UCR | Bourns College of Engineering

CELL CULTURE FACILITIES

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BCOE Cell Culture Facilities Operating Policies

Locations: Materials Science and Engineering Building Room 279
Bourns Hall, Rooms B360

Effective Date: January 1, 2015

Preface

In 2012, two ad hoc BCOE committees consisting of six faculty members working together were formed to establish policies for the two cell culture suites in the MSE building (mammalian cell culture) and Bourns Hall (mammalian and microbial cell culture). Professor Victor Rodgers was appointed as the chair for these committees. Over this period of time, the committees have collected data and interacted with the users of these facilities as well as other interested users. In addition, they considered options to accommodate future faculty members.

The purpose of these policies is to:

- Ensure that all BCOE faculty members and their researchers have equal opportunity to access cell culture facilities in support of their research efforts.
- Provide a framework for maintaining and replacing major pieces of equipment associated with the BCOE cell culturing such as the biological safety cabinets (BSCs) and autoclaves.
- Establish laboratory safety protocols and training consistent with campus and CDC guidelines for the relevant Biosafety Level (BSL).

Available Facilities

BCOE has two cell culture suites, MSE 279, and Bourns B360.

Materials Science and Engineering Room 279 is a Biosafety Level 2 facility dedicated to mammalian cell culture. MSE 279 totals 651 assignable square feet (asf) that consists of a 227 asf outer common room equipped with a wash sink and three individual sterile culture rooms:

- 279A (187 ft^2) contains two Class II BSCs – 6-ft and 4-ft– and four incubators
- 279B (154 ft^2) contains two Class II BSCs – 6-ft and 4-ft– and two incubators
- 279C (96 ft^2) contains one 4-ft Class II BSC and two incubators

The outer common room (227 ft^2) contains one -20 °C freezer and one 4°C refrigerator, one autoclave, and supplies for sterile techniques.

Bourns Hall B-wing Room 360 is a Biosafety Level 2 facility containing both mammalian and microbial cell culture rooms. B360 totals 800 asf of space comprised of a 189 asf outer common room equipped with a wash sink and 5 individual cell culture rooms including:

- 360A – 1 level 2 BSC
- 360B – 1 level 2 BSC
- 360C – 2 level 2 BSCs
- 360D – 2 BSCs – approval level to be determined[1]
- 360E – 1 BSC – approval level to be determined

[1] Per campus policy, determination is made by the IBC via the Campus Biosafety Officer

General Access

Access to the culture facilities will be approved and monitored by the two ad hoc committees. PIs wishing to use the cell culture facilities must complete and submit a Cell Culture Facility Use Form (see Attachment 1) to the Safety and Facilities Coordinator, which then must be approved by the relevant cell culture facility committee. PIs must include the relevant campus approvals with their Facility Use Form. These may include:

- Biological Use Authorization (BUA) – ***required for all cell culture work***; PIs with existing BUAs (Biological Use Authorization) must amend their BUA to include the Facility as a location.
- Human Research Review Board approval – required for human cell work
- Stem Cell Use Authorization – required for human pluripotent stem cell work
- International Animal Care and Use Committee protocol – required for non-human cell work

NOTE: Because it may take several months to complete the above reviews, it is recommended that PIs obtain the necessary approvals listed above well in advance of the time they anticipate beginning their project.

Card key access will be granted to users upon: 1) approval by the relevant cell culture committee, the Safety/ Facilities Coordinator, and Lab Manager and 2) documentation that all required training has been completed by each individual facility user. At a minimum, users must have completed:

- Laboratory Safety Fundamentals,
- Biosafety Training,
- Cell Culture Facility training, and
- Project-specific training.
- Other training may include but is not necessarily limited to Bloodborne Pathogens training and additional training specified in the BUA for that PI/project.

Training: documentation of all required training for each research team member must be provided prior to room access being provided to each individual.

Cost

Fees are applied to users to maintain the cell culture facilities, equipment, and sterile conditions. Rates are established based on total facilities operations costs and the number of users. Fees are charged per PI, per hood, per month. The number of users per PI per hood is limited to 5. PIs with more than 5 users may elect to reserve more than one hood at an additional cost. An FAU is required from all users prior to obtaining facility access. Users are not guaranteed sole use of hoods. Users having projects with limited duration should inquire about proration.

Facility Access and Use Guidelines
The following guidelines are to be followed by facility users:

- Card access will be granted to researchers who are listed on their approved BCOE Cell Culture Facility Use Form. Access will be cancelled at the end of the requested use time.
- Facility doors are to be kept closed and locked at all times. Keeping doors propped open is a security and contamination concern.
- Users may utilize hoods only during reserved times.
- Users agree to follow documented standard operating procedures to ensure maintenance of sterile conditions both before and after completion of space use.
- Each user must sign the login sheet when entering the cell culture suite.
- Individuals linked to contamination must successfully repeat cell culture training; if contamination continues to occur, user access can be revoked.

Additional facility use guidelines can be found in Attachment 2.

User training procedures and documentation information are included in Attachment 3.

Questions regarding administration or reservations may be directed to the BCOE Safety & Facilities Coordinator at (951) 827-1241. Technical or training questions may be directed to or the Bioengineering Senior Development Engineer at (951) 827-7235.

ATTACHMENTS

ATTACHMENT 1

Cell Culture Facility Use Request Form

Cell Culture Facility Use Request Form

PI Name: _____

Department: _____ Email: _____

Phone (work): _____ Phone (cell): _____

BSCs needed: _____ FAU: _____

BUA #: _____ (**REQUIRED for all cell culture work**)

HRRB #: _____ (Required for human cell work)

SCUA #: _____ (Required for human pluripotent stem cell work)

IACUC Protocol: _____ (Required for non-human cell work)

Project Start Date: _____ Project End Date: _____

Indicate preferred work hours for each weekday (NOTE: every effort will be made to accommodate desired days/times)

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY

List cell lines to be used in facility (please print/type clearly):

List personnel who will work in facility (please print/type clearly):

Incubator needed? YES ☐ NO ☐ # SHELVES NEEDED_____

List items you plan to bring into the Facility:

By signing I acknowledge I have read, understand, and agree to abide by the BCOE Cell Culture Facility Operating Policy and Procedures and agree to ensure all required training is successfully completed and documented for all of my research personnel.

PI Signature Date

SUBMIT COMPLETED FORM ALONG WITH YOUR APPROVED BUA TO BCOE DEANS OFFICE – ATTN: SAFETY & FACILITIES COORDINATOR

OFFICIAL USE ONLY:

APPROVAL ROUTING

	Review Status (approved/denied)	Date	Signature
Microbial Cmte Chair			
Mammalian Cmte Chair			
Administrative Oversight			
Technical Oversight			

DEANS OFFICE APPROVAL: _____ DATE: _____

LOCATION(S) ASSIGNED:

PRIMARY			SECONDARY (IF APPLICABLE)		
Building	Room #	Hood #	Building	Room #	Hood #

DAYS/TIMES ASSIGNED:

HOOD 1

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY

HOOD 2

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY

ATTACHMENT 2

Cell Culture Facility Operating Procedures

BCOE Cell Culture Facilities Operating Procedures

Access Procedures/Policies

1. All users must log in when using Cell Culture facilities.
2. All materials brought into the facility must be sterile.
3. All cell lines brought into or used in the Facility must be listed on the approved BCOE Cell Culture Facility Use Form.
4. Sterile and aseptic cell culturing techniques shall be practiced at all times.
5. Only listed, trained, and approved personnel may use the BCOE Cell Culture Facility.
6. Users of the culture facilities shall not consume yeast-based beverages (such as beer), 24 hours prior to working in the culture labs.
7. Individuals who bake at home should not do so for 3 days prior to using the culture facilities.
8. Dedicated lab coats and gloves must be worn at all times when entering or working in the tissue culture rooms. They must be removed when leaving the facility.
9. Users working with retroviruses, lentiviruses, and/or adenoviruses-transfected cells must follow special procedure of sterilization as noted in supplemental documents.
10. Any cells that show signs of contamination will be discarded with special instruction (see Contamination Containment Plan for details) and users need to report to the Cell Culture Facility Manager.
11. All incubators will be monitored once a month for signs of fungal contamination (see Contamination Containment Plan for details). Detected contamination will be reported to the Safety Manager.
12. Individuals who do not comply with the sterile operating procedures of the Facility will not be allowed to perform experiments in the Facility until they have successfully repeated the cell culture training program. If contamination continues to occur, user access can be revoked.
13. Users who share the same culture room are encouraged to share their experimental procedures with other co-users.

Tissue Culture Suites

1. The tissue culture rooms are sterile areas.
2. The culture suite foyers are semi-sterile areas.
3. Only individuals with approved projects and training are admitted in the Cell Culture Facility.
4. Upon entering the Facility, users must step on a blue sticky pad and put on a lab coat, which will be provided by the Facility.
5. Users are not allowed to bring lab coats in and out of the Facility.
6. A minimum of 30 minutes must be allowed between hood users.

Contamination Prevention and Containment Plan

Sterile environments are essential for any mammalian or microbial cell culture. The Facility will follow a zero tolerance policy for un-sterile culture.

Prevention

1. The biosafety cabinet must be UV-sterilized for at least 15 minutes prior to use.
2. Wash hands extensively with hand soap prior to putting on gloves.
3. Spray 70% ethanol on your gloved hands.
4. After UV sterilization, sterilize the working surfaces with 70% ethanol before and after the use.
5. Incubator maintenance:
    a. Biweekly: water pan cleaning and incubator surface sterilization with 70% ethanol
    b. Monthly: autoclave shelves and incubator sterilization accordingly to manufacturers' instruction
6. Once a cell line is established in a cell culture room, the cells should not be transferred to other cell culture rooms (e.g. to use microscope or centrifuge, etc.)
7. Biosafety cabinets should be wiped down with approved disinfectant after each use.

Containment

1. Once bacterial or fungal contamination is found in a culture, the culture should be discarded after sterilizing it with 10% bleach for 20 minutes.
2. The observer must alert the shared users of the tissue culture room that the contaminated culture was manipulated and maintained in.
3. Any cultures in the contaminated incubator must not be transferred to other incubators.

Waste Disposal

1. All liquid waste is to be aspirated with aspirating pipettes to a vacuum aspirator bottle that is prefilled with 10% bleach solution. When the waste reaches the marked level (3/4 full), the liquid waste must be emptied, vacuum aspirator bottle must be rinsed, washed and prefilled with 10% bleach solution by the user.
2. Consumable tissue culture waste, such as serological pipettes and tissue culture vessels that have been in contact with cells and their supernatant must be discarded in the biohazard bin designated for each tissue culture cabinet.
3. Other consumable waste that has not been in contact with any cells and their supernatant, such as serological pipettes and their wrappers is to be discarded in the trash bin located next to every tissue culture cabinet.
4. Pipette tips must be discarded in an empty plastic bottle (e.g. empty plastic solid DMEM bottle) instead of in the waste bags to avoid puncturing of the bags and injury.
5. Other sharp items such as needles must be discarded in the sharps bins.
6. Any chemicals that meet the definition of a hazardous material, such as paraformaldehyde and methanol, must be discarded in the designated, labeled waste container in the fume hood. These materials require special disposal by Department of Environmental Health and Safety (EHS) and must be labeled with an approved waste label.
7. Please notify the Facility Manager if assistance is needed with waste.

Emergency Procedures

For police, medical emergencies, fire, and earthquake:

- Dial 951-827-5222 from a cell phone or Dial 911 from campus phones. This connects you directly to UC Police (UCPD) who can then guide emergency responders to you when needed. Dialing 911 from your cell phone may delay the arrival of emergency responders.
- If necessary, the campus fire alarm pull-boxes can be used both to summon emergency responders and to help evacuate the building.
- Report the event to your supervisor and department FAO as soon as possible.

Skin contact: external exposure (splash):

- Call 911 from a campus phone or 951-827-5222 from a cell phone. Ask for assistance from your co-workers if necessary and they are available.
- Start shower/eye wash
- Remove contaminated clothing, if appropriate
- Wash for at least 15 minutes
- Seek additional assistance as necessary
- Report the event to your supervisor and department FAO as soon as possible.

Inhalation:

- Leave the area and go to an area with fresh air
- If you have shortness of breath or trouble breathing, call 911 for help.
- Report the event to your supervisor and department FAO as soon as possible.

Ingestion:

- Call 911 for help
- Call the National Capital Poison Center (800) 222-1222 for poison emergencies.
- Report the event to your supervisor and department FAO as soon as possible.

For hazardous material spill or release:

- If you have been trained and have the proper materials, contain or clean up spill, and call EH&S at (951) 827-5528.
- If you have not been trained, cannot contain the spill, call EHS or (951) 827-5222.
- Report the event to your supervisor and department FAO as soon as possible.

Emergency Contacts:

- First Contact: Principal Investigator
- Second Contact: Hong Xu, Development Engineer at 2-7235; then cell phone at 951-403-0932
- Third Contact: BCOE Deans Office, Maggie Souder, Safety & Facilities Coordinator at 2-1241, then cell phone at 760-846-3458

ATTACHMENT 3

Facility Training and Orientation

BCOE Cell Culture Facility Training and Orientation

All users agree abide by the "BCOE Cell Culture Facilities Operating Policies."

Before working in the cell culture facility, all users must first successfully complete:

1. Laboratory Safety Orientation/Fundamentals,
2. Biosafety training,
3. Cell Culture Facility Orientation (below), and
4. Project-specific training (from the PI listed on the Biological User Authorization).
5. Other training may include but is not necessarily limited to Bloodborne Pathogens training and additional training specified in the BUA for that PI/project.

Cell Culture Facility Orientation:

Essential Sterile Cell Culture Techniques

1. Prior to beginning cell culture work, put on all appropriate, dedicated personal protective equipment (PPE) (e.g., lab coats, gloves, and protective eyewear) as directed by the Facility Manager.
2. Sterilize the laminar hood surface by turning on UV lamp at least 15-20 min prior to use.
3. To prevent hood contamination, each time non-sterile items outside the hood are handled, rinse gloves with 70% ethanol (EtOH) prior to resuming work in the sterile laminar hood.
4. Before using the microscope, spray 70% EtOH on paper towel and wipe off the microscope stage;
5. Once the laminar hood sash has been raised to the recommended mark, close it only when your work is completed. You do not need to close the hood if you have an incubation period during your cell culture. The vertical air barrier that is established in the hood prevents any cross-flow.
6. Always spray pipettes, tip boxes, media/PBS/H_2O bottles with 70% EtOH and wipe off with paper towels before bringing it inside the laminar hood.
7. Never spray EtOH on tissue culture dishes/flasks.
8. Never place the caps/lids of cell culture tubes/bottles/dishes facing downward on any surface.
9. Clean up any spilled media/buffer on the hood surface with EtOH-sprayed paper towel/kimwipe right away – the phenol red dye in media leaves a permanent stain if not cleaned up immediately.
10. Dispose the used serological pipettes in dedicated waste container and later into the biohazard (red) container for autoclaving; autoclaving must follow UCR safety practices and must occur before disposal.
11. Hazard-free trash (not a hazardous material, biohazard, or radiation hazard) that has not touched any cell culture media can be disposed in regular trash bin.
12. Never talk/cough/sneeze with the incubator door open - this will prevent contamination by mycoplasma, which is found in saliva.
13. Aspirating glass Pasteur pipettes must be autoclaved in stainless steel boxes and, following sterilization, these pipette boxes should be left in the culture hood (they should never be opened outside the hood).
14. Dispose used glass Pasteur pipettes into the provided specialized "sharps" container.
15. Make sure the aspirating glass Pasteur pipette never touches the hood surface. If so, discard it and use a fresh one.
16. Sterile PBS and H_2O bottles may be left outside the laminar hood after use.
17. Never use other user's media, PBS buffer, or Trypsin unless permission is granted by the user.
18. Never transfer culture plates/flask to other tissue culture rooms, unless cell lines are compatible and permission is obtained from the Cell Culture Facility Manager.

Cell Culture Waste Management

1. All waste must be managed according to campus waste policies and guidelines.
2. Biohazardous and Medical wastes may be submitted to campus EH&S using the Biohazardous/Medical Waste Pickup Request form (note: this form is expected to be merged into the UC WASTe System by October 2014). To submit waste for autoclave in-house, contact the Cell Culture Facility Manager.
3. Non-biological hazardous waste must be labeled using the UC WASTe program.
4. Radioactive materials are not currently allowed in the Cell Culture Facilities.

Questions regarding administration or reservations may be directed to the BCOE Safety & Facilities Coordinator at (951) 827-1241. Technical or training questions may be directed to or the Bioengineering Senior Development Engineer at (951) 827-7235.

ATTACHMENT 3

Training Documentation

BCOE Cell Culture Facility Training Documentation

Please complete a separate form for each person listed on your "BCOE Cell Culture Facility Use Request" form. Required training is noted in ***bold italics*** and must be current in order for Facility access to be provided. Incomplete or lapsed training may result in revoked access. Additional training may be required depending upon cell lines used. Training questions should be directed to the Bioengineering Senior Development Engineer at (951) 827-7235 or hong@engr.ucr.edu . Please submit copies of this form both to EHS Training (fax 951-827-5122 or ehstraining@ucr.edu) and to the BCOE Deans office (Attn: Safety & Facilities Coordinator or msouder@engr.ucr.edu)

TRAINEE

Name: (print clearly) _____

Identification[2]: _____

Email: _____

Phone: _____

INSTRUCTOR

Name: _____

Department: _____

Email: _____

Phone: _____

Signature[3]: _____

Course Description	Date Trained	Student Signature	Instructor Signature (when applicable)
Laboratory Safety Fundamentals			Course online
Biosafety Training (Online via EHS)			Course online
Cell Culture Facility Orientation			
Project-specific Training			
Please note below any additional training provided:			
Bloodborne Pathogens Training			Course online

[2] ***Identification Number****: Enter your Employee ID, Student ID, UCR NetID, or Date of Birth*

[3] ***Instructor Signature****: By my signature I certify that the individual on this form has completed and passed the noted courses.*