseases*, (1 credit) – Co-Instructor of the Journal club portion of this graduate directed research course (16 hr)
2007	TRMD 699, `*Inflammation and human diseases*' (1 credit) – Co-Instructor of the Journal club portion of this graduate directed research course
2009	TRMD 690 (1 credit) – Instructor, Seminar of Tropical Medicine, Medical Microbiology and Pharmacology TRMD 604, guest lecture on `*Inflammasomes as guardian of cytosolic sanctity in infection and immunity*'
2010	TRMD 699, Directed research mentoring (1 M.S. student, 3 credits) TRMD 609, Advanced Medical Immunology- Co-instructor of this graduate advanced level course (3 credits). Topic covered was `*Vaccine adjuvants and innate immune responses'* (8 hr) TRMD 604, guest lecture on `*Inflammasomes as guardian of cytosolic sanctity in infection and immunity*' TRMD 601, `*Inflammation and human diseases*' (1 credit)
2011	TRMD 705, Infection and Immunity (3 credit hrs)- Instructor of this advance immunology course TRMD 604, guest faculty to teach advances in innate immunity TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring (2 M.S. students, TMMMP and MBBE, 6 credits)
2012	TRMD 604, guest faculty to teach advances in innate immunity TRMD 699, Directed research mentoring to 2 Ph.D students (total 6 credits)

2013	TRMD 610, Infection and Immunity (3 credits)- Instructor of advance immunology course TRMD 604, guest faculty to teach advances in innate immunity TRMD 699, Directed research mentoring to 2 Ph.D students (total 8 credits)
2014	TRMD 609, Advanced Medical Immunology- Co-instructor of this graduate advanced level course (3 credits). Topic covered was `*MyD88 signaling'* (8 hr) TRMD 604, guest faculty to teach advances in innate immunity TRMD 699, Directed research mentoring to 2 Ph.D students (total 8 credits)
2015	TRMD 610, Infection and Immunity (3 credits)- Instructor of advance immunology course TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2016	TRMD 604, guest faculty to teach advances in innate immunity TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2017	TRMD 610, Infection and Immunity (3 credits)- Instructor of advance immunology course TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2018	TRMD 609, Advanced Medical Immunology- Co-instructor of 3credit course Topic covered was `*cross-talk between PRRs* (10 hr) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2019	TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2020	TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits) TRMD 610, Infection and Immunity (3 credits)- Instructor of advance immunology course
2021	TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits)
2022	TRMD 601, `*Inflammation and human diseases*' (1 credit) TRMD 699, Directed research mentoring to 2 graduate students (total 8 credits) TRMD 610, Infection and Immunity (3 credits)- Instructor of advance immunology course
2023	TRMD 699, Directed research mentoring to 3 graduate students (total 10 credits) TRMD 654, Advances in HIV/AIDS (2 credits)- Instructor of advance immunology course

Course developed and approved in 2011: TRMD 610 (Infection and immunity) for PhD level students: A three credit advanced course covering special topics in (i) Recognition of pathogen (ii) Cross talk between innate and adaptive immunity (iii) Cutting edge topics in infectious disease immunity and (iv) CNS infections

Problem-based learning (PBL) lectures for JABSOM

2009-present	Biochemistry unit MD3 course for the JABSOM First year medical students on `*Lysosome function and Disorders'* Biochemistry unit MD3 course for the JABSOM First year medical students on `*Vitamins*'

Biochemistry unit MD4 course for the JABSOM First year medical students on `*Steroid metabolism'*
Biochemistry unit MD3 course for the JABSOM First year medical students on `*RNA replication and translation'*
Biochemistry unit MD4 course for the JABSOM First year medical students on `*Vitamin D metabolism'*
Gastroenterology sub-unit MD4 course for the JABSOM First year medical students on ` *Immunopathology of Inflammatory Bowel Disease'*

Invited lectures in other departments

Fall 2007-2014	MBBE 651, `*Toll like Receptors as Sensors of Microbial Pathogens: Bringing Specificity to Innate Immunity'*
Spring 2008	MICR 690, `*Analysis of Disease Related Genes in Kawasaki Disease'*
Spring 2010	MICRO 690 on `*Can blocking the blood-brain barrier disruption improve WNV-associated neurological sequeale'*
Spring 2015	TROPMED 431 `Immunology of parasitic infection'
Spring 2013-present	CMB 622 on 'Neuroinflammation and blood-brain barrier'
Fall 2022	CMB621 on 'Introduction to Virology and antiviral immunity'

Invited talks in scientific and public meetings

Spring 2008	Invited lecture at the Pediatric CME program on `Kawasaki Disease: Blood-based markers for KD diagnosis'
Spring 2009	Invited public lecture `*Biology of West Nile virus'* for the JABSOM faculty and staff (1 hr lecture)
Spring 2010	Guest lecture RCMI forum on `*Can blocking the blood-brain barrier disruption improve WNV-associated neurological sequeale'*
Fall 2012	Invited oral presention at the Keystone Symposium 'Innate Immunity: Sensing the Microbes and Damage Signals'
Spring 2013	Invited oral prestention at the block symposium of the Annual Meeting of American Association of Immunologists
Summer 2013	Invited speaker at the special seminar series at Georgetown University, DC Invited speaker at the John Hopkins Bloomberg School of Public Health, DC
Summer 2014	Invited speaker at the seminar at the National Brain Research Institute, Manesar, India Invited speaker at the seminar at the Trsnslation Health Science Technology Institute, Gurgaon, India Invited speaker at the seminar at the School of Biotechnology, Jawaharlal Nehru University, India
Fall 2016	Visiting Professor at Jawaharlal Nehru University, India Invited speaker at the Viral Disease Biology program at the Rajiv Gandhi Center of Biotechnology, India
Fall 2017	Invited speaker at the *'NIH/NIAID Symposium: Molecular Mechanisms and Immune Consequences of Pathogen Reservoirs'* at Rockville, Maryland
Fall 2018	Invited speaker at the seminar at the Wake Forest Intitute of Regenerative Medicine, Winston-Salem, NC Invited speaker at the seminar at the United State Army Research Institute of Infectious Disease (USAMRIID), Fort Detrick, Maryland
Spring 2019	Keynote speaker at the prestigious joint meeting of North American Testis Workshop/American Socciety of Andrology held in April 2019 in Chicago

Summer 2019	Invited speaker at the special seminar series at Center for Craniofacial Molecular Biology, University of Southern California, California
2020	Invited speaker at the meeting 'Viruses' at Barcelona, Spain, February 2020
2021	Invited speaker at the annual meeting of Society of Study of Reproduction, December 2020

Students Mentored for research:
Graduate students directly mentored as Chair: 9
Graduate students mentored as committee members: >20
Undergraduate students directly mentored: 10
International medical school students directly mentored: 2
Post-doctoral fellow mentored: 4

Research support

Hawaii Community Foundation (PI: Verma) 12/1/2023-6/30/2025
Understanding long-term effects of SARS-CoV-2 on testicular complications in hACE2 mice
Goal of this study is to define the short and long-term effects of SARS-CoV-2 infection on testicular injury and testis function in hACE2 mouse model

Hawaii Community Foundation (PI: Tallquist and Verma) 12/1/2020-6/30/2023
Modeling SARS-CoV-2 cardiac complications in hACE2 mice
Goal of this study is to define how SARS-CoV-2 infection affects cardiac fibroblasts, especially in the presence or absence of fibrosis from myocardial infarction in hACE2 mouse model

PIKO Pilot Project- Year 1 (PI: Verma) 6/1/2022-5/30/2023
Association of SARS-CoV-2 proteins with COVID-19 disease in Hawaii
Goal: To assess the levels of circulating virus antigens in COVID-19 patients and its association with disease severity

INBRE Collaborative Research Opportunity Award (MPI: Horgen and Verma) 7/1/2022- 6/30/2023
Screening for anti-SARS-CoV-2 activity of marine extracts from Hawaii
Goal: To screen natural marine extracts library from Hawaii for anti-SARS-CoV-2 properties

NIH-R21AI140248-01 (PI: Verma and Sadri-Ardekani) 5/18/2019-5/17/2022
Human testicular organoids as a model to dissect cell-type specific tropism and immune response to ZIKV
Goal is to use single-cell RNA sequencing approach to identify aniviral and cell death pathways modulated by ZIKV

Hawaii Community Foundation (PI: Verma) 5/1/2018-12/30/2022
AXL Receptor Regulates Zika Virus Entry and Immune Response in Human Testicular Cells
Goal of this study is to define mechansism of ZIKV entry in the testicular cells

FERRING COVID-19 Investigational Grant in Reproductive Medicine (PI: Sadri-Ardekani)
SARS-CoV-2 infection in human testis 3D organoid model 8/1/2020-7/31/2021
Goal is to assess if ACE2 expressing cells in the human testes can support SARS-CoV-2 infection
Role: Collaborator

NIH R21 AI129465-02 11/01/16 - 10/31/2019

Under attack: Modulation of the blood-testes barrier by Zika virus
Goal: To understand the mechanisms by which ZIKV establishes infection in the testes
Role: Principal Investigator

COBRE pilot grant 08/01/2017 - 07/30/2018
RNAseq analysis of the persistently infected tissue-reservoirs of the Zika virus
Goal: To understand tissue-specific responses to ZIKV' by using highly relevant nonhuman primate model that mimics human ZIKV disease
Role: Principal Investigator

American Association of Immunology 09/01/15-9/30/16
NLRC5 in immune control of WNV
Goal: To understand how NLRC5 affects innate-adaptive interface, specifically expression of MHC class I molecules in WNV infection
Role: Principal Investigator

Hawaii Community Foundation 07/01/14-12/30/16
Role of NLR Family protein 5 in flavivirus pathogenesis
Goal: To understand the mechansims associated with cytokine production and inflammatory response to WNV
Role: Principal Investigator

Pacific Center for Emerging Infectious Disease Research 08/01/10-07/30/15
NIH - COBRE (Yanagihara)
Project 2: Molecular mechanisms of West Nile virus neuroinvasion (PI: Verma)
The major goals of this project are to analyze matrix metalloproteinase and urokinase plasminogen activator signaling pathway in blood-brain barrier disruption after West Nile virus infection using both, in vitro and in vivo models.

NIH - R01AI089999-01 (Hoffman) 08/01/10-07/30/15
Selenoprotein K modulates calcium-dependent signaling in immune cells
The major goals of this project are to determine selenoprotein K (SelK) dependent immune responses and use WNV infection model to characterize role of SelK in disease pathogenesis.
Role: Collaborator

RTRN- RCMI 07/01/13-06/30/14
Epigenetic regulation of innate immune responses to flaviviruses
The goal of this project is to understand the role of epigenetic modulation, specifically histone deacetylases in regulating production of WNV-induced inflammatory cytokines.
Role: Principal Investigator

5 G12 RR/AI03061-21 (Shomaker/Yanagihara) 09/01/06-08/31/11
NIH/NCRR
Research Centers in Minority Institutions Program
Research Outcomes Accelerating Discoveries for Medical Applications and Practice
Activity 3 Tropical Infectious Diseases and Prevention
The proposed Tropical Infectious Diseases Detection and Prevention Core activity consisting of the Pathogen Reference and Reagent Core Facility, Molecular Pathology and Histology Core Facility and the Microarray Core Facility responds to an urgent local, regional and national need to position UHM in a leadership role to detect exotic infectious diseases that may be introduced to Hawaii and the continental United States from Asia. Our expectations are that at the end of the grant period, UHM will

be one of the premier institutions for tropical infectious diseases research and training in Asia and the Pacific.
Role: Molecular Virologist

Clinical and Translational Science Bridging Fund 09/01/09-08/30/10
NIH-RCMI (Yanagihara)
Cyclooxygenase 2 and glial cells – Role in WNVE-associated neuroinflammation
The major goal of this project is to determine the role of WNV-induced COX-2 in triggering pro-inflammatory cytokines and MMPs in the brain glial cells
Role: Principal Investigator

20050001 (Verma) 03/01/08-10/30/09
Hawaii Community Foundation
Role of Human Brain Microvascular Endothelial Cells in West Nile Virus Central Nervous System Invasion
The major goals of this project are to determine and delineate the mechanism(s) of infection and injury induced by West Nile virus to human brain microvascular endothelial cells and the trafficking of cell-free WNV into the CNS.
Role: Principal Investigator

Role: Principal Investigator
20050405 (Verma) 07/01/05-04/30/08
Hawaii Community Foundation
Oxidative Stress and its Implications in the Pathogenesis of West Nile Virus Infection
The goal of this project was to delineate the pathophysiological mechanisms underlying oxidative stress-induced disease pathogenesis.
Role: Principal Investigator

Pacific Center for Emerging Infectious Disease Research 01/01/04-06/31/05
NIH- P20 RR 018727 (Yanagihara)
Effects of Selenium deficiency on genomic mutations of RNA viruses.
The primary goal of this project was to investigate how West Nile virus may be affected by selenium deficiency within the cells in which they replicate.
Role: Principal Investigator

20050411 (Melish) 06/01/05-06/07/07
Hawaii Community Foundation
Kawasaki Disease: The Diagnosis Project- Host response
The goal of this project is to employ gene array technology to analyze specific host genes and signaling pathways involved in the pathogenesis of Kawasaki syndrome.
Role: Co-Investigator

Service activities

DTMMMP service

2010-present	Member of Department of TMMMP Graduate Student Selection Committee
2010	Served in the Selection Committee for the recruitment of `Infectious Disease Pathologist' in the TMMMP
	Served in the Selection Committee for the recruitment of `HIV Immunologist' in the TMMMP
2012	Served in the Selection Committee for the recruitment of 'Institutional Support Position # 79325' in the TMMMP
2015-present	Reviewer and Interviewer of the applicants for MHIRT funded program, DTMMMP

2016 Served in the Selection Committee for the recruitment of 'Virologist' in the TMMMP
2018-present Graduate Co-Chair, TMMMP

UH Manoa campus wide service

2013-2019 Voting member of the University of Hawaii's Institutional Animal Care and Use Committee
2015-2020 Reveiwer of the proposals for Undergraduate Research Opportunities Program (UROP) funding
2019- Member of the Honors Council that guides 60+ yr old Honors Program at the UH at Manoa

JABSOM service

2010-2013 Member of the Medical Students Selection Committee of the JABSOM, UHM (>80 hr per year)
2013-present Judge for the poster session at the JABSOM Biomedical Symposium
2014-2017 Interveiwer for the JABSOM Medical student applicants (30 hrs per year)
2015 Member of the committee for selecting speakers for 50th Anniversary celebration of the JABSOM
Served in the Selection Committee for the recruitment of `Associate Specialist' in the Dean's office
2014 Pilot grant reviewer for Hawaii Pediatric Association Research and Education Foundation, HI
2015-present Reviewer and Interveiwer for the Northern Pacific Global Health (NPGH) program Fellowship applicants
2017-18 Member of the committee for selection of the Associate Director of Research, JABSOM
2019 Member of the Medical Students Selection Committee of the JABSOM, UHM (>80 hr per year)
2021- Co-organizer of the JABSOM Annual BioMed Symposium

Public and professional services

Editorial Board

2013-present Editorial Board Member of the journal *Clinical Microbiology* published by OMICS Group Inc, NY, USA.
2019- Editorial Board Member of the journal *Scientific Reports*, Nature pulishing Group

Ad hoc Reviewer

Journals 2009-present-

Adhoc Reviewer for PNAS, Journal of Virology, Journal of General Virology, PLoS ONE, Virology, Japanese Journal of Infectious Diseases, Journal of Neuroinflammation, Journal of Genetics, Journal of Functional Foods, Mediators of Inflammation, Journal of Neurochemistry, Journal of Immunology, Microrganisms, PloS Neglected and Tropical Diseases, Journal of Virology, Plos Pathogens, Frontiers in Microbiology, AIDS Research and Human Retroviruses, Viruses, Scientific Reports and Nature Communications

Grant Reviews

2023 External reviewer of grant from French National Research Agency
2021-22 Member, NIH/CSR of **Virology B** (February 2021, June and October 2022)
2020 Member, NIH/CSR of **Clinical Neuroimmunology and Brain Tumor** (February 2020)

	Member, NIH/CSR of special emphasis panel **ZRG1-IDM-W-02** (August 2020)
2019	Member, NIH/CSR of **Topics in Virology, ZRG1 IDM-W** (November)
	Member, NIH/CSR of **Clinical Neuroimmunology and Brain Tumor** (June)
	Member, NIH/CSR of **Development and Brain Disorder** (June)
	Member, NIH/CSR of **ZRG1 IMM-R (50)** (July)
2018	Member, NIH/CSR of **Clinical Neuroimmunology and Brain Tumor** (February)
	Member, NIH/CSR of **ZRG1 BDCN-Q** (June)
	Member, NIH/CSR of **ZRG1 BDCN-M (91) S** (December)
2017	Member, NIH/CSR of **Clinical Neuroimmunology and Brain Tumor** (June)
	Member, NIH/CSR of **Clinical Neuroimmunology and Brain Tumor** (October)
	Member, NIH/CSR of **ZRG1 BDCN-Q** (November)
2016	Member, NIH/CSR of **Development and Brain Disorder** (October)
2015-16	Pilot Grant proposals for Clinical Translational Research-Infrastructure Network, USA
2014	COBRE Pilot small grant proposals
2015-	Fellowship proposals for the Northern Pacific Global Health program, USA
2015-	Research proposals for the UROP Program, UH
2014	RCMI Translational Research Networks Small Grants Pilot Program award, USA
2008-13	Hawaii Community Foundation, HI
2013	The Deutsche Forschungsgemeinschaft (German Research Foundation), Germany

Others

2008-2013	Member of the Scientific Advisory Committee of the Hawaii Community Foundation
2014-2016	President of American Society of Microbiology (Hawaii Chapter)
2019	External Doctorate Thesis Examiner, Monash University, Australia,

Publications in peer reviewed journals/Books

Book chapters

1. Kelly J and **Verma S.** Book chapter 'Flaviviruses' in the book 'Neuroinfections', edited by Paweł P. Liberski, Wojciech Kozubski and Michael Katz Warsaw, Poland, 2015, pp. 167-175.
2. GKaur, KWright, **S Verma**, A Haynes, JM Dufour, *The good, bad and ugly of testicular immune privilege* published in 'Molecular mechanisms in Spermatogenesis' (2021, Publisher: Springer Nature)

Original articles:

1. Giannakopoulos S, Pak J, Ward M, Bakse J, Tallquist M, and Verma S. SARS-CoV-2 infection leads to persistent testicular injury and functional impairments that resolve within a month of recovery in K18 hACE2 mice. 2024, in review *Nature Communications.*
2. Giannakopoulos S, Strange D, Jiyarom B, Abdelaal O, Bradshaw AW, Nerurkar VR, Ward M, Bakse J, Yap J, Vanapruks S, Boisvert W, Tallquist M, Sadri-Ardekani H, Clapp P, Murphy SV, **Verma S.** In vitro evidence against productive SARS-CoV-2 infection of human testicular cells: Bystander effects of infection mediate testicular injury. PLoS Pathogens 2023 May 18;19(5):e1011409.
3. Jiyarom B, Giannakopoulos S, Strange DP, Panova N, Gale M Jr, **Verma S.** RIG-I and MDA5 are modulated by bone morphogenetic protein (BMP6) and are essential for restricting Zika virus infection in human Sertoli cells. Front Microbiol 2022: 13: 1062499 doi: 10.3389/fmicb.2022.1062499
4. **Verma S,** Saksena S and Sadri-Ardekani H. ACE2 receptor in testes: Implications in COVID-19 pathogenesis. *Biology of Reproduction*, 2020 PubMed PMID 32427288

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Press articles and community Interveiws since 2013

January 2018: Interviewed by Shraddha Chakradhar, Associate News Editor, *Nature Medicine* for her story on testes immune privilege and our research on Zika virus
June 2018: Interviewed by the JABSOM and UH News team for second Zika virus grant funding

April 2017: Presented talk as guest speaker on the World Malaria Day celebrated by the Student Immunization Initiative, Hawaii Chapter
May 2017: Interviewed by the *Hawaii Business Magazine* for the story on recent Zika virus research. Story published in the June edition of the magazine
December 2016: Interviewed by the JABSOM and UH News team for first Zika virus grant funding at UH
August 2014: Interviewed by the Assitant News Editor of a national TV channel in India, RajyaSabha TV

Centers for Disease Control and Prevention

Mpox

Mpox Home

Biosafety Laboratory Guidance for Handling and Processing Mpox Specimens

Updated December 8, 2023

> Category B (UN3373) categorization includes infectious substances transported for diagnostic or investigational purposes. Submitters should follow all appropriate Category B regulations for packaging and transporting specimens from suspect mpox patients for diagnostic testing.

All clinical specimens may contain potentially infectious agents or organisms. Take precautions when handling specimens suspected or confirmed positive for mpox virus. Timely communication between clinical and laboratory staff is essential to minimize the risk of laboratory transmission when handling and testing specimens from patients with possible mpox. Label specimens accordingly and alert the receiving laboratory to ensure that specimens are appropriately handled. Correct handling and storage of specimens during transportation are essential for accurate diagnostic testing.

General Guidance

Mpox virus is a member of the *Orthopoxvirus* genus within the *Poxviridae* family. Some federal regulations and guidelines apply to work conducted with the mpox virus. See Select Agent Regulations.

According to Advisory Committee on Immunization Practices (ACIP) recommendations, employers should offer pre-exposure orthopoxvirus vaccination to workers at risk of occupational exposure. Two vaccines may be used to prevent mpox disease, JYNNEOS and ACAM2000. Individuals are considered fully vaccinated 14 days after the second dose of the JYNNEOS vaccine or four (4) weeks after the ACAM2000 vaccination. The Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th edition recommends vaccination for laboratorians who work directly with viral cultures or animals contaminated or infected with replication-competent orthopoxvirus (e.g., mpox virus). The BMBL and the ACIP recommend booster doses of JYNNEOS every 2 years and ACAM2000 every 3 years for people at occupational risk for virulent replicating orthopoxviruses (e.g., mpox virus). They also recommend booster doses at least every 10 years for those at occupational risk for less virulent orthopoxviruses (e.g., cowpox virus and vaccinia virus).

As with all procedures, laboratories should perform a site-specific and activity-specific risk assessment to identify and mitigate risks. Risk assessments and mitigation measures depend on the following:

- The procedures performed
- The hazards involved in the processes and procedures
- The competency level of the personnel who perform the procedures
- The laboratory equipment and facility
- The resources available
- The vaccination status of the personnel who perform the procedures

Follow Bloodborne Pathogens – Worker protections against occupational exposure to infectious diseases | Occupational Safety and Health Administration (OSHA) when handling clinical specimens, all of which may contain infectious agents or organisms. These recommendations include hand hygiene and specific personal protective equipment (PPE) determined by

the potential for exposure to blood, body fluids, and infectious material. PPE, such as laboratory coats or gowns, gloves, eye protection, respiratory protection, and face shield, can help protect the skin and mucous membranes of the eyes, nose, and mouth. Avoid procedures that could generate infectious aerosols.

For more information, see:

- Biological Risk Assessment: General Considerations for Laboratories
- Core Infection Prevention and Control Practices for Safe Healthcare Delivery in All Settings
- Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard
- Occupational Safety and Health Administration (OSHA) Personal Protective Equipment Standard
- Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition, Section II – Biological Risk Assessment, pages 9-20 and Section IV -Laboratory Biosafety Level Criteria, pages 32-69
- Mpox: Experts Give Virus Variants New Names

Select Agent Regulations

Specimens specifically identified as Clade I **monkeypox virus** are regulated as a select agent (SA). Entities that possess, use, or transfer this material must comply with the HHS Select Agent and Toxin Regulations [42 CFR § 73]. Specimens specifically identified as Clade II monkeypox virus are excluded from SA regulations. However, if a generic mpox test that does not identify the clade was used, the material is regulated unless another exemption or exclusion applies.

Specimens identified as orthopoxvirus or non-variola orthopoxvirus are not select agents and, thus, are not regulated material. See SA Grams – 2022 | Resources | Federal Select Agent Program (selectagents.gov) for more information on the 2022 U.S. Mpox Outbreak & FSAP Regulations.

Biosafety Considerations for Diagnostic Testing

Facilities that process and test mpox lesion materials including swabs of lesion surface and exudate, and lesion crusts, should have the necessary equipment, engineering controls, personal protective equipment, appropriate diagnostic assays, and properly trained personnel. If the appropriate safety equipment or protocols are unavailable, consider referring specimens to an equipped reference laboratory that meets the recommendation above.

- Perform routine diagnostic specimen processing in Biosafety Level 2 (BSL-2) laboratory facilities following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments. Additional precautions to reduce exposure risk may include, but are not limited to:
  - Solid-front gowns with cuffed sleeves
  - Double gloves
  - Eye protection (safety glasses, snugly fitting goggles) or face protection (face-shield)
  - NIOSH-approved particulate respirator equipped with N95 filters or higher
  - Limiting the number of laboratory personnel who work during specimen manipulation
  - Laboratory with directional airflow
- Manipulate diagnostic specimens in a certified Class II Biosafety Cabinet (BSC) or other containment devices, especially if there is a potential to generate aerosols (e.g., vortexing or sonication of specimens in an open tube). Do not work with open vessels on the bench top unless it is safe to do so based on site and activity-specific risk assessments (i.e., the specimen has been fully inactivated utilizing an approved inactivation method).
- If you cannot perform a procedure within a BSC, use a combination of PPE and other containment devices (e.g., glove box, centrifuge safety cups, or sealed rotor) designed to create a barrier between the specimen and the laboratory personnel. Perform site-specific and activity-specific biosafety risk assessments to determine if your situation warrants additional biosafety precautions.

For further details, see:

- Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, Section IV – Laboratory Biosafety Levels, pages 37-43 and Appendix N – Clinical Laboratories, pages 529-541

Routine Diagnostic Testing

If a patient is suspected or confirmed for mpox virus infection, testing to evaluate other illnesses on the clinical differential should continue while awaiting orthopoxvirus test results. Implement specific biosafety precautions depending on the specimen tested.

- For routine clinical procedures and testing of non-lesion specimens such as urine for urinalysis, blood for analysis [e.g., complete blood count (CBC), chemistries, microbiology] from suspected or confirmed mpox patients:
  - Perform in Biosafety Level 2 (BSL-2) laboratory facilities following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments. For additional routine diagnostic testing information, see BMBL Appendix N – Clinical Laboratories .
  - The quantity of orthopoxvirus in clinical specimens, such as blood and body fluids, is likely low. Take standard universal precautions to protect against potential infectious agents in any specimen. Consistently adhering to Standard Precautions | Section IV and biosafety protocols for protecting laboratory workers will prevent possible exposure to the mpox virus in clinical specimens. Limit the number of staff who test specimens and avoid any procedures that have the potential to generate infectious aerosols. See precaution guidance below to prevent exposures for Procedures with a High Likelihood of Generating Aerosols.
- For lesion specimens (including swabs of lesion surface and exudate, and lesion crusts) from patients who are suspected of having mpox and who are being concurrently tested for orthopoxvirus and other differentials [e.g., herpes simplex virus (HSV) or varicella-zoster virus (VZV), which are known to have the highest quantity of mpox virus]:
  - Perform in Biosafety Level 2 (BSL-2) laboratory facilities, following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 according to site-specific and activity-specific biosafety risk assessments.
  - Additional PPE, mitigation, and practices should be assessed during the risk assessment process to reduce exposure risk. See Biosafety Considerations for Testing.
- For viral culture of lesion specimens from patients suspected to have mpox for diagnostic purposes other than mpox virus (e.g., HSV or VZV):
  - Perform in BSL-2 laboratory facilities, using additional precautions based on the laboratory's site-specific and activity-specific risk assessment to identify and mitigate risks. See Biosafety Considerations for Testing.
  - As stated above, lesions are known to have the highest quantity of mpox virus. Once laboratory personnel extract the viral DNA using a validated extraction protocol, the viral DNA is non-infectious. Laboratory personnel can work in a BSL-2 laboratory facility following standard and special practices, safety equipment, and facility specifications recommended for BSL-2 with this material. Instead of culturing lesion specimens, laboratory personnel should consider using diagnostic techniques that extract DNA or RNA, if possible. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition , Section IV -Laboratory Biosafety Level Criteria, and Section VIII-E Viral Agents.

Culturing Specimens for Mpox Virus

Culture-based testing for mpox virus should not be performed as a routine diagnostic procedure in clinical or diagnostic laboratories. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition , Section IV - Laboratory Biosafety Level Criteria BSL-3, and Section VIII-E Viral Agents.

Molecular Testing and Analysis of Bacterial or Mycotic Cultures

Perform the following procedures in a BSL-2 laboratory facility following standard and special practices, safety equipment, and facility specifications recommended for BSL-2[1] :

- Molecular analysis of extracted nucleic acid preparations
- Routine examination of bacterial and mycotic cultures for diagnostic purposes

[1]BSL-2 procedures apply, unless the viral cultures are being done with lesion specimens awaiting orthopoxvirus test confirmation. Refer to the Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th edition, Section IV - Laboratory Biosafety Level Criteria BSL-3, and Section VIII-E Viral Agents, when performing culturing of lesion specimens for diagnostic purposes other than mpox virus from an individual suspected of having mpox.

Clinical and Anatomic Pathology

The practice of pathology plays a critical role in determining accurate disease diagnoses by studying organ tissues and fluids. This includes microscopic evaluation and testing of cytology, surgical biopsy, and autopsy specimens.

Risks associated with surgical pathology and some cytology procedures occur when manipulating fresh tissue and body fluids from patients who may have an unknown or known infectious disease or virus, such as the mpox virus. Risks are increased in the surgical grossing room during manual specimen handling, tissue dissection, and the preparation of frozen tissue sections using a cryostat. These procedures can result in percutaneous exposures from punctures or cuts, droplet or aerosol exposures from blood and body fluid splashes, and surfaces contaminated with the virus. Clinical laboratory and support staff must be aware of these risks and provide effective mitigation procedures.

The following pathology specimen types are considered inactivated and can be handled in accordance with BSL-2 guidelines:

- Fixed fluid or tissue smears for routine diagnostic staining and microscopic analysis
- Formalin-fixed biopsy or autopsy tissues
- Glutaraldehyde-fixed grids for electron microscopic study

Sufficient incubation time in fixative should be utilized, dependent on tissue/biopsy size, to allow adequate fixative penetration. Orthopoxviruses (such as vaccinia virus and mpox virus) may require additional incubation time in the fixative. For larger tissue samples, additional incubation time should be utilized to ensure complete inactivation of the virus.

For information, see:

- Evaluation of Virus Inactivation by Formaldehyde to Enhance Biosafety of Diagnostic Electron Microscopy
- Reassessment of the rate of fixative diffusion
- Autopsy and Handling of Human Remains | Mpox | Poxvirus | CDC

Anatomic pathology uses different procedures and workflows than clinical pathology, so the risks and mitigation control needed to protect personnel may differ. At a minimum, all personnel practicing anatomic or clinical pathology should follow Standard Precautions | Section IV when handling clinical specimens, including hand hygiene and using PPE, such as laboratory coats or gowns, gloves, eye protection, or a disposable mask and face shield, to help protect the skin and mucous membranes of the eyes, nose, and mouth. See precaution guidance below to prevent exposures for Procedures with a High Likelihood of Generating Aerosols.

Site- and activity-specific biosafety risk assessments should be performed to determine if additional biosafety precautions are warranted.

Environmental Testing

At this time, the National Wastewater Surveillance System team recommends that untreated wastewater samples be pasteurized (60°C for 1 hour) before processing if they are suspected of containing mpox virus. This is due to the potential exposure of laboratory personnel during untreated wastewater processing.

Procedures with a High Likelihood of Generating Aerosols

Laboratory exposures to poxviruses occur primarily through needle-stick injuries, direct contact with the specimen, or aerosols that laboratory procedures may generate. Conduct procedures with a high likelihood of generating aerosols (e.g., vortexing or sonication) in a certified Class II BSC. Use additional precautions to create a barrier between the specimen and personnel. These additional precautions can include centrifuge safety cups, sealed centrifuge rotors, and additional PPE to reduce the risk of exposure to laboratory personnel. Perform site-specific and activity-specific biosafety risk assessments to identify and mitigate risks and to determine if your situation warrants additional biosafety precautions. Situations that may warrant additional biosafety precautions include high testing volumes, use of pneumatic tube systems, and automated testing platforms (e.g., laboratory robotic platforms, etc.). If testing a lesion specimen from a suspected mpox patient, CDC recommends that laboratory personnel perform complete viral inactivation before putting the specimen on any automated platform or placing the platform within a Class II BSC, if available, to perform the work.

If laboratory personnel cannot perform procedures that may generate aerosols in a BSC, acceptable methods of respiratory protection include NIOSH-approved respirators with N95 filters or higher. N95 filtering facepiece respirators provide the minimum level of respiratory protection. Facilities may consider using higher levels of respiratory protection, particularly if personnel cannot be correctly fitted to tight-fitting respirator models. These higher levels may include using loose-fitting NIOSH-approved powered air-purifying respirators equipped with particulate filters.

Decontamination

Perform routine cleaning and disinfection procedures using an EPA-registered, hospital-grade disinfectant with emerging viral pathogens claim. Products with Emerging Viral Pathogens claims may be found on EPA's List Q. Follow the manufacturer's directions for concentration, contact time, and care and handling.

Reevaluate current protocols for cleaning, use of PPE, patient placement, and hand hygiene; see Standard Precautions | Section IV. For example, high-touch surfaces such as patient waiting rooms and equipment present a higher probability of contamination in the work area and should be disinfected frequently. Increase the number of available cleaning supplies, distribute them throughout the laboratory and waiting areas, and encourage staff to clean surfaces and equipment frequently. Reusable PPE should be cleaned and disinfected according to manufacturer instructions because not all disinfectants are compatible, and some may degrade the PPE.

Laboratory Waste Management

Dispose of sharps in appropriate puncture-resistant containers to autoclave as infectious waste. All cultures, stocks, residual specimens, and mpox virus waste should be decontaminated before on-site disposal using an approved method, such as autoclaving. Materials decontaminated outside the immediate laboratory should be placed in a durable, leak-proof container and closed for transport from the laboratory. Follow local, regional, state, national, and international regulations for waste disposal. State and local waste disposal regulations vary; for more information, see:

- Environmental Protection Agency Regulations
- State Universal Waste Programs in the United States
- U.S. Department of Transportation's: Managing Solid Waste | F-2 pages 94–97
- Notice of Enforcement Discretion Regarding Mpox Medical Waste

Resources for Monitoring Healthcare Workers Exposed to Mpox Virus

Infection Control: Healthcare Settings

Pages 269-270 from the Chapter VIII-E: Viral agents, BMBL 6th edition book that explains biosafety level recommendations

Natural Modes of Infection

The most well-known orthopoxvirus is variola virus, which causes smallpox. After an extensive vaccination campaign, smallpox was declared eradicated in 1980. Monkeypox occurs sporadically in several West and Central African countries but remains endemic in the Democratic Republic of Congo. The importation of wild-caught animals from Ghana into the United States resulted in a 2003 monkeypox outbreak that affected multiple states. Vaccinia virus is used to make the current smallpox vaccine. Naturally-acquired infections with vaccinia virus exist outside of the United States.[104] Cases of human cowpox occur in Europe and Asia. Rodents are known or suspected to play a part in the transmission of monkeypox, cowpox, and vaccinia viruses.[99-101]

Laboratory Safety and Containment Recommendations

Vaccination with vaccinia virus can afford protection against infection from other species of orthopoxviruses. Smallpox vaccination occurs via scarification using a multi-puncture method with a bifurcated needle. The current U.S.-licensed smallpox vaccine, ACAM2000, uses a replication-competent vaccinia virus strain. Symptoms such as fever, headache, and swollen lymph nodes are prevalent following vaccination. Adverse events include localized reactions (e.g., robust take), unintentional transfer of virus (e.g., self-inoculation, ocular vaccinia), diffuse dermatologic complications (e.g., eczema vaccinatum, non-specific post-vaccination rash), progressive vaccinia, cardiac complications, fetal vaccinia, and postvaccinial central nervous system disease. Due to the severity of complications that can arise from vaccination, the vaccine is not recommended for persons with certain contraindications.[99,103,105,106]

Orthopoxviruses are stable in a wide range of environmental temperatures and humidity. Virus may enter the body through the mucous membranes (e.g., eye splashes, inhalation of droplets or fine-particle aerosols), broken skin (e.g., needlesticks, scalpel cut), ingestion, or by parenteral inoculation. Sources of exposure include fomites, infected human or animal tissue, excretions or respiratory secretions, or infectious cultures.[106]

Routine vaccination with ACAM2000 is recommended for laboratory personnel who directly handle cultures or animals contaminated or infected with replication-competent vaccinia virus, recombinant vaccinia viruses derived from replication-competent vaccinia strains (i.e., those that are capable of causing clinical infection and producing infectious virus in humans), or other orthopoxviruses that infect humans (e.g., monkeypox, cowpox, and variola).[106] Vaccination is advised every three years for work with monkeypox and variola viruses, and every 10 years for cowpox and vaccinia viruses. Vaccination is not required for individuals working in laboratories that only manipulate replication-deficient strains of vaccinia virus (modified virus Ankara [MVA], NYVAC, TROVAC,

and ALVAC). Vaccination may be offered to healthcare workers, animal care personnel, and vaccinators who have contact with contaminated materials. Vaccination does not protect against non-Orthopoxvirus species.[103,106]

Research with variola virus is restricted to two WHO-approved BSL-4 and ABSL-4 facilities; one is the CDC in Atlanta, GA, and the other is the State Research Center of Virology and Biotechnology (VECTOR) in Koltsovo, Russia. ABSL-3 practices, containment equipment, and facilities are recommended for monkeypox work in experimentally or naturally infected animals. 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. BSL-2 and ABSL-2 plus vaccination are recommended for work with most other poxviruses. Note that for research subject to the *NIH Guidelines*, approval to lower containment from BSL-2 must be requested from NIH Office of Science Policy.[107]

Special Issues

The CDC provides information on a variety of topics relating to variola, monkeypox, and vaccinia viruses online at https://www.cdc.gov. For non-emergency information on potential human infections, smallpox vaccination, or treatment options, the CDC Poxvirus Inquiry Line can be contacted at 404-639-4129 or CDC-Info can be reached at 800-232-4636. To obtain smallpox vaccine, CDC Drug Services can be reached by phone at 404-639-3670 or by email at drugservice@cdc.gov. Clinicians or health departments may contact the CDC Emergency Operations Center in critical circumstances.

Select Agent Congo Basin monkeypox, Variola major, and Variola minor are Select Agents requiring registration with CDC for possession, use, storage, and/or transfer. See Appendix F for additional information.

Transfer of Agent The importation of poxviruses into the United States and/or their interstate transport may be subject to the rules and regulations of the CDC Import Permit Program, CDC Division of Select Agents and Toxins, and/or the USDA Animal and Plant Health Inspection Service. The exportation of poxviruses may require a DoC permit.

Mpox Monitoring and Risk Assessment for Persons Exposed in the Community

Updated November 25, 2022

This guidance is intended for people who have had mpox **exposures in the community.** Guidance for exposures in healthcare settings can be found here: Infection Prevention and Control of Mpox in Healthcare Settings.

Who should be monitored and for how long?

Anyone with an exposure to people or animals with mpox should monitor their health or be monitored for signs or symptoms consistent with mpox for 21 days after their last exposure. Information about human-to-human transmission of monkeypox virus is described in How it Spreads | Mpox | Poxvirus | CDC.

What to monitor

Monitoring should include assessing the person for signs and symptoms of mpox, including a thorough skin and mouth (oral) exam in good lighting. Skin examination can be performed by the person in isolation, a caregiver, or a healthcare provider and should include examination of the genitals and anus for rash or lesions.

Development of rash, signs, or symptoms

During the 21-day monitoring period:

- If a rash occurs:
  - An individual should follow isolation and prevention practices until (1) the rash can be evaluated by a healthcare provider, (2) testing is performed, if recommended by their healthcare provider, and (3) results of testing are available and are negative.
- If other signs or symptoms are present, but there is no rash:
  - An individual should follow isolation and prevention practices for 5 days after the development of any new sign or symptom, even if this 5-day period extends beyond the original 21-day monitoring period. If 5 days have passed without the development of any new sign or symptom and a thorough skin and oral examination reveals no new skin changes such as rashes or lesions, isolation and prevention practices for mpox can be stopped.
  - If a new sign or symptom develops at any point during the 21-day monitoring period (including during a 5-day isolation if applicable), then a new 5-day period should begin where the individual follows isolation and prevention practices.

Isolation and prevention practices can be ended prior to 5 days if a healthcare provider or public health authority believes the rash, signs, or symptoms are not due to mpox and there is a clear alternative diagnosis made that doesn't require isolation. The decision on when to end symptom monitoring and home isolation, either during the 21-day monitoring period or any 5-day extension, should be made with input from public health authorities.

Activity restriction during monitoring

Individuals exposed to monkeypox virus can continue their routine daily activities (e.g., go to work or school) as long as they do not have signs or symptoms consistent with mpox.

To date, there have been no cases of mpox transmitted by blood transfusion, organ transplantation, or implantation, transplantation, infusion, or transfer of human cells, tissues, or cellular or tissue-based products (HCT/Ps). As a precaution, patients with exposures should not donate blood, cells, tissue, breast milk, or semen while they are being monitored for symptoms. Given the morbidity and mortality among individuals awaiting organ transplantation, persons who have been exposed, but who are asymptomatic and without evidence of monkeypox virus infection, could be considered for organ donation following appropriate risk-benefit considerations.

How to monitor

Decisions on how to monitor exposed persons are at the discretion of public health authorities. In general, the type of monitoring recommended reflects the risk for transmission, with more active-monitoring approaches used for people who have had higher-risk exposures. Self-monitoring approaches are usually sufficient for people with exposures that carry a lesser risk for transmission. Evsen higher-risk exposures may be appropriate for a self-monitoring strategy if public health authorities determine that it is appropriate. Ultimately, the person's exposure risk level, their reliability in reporting signs or symptoms that might develop, the number of people needing monitoring, time since exposure, and receipt of post exposure prophylaxis (PEP) are all factors when determining the type of monitoring to be used.

How to monitor people unable to communicate onset of symptoms

Some people may be unable to communicate onset of symptoms, such as newborns, young children, or people with cognitive disorders. Parents and other caregivers should watch for changes in behavior and temperament that could signal that the person is experiencing uncomfortable symptoms such as fatigue or headache.

- Exposed people do not need to quarantine, but on a case-by-case basis, clinicians or public health officials could consider restricting programs, activities, or events that would pose high risk of transmission to other people (e.g., group play/education environments).
- Decisions about whether to limit activities in people who have been exposed to mpox but are unable to communicate onset of symptoms should consider the risk of their exposure incident (how likely they are to develop mpox infection) and the risk that transmission would pose to other people (e.g., immunocompromised family members, young children).

Exposure risk assessment for community settings

Each risk level category in the table below is intended to highlight the need for monitoring and assist with determining the need for postexposure prophylaxis (PEP). The exposure risk level of any incident may be recategorized to another risk level at the discretion of the treating clinician or public health authorities due to the unique circumstances of each exposure incident.

Mpox typically spreads through prolonged close, skin-to-skin contact with a person who has mpox, or their contaminated materials (e.g., clothing, bed sheets). Transmission during quick interactions (e.g., brief conversation), between people in close proximity has not been reported for any persons with mpox.

There may be settings in which contact tracing is not feasible due to the characteristics of the setting (e.g., level of crowding, types of interactions occurring). In settings where contact tracing is not feasible, people who spent time in the same area as someone with mpox should be considered to have intermediate or lower degree of exposure.

Interim Community Exposure Risk Assessment and Recommendations

For Monitoring and Postexposure Prophylaxis (PEP) in Individuals Exposed to Mpox Virus in a Community Setting

High Degree of Exposure

Exposure Characteristics

- Contact between an exposed individual's broken skin or mucous membranes with the skin lesions or bodily fluids from a person with mpox **-OR-**
- Any sexual or intimate contact involving mucous membranes (e.g., kissing, oral-genital, oral-anal, vaginal, or anal sex (insertive or receptive)) with a person with mpox **-OR-**
- Contact between an exposed individual's broken skin or mucous membranes with materials (e.g., linens, clothing, objects, sex toys) that have contacted the skin lesions or bodily fluids of a person with mpox (e.g., sharing food, handling or sharing of linens used by a person with mpox without having been disinfected† or laundered)

Recommendations

- Monitoring: Yes
- PEP¶: Recommended

Intermediate Degree of Exposure

Exposure Characteristics

- Being within 6 feet for a total of 3 hours or more (cumulative) of an unmasked person with mpox without wearing a surgical mask or respirator **-OR-**
- Contact between an exposed individual's intact skin with the skin lesions or bodily fluids from a person with mpox **-OR-**
- Contact between an exposed individual's intact skin with materials (e.g., linens, clothing, sex toys) that have contacted the skin lesions or bodily fluids from a person with mpox without having been disinfected† or laundered **-OR-**
- Contact between an exposed individual's clothing with the person with mpox's skin lesions or bodily fluids, or their soiled linens or dressings (e.g., during turning, bathing, or assisting with transfer)

Recommendations

- Monitoring: Yes
- PEP¶: Informed clinical decision making recommended on an individual basis to determine if the benefits of PEP outweigh the risks

Lower Degree of Exposure

Exposure Characteristics

- Entry into the living space of a person with mpox (regardless of whether the person with mpox is present), and in the absence of any exposures above

Recommendations

- Monitoring: Yes
- PEP¶: None

No Risk of Exposure

Recommendations

- Monitoring: No
- PEP¶: None

Exposure Characteristics

- No contact with the person with mpox, their potentially infectious contaminated materials, nor entry into their living space

¶ JYNNEOS and ACAM2000 are available for PEP

† Disinfection using a disinfectant registered with the U.S. Environmental Protection Agency (EPA), such as those with an emerging viral pathogens claim found on EPA's List Q

Factors that may increase the risk of mpox transmission include (but are not limited to): the person with mpox had clothes that were soiled with bodily fluids or secretions (e.g., discharge, skin lesion crusts or scabs on clothes) or was coughing while not wearing a mask or respirator, or the exposed individual is not previously vaccinated against smallpox or mpox. People who may be at increased risk for severe disease include (but are not limited to): young children (<1 year of age), individuals who are pregnant or immunocompromised, and individuals with a history of atopic dermatitis or eczema.

Last Reviewed: November 25, 2022

Having technical issues or questions regarding this product?
Please Contact Us.

BEI Resources currently limits the total number of "Made to Order" items which can be ordered by a registrant to 10 "Made to Order" items per 6 month period. Please check the Availability Status on the items are ordering and limit orders appropriately.

NR-58622 Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb) *(Viruses)*

Price: All BEI Resources products are provided at no cost to registered researchers.

Description:	hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb)
Organism:	Monkeypox Virus
Biosafety Level:	3
Availability Status:	In Stock
Store at:	-60°C or colder
Contributor:	CDC
Comments:	Quantity limit per order for this item is 1. This item can be ordered twice a year. Orders over this limit will be sent to NIAID for approval before shipment. Monkeypox virus, hMPXV/USA/MA001/2022 was isolated from a human in Massachusetts, USA in May of 2022, during an outbreak of monkeypox. Monkeypox virus, hMPXV/USA/MA001/2022 belongs to Clade IIb (previously west African clade) and lineage B.1. The complete genome of monkeypox virus, hMPXV/USA/MA001/2022 has been sequenced (GenBank: ON563414.3 and GISAID: EPI_ISL_13052289). Each vial contains approximately 0.5 mL of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (BSC-40; ATCC® CRL-2761™) infected with monkeypox virus, hMPXV/USA/MA001/2022. Additional information and tools are available at the Bacterial and Viral Bioinformatics Resource Center (BV-BRC). **The MATERIAL can be used for any legitimate purpose required to rapidly prevent, detect, prepare for, and respond to, the spread or transmission of MPXV, and to include use and sharing of the MATERIAL in conjunction with other public health institutions and like partners.**
Citations:	Acknowledgment for publications should read "The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb), NR-58622."

ATTACHMENTS

- Product Information Sheet
- Certificate of Analysis by Lot

PERMITS

For a list of permits that may be required for shipping this product and to set the permit information preferences; please select a country from the drop down below.

Country : USA

*
If shipping to the U.S. State of Hawaii, you must provide either an import permit or documentation stating that an import permi

Click to read more

KNOWLEDGE BASE

- How much do the reagents cost?
- How are viruses propagated at BEI Resources?
- Is the passage history available for viruses?

All compliant version documentation requests will be addressed within two business days of receipt.

Return to

Notices and Disclaimers

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Product Information Sheet for NR-58622

Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb)

Catalog No. NR-58622

This reagent is the property of the U.S. Government.

For research use only. Not for use in humans.

Contributor:

Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Manufacturer:

The University of Texas Medical Branch, Galveston, Texas, USA

Product Description:

Virus Classification: *Poxviridae*, *Orthopoxvirus*
Species: Monkeypox virus
Strain/Isolate: hMPXV/USA/MA001/2022
Original Source: Monkeypox virus, hMPXV/USA/MA001/2022 was isolated from a human in Massachusetts, USA in May of 2022, during an outbreak of monkeypox.[1]
Comments: Monkeypox virus, hMPXV/USA/MA001/2022 belongs to Clade IIb (previously west African clade) and lineage B.1.[2] The complete genome of monkeypox virus, hMPXV/USA/MA001/2022 has been sequenced (GenBank: ON563414.3 and GISAID: EPI_ISL_13052289).

Material Provided:

Each vial contains approximately 0.5 mL of cell lysate and supernatant from *Cercopithecus aethiops* kidney epithelial cells (BSC-40; ATCC® CRL-2761™) infected with monkeypox virus, hMPXV/USA/MA001/2022.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

NR-58622 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

Growth Conditions:

Host: *Cercopithecus aethiops* kidney epithelial cells (BSC-40; ATCC® CRL-2761™)
Growth Medium: Eagle's Minimum Essential Medium (EMEM; HyClone) supplemented with 2% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin solution, or equivalents
Infection: Cells should be 70% to 80% confluent
Incubation: 3 to 5 days at 37°C
Cytopathic Effect: Cell rounding and sloughing

Citation:

Acknowledgment for publications should read "The following reagent was deposited by the Centers for Disease Control and Prevention and obtained through BEI Resources, NIAID, NIH: Monkeypox Virus, hMPXV/USA/MA001/2022 (Lineage B.1, Clade IIb), NR-58622."

Biosafety Level: 3

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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Use Restrictions:

The MATERIAL can be used for any legitimate purpose required to rapidly prevent, detect, prepare for, and respond to, the spread or transmission of MPXV, and to include use and sharing of the MATERIAL in conjunction with other public health institutions and like partners.

References:

1. GenBank
2. GISAID

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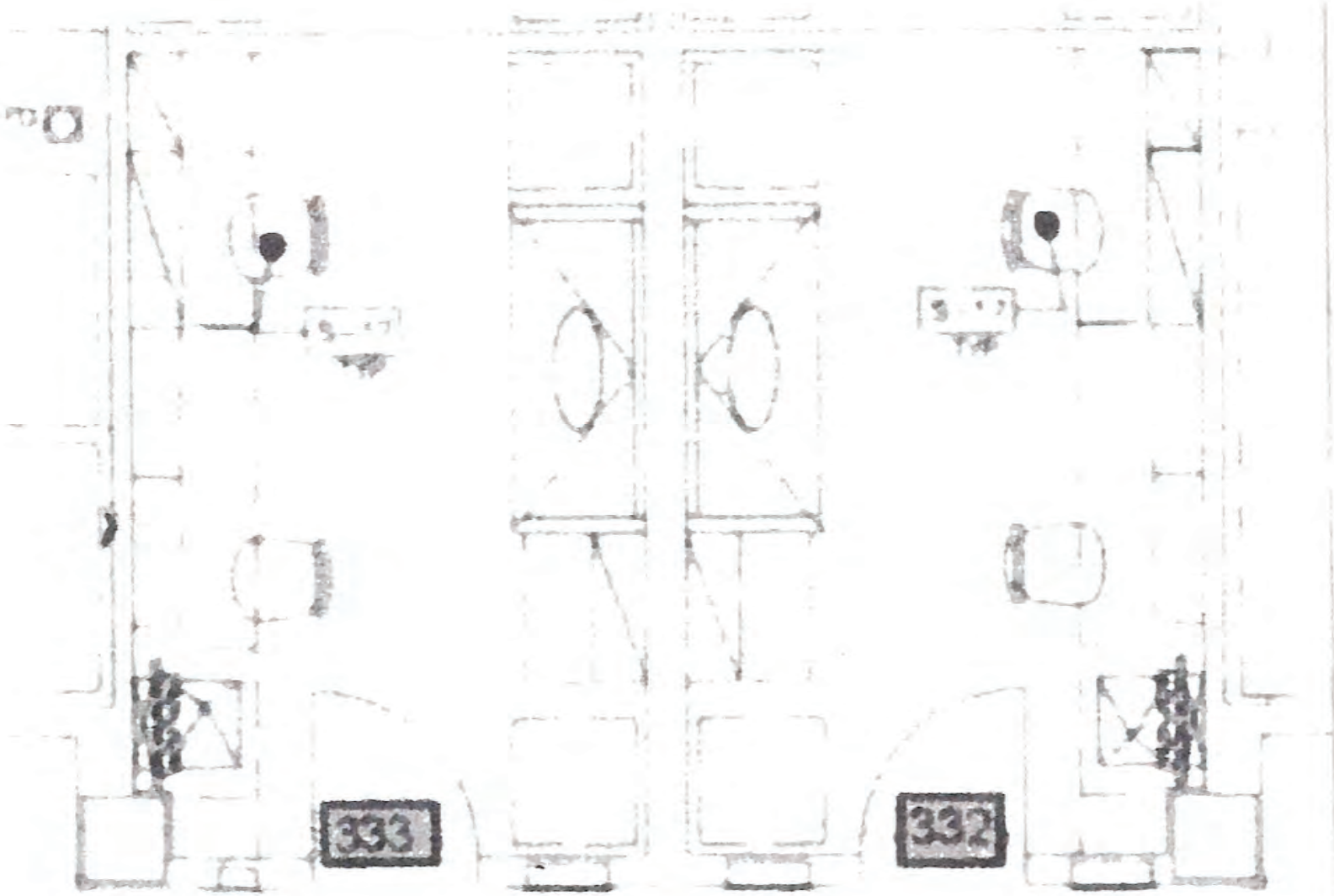
Support Provided by NIAID

BEI Resources
www.beiresources.org

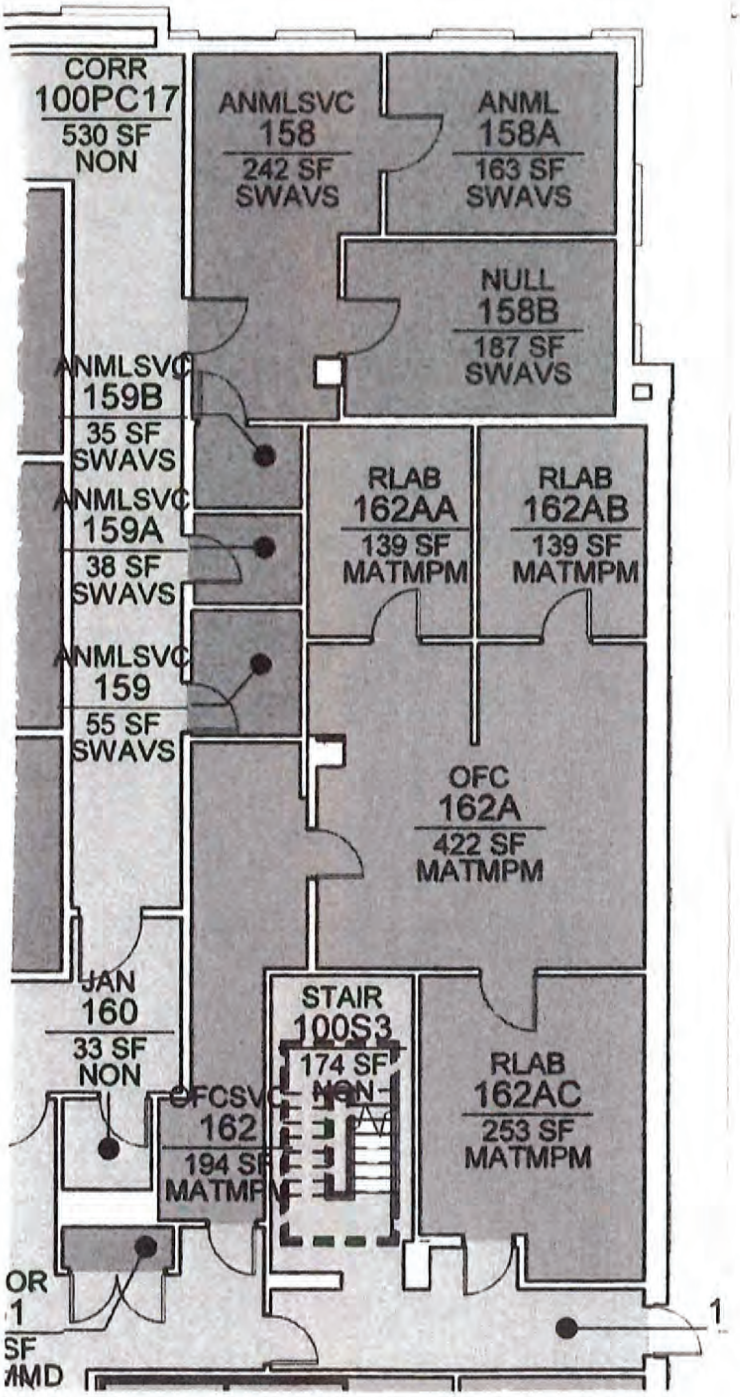
E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

BSL2+ labs layout: Room # 333 and 332



JABSOM BioContaiment Facility

ABSL3 rooms: 158, 158A, 158B, 159, 159A, 159B
BSL3 rooms: 162, 162A, 162AA, 162AB, 162AC



List of BSL3 SOPs included in this document

These SOPs are specific for Monkeypox (MPXV) research in the BSL-3. You must read and understand general SOPs in the JBF Biosafety manual to conduct research in the JBF.

SOP 1: Entry into the BSL3 laboratory

SOP 2: Exiting BSL3 laboratory

SOP 3: Working with MPXV in the BSL-3 Suites

SOP 4: MPXV infection in the BSL3

SOP 5: Daily decontamination procedure for liquid waste

SOP 6: Spill Clean up

SOP 7: Kakaako BSL-3 sharps management

SOP 8: Emergency responses to HVAC and power failure

Appendix 1: Pest management plan specific for Kakaako BSL-3

Appendix 2: SOP for tsunami

Appendix 3: Bomb threat/suspicious package SOP

SOP 1: ENTRY INTO THE BSL-3 LABORTORY

Entrance in to the BSL-3 Anteroom

1. Pass keycard over reader at hallway door and touch finger on biometric sensor.
2. Enter hallway and proceed to end of 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 keycard on the biometric reader.
6. Place finger or thumb on top of biometric surface 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 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 keycard next to the Biometric Palm reader.
4. Place your palm on Biometric reader to gain access into preparation room.
5. Open door and enter preparation room area.
6. Immediately don 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.

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 biometric reader. NO PIGGYBACKING!
4. Enter Suite and place carrier container on to the counter. If using 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 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 sink to fill trap.
4. Remove N95 masks.
5. Discard N95 mask if doing high-risk procedures. Save N95 mask appropriately if dong low-risk procedures. There is a shortage of masks and PPE. Consult your supervisor.
6. Remove sleeves and outer gloves and place in wastes 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 in the BSC.
4. Infect T75flask (10mL media) with 100uL and T125flask (20mL 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 minutes 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 (~15mL 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 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 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*.

SOP 5: 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 similar type of disinfectant will be added to the sink trap before and after each use.

SOP 6: SPILL CLEAN UP

Biological Spill/Release inside a Biosafety Cabinet (BSC)

- Notify others in the area about the spill.
- Leave the BSC blower on.
- Secure any biological materials.
- If there is gross contamination of your PPE, wipe down with paper towels soaked in disinfectant solution, carefully remove and properly dispose of contaminated PPE. Don fresh PPE before beginning clean up.
- Remove any sharps that might be present in the spill area using forceps or tools. Do not use your hands, even if gloved.
- Cover spill with paper towels/absorbent material. Apply appropriate disinfectant proceeding from the outside toward the center. Allow to remain for the required contact time.
- Wipe down all surfaces and equipment in the BSC with appropriate disinfectant solution.
- Dispose of all contaminated materials into red biohazard bag. Change outer gloves after collecting materials.
- Autoclave/decontaminate waste.
- Follow the phone tree and notify your PI and JBF Supervisor regarding the spill, and then complete a JBF Incident Report.
- The JBF Supervisor will notify the appropriate individuals on the JBF Incident Response Phone Tree.
- The JBF Director will inform the BSO, Kaka'ako EHSO, AVS Manager/Manager (if applicable), and Facilities Director of the spill.

Biological Spill/Release outside a BSC

Any spill of infectious material should have occurred in a BSC. Otherwise, you have already violated these requirements.

A spill outside of a BSC is unacceptable and presents a number of problems. First, all staff members in the room where the spill has occurred are now considered potentially exposed and, thus, must be considered for a self-quarantine for 14 days. Second, the spill must be immediately decontaminated to prevent the potential spread of infectious material. The survival of MPXV on surfaces is expected to be a number of hours so shoes, socks, pants, etc are all suspect in the event of a spill outside the BSC and must be removed carefully and bagged to minimize the chance of generating inhalable aerosols. Follow the JBF spill containment procedures.

If the spill is **SMALL** (<100 mL) and can be easily contained and cleaned up:

- Notify others in the area about the spill.
- Spray down any potentially contaminated PPE with decontamination solution and carefully remove and properly dispose.
- Don fresh PPE. Obtain spill kit materials.
- Remove any sharps present in the spill area using forceps or tools to collect sharp pieces. Do not use hands, even if gloved.

- Place absorbent material over the spill, starting from the outside and working toward the center. Add extra absorbent material beyond the edges of the spill.
- Soak the absorbent material, outside toward the center, with the appropriate disinfectant also spray area around spill.
- Allow the required time for absorbent material soaked with disinfectant to remain on the spill. Carefully collect and dispose of all contaminated materials in red biohazard bag
- Wipe down spill area again with disinfectant soaked paper towels.
- Disinfect any equipment, walls, or other areas around spill that might have been splashed. Discard paper towels used in cleanup/wipe down into a red biohazard bag.
- Change outer gloves after collecting materials.
- Autoclave/decontaminate waste.
- Notify your PI and JBF Supervisor that clean-up was completed and complete a JBF Incident Report.
- The JBF Supervisor will notify the appropriate individuals on the JBF Incident Response Phone Tree.
- The JBF Director will inform the BSO, Kaka'ako EHSO, AVS Manager/Manager (if applicable), and Facilities Director of the spill.

If a **LARGE** spill (greater than 100 mL) should occur and cannot be handled easily or contained:
- Immediately evacuate the laboratory.
- Post signs at the Suite and Anteroom entrances prohibiting entry.
- Notify your PI and JBF Supervisor once outside of the laboratory and complete a JBF Incident Report.
- The JBF Director will inform the BSO, Kaka'ako EHSO, AVS Manager/Manager (if applicable), and Facilities Director of the incident.
- The JBF Supervisor, JBF Director, JBSO, Kaka'ako EHSO, and JABSOM Facilities Director will discuss the situation, conduct a risk assessment, and determine the course of action.
- Once the plan has been discussed and approved, the spill will be cleaned up by approved staff.

Biological Spill/Release Inside a Centrifuge

- If a breakage or spill is known or suspected while the machine is running.
- Immediately stop the cycle and turn off the centrifuge.
- Allow the centrifuge to come to a complete stop.
- If the centrifuge is small and can be placed into the biosafety cabinet, (e.g., microcentrifuge) place it in the biosafety cabinet before opening. If it cannot be placed in the biosafety cabinet, contact your PI and JBF Supervisor, before proceeding.
- Do not open the centrifuge for at least 30 minutes to allow any aerosols to settle.
- Once approval has been given, use appropriate PPE (e.g., thick rubber gloves, safety goggles) to clean spill.
- Lay towels soaked in disinfectant over the spill area. Allow the required time for absorbent material soaked with disinfectant to remain on the spill.
- Use forceps, or cotton swabs held in forceps, to pick up small pieces of sharps.
- All broken tubes, sharps fragments, buckets, trunnions, and rotors must be properly decontaminated (treated with an appropriate disinfectant or autoclaved). If safety cups (sealed buckets) or sealed rotors were used, they must be opened in a biosafety cabinet.

- Unbroken, capped tubes can be disinfected in a separate container if the contents are to be recovered.
- All cleaning materials shall be collected and decontaminated.
- Contact the JBF Supervisor when cleanup is completed and document the incident by completing a JBF Incident Report.
- If, upon opening the rotors inside of the BSC, you notice evidence that a spill has occurred during centrifugation (e.g., cracks in the containers), follow the instructions for a spill inside of the BSC.
- The JBF Director will inform the BSO, Kaka'ako EHSO, AVS Manager/Manager (if applicable), and Facilities Director of the incident.

Spill or Release Outside of Containment

- Biological materials leaving the BSL-3 facility must be properly packaged as per Department of Transportation (DOT), the Federal Aviation Authority (FAA), and the International Air Transport Association (IATA) dangerous goods regulations. Federal and international regulations require that the shipper successfully complete job-specific training and be certified to ship infectious materials. If proper packaging fails and if there is a spill outside of containment, then follow the same rules and guidelines for response to Spill/Release outside of a BSC.

SOP 7 - KAKA'AKO BSL-3 SHARPS MANAGEMENT

1. For conducting research on MPXV in the BSL3, the use of glass will be kept minimal. Risk assessment must be conducted with the JBF Director and the Biosafety Officer. Alternative methods must be taken into consideration before using any sharps. Buddy system must be followed. **For stock preparation of MPXV, no glass containers will be used.**
2. Place a biohazard sharps container within an arm's reach of the area where sharps are used. Position the biohazard sharps container low enough in the work area so that you can readily visualize the opening. Biohazard sharps containers are available for purchase from laboratory supply companies (e.g., Fisher, VWR, etc.).
3. The disposal of any broken glassware with biohazardous materials directly into a biohazard sharps container. Store reusable sharps in a puncture resistant container, to prevent accidental or unintentional contact. If contaminated with biohazardous materials, reusable sharps should be stored in disinfectant solution until processed.
    a. Sharps MUST NOT be disposed in the regular trash.
4. Avoid handling any broken, contaminated glassware directly by hand, even if wearing gloves. Use a device such as tongs, forceps, brush and dustpan, or even two pieces of cardboard.
5. Biohazard sharps containers will be disposed when they are 3/4 full. Secure the containers to prevent leakage, punctures, and spillage during transport. Sharps containers must be disposed through the UH-EHSO program.
    a. Sharps containers must not enter the regular solid waste stream.
    b. DO NOT overfill biohazard sharps containers.
    c. DO NOT force a sharps item into a container, or retrieve a discarded it

SOP 8 - EMERGENCY RESPONSES TO HVAC AND POWER FAILURE

Should a power outage or an HVAC failure occur at the University of Hawai'i John A. Burns School of Medicine at Kaka'ako, these procedures will be followed:

POWER FAILURE

If a power outage does occur while you are working in the BSL3 lab, these labs are designed to run on back-up emergency power so you should not be affected. The back-up power should maintain the negative pressure in the suites as well as in the Isocage™ system. Additionally, the Isocage™ system is an independent unit, which has its own back-up power—the UPS (Uninterrupted Power Supply) has the ability to supply power to the Isocage™ system for 3 to 6 hours, independent of the building systems, which will allow the Isocage™ to maintain negative pressure in the cages. If building power is not restored within the 3 to 6 hour timeframe, the exhaust fans of the Isocage™ will no longer function and the negative pressure of the Isocage™ will continue to draw in environmental air until the pressure is equalized (becomes static, NOT POSITIVE), at which point, the mice will have approximately 15 minutes of Oxygen, and will then suffocate and perish. Note that no air is being exhausted from the cage in this scenario and the DOUBLE HEPA filtration is still in place and functional.

HVAC FAILURE

The HVAC system is a redundant system consisting of two exhaust fans. When one fan becomes inoperable, the backup fan should engage and become operational in just moments. However, if both fans are not operating, the situation will be immediately noticeable, as the suites will no longer hold negative pressure. You will immediately hear the audible alarms, but also see the visual strobe alarms as well in all of the different rooms making up the BSL3 Labs. In addition the BSCs will also sound their individual, independent alarms as well. The only exception will be seen in the Isocage™ system. The HVAC system failure will have NO effect on the Isocage™ itself. The Isocage™ system has complete containment and filtration independent of the building HVAC. The building HVAC serves as to help control the odors from the Isocages™ only, and is not necessary for Isocage™ biocontainment. In fact, there is a special "thimble" connection between the Isocage™ exhaust and the building HVAC specifically designed not to be airtight to prevent the interference of the building HVAC with the Isocage™ function. Again, it is important NOT to have an airtight connection between building exhaust and Isocage™ exhaust in order to prevent interference with the Isocage™ airflow rates and pressure balance.

RESPONSE

However, regardless of the nature of the failure, you should immediately stop working and start to secure any biological agents, animals, and/or chemicals. Depending upon the ongoing procedures following steps should be taken:

1. While conducting following procedures, it is mandatory to wear N95 mask.

2. Remember to close all BSC sashes.
3. Notify Security (692-0911 or 692-1911).
4. Disconnect all equipment that could be damaged by a power surge when electricity is restored.
5. Turn off all appliances and other energy users to reduce the power requirements for restoration.
6. Do not evacuate the building unless instructed to do so by emergency services (HPD, HFD, and JABSOM Security). If you are instructed to evacuate the building, use the emergency EXITs and go to a designated Evacuation Gathering Area to await further instructions from emergency services. (See Diagram for Designated Evacuation Gathering Area below).
7. If instructed to evacuate, follow SOP to exit the lab.

APPENDIX-1: PEST MANAGEMENT PLAN SPECIFIC FOR KAKA'AKO BSL-3

The Kaka'ako, JABSOM, BSL-3 facilities infrastructure meets the standards defined in the 5th edition of the BMBL. Similarly, all BSL-3 experimental practices, and containment practices will be adhered to as prescribed in the 5th edition of the BMBL. The SOP for conducting research with MPXV, is described in detail in the attached SOPs. The BSL-3 facilities at Kaka'ako are under negative pressure and are built according to the CDC and USDA standards as defined in the 5th edition of the BMBL.

General Pest Management Plan

The following describes the JABSOM Kaka'ako Plan for minimizing the risk of insects and rodent vector transmission of hazardous agents from the Level 3 Facilities. Many pests can mechanically transmit disease pathogens and compromise the research environment. As such, integrated pest management (IPM) is an important part of managing a Research Facility. The Plan relies heavily on the education and assistance of JABSOM Facilities Staff who care for JABSOM buildings and grounds. Consequently, it is the policy of the JABSOM Facilities to reduce or eliminate the potential for pest breeding, harborage, and entrance to the research facility. Proper sanitation, good housekeeping, and good building maintenance are key factors to keeping insects and rodents from entering the buildings.

Insects

1. Users of BSL-3 will routinely inspect the labs for signs of insect infestations and notify JABSOM Facilities through a Work Order Request to investigate and trap or treat insects that are found in the building or the surrounding campus grounds.
2. Facilities will contact a Licensed Pesticide Contractor on an as-needed basis to inspect, verify and take proper treatment to eradicate insects in the BSL-3 areas that are affected as well as other areas of the campus. The Contractor shall meet with EHSO to discuss the Pesticide to be used and application to ensure all EPA FIFRA requirements and guidelines are followed and to ensure the safety of the JABSOM Kaka'ako occupants.

Pesticides may only be applied when the lab is closed down for maintenance, i.e. there are no active research being conducted.

Safety: Every effort must be made to insure that pesticide application/exposure is held to a minimum to insure the safety and welfare of JABSOM Kaka'ako Occupants and Research Projects.
Preventive applications of pesticides are not encouraged in any Research Facility. As such, pesticide application(s) are restricted to areas where pest populations cannot be controlled by other means.

Facilities shall monitor the Pesticide Contractor to insure treatment was administered effectively.

Rodents

1. If there are signs of rodent infestations in the Level 3 Labs, the Users shall inspect the areas and capture any rodents. Traps shall be placed throughout the lab. The rodent shall be contained in the Level 3 Lab until an investigation conducted by EHSO is concluded and decisions are made regarding testing the rodent for infection, destruction of the rodent, surveying the facility for ports of entry for rodents, decontamination of the facility, SOP evaluation and revisions as necessary.
2. Careful records of all animals shall be maintained and any unaccounted for rodents shall be reported to the Level 3 Manager and Supervisors, LAS, and EHSO immediately

Mosquitoes

1. JABSOM facilities shall maintain the buildings and grounds according to the general JABSOM Kaka'ako Pest Management Plan to prevent, identify, monitor, and eliminate mosquito-breeding sites and prevent wild mosquitoes from entering the research building. Refer to the General Plan. If wild mosquitoes are able to enter the building, there are several safeguards that prevent the wild mosquitoes from contacting infecting animals or inoculating cultures refer to specific protocol SOPs.

SPECIFIC PROTOCOL IPM

Specific IPM shall be protocol driven.

Moreover, JABSOM Facilities provide building, campus wide monitoring, and protection. The following is the Facilities Management Plan.

Many pests can mechanically transmit disease pathogens and compromise the research environment. As such, integrated pest management (IPM) is an important part of managing a Research Facility. Consequently, it is the policy of the JABSOM Facilities to reduce or eliminate the potential for pest breeding, harborage and entrance to the research facility.

JABSOM KAKA'AKO CAMPUS BUILDINGS AND GROUNDS

Building occupants should routinely inspect their work areas for signs of insect infestations and notify JABSOM Facilities through a Work Order Request to investigate and trap or treat insects that are found in the building or the surrounding campus grounds.

1. JABSOM Facilities will contact a Licensed Pesticide Contractor on an as-needed basis to inspect, verify and take proper treatment to eradicate insects in areas that are affected as well as other areas of the campus. The Contractor shall meet with JABSOM EHSO to discuss the pesticide to be used and application to ensure all EPA FIFRA requirements and guidelines are followed and to ensure the safety of the JABSOM Kaka'ako occupants.

   Safety: Every effort must be made to insure that pesticide application/exposure is held to a minimum to insure the safety and welfare of JABSOM Kaka'ako Occupants and Research Projects.

   Preventive applications of pesticides are not encouraged in any University Facility. As such, pesticide application(s) are restricted to areas where pest populations cannot be controlled by other means.
2. JABSOM Facilities shall monitor the Pesticide Contractor to insure treatment was administered safely and effectively.
3. If there are signs of rodent infestations in the buildings or on the grounds, contact Facilities and complete a Work Order Request. All unaccounted for lab rodents shall be reported to EHSO, Security, and Facilities immediately.

REDUCING THE NUMBER OF MOSQUITOES ON THE JABSOM KAKA'AKO GROUNDS, NEAR BUILDING ENTRANCES, AND IN THE BUILDINGS

Proper sanitation, good housekeeping, and good building maintenance are key to keeping insects and rodents from entering the buildings.

Draining sources of standing water reduces possible breeding areas. In addition, larvicides (to control breeding areas) and other pesticides may be used to reduce mosquito populations.

- JABSOM Facilities shall respond to any standing water problems in the landscape on campus.
- JABSOM Facilities shall schedule preventive maintenance on gutters and downspouts and the fountain in front of the Medical Education Building.
- Potential mosquito breeding areas (wetter areas of landscaping, etc.) shall be inspected periodically and treated with pesticides when mosquito presence is reported. These "trouble" areas shall be monitored periodically to ensure there are no breeding mosquitoes.
- Trash, recyclables, and discarded equipment and materials must be contained in the trash compactor or promptly removed from all collection areas. Any spilled trash outside of the trash compactor shall be reported to JABSOM Facilities and Custodial staff shall promptly remove the trash.

RECORD KEEPING AND PROGRAM EVALUATION

1. Visual sightings or other evidence of any pests shall be reported to the Lab Manager/Supervisors and EHSO immediately and shall be documented.
2. Reports communicated verbally and in writing concerning pest activity will be recorded and kept on file by Lab Manager/Supervisors, Facilities, and EHSO.
3. The Laboratory Director, JABSOM Facilities, and EHSO shall also maintain inspection results for review.

Quality assurance and program review must be performed to provide an objective, ongoing evaluation of pest management activities. EHSO is responsible for evaluating the effectiveness of all pest control procedures implemented and approving or redirecting efforts to control pests found.

APPENDIX-2: SOP FOR TSUNAMI

1. If working in a Biosafety Cabinet (BSC)
    a. Seal all open cultures (this includes putting animal carcasses in a sealed or disinfectant filled container).
    b. Cover pipette trays.
    c. Carefully remove your outer gloves, and leave them in the BSC.
    d. Close the sash to the lowest possible position.
    e. If time permits, animals may be sacrificed and infected cultures may be autoclaved. **NOTE**: If you believe that you are in immediate danger from tsunami, DO NOT perform the procedures described for the BSC and immediately exit the building. The Isocage™ systems which house the animal are waterproof and without power supply, the animal will die in 15 minutes due to suffocation. Freezer and incubator are waterproof. Therefore infected material will not spread into the environment. In worse case scenario when salt water can get into these equipment's, salt water will kill all these infected cultures and animals.
2. If you are working outside of a cabinet, close up any equipment/appliances you are working with.
3. Meet at the designated evacuation points.
4. Stay at the evacuation point and ensure there is full accountability for all personnel that were in the facility. If individuals are missing do not re-enter the facility to find them, but notify police or fire personnel of the number and identity of individuals missing and where they were working in the facility. DO NOT LEAVE ; until you are given the clearance to do so from Emergency Management.
5. If the situation is cleared, and the building can be re-occupied, return to your labs (under the appropriate conditions), and finish/clean up your work.
6. At your earliest possible convenience fill out an Incident Report if a potential exposure occurred.

APPENDIX-3: BOMB THREAT/SUSPICIOUS PACKAGE SOP

BOMB THREAT/SUSPICIOUS PACKAGE

- Called in Bomb Threat:
  - DO NOT hang up on caller
  - Keep them on phone for as long as possible and get as much info as possible
  - Once caller hangs up, contact Security
- Suspicious Package
  - DO NOT open or move package
  - Vacate area and notify Security and Biosafety Offices

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