


## STANDARD OPERATING PROCEDURE

	<b>Ragon Institute Tissue Culture Training</b>		<b>SOP #</b>	TC_SOP_001
	Originated by:	Alicja Trocha	Date:	June 2017
	Laboratory:	Walker Lab	Pages:	1 of 3
	Approved by:	Alicja Trocha		

**I. PURPOSE:**

The purpose of this procedure is to outline the procedure to be carried out uniform training for all new Ragon employees.

**II. SAFETY:**

This protocol needs to be carried out in the BSL2 laboratory following all BL2+ regulations.

**III. REQUIREMENTS:** Qualified trainer

**IV. PROCEDURE:**

**Each new Person hired who will be working in one of our Tissue Culture rooms must go through training by an already trained, proficient lab member (technician or postdoc) and approved by Floor Lab Manager. Collaboration Scientist will work under the supervisor of the lab they are associated with and all items in Bold must be done as well, the rest is under the discretion of PI.**

Name: \_\_\_\_\_  
 PI Name: \_\_\_\_\_  
 Date of Hire: \_\_\_\_\_  
 Badge ID# \_\_\_\_\_

Each new person will be introduced to the Ragon Tissue Culture (TC) rooms by watching an already trained and proficient person working. Explanations will include sterile technique, how to clean the laminar flow hoods, how to change buckets with D-125, how and where to dispose of all biohazard waste and a proper introduction to autoclaving and disposing of autoclaved waste.

List of procedures that must be performed to a level of proficiency under supervision before you will be able to perform these procedures independently:

1. **Verbal introduction by PI/Fellow/PostDoc or training person.**
2. **General room stocking and cleaning /media preparation/ disposal of discarded waste/location of waste collection on floor.**
3. **Autoclaving and proper disposal of waste /proper dress code doing that/learning usage of the autoclave**
4. Blood separation for those who will be handling samples (/this process must be done several times to give student enough time to learn that. Preliminary training can be done on the bench outside the TC with the Ficoll and PBS
5. Long term Culture cultivation for 2-3 weeks, counting them, expanding them freezing and thawing to master sterile technique.
6. Freezing cells
7. Thawing cells
8. **Emergency response – what to do in case of exposure or spill.**


Each procedure must be sign off by Floor manager – **the procedures in bold must be done by everybody asking for access to TC** and rest depending on experience and PI input **(must be sign off by PI if not needed)** Each new RI Employee must go through all points (BCL might be substituted with another procedure verifying mastering sterile techniques)

**#2 Stocking , cleaning.**

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Cleaning of hoods				
Changing of blue pads				
D125 change / Buckets				
Routine stocking				
Final sign off by Lab Manager				

Name: \_\_\_\_\_

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**#3 Autoclaving**

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Proper cycle selection				
Disposal of liquid waste				
Disposal of solid waste				
Final sign off by Lab Manager				

**#4 Blood separation (Ficoll) if applicable ( PI /postdoc sign off if N/A)**

Must observe protocol at least once.  
 Must perform protocol at least twice with HIV negative blood while being observed.  
 Must perform protocol at least twice with HIV positive blood while being observed.  
 One final protocol must be observed by Lab Manager.

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Observe				
Protocol #1 with HIV negative blood				
Protocol #2 with HIV negative blood				
Protocol #1 with HIV positive blood				
Final protocol with sign off by Lab Manager				

**#5. BCL cultivation ( or other long term cultivation)**

New Employee will be given 4-5 BCL lines to maintain for approximately three to four weeks. This longer term culturing will validate sterile technique and proper culture maintenance. Each line must be expanded at least once from a T-25 to a T-75, carried to a second T-75. Two lines must be frozen then thawed a week later to check for viability.

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Expanded to 1 <sup>st</sup> T-75				
Carried to 2 <sup>nd</sup> T-75				


**#6. Freezing**

Must observe protocol at least once.  
 Must perform protocol at least twice on BCL lines while being observed. Must perform protocol at least twice on HIV negative PBMC while being observed.

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Observe freezing				
Freeze BCL				
Freeze BCL				
Protocol #1 with HIV -				
Final protocol with sign off by Lab Manager				

**Name:** \_\_\_\_\_

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**#7 Thawing**

Must observe protocol at least once. Must perform protocol twice on BCL (frozen on 2 separate occasions)

Must perform protocol at least twice on HIV negative PBMC while being observed.

	Date	Signature of tech receiving training	Name of proficient trainer	Signature of proficient trainer
Observe				
Protocol #1 with BCL				
Protocol #2 with BCL				
Freezer HIV- PBMC				
Freezer HIV+ PBMC				
Final protocol with sign off by Lab Manager				

**#8 EMERGENCY PROCEDURE FOR ACCIDENTAL EXPOSURE TO INFECTIOUS AGENTS – refer to SOP**

**V. REFERENCES/ ADDITIONS/ NOTES**

**Name:** \_\_\_\_\_