FOOD SAFETY LABORATORY
CORNELL UNIVERSITY

Spiral Plater Operation Start-up Check Sheet

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for operating the spiral plater and the Q-Count.

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases.

1.3 Definitions

N/A

1.4 Safety

Appropriate protective measures need to be taken when working with bacteria for plating. All waste from these experiments needs to be treated as BSL-2 waste.

SECTION 2 MATERIALS

- **Spiral Plater**
- **Q-count**

SECTION 3 PROCEDURE

3.1 Spiral Plater Operation Start-up Check Sheet

1. Before plating:

1. Fill the bleach and water trays to the top of the "black box" in each reservoir. Do not completely fill the bleach tray. Always use fresh bleach and sterile water.
2. From LEFT to RIGHT (facing the machine), the order of the reservoirs are:
   Water #2------Water #1------Disinfectant
3. Turn on the vacuum source and machine; pump should reach 18psi.
4. Flush the tubing system. Move the stylus to a water tray, lower the stylus into the water and open pinch valve (push "valve"). Observe the water flowing through the tubing from stylus to pinch valve and watch for air bubbles being flushed from the system. When water reaches pinch valve, push "valve" again and run a "clean" cycle.
5. While running the cleaning cycle, LISTEN for any of the following sounds, which are indicative of issues:
   - Excess running of vacuum pump
   - Popping" sounds as the stylus unit slides left and right.
6. LOOK at the syringe to verify there is glycerol residue on the stainless steel plunger

2. While plating:

1. Upon lowering the stylus, you should observe a dry gap (~ ¼") at the tip of the plastic portion of the stylus. If you do not see a gap, get help immediately.
2. Never dip more than plastic portion of the stylus into the liquid you are plating.
3. Make sure that the bleach and two water trays remain at appropriate levels --- above the bottom of the black box.
4. See # 5 above. Also watch that sample drawn up into the syringe fills the stylus completely and make sure liquid is deposited to your plate. If you notice a problem, STOP and find Matt S.
5. If plating "chunky" or non-clear samples, e.g. fish, milk, or homogenized organs, select "expel" to prevent clogging the machine. Otherwise, use a normal "clean" cycle.

3 After plating:

1. Run a "power clean" cycle.
2. Turn off vacuum pump and flush tubing system for 1 minute to leave the tubing full of water (see #4 under before plating).
3. Empty, rinse, and leave the three trays to dry inverted next to the spiral plater.
4. Let plates dry at least 30 min before incubating. Tell Matt if they look bad the next day.

3.2 Q-Count Operation Check Sheet

1. Before counting:

1. Let your plates warm up so that condensate does not affect the count.
2. Verify the Q-Count settings match your plates and preferences
   a. Plating Mode: 50-exponential is standard
   b. Reduce Region: If checked, the outer rim of the plate is NOT counted
   c. Light Colonies: Checked is standard, e.g. LM on BHI
   d. Threshold: 0.1-20mm is standard
   e. Light: Top or Bottom, try to stay constant for a data set
3. Ensure the glass stage is clean and free of any unwanted background disk
4. Make sure the plate is centered inside the purple circle on the screen.

2. While counting:

6. Enter the following information for each plate
    a. Description: Limited to 20 characters. Standard coding systems can easily be split by Excel using 'Text to columns", e.g. strain-treatment-rep
    b. Dilution: In positive log10 units, i.e. '1' for a 1:10 serial dilution
7. Count the plate automatically using 'count'
    a. Adjust the shutter for optimal brightness
    b. Ensure single green dots count only single colonies
    c. Manually edit the count if there is a problem (use sparingly, knowingly)
        i. This can ONLY be used to count the entire plate. DO NOT use this to 'correct' a reduced region, or when the plate has >250 colonies.
        ii. Note calculated CFU/ml. Right click on the image -> 'edit colonies'
        iii. Click unmarked colonies to add, click marked colonies to delete.
        iv. Compare new CFU/ml to previous
8. Record the plate count
    a. 'rep' records the data, maintains description and dilution
    b. 'new' record the data, clears description and dilution, averages all reps.
9. Repeat for all plates

3. After counting:

5. Export data by right clicking on the spreadsheet and 'save as' excel file
6. Exit the software. All data is automatically saved.

SECTION 4 TROUBLESHOOTING

If you have any problems, see Matt S.

SECTION 5 REFERENCES