



FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM

Standard Operating Procedure

Title: Spiral Plater and QCount Operation

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Spiral Plater and QCount Operation

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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth standard guidelines for enumerating microbial cell populations in diluted culture using the Autoplate 4000 and Autoplate 5000 spiral platers and the associated QCount and QCount Color automated colony counters.

1.2 Scope

This SOP applies to activities within the Cornell Food Safety Lab (FSL), Laboratory for Molecular Typing (LMT), the Milk Quality Improvement Program (MQIP), and the Worobo Laboratory. This SOP also applies to guests from other laboratories who have undergone lab safety training and have been permitted to and trained on the correct use of the spiral platers and QCount.

1.3 Definitions

Autoplate 4000: The older spiral plater, nicknamed “Woody.” Requires a vacuum source for operation.

Autoplate 5000: The newer spiral plater, nicknamed “Buzz.”

Disinfectant: A bleach solution used to flush the stylus, tubing, and syringe between samples to prevent cross-contamination of organisms

QCount: Name for both the piece of equipment and the software that uses imaging technology to automatically count the colonies on a petri dish and estimate the concentration of the original culture

Spiral Plater: A machine that deposits a microbial culture on a petri dish in a spiral pattern for the purposes of enumeration. Often, the volume deposited in different regions of the spiral is altered to avoid performing dilutions on the culture.

Stylus: Aspirates and deposits the sample in a spiral pattern onto the surface of the rotating agar plate.

Stylus Tubing: The transparent PTFE tubing that is comprises the stylus and connects to the syringe

Syringe: The cylindrical glass tube and plunger visible on the right side of the instrument that is responsible for metering the correct volume of sample onto the petri dish

Turntable: The rotating platform that holds the petri dish during plating

Vacuum Source: A vacuum pump and trap flask that aspirates sample from the stylus and syringe during the cleaning cycle of the Autoplate 4000



1.4 Safety

Operation of the spiral platers may require work with open tubes of organisms that are either known or unknown but potentially BSL-2 organisms. Proper PPE, as outlined in lab safety training, must be worn, and proper aseptic technique must be followed. Any spills must be safely and correctly contained and cleaned.

Bleach (sodium hypochlorite) solutions are used as disinfectants for both spiral platers. Bleach is a powerful oxidizer that will severely damage human tissue and clothing. Proper PPE is required when handling bleach (gloves, lab coat, safety glasses).

Decon90 contact with the skin and eyes may cause severe irritation. Exercise caution and wear appropriate PPE when handling Decon90.

SECTION 2 MATERIALS

For Spiral Plating:

Autoplate 4000	Autoplate 5000
<ul style="list-style-type: none"> • Sterile, ultrapure water (<i>Room 350A</i>) • <u>5%</u> sodium hypochlorite solution (<i>Shelf above spiral plater, recipe in Appendix</i>) • 70% ethanol • 4% Decon90 solution (<i>Shelf between the two spiral platers, recipe in Appendix</i>) • Disposable sample cups (<i>Cabinet below spiral plater</i>) • Permanent marker • Appropriately concentrated microbial culture in a 1.5 or 2 mL microcentrifuge tube • Vortex mixer • Sterile 100 mm petri dishes containing appropriate medium 	<ul style="list-style-type: none"> • Sterile, ultrapure water (<i>Room 350A</i>) • <u>1%</u> sodium hypochlorite solution (<i>Shelf above spiral plater, recipe in Appendix</i>) • 4% Decon90 solution (<i>Shelf between the two spiral platers, recipe in Appendix</i>) • Disposable sample cups (<i>Cabinet below spiral plater</i>) • Permanent marker • Appropriately concentrated microbial culture in a 1.5 or 2 mL microcentrifuge tube • Vortex mixer • Sterile 100 mm petri dishes containing appropriate medium

For QCounting:

Autoplate 4000	Autoplate 5000
<ul style="list-style-type: none"> • Incubated inoculated petri dishes: spread, pour, or spiral plated • Ethanol • Kimwipes • USB flash drive 	<ul style="list-style-type: none"> • Incubated inoculated petri dishes: spread, pour, or spiral plated • Ethanol • Kimwipes



For Preventive Maintenance and Validation:








Autoplate 4000	Autoplate 5000
<ul style="list-style-type: none"> • Glycerol • Transfer pipette • White lithium grease • Kimwipes • Validation test fixture • Sterile ultrapure water • Acid cleaner (0.5 N H₂SO₄) • Crystal violet dye • Disposable sample cups 	<ul style="list-style-type: none"> • Glycerol • Transfer pipette • White lithium grease • Kimwipes • Validation test fixture • Sterile ultrapure water • Acid cleaner (0.5 N H₂SO₄) • Crystal violet dye • Disposable sample cups

SECTION 3 PROCEDURES









Spiral Plating

Autoplate 4000	Autoplate 5000
<p>1. Place your initials and the time of use on the spiral plater usage calendar. Usage logging is required, even if the machine is free, as it assists with scheduling multiple experiments as well as preventive maintenance and troubleshooting.</p>	
<p>2. Agar plates for spiral plating should be dry and free of visible condensation. This typically requires ~48 hours of drying while inverted after the plates are made, and 24-48 hours of drying while inverted after the plates are removed from the cold room.</p>	
<p>3. Label the plate lids with pertinent information to your experiment, which should, at minimum, include:</p> <ul style="list-style-type: none"> • Organism • Medium • Date • Initials of experimenter <p><i>Plates with writing on the bottom will not be counted accurately by the QCount!</i></p>	
<p>4. Mark the side of each dish with a single vertical line. This will be used to align the dish during plating and counting.</p>	
<p>5. Ready all cultures for plating by transferring the cultures into 1.5 or 2 mL microcentrifuge tubes and performing any necessary dilutions. Using default settings, cultures must have a concentration in the range of 4.0×10^2 - 4.0×10^5 to be countable. Take the amount of time that will be spent plating into consideration; it may be advisable to store the cultures and dilutions on ice.</p>	
<p>6. Remove the dust cover from the spiral plater. Disinfect the reservoirs labeled <i>Water 1</i> and <i>Water 2</i> by spraying with</p>	<p>6. Remove the dust cover from the spiral plater. Power on the spiral plater using the labeled switch on the lower left side of the instrument.</p>



Autoplate 4000	Autoplate 5000
70% ethanol and wiping with a Kimwipe. Allow to air-dry.	
7. Place the reservoirs in the cleaning station on the left side of the instrument in the following order, from left to right: <i>Water 2.....Water 1.....Disinfectant</i>	7. Check the liquid levels in the water bottle and the disinfectant bottle. The bottles should be full from the previous user.
8. Fill the reservoirs up to the top of their liquid level bands either with sterile ultrapure water for the <i>Water</i> reservoirs or with 5% sodium hypochlorite for the <i>Disinfectant</i> reservoir.	8. Refill the water and disinfectant bottles if they are not full. 8.1. Pull one or both bottles gently from their compartment and place upright on the benchtop.
9. Place a sample cup in the upper left well of the block on the right side of the instrument to receive expelled sample.	8.2. To open, turn the bottle while holding the lid stationary to unscrew it without twisting the attached tubing. Leave the lid sitting on top of the bottle.
10. Power on the vacuum source using the switch on the front of the instrument. The pressure gauge on the vacuum source will reach a reading of ~18 psi, and then the pump will shut off.	8.3. Top off the water bottle with sterile ultrapure water, and/or top off the disinfectant bottle with 1% sodium hypochlorite solution.
11. Remove the dust cover from the spiral plater. Power on the spiral plater using the <i>POWER</i> switch on the left side of the instrument keypad.	8.4. Screw the lid back onto the bottle and return the bottle(s) to their compartment. 8.5. To reset the water level indicator and purge any air that has been introduced into the tubing, the system must be primed. 8.5.1. Touch the  button. 8.5.2. Touch the  button. 8.5.3. Touch the  button 8.5.4. Touch the  button.
12. The spiral plater must be cleaned before use. 12.1. Press the  button. The LED next to the button will light to indicate that this setting is selected.	8.6. When priming has completed, touch the  button to return to the home screen. 9. The spiral plater must be cleaned before use. 9.1. Press the  button beneath the touch screen to start the clean cycle.




















Autoplate 4000	Autoplate 5000
<p>12.2. Press the  button to start the clean cycle.</p> <p>12.3. When the clean cycle has completed, press the  button again to deselect this option.</p>	
<p>13. Both the machine and lab default plating settings are exponential deposition of 50 μL onto 100 mm plates. This is indicated by the lit LEDs next to the buttons  and . Details on alternative plating modes can be found on pages 22-24 of the user manual.</p>	<p>10. The home screen displays the main six plating options, which can be changed by touching them:</p> <div style="display: flex; flex-wrap: wrap; justify-content: space-around;"> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Clean mode</div> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Depositor mode</div> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Fill Volume</div> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Plate size</div> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Replicates</div> <div style="border: 2px solid black; padding: 5px; margin: 5px;">Sample container</div> </div> <p>10.1. Clean Mode should always be set to <i>Normal Clean</i></p> <p>10.2. Deposition Mode will most normally be set to the lab default of  (50 μL exponential). Information on alternate deposition modes can be found on pages 31-33 of the manual.</p> <p>10.3. Fill Volume</p> <p>10.3.1. <i>Max</i> will draw up 500 μL of sample.</p> <p>10.3.2. <i>Min</i> will draw up the appropriate volume of sample given the settings for Deposition Mode and Replicates.</p> <p>10.4. Plate Size will always be set to 100 mm.</p> <p>10.5. Replicates can be set to any number between 1 and 10.</p> <p>10.6. Sample Container should be set to  (test tube).</p>
<p>14. Lower the stylus by pressing the STYLUS  button.</p>	<p>11. Press the  button below the touch screen to begin the plating cycle.</p>
<p>15. Briefly vortex the microcentrifuge tube containing the sample to be plated. Open</p>	<p>12. Briefly vortex the microcentrifuge tube containing the sample to be plated. Open</p>











Autoplate 4000	Autoplate 5000
<p>the tube and place the tip of the stylus beneath the surface of the sample, so that no more than half of the exposed stylus tubing is immersed.</p>	<p>the tube and place the tip of the stylus beneath the surface of the sample, so that no more than half of the exposed stylus tubing is immersed.</p>
<p>16. Draw the sample into the stylus tubing using one of the following two methods:</p> <p>16.1. Press the FILL <input type="button" value="MAX"/> button to draw up the maximum volume of sample, 250 μL.</p> <p>16.2. Press the FILL <input type="button" value="MIN"/> button to draw up the minimum volume of sample to plate one replicate, typically 50 μL. Once the sample has been drawn up, the button can be pressed up to four more times to draw up sample for additional replicates.</p> <p>16.3. Remove and cap the sample tube.</p>	<p>13. Draw the sample into the stylus tubing.</p> <p>13.1. Touch the <input checked="" type="checkbox"/> button to draw a small amount of sample into the stylus tubing.</p> <p>13.2. Expose the tip of the stylus to air by removing the sample tube. Touch the <input checked="" type="checkbox"/> button to draw up a small volume of air, creating an air gap.</p> <p>13.3. Return the sample tube to its position with the stylus tubing inserted. Touch the <input checked="" type="checkbox"/> button to draw up the sample to be plated.</p> <p>13.4. Remove and cap the sample tube.</p>
<p>17. Load the petri dish onto the turntable</p> <p>17.1. Use the bottom edge of the petri dish to push the spring-loaded adaptor brace back.</p> <p>17.2. Center the plate on the turntable against the spring-loaded brace and the fixed brace.</p> <p>17.3. Align the line on the side of the plate with the radial line engraved on the turntable surface.</p> <p>17.4. Remove the lid from the petri dish.</p>	<p>14. Load the petri dish onto the turntable</p> <p>14.1. Use the bottom edge of the petri dish to push the spring-loaded adaptor brace back.</p> <p>14.2. Center the plate on the turntable against the spring-loaded brace and the fixed brace.</p> <p>14.3. Align the line on the side of the plate with the radial line engraved on the turntable surface.</p> <p>14.4. Remove the lid from the petri dish.</p>
<p>18. Press the <input type="button" value="PLATE"/> button to begin plating. As the turntable spins, the stylus will lower onto the agar and move radially outward. Simultaneously, the downward movement of the syringe plunger deposits sample onto the plate.</p>	<p>15. Touch the <input checked="" type="checkbox"/> button to begin plating. As the turntable spins, the stylus will lower onto the agar and move radially outward. Simultaneously, the upward movement of the syringe plunger deposits sample onto the plate.</p>
<p>19. Replace the petri dish lid. Remove the dish from the turntable. Do not invert the plate until it has had a chance to dry.</p>	<p>16. Replace the petri dish lid. Remove the dish from the turntable. Do not invert the plate until it has had a chance to dry.</p>



Autoplate 4000	Autoplate 5000
<p>20. If multiple replicates are desired, repeat steps 17-19. Top off the reservoirs if at any point their levels drops below the bottom of their liquid level bands.</p>	<p>17. If multiple replicates are desired, load the next petri dish onto the turntable and then touch the  button to plate the next petri dish.</p>
<p>21. The spiral plater must be cleaned between samples.</p> <p>21.1. If more sample was drawn up than was used for plating, press, the  button to prevent this sample from being drawn through the syringe.</p> <p>21.2. Press the  button to initiate the cleaning cycle.</p>	<p>18. Following the plating of the final replicate, the spiral plater will automatically run a clean cycle.</p> <p>18.1. If you wish to run a cleaning cycle early and discard the remaining sample, touch the  button.</p>
<p>22. Once the cleaning cycle is complete, repeat steps 14-21 to plate additional samples.</p>	<p>19. Once the cleaning cycle is complete, the home screen is displayed. Repeat steps 10-18 to plate additional samples.</p>
<p>23. For samples other than broth cultures and dilutions (milk, fish, chocolate, pet food, etc), a Decon90 rinse must be performed prior to shutting down the spiral plater.</p> <p>23.1. Fill a sample cup with 4% Decon90 solution: Press the STYLUS  button to lower the stylus into the solution.</p> <p>23.2. Press the  button to open the valve, and wait 5 seconds. Press the  button again to close the valve. This fills the stylus, stylus tubing, and syringe with detergent.</p> <p>23.3. Wait 1-5 minutes.</p> <p>23.4. Press the STYLUS  button to raise the stylus.</p> <p>23.5. Open and close the valve as described in step 23.2 to purge the system with air.</p>	<p>20. For samples other than broth cultures and dilutions (milk, fish, chocolate, pet food, etc), a Decon90 rinse must be performed prior to shutting down the spiral plater.</p> <p>20.1. Navigate to the manual operation screen by touching , then .</p> <p>20.2. Fill a sample cup with 4% Decon90 solution. Touch the Stylus  and  buttons to move the stylus to the filling station and lower it into the solution.</p> <p>20.3. Touch the Syringe Fill  button to fill the stylus and tubing with Decon90 solution.</p> <p>20.4. Wait 1-5 minutes.</p> <p>20.5. Touch the Syringe  button until the Syringe Volume on the screen reads 0.</p> <p>20.6. Touch the Stylus  and  buttons to move the stylus to the wash station. Touch the stylus .</p>



Autoplate 4000	Autoplate 5000
	<p>button to lower the stylus into the wash station.</p> <p>20.7. Touch the  Flush button to begin flushing the system with water. Wait 30 seconds.</p> <p>20.8. Touch the  Flush button a second time to turn off the water.</p> <p>20.9. Press the  button to return to the main menu screen.</p>
<p>24. Once plating of all samples is complete, the spiral plater must be shut down.</p> <p>24.1. Press the STYLUS  button three times to move the stylus to the <i>Water 2</i> station.</p> <p>24.2. Press the STYLUS  button to lower the stylus into the water.</p> <p>24.3. Press the  button to open the valve and fill the stylus, syringe, and tubing with water. Leave the valve open until all air has been purged from the tubing and a steady stream of water drops enters the trap flask.</p> <p>24.4. Press the  button again to close the valve.</p> <p>24.5. Press the STYLUS  button to raise the stylus.</p>	<p>21. Once plating of all samples is complete, the spiral plater must be shut down.</p> <p>22. Refill the water and disinfectant bottles as described above in step 8.</p>
<p>25. Power off the spiral plater using the <i>POWER</i> switch on the left side of the instrument keypad.</p>	<p>23. Power off the spiral plater using the labeled switch on the lower left side of the instrument.</p>
<p>26. Empty the contents of the reservoirs down the drain. Rinse the <i>Disinfectant</i> reservoir with tap water. Leave the reservoirs to dry, inverted, on a paper towel.</p> <p>27. Replace the dust cover.</p>	<p>24. Empty the cleaning station waste container down the drain using the built-in spout.</p>
<p>28. Empty the trap flask if it contains more than 500 mL of waste.</p> <p>28.1. Disconnect the inlet and outlet tubing from the trap flask.</p>	<p>25. Replace the dust cover.</p>



Spiral Plater and QCount Operation
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Autoplate 4000	Autoplate 5000
28.2. Dispose of the flask contents down the drain. 28.3. Reconnect the inlet and outlet tubing. 29. Replace the dust cover.	



SECTION 4 TROUBLESHOOTING

Troubleshooting for common spiral plater issues is covered in the Autoplate User Guides and the VS2 user guide stored in the drawers next to each spiral plater (Autoplate 4000 – pg. 77; Autoplate 5000 – pg. 85). Specific issues we have encountered that are not discussed in the user manuals are covered below. **Any deviation from expected spiral plater operation should not be ignored, and should be reported immediately to an individual responsible for spiral plater maintenance. All plates prepared on a spiral plater experiencing any issues should be expected to be inaccurate and unreliable.**

Spiral plater technical support is provided by Advanced Instruments free of cost through the year 2020. Technical support can be reached by calling 1-800-225-4034 and dialing ext. 2123 or by asking for Mike Talbot if an operator answers. Mike’s e-mail address is miket@aicompanies.com. Make sure you know the serial number of the instrument in question:

- Autoplate 4000 - AP4A255 (tech support is no longer provided for this instrument)
- Autoplate 5000 - 13095599C
- QCount – 05090956B (tech support is no longer provided for this instrument)
- Color QCount – 13115872D

Autoplate 4000

Issue	Possible Cause	Other Symptoms	Solution
Vacuum pump will not shut off because pressure has not reached 15” Hg	Condensation formation within pump	Pump sounds quieter than usual	Disconnect the vacuum tube from the side arm of the waste flask. Turn on the pump, and allow it to run continuously for 15 minutes. Turn off the pump, reconnect the vacuum tube, and turn the pump back on.
	Pinch valve is open	Valve indicator light is lit	Close the valve
	Tubing is not securely connected	None	Ensure that the stopper is seated firmly inside the flask and that the vacuum tube is secured firmly onto the side arm. Replace the tubing, if necessary.



Issue	Possible Cause	Other Symptoms	Solution
	Waste flask is overfilled	None	Empty the waste flask and reattach the tubing. Do not allow more than 500 mL of liquid to accumulate in the waste flask.
	Waste flask is cracked	Bubbles forming inside the flask, hissing sound coming from flask	Replace flask
Large bubbles appear in the syringe during plating (bubbles during cleaning are normal)	Clogged or leaky stylus tubing	Uneven deposition of sample on plate, irregular pattern of bubbles through the syringe during cleaning or no movement through syringe during cleaning	Backflush the stylus tubing as described in the User Guide. Replace the stylus tubing, if necessary.
	Clogged or leaky syringe	Uneven deposition of sample on plate, irregular pattern of bubbles through the syringe during cleaning	Backflush the syringe as described in the User Guide. Replace the syringe, if necessary.
	Water reservoir(s) are underfilled or empty	Uneven deposition of sample on plate, irregular pattern of bubbles through the syringe during cleaning	Refill water reservoirs and replate all samples that were plated with empty reservoirs.
	Vacuum source is turned off	Uneven deposition of sample on plate, no movement through syringe during cleaning	Turn on vacuum source and replate all samples that were plated without the vacuum turned on.



Autoplate 5000

Issue	Possible Cause	Other Symptoms	Solution
Replicate plates have counts significantly lower than the first plate	Bleach contamination of water or sample	Chlorine test strips show high levels in water bottle or water from stylus	Replace reagent bottle quick connects, umbrella valves, o-rings, and tubing, check for broken valves (see below)
	Broken isolation valve and/or pump supply valve	Visible bubbles in valve tubing or coming from stylus during cleaning cycles, liquid creeping up stylus tip, chlorine contamination of water supply	Replace the broken valve(s)
	Leaky syringe	Bubbles forming in the syringe during plating that are not solved by (i) backflushing of the syringe and stylus, (ii) by purging the syringe of air, or (iii) refilling the empty reagent bottles	Replace the syringe
	Leaky stylus tubing	Bubbles forming in the syringe during plating that are not solved by (i) backflushing of the syringe and stylus, (ii) by purging the syringe of air, or (iii) refilling the empty reagent bottles	Replace the stylus tubing
Large bubbles appear in the syringe	Empty water bottle	No water exits the stylus at the cleaning station	Refill the water bottle, run a priming cycle, and repeat all



Issue	Possible Cause	Other Symptoms	Solution
			plating that has been performed without water. If large bubbles still appear, follow troubleshooting instructions in User Guide (pp. 86-87)
	Leaky syringe	Bubbles forming in the syringe during plating that are not solved by (i) backflushing of the syringe and stylus, (ii) by purging the syringe of air, or (iii) refilling the empty reagent bottles	Replace the syringe
Loud, grinding noise when performing a max fill	Rust on the syringe lead screw	None	Lubricate syringe lead screw with lithium grease and perform several max fills to work it in
Unresponsive or inaccurate touch screen	Touch panel has become misaligned	None	Remove right side cover and splash panel, adjust touch screen position, and tighten screws.



SECTION 5 REFERENCES

- Manuals from the Autoplate 4000, Autoplate 5000, VS2, QCount, and Color QCount
- Communication with Mike Talbot at Advanced Instruments

SECTION 6 APPENDIX

Disinfectant Solutions – Yields 3.5 L

Ingredient	Autoplate 4000 (5% bleach)	Autoplate 5000 (1% bleach)
ddH ₂ O	1.38 L	3.076 L
Commercial bleach (8.25% sodium hypochlorite)	2.12 L	0.424 L

Store in an opaque container away from heat and direct sunlight.