


STANDARD OPERATING PROCEDURE				
	Guidelines for working with <i>M.Bovis</i> BCG in BL2+ Laboratory		SOP#	TC-SOP-008
	Originated by:	Alicja Trocha	Date:	23 November 17
	Laboratory:	Walker Laboratory	Pages:	1 of 3
	Approved by:	Alicja Trocha		

## I. PURPOSE

The purpose of this procedure is to outline the guideline for working with *Mycobacterium bovis* BCG in BSL2+ tissue culture laboratories at Ragon Institute.

## II. SAFETY

All users (trainers and trainees) are required to observe the listed guidelines in this SOP. Also, additional requirements may be introduced by the trainer, subject matter expertise (SME) or Ragon Institute Managers.

## III. SCOPE

This procedure applies to all employees, students, contractors and visitors that work with *Mycobacterium bovis* BCG in BSL2+ tissue culture laboratories at Ragon Institute.

## IV. REQUIREMENTS

Training to be received by qualified and approved list of trainers only.

## V. RESPONSIBILITIES

- A. The Ragon Institute **qualified trainers** are responsible for the overall implementation of this procedure and ensuring compliance and for periodic review of this procedure. Updates if any may be initiated by the qualified trainers or Subject Matter Expertise (SME).
- B. **All employees, students, contractors and visitors** are required to follow this procedure.


## VI. GUIDELINES

Mandatory guidelines for working with *M. bovis* BCG in the BL2+(adapted from Fortune lab BCG work guidelines and Ragon SOP#101)

### **Background**

*Mycobacterium bovis* BCG is an attenuated strain of *M. bovis* that was derived by repeated in vitro passage of a bovine tuberculosis isolate at the Pasteur Institute between 1908 and 1919. The resulting attenuated strain has been used as a live vaccine against human tuberculosis in many locations around the world since 1921. The BCG vaccine is not routinely used in the United States. Its efficacy in preventing adult tuberculosis appears limited at best, but vaccination of infants with BCG is effective in preventing tuberculous meningitis in children living in TB-endemic areas.


### **Risks**

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Although BCG does not cause disease in healthy adults, it can establish a limited infection if aerosolized bacteria are inhaled or if bacteria enter the body parenterally via accidental injury. Such an infection may lead to a positive Mantoux (PPD) test, which is used to screen for TB infection. Since conversion due to BCG infection cannot be distinguished from conversion due to *M. tuberculosis* infection, development of a positive test will necessitate lengthy prophylaxis with antibiotics. Transmission of BCG to immunocompromised individuals can potentially result in disease. Because of the risk of aerosol transmission of BCG, all work with this organism must be conducted in an approved and certified biosafety cabinet.

All work with *M. bovis* BCG (BCG) will take place in the BL2+. All general guidelines for work in the BL2+ have to be followed (SOP #1-01). Further mandatory guidelines for the work with BCG are listed below:

1. Always wear disposable lab coat in the Tissue Culture room. Wear it for one day only and dispose in biohazard waste to be autoclaved.
2. Always wear one pair of gloves in the Tissue Culture room. Put second pair when entering hood. This pair **MUST** be removed every time your hands are out the hood. Wear a disposable N95 mask when working with BCG cultures or samples.
3. Inspect your gloves regularly!
4. Wear safety glasses when working in the Tissue Culture Room – **MANDATORY ALL THE TIME.**
5. Before you begin any work in the hood wipe all area with 70% alcohol thoroughly and diligently. Wipe all equipment and tools inside the hood. Keep bottle of fresh made D125 detergent or vesphene in the hood.
6. Spillage outside the hood: few layers of gauze must be put on the spill and soaked in D125 detergent or vesphene. The room where the spill occurs must be evacuated for 1 hour. After 1 hour clean the spill while wearing a N95 mask. Discard contents from the spill into a small red biohazard bag which is then discarded into the large biohazard box and autoclaved ASAP.  
Spillage inside the hood: few layers of gauze must be put on the spill and soaked in D125 detergent or vesphene for 15 minutes. Place gauze into a small red bag wipe all surfaces inside the hood with 70% ethanol and discard waste into a large biohazard bag.
7. Never open exit doors with gloves on, always take gloves off.
8. Keep the area around you clean and tidy. Use centrifugation time to clean the Tissue Culture room: wiping with alcohol all doors, centrifuge, benches etc.

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9. Use a designated hood for all BCG work and label the hood with a sign accordingly.

10. In the hood – make sure that the small bucket and vacuum flask is not full and not over dated. Expose MUST be changed every 7 days, so place tape with date and initials when doing so. Big bucket under the hood must be changed as well. Follow dilution instruction for D125 listed below each sink.

**11. Follow all precautions when using laminar hood: do not work very fast, have your chair sit adjust to high level, so you will not breathe air from the hood, do not keep any obstruction on the hood grid to disturb air flow.**

12. Always spin BCG positive sample in safety centrifuge canisters with the covers on. Do NOT exceed 45 ml in a 50 ml conical tube for centrifuge spins. (Leakage can easily occur from under the tube top if the tube is overfilled.) Do not open the bucket covers outside the hood. Load and unload canisters inside the hood. After spinning inspect, take the closed canisters to the hood and check the inside of the containers for possible leakages. If this occurs, add disinfectant (D125) and allow standing for 15 minutes. Discard all liquid in container into small waste bucket and rinse containers with 70% ethanol. Allow bucket to air dry.

13. Always spin with COVERS on the buckets! Always put tops on the conical and falcon tubes and tie them up. Never spin glass blood tubes – transfer to plastic tubes first.

14. Growing BCG cultures: All BCG stock will be grown in a roller bottle on a shaker at 37°C degrees. BCG cultures will be grown in a designated incubator in the BL2+ TC room only. All growing BCG cultures outside of the hood must be well-labeled as “BCG”. Prior to using roller bottles check the integrity of each bottle by adding some media, turning the cap until finger tight, shaking for 30 minutes, and visually checking for leaks.

**1.** 15. Transport of BCG samples inside the BL2+: Use sealed zip-lock bags to transport BCG samples from the hood to another hood or the incubator. Open bags only inside the incubator and in the hood. For Transport of BCG cultures between BL2+ TC rooms place the sealed bag containing the BCG sample in a Ragon approved transport box for unfixed samples and follow the Ragon guidelines for unfixed infected samples. For further details read (Sample and Specimen transport proto