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

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.

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

Name: ________________________________
#3 Autoclaving

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<th>Date</th>
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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.

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<th>Date</th>
<th>Signature of tech receiving training</th>
<th>Name of proficient trainer</th>
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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.

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Expanded to 1st T-75

Carried to 2nd 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.

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<th>Date</th>
<th>Signature of tech receiving training</th>
<th>Name of proficient trainer</th>
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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.

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<th>Practice</th>
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<th>Signature of technician receiving training</th>
<th>Name of proficient trainer</th>
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<td>Observe</td>
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<tr>
<td>Protocol #1 with BCL</td>
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<td>Protocol #2 with BCL</td>
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<td>Freezer HIV- PBMC</td>
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<td>Freezer HIV+ PBMC</td>
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<td>Final protocol with sign off by Lab Manager</td>
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#8 EMERGENCY PROCEDURE FOR ACCIDENTAL EXPOSURE TO INFECTIOUS AGENTS – refer to SOP

V. REFERENCES/ADDITIONS/NOTES